PART I

Prefatory

A large meal such as this breakfast contains representatives of most types of biological molecules, whose study forms the subject matter of Biochemistry.

> "There is no greater object of wonder, no greater thing of beauty, than the dynamic order, the organized complexity of life."
>
> *—Ariel G. Loewy and Philip Siekevitz : Cell structure and Function, 1969.*

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THE DIVERSITY OF LIVING SYSTEMS

The distinct morphology of the 3 organisms – a plant (*Amborella trichopoda*), an animal (frog, *Rana* sp.) and a microorganism (bacteria)– might suggest that they have little in common. Yet, biochemically they display a remarkable commonality that attests to a common ancestry.

C H A P T E R

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Introduction

DEFINITION

The term **Biochemistry** (*bios*[G] = life) was first introduced by a German chemist Carl Neuberg in 1903. Biochemistry may be defined as a science concerned with the chemical nature and chemical behaviour of the living matter. It takes into account the studies related to the nature of the chemical constituents of living matter, their transformations in biological systems and the energy changes associated with these transformations. Such studies have been conducted in the plant and animal tissues both. Broadly speaking, biochemistry may thus be treated as a discipline in which biological phenomena are analysed in terms of chemistry. The branch of biochemistry, for the same reason, has been variously named as **Biological Chemistry** or **Chemical Biology**.

In fact, biochemistry originated as an offshoot from human physiology when it was realized that the chemical analysis of urine, blood and other natural fluids can assist in the diagnosis of a particular disease. Hence in its infancy, biochemistry was accordingly known as **Chemical Physiology**. But physiology now covers the study of normal functions and phenomena of living beings. And biochemistry is concerned particularly with the chemical aspects of these functions and phenomena. In other words, biochemistry is but one of the many ways of studying physiology. The two may be compared by watching the monkeys in a zoo which means studying the physiology of behaviour. But if the behaviour of animal molecules is studied, rather than the whole animals, it would form the study of biochemistry.

Modern biochemistry has two branches, descriptive biochemistry and dynamic biochemistry. **Descriptive biochemistry** is concerned with the qualitative and quantitative characterization of the various cell components and the **dynamic biochemistry** deals with the elucidation of the nature and the mechanism of the reactions involving these cell components. While the former branch is more a concern of the organic chemist, the latter branch has now become the language of modern biochemistry.

However, as the knowledge of biochemistry is growing speedily, newer disciplines are emerging from the parent biochemistry. Some of the disciplines are **enzymology** (science of the study of enzymes), **endocrinology** (science dealing with the endocrine secretions or the hormones), **clinical biochemistry, molecular biochemistry** etc. Along with these branches certain link specialities have also come up such as agricultural biochemistry, pharmacological biochemistry etc.

HISTORICAL RESUME

In terms of history, biochemistry is a young science. It started largely as an offshoot of organic chemistry and later incorporated ideas obtained from physical chemistry. In fact, the science of biochemistry may be regarded to have begun with the writings of **Theophrastus Bombastus von Hohenheim,** better known as **Philippus Aureolus Paracelsus** (Lifetime or LT, 1493–1541), a Swedish physician and alchemist who laid the foundation of chemotherapy as a method of treating diseases and also the promoter of '*doctrine of signatures*.' He first acquired the knowledge of chemistry of his time and then entered the field of medicine to apply his knowledge of chemistry. He proclaimed, "Life processes are essentially of chemical nature and diseases can be cured by medicines". Later, his followers notably **Jan Baptist van Helmont** (LT, 1577–1644) amalgamated the science of chemistry with medicine which emerged under the name of 'medical chemistry' (or Iatrochemistry).

The basis of biochemistry was, in fact, laid down by chemists like Scheele and Lavoisier. **Karl Wilhelm Scheele** (LT, 1742–1786), a swedish pharmacist, discovered the chemical composition of various drugs and the plant and animal materials. He also isolated a number of substances such as citric acid from lime juice, lactic acid from sour milk, malic acid from apple and uric acid from urine. Scheele, thus, laid the foundation of descriptive biochemistry. Similarly, **Antoine Lavoisier** (LT, 1743–1794), a French chemist, who studied the composition of air and propounded the theory of conservation of matter, put dynamic biochemistry on firm standfootings. He developed the concept of oxidation and also clarified the nature of animal respiration. He had concluded that respiration could be equated with combustion and that it was slower but not essentially different from the combustion of charcoal. Lavoisier is often spoken of as '*father of modern biochemistry*'.

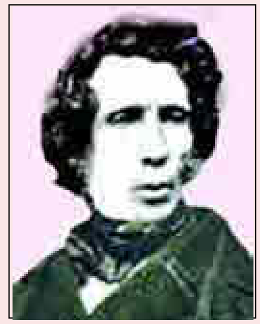
Scheele can probably be called the unluckiest chemist in History, for although he discovered several elements such as barium, chlorine, manganese and many others, he does not receive undisputed credit for having discovered even a single one.

The earliest book relating to Biochemistry was *'Lectures in Animal Chemistry'* published by the famous Swedish chemist **Jöns Jacob Berzelius** (LT, 1779–1848), in 1806.

In 1828, **Friedrich Wöhler**, a German chemist, synthesized urea, a substance of biological origin, in the laboratory from the inorganic compound ammonium cyanate. This achievement was the unexpected result of attempts to prepare ammonium cyanates through the treatment of metal cyanates with ammonium salts. As Wöhler phrased it in a letter to a colleague, "I must tell you that I can prepare urea without requiring a kidney or an animal, either man or dog." This was a shocking statement in its time, for it breached the presumed barrier between the living and the nonliving. Consequently, this rendered the vitalistic theory of organic materials untenable. *Vitalistic theory* (= *the doctrine of vitalism*) maintained that organic compounds could be synthesized only through the agency of a vital force, supposed to be present only in living tissues.

Wöhler's work was followed by the synthesis of acetic acid from inorganic materials by another German chemist **Adolf Wilhelm Hermann Kolbe** (LT, 1818–1884), in 1845. However, the final blow to the theory of vital force was given by a French Chemist **Pierre Eugene Marcellin Berthellot** (LT, 1827–1907) who synthesized a host of organic compounds (such as methyl alcohol, ethyl alcohol, methane, benzene, acetylene) from inorganic compounds in 1850s. Vitalism was, thus, quietly laid to rest. Organic synthesis remains very much alive.

FRIEDRICH WÖHLER
(LT, 1800-1882)



Wöhler, a German chemist, is well-known for the historical synthesis of urea. He worked with Liebig on the benzoyl derivative. Wöhler earned worldwide acclaim for his teaching during his 46 years at the University of Göttingen. Between 1845 and 1866, he lectured to some 8,250 students. In his lifetime, 13 and 15 editions of his Organic and Inorganic texts, respectively, were published. He is also credited with the discovery of calcium carbide and isolation of beryllium and yttrium.

Justus von Liebig, a German chemist and discoverer of chloroform and who is often termed as '*father of agricultural chemistry*', arrived at the conclusion that "the nutritive materials of all green plants are inorganic substances." He wrote many books which provided an impetus in the early development of biochemistry. Of special interest is his book '*Organic Chemistry in Its Applications to Physiology and Pathology*', published in 1842. **Michel Chevreul** (LT, 1786–1889) demonstrated through studies on saponification that fats were composed of glycerol and fatty acids, of which he isolated several. The excellent researches conducted by the great German biochemist **Hermann Emil Fisher** (LT, 1852–1919) may be regarded as landmark in the development of structural biochemistry. In the course of his studies, this remarkable man completely revolutionized research concerning the structures of carbohydrates, amino acids and fats. Although nucleic acids are the newest of the 4 great groups of biochemical materials, their discovery goes back to observations by **Friedrich Miescher** (LT, 1844–1895) in 1869. His discovery of nucleic acids in the nuclei of pus cells, obtained from discarded surgical bandages, led him to investigate the distribution and the properties of these compounds.

JUSTUS VON LIEBIG
(LT, 1803–1873)

Justus von Liebig, by age 36, headed the world's largest laboratory and school for training chemists. He was one of the early investigators of large-scale research. Today, his laboratory with its furnishings is preserved as a museum in Giessen, Germany. Liebig's oft-repeated quotation, for which he is famous, reads as :

"The secret of all those who make discoveries is to look upon nothing as impossible."

During the first half of the nineteenth century, the studies on heat conducted mainly by **V. Mayer** and **Ludwing von Helmholtz** (LT, 1821–1894) led to the formulation of the 'laws of thermodynamics' which are essential to the understanding of energy relations in biological systems.

Besides respiration, the other physiological process to attract the attention of biochemists was that of digestion. Main contributions towards this were made by **van Helmont, Abbé Lazaro Spallanzani** (LT, 1729–1799), **René Antoine de Réaumur, William Beaumont** and Claude Bernard. **Claude Bernard** (LT, 1813–1878) of Paris was perhaps the greatest of these. His contributions included the discovery of liver glycogen and its relation to blood sugar in health and disease. He noted the digestive properties of pancreatic juice and began research in muscle and nerve physiology.

The process of fermentation is probably the single most important process around which the interest of biochemists persisted for a considerable period. By about 1780, fermentation had been recognized by **Theodor Schwann** (LT, 1810–1882) as a biological process. He established that yeast was a plant capable of converting sugar to ethanol and carbon dioxide. However, many of the leading chemists of the day, including Berzelius, Wöhler and Leibig, considered yeast to be nonliving and fermentation to be caused solely by oxygen. Ridicule by Liebig and Wöhler delayed acceptance of Schwann's views until the illustrious French microbiologist **Louis Pasteur** (LT, 1822–1895) presented evidence overwhelming all objections. He founded the useful branch of Microbiology in 1857 and identified several organisms that carried out various fermentations, including that leading to butyric acid, a type performed by organisms that function without oxygen. He defined fermentation as "*la vie sans l'air*" (life without air). Pasteur, thus, introduced the concept of aerobic and anaerobic organisms and their associated fermentations. These conclusions again aroused the ire of Liebig who again took sharp exception but this time with less effect. Such studies on fermentations climaxed by the demonstration in 1887 of **Eduard Buchner** (LT, 1860–1917) that sugars could be fermented by cell-free extracts of yeast. This discovery represents the cornerstone of much of the enzymological and metabolic study of the twentieth century since it led to techniques of isolation and identification that ultimately permitted the study of enzymes and the individual reactions concerned. It also expunged, yet once and for all, any traces of vitalism (or vitalistic theory) still lingering.

Researches conducted by pioneers such as Arrhenius, van't Hoff and Ostwald on electrolytic dissociation and osmotic pressure led physical chemists to turn their attention to biological phenomena. **Soren Sörensen** (LT, 1868–1939), a Danish chemist, developed our concept on pH, **Jacques Loeb** (LT, 1859–1924) studied the colloidal behaviour of proteins and their effect on the cell, **Leonor Michaelis** placed the concept of chemical compound formation between enzyme and substrate on an experimental basis and **Wendell Stanley,** a biochemist working at the Rockfeller Institute, New York, showed that viruses are nucleoproteins. For his fundamental research on TMV, Stanley was awarded a share of the 1946 Nobel Prize in Chemisty. Later, many instruments were invented such as Van Slyke blood gas apparatus, the ultracentrifuge of **Theodore Svedberg** (LT, 1884-1971) and the electrophoresis apparatus of **Arne W.K. Tiselius**. The use of isotopes in biochemical research by **Urey** and **Schoenheimer** and the application of chromatography, first developed by **Martin** and **Synge,** opened a new chapter in modern biochemistry.

With the advent of twentieth century, biochemistry burst into full bloom. Important developments took place rapidly on several fronts including nutrition. The significance of unknown food factors was clearly recognized by **Frederick Gowland Hopkins** at Cambridge University and his associates, who developed the *concept of deficiency diseases*. Extensive series of feeding experiments utilizing synthetic diets were conducted mainly by Babcook McCollum, Osborne, Mendel and Sherman. As a result, many deficiency diseases such as scurvy, rickets, beriberi and pellagra were recognized and their curative agents, which were called vitamins by a Polish biochemist **Casimer Funk,** were isolated and subsequently characterized.

Buchner's work on cell-free fermentation of sugars was actively extended in many laboratories, including those of **Harden and Young, Embden and Meyerhof,** with the result the complete biochemical pathway known as *Embden-Meyerhof-Parnas pathway* (or *glycolysis*) was elucidated. The researches conducted by **Warburg, Heinrich Wieland, Keilin** and **Theorell** led to the discovery of enzymes and cofactors involved in cellular oxidation. Later, **Fritz Albert Lipmann** (LT, 1899–1986) and **Kurt Henseleit** made notable observations on the significance of the terminal pyrophosphate linkages of ATP as an energy storage reservoir. **Albert Szent-Györgyi** and **Hans Adolf Krebs** (LT, 1900–1981) of England studied the fate of lactate (or pyruvate) during aerobic oxidation. This led to the development of a sequence of reactions known as *Krebs cycle* (or *citric acid cycle*). Later studies revealed that the fatty acids and amino acids, upon oxidation, also yield intermediates that are identical with those in the Krebs cycle, thus providing a common mechanism for the liberation of energy from all foodstuffs. **Frederick Sanger** established the complete amino acid sequence of the protein hormone insulin and **du Vigneaud** proved the structure of the nonapeptide hormones of posterior pituitary by direct synthesis.

The brilliant studies by **Linus Carl Pauling** (LT, 1901–1994) and **Robert Corey** (LT, 1897–1971) led to the concept of a secondary structure of protein molecules in the form of an α-helix. A similar kind of structure for the nucleic acids was also elucidated. **James Dewey Watson** and **Francis Harry Compton Crick,** in 1953, proposed that a double-stranded DNA molecule could be made by binding bases on adjacent strands to each other by hydrogen bonding. This *base-pairing hypothesis* was confirmed by the quantitative data of an Austrian refugee biochemist, **Erwin Chargaff** and was soon followed by the enzymatic synthesis of DNA by **Arthur Kornberg.** These synthetic macromolecules have properties that suit the Watson-Crick hypothesis. Soon after, the base sequence of transfer RNA molecules specific for different amino acids was determined by many workers such as **Holley, Medison, Zachan** and others. A final and accurate list of the base sequences in messenger RNA that code for each of the amino acids was made available as a result of the brilliant researches by **Marshall Nirenberg**, who used synthetic nucleotides as messenger molecules to identify the coded base sequences for each of the amino acids.

By 1835, **Jönes Jacob Berzelius,** a Swedish chemist, had clearly recognized the importance of catalysis in controlling the rates of chemical processes. He suggested that the formation of biological materials was controlled by catalytic actions and cited an enzyme from potato (*i.e.*, *potato diastase*) as an example of a biological catalyst effective in the hydrolysis of starch. He reasoned that all materials of living tissues are formed under the influence of catalytic action, a conclusion thoroughly established by subsequent work. Later, many catalysts were isolated, purified to some extent and the associated reactions investigated kinetically. However, the chemical nature of these so-called biocatalysts or enzymes remained unknown until 1926, when **James B. Sumner** (LT, 1887–1955) at Cornell University, for the first time, crystallized the enzyme *urease* from the extracts of Jack bean and demonstrated its protein nature. For many years, Sumner's discovery was greeted with skepticism and derision, most particularly by the renowned German biochemist **Richard Willstätter**, an authoritative figure, who insisted that enzymes are low-molecular-weight compounds and that the protein found in urease crystals was merely a contaminant. Sumner was a tenacious man, armed with a body of convincing evidence and did not surrender to authority and even successfully repeated his experiments in Willstätter's laboratory. Subsequently, **John H. Northrop** and **Kunitz** crystallized a series of pancreatic and gastric enzymes. This work and subsequent isolation studies clearly confirmed that enzymes are proteins and established Sumner as the '*father of modern enzymology*'. Invaluable work in this field has been accomplished by **Stanford Stein**, **William Moore, Max F. Perutz, John C. Kendrew and David C. Phillips.**

Perhaps the greatest impetus in biochemical researches is nowadays being given to the mechanisms for the regulation of the synthesis of cellular compounds. The phenomenon of feedback inhibition of enzyme activity by the end product of a reaction sequence illustrates a self-regulating mechanism. Enzyme induction and repression—the acceleration or inhibition of synthesis of an enzyme—have been described. These discoveries have led to a hypothesis proposed in 1961 by two Frenchmen, **Francois Jacob** and **Jacques Monod,** suggesting that the DNA molecules consist of areas in which genes are maintained in an inactive state (by repressors) until they need to be activated for the production of messenger RNA molecules. In 1963, Jacob and Monod, with **Jean-Pierre Changeux**, also

> Both **Jacob** and **Monod** shared the 1965 Nobel Prize for their work on the discovery of a class of genes which regulate the activities of other genes, along with **A. Lwoff**, their compatriot. Jacob, whose medical studies were interrupted by World War II, was seriously wounded while serving in the Free French forces and was a decorated veteran. After the war, he finished his medical training, but his physical disabilities prevented him from fulfilling his original desire to practice surgery.

proposed a theory to explain the molecular aspects of regulation of the catalytic activity of enzymes. These two findings introduced scientific concepts about the control of genetic andmetabolic functions of organisms which soon established a new field of scientific enquiry — the study of biological regulation.

At about this point, the strands of scientific development (shown in Fig. 1–1) –biochemistry, cell biology, and genetics–became inextricably woven, and the new science of **molecular biology** emerged. The distinction between molecular biology and biochemistry is not always clear, because both disciplines take as their ultimate aim the complete definition of life in molecular terms. The term

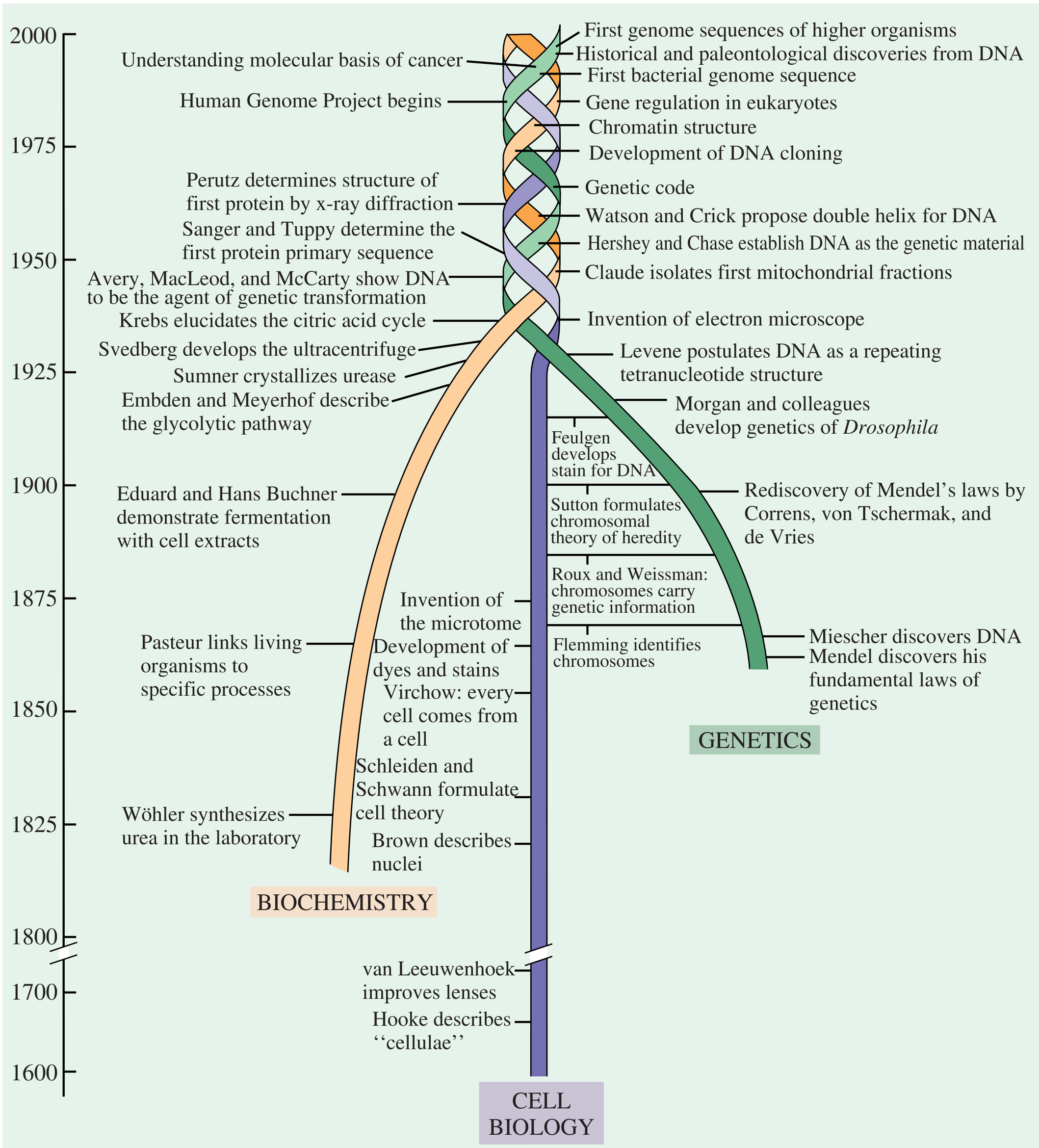


Fig. 1–1. Intertwining of historical traditions of biochemistry, cell biology and genetics

These 3 disciplines of science, originally considered quite distinct among each other, have become interweaved to yield a true molecular biology, which is the subject matter of present-day biochemistry.

(*Adaped from Becker WM, Kleinsmith LJ and Hardin J, 2000*) *molecular biology* is often used in a narrower sense, to denote the study of nucleic acid structure and function and the genetic aspects of biochemistry, an area which one might more accurately call *molecular genetics.* Molecular biology and biochemistry are perhaps differentiated more easily by the orientations of their users than by the research problems being addressed. It may be said that while a biochemist thinks like chemist, a molecular biologist thinks like biologist. Even this distinction is somewhat artificial, since scientists in either field must use the approaches of all relevant disciplines, including chemistry, biology, and physics. In fact, 3 of the most powerful research techniques used by biochemists were developed by physicists : **electron microscopy (EM)**, which has revealed the minutest details of cellular structure, and **X-ray diffraction** and **nuclear magnetic resonance (NMR),** which have revealed the precise 3-dimensional structure of huge biological molecules (=biomolecules).

In the 1970s, molecular biology reached a new zenith with the introduction of recombinant DNA technology, which has brought about a revolution in biological research and has been instrumental in the emergence of a new branch of science called biotechnology. Use of this technique in science has allowed researchers to isolate eucaryotic genes to study their structure, regulation and expression.

In this era of biotechnology, biochemistry will continue to occupy a position of central importance. As a basic science, it is in the foreranks of many scientific endeavours that stand to make a reality of the statement that "the twentieth century belongs to the biologists." It was about 125 years ago that a German **Ernst Hoppe–Seyler,** one of the foremost chemists (and possibly the first biochemist) of the period, established a journal for those studies in physiological chemistry he believed should be recognized as the new discipline of biochemistry. If alive today, he would be justifiably proud of the science whose niche he helped define.

BIOCHEMISTRY AS MOLECULAR LOGIC OF LIVING ORGANISMS

Biochemistry operates at a molecular level and brings to light the hidden secrets of life. Therefore, in considering the molecules of various biological compounds, it is necessary to have an idea of scale (Fig. 1–2). The angström (Å)unit (1Å = 10^{-10} meter or 10^{-8} centimeter or 0.1 nanometer) is customarily used as the measure of length at atomic level. Small biomolecules such as amino acids, sugars etc., are many angströms long whereas biological marcromolecules are much larger : for example, hemoglobin, an oxygen-carrying protein in red blood cells, has a diameter of 65 Å. Ribosomes, the protein-synthesizing organelles of the cell, have diameters of about 300 Å. Most viruses fall within a range of 100 Å (= 10 nm) to 1,000 Å(= 100 nm). Cells are mostly a hundred times as large, in the range of micrometers (µm). For example, a red blood cell is 7 µm (= 7×10^4 Å) long. As the limit of resolution of the light microscope is about 0.2 µm (= 2,000Å), most of the studies of biological structures in the range between 1 Å (= 0.1 nm) and 10^4 Å (= 1µm) have been conducted with the help of electron microscope and *x*-ray diffraction.

The term angström is named after **Anders J. Ångström** (LT, 1814–1874), a spectroscopist. Å is used for lengths shorter than 100 Å, whereas nm or µm is used for longer dimensions.

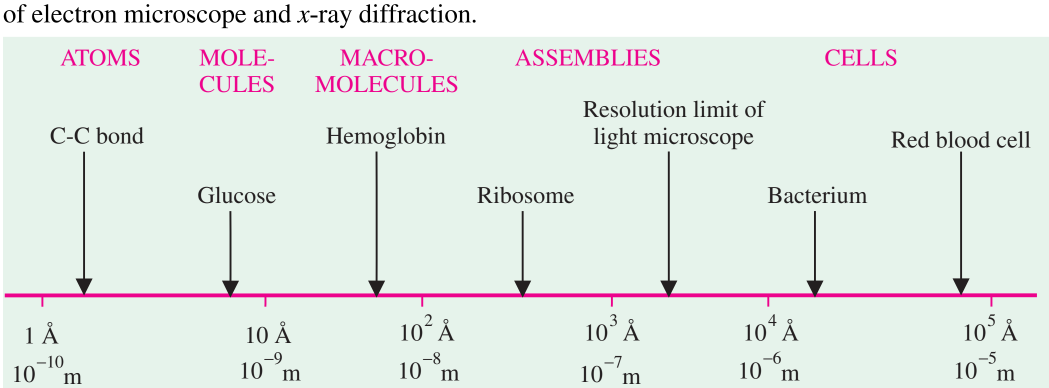


Fig. 1–2. Dimensions of biomolecules, assemblies and cells

The approximate dimensions of the components of the hierarchy of organization in cells is as follows :

Atoms	Å
Micromolecules (Amino acids)	1 nm
Macromolecules (Proteins)	5 to 500 nm
Organelles	nm to µm
Cells	µm to cm

The biomolecules are in a state of flux (Fig. 1–3). The enzymes change their substrate into product in milliseconds (1 m sec = 10^{-3} second). Some enzymes are even more efficient and catalyze their substrate in even few microseconds (1 µ sec = 10^{-6} sec). The unwinding of the DNA double

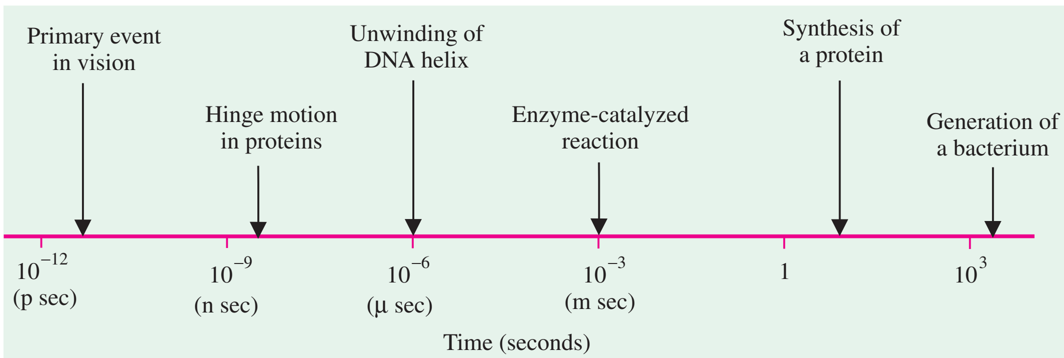


Fig. 1–3. Rates of some biological processes

helix, which is essential for its replication, is completed in a microsecond. The rotation of one sphere of a protein with respect to another takes place in nanoseconds (1 n sec = 10^{-9} sec). It is remarkable to note that the primary event in vision — a change in structure of the light-absorbing group — occurs within a few picoseconds (1 p sec = 10^{-12} sec) after the absorption of a photon.

The molecular events are associated with energy changes (Fig. 1–4). The ultimate source of energy for the living objects is the sun. The energy of visible light, say green, is 57 kilocalories per mole (kcal/mol). ATP, which is the universal currency of energy, has a usable energy content of about 12 kcal/mol. The amount of energy needed for the cleavage of a covalent C—C bond is 83 kcal/mol. Hence, the covalent skeleton of biomolecules is stable in the absence of enzymes and inputs of energy. On the contrary, thermal energy is enough to make and break noncovalent bonds in living systems, which typically have energy contents of only a few kilocalories per mole.

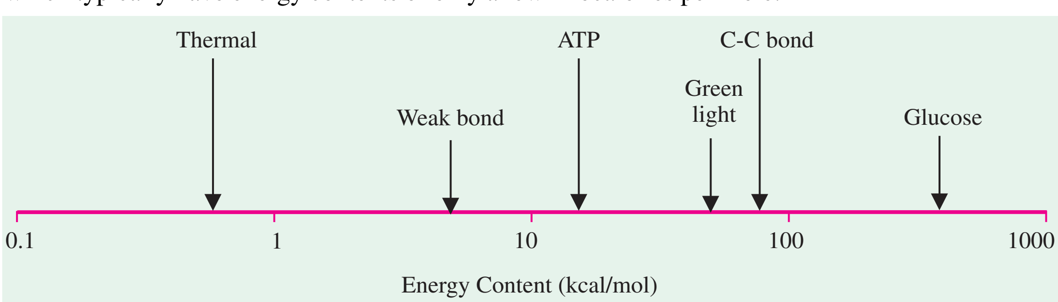


Fig. 1–4. Some biologically important energies

NATURE

Biochemistry has been defined as **"the chemistry of living things"**. The living things are composed of lifeless molecules which, when examined individually, exhibit all the physical and chemical laws that are characteristic of inanimate bodies. Yet living organisms possess extraordinary attributes, not shown by inanimate molecules. At this stage, henceforth, it shall be useful to distinguish 'living' from 'nonliving'. Although the word 'life' is difficult to define but it can be associated with certain characteristics which are common to the living objects and are usually not found in the nonliving or lifeless objects. All **living objects** are characterized by their capacity of movement, power of growth, respiratory activity, sense of irritability and above all the power of reproduction.

Certain identifying characteristics of the living matter or the '*signs of life*', as they are also called, are enumerated below :

1. Living organisms are *highly complicated and organized structures* and contain a very large number of different organic molecules. For example, a single bacterial cell of *Escherichia coli* contains 5,000 different kinds of organic compounds, including as many as 3,000 different kinds of proteins and 1,000 kinds of nucleic acids. In contrast, the inanimate objects such as clay, sand, rock, sea water etc., consist of random mixtures of simpler chemical molecules.

2. Each component unit of a living object appears to have a *specific purpose or function*, whether it be a macroscopic structure (heart, lungs, brain) or a microscopic intracellular structure (nucleus). Even the individual chemical compounds in cells (carbohydrates, proteins, lipids) have specific functions to perform. But it is meaningless or futile to ask about the function of various chemical compounds present in inanimate bodies. They just happen to be there.

3. The living organisms have the *ability to extract, transform and use energy* from their environment, either in the form of organic nutrients or the radiant energy of sunlight. Living organisms are never at equilibrium within themselves or with their surroundings. On the other hand, the inanimate bodies do not use energy to maintain its sturcture or else to do work. Rather when left to itself, it tends to come to equilibrium with its surroundings.

4. The most remarkable attribute of the living organisms is their *capacity for self- replication*, a characteristic which can be regarded as the very quintessence of the living state. Lifeless objects, on the contrary, do not grow and reproduce in forms identical in mass, shape and internal structure.

It should, however, be realized that the boundary between the living and nonliving objects is not always well demarcated. For example, certain diseases are caused by extremely small filterable substances called viruses. These can also reproduce when introduced into the environment of living cells and a few of them have been isolated in a purified cyrystalline form. Chemically, the crystalline viruses such as tobacco mosaic virus (TMV) are nucleoproteins and have no apparent features of living things. Yet TMV, when inoculated into a healthy leaf of a tobacco plant, multiplies rapidly and causes the onset of a disease termed tobacco mosaic disease. Thus, an inanimate crystalline substance, on inoculation, starts behaving as if it were living.

AXIOMS OF LIVING MATTER

The living objects are endowed with certain remarkable attributes. These attributes are due to the typical nature, function and interactions of the **biomolecules**, the kinds of molecules found in living organisms. **Albert L. Lehninger** (1984) lists some of the *axioms* or 'principles' which are characteristic of the living state. These are :

1. There is a basic simplicity in the structure of biological molecules.
2. All living organisms use the same kinds of building block molecules and thus appear to have a common ancestry.
3. The identities of each species or organism is preserved by its possession of distinctive sets of nucleic acids and of proteins.
4. All biomolecules have specific functions in cells.
5. Living organisms create and maintain their comnplex, orderly, purposeful structures at the

expense of free energy from their environment to which they return energy in less useful forms.

6. Living cells are chemical engines that function at constant temperature.
7. The energy needs of all organisms are provided, directly or indirectly, by solar energy.
8. The plant and animal worlds – indeed,all living organisms – are dependent on each other through exchanges of energy and matter *via* the environment.
9. Living cells are self-regulating chemical engines, tuned to operate on the principle of maximum economy.
10. Genetic information is encoded in units that are submolecular in dimensions; these units are the four kinds of nucleotides, of which DNA is composed.
11. A living cell is self-assembling, self-adjusting, self-perpetuating isothermal system of organic molecules which extracts free energy and raw materials from its environment.
12. It carries out many consecutive organic reactions promoted by organic catalysts, which it produces itself.
13. It maintains itself in a dynamic steady state, far from equilibrium with its surroundings. It functions on the principle of maximum economy of parts and processes.
14. Its nearly precise self-replication through many generations is ensured by a self-repairing linear coding system.

MAJOR ORGANIC COMPOUNDS OF ANIMATE OBJECTS

Living beings contain a wide variety of organic compounds, besides the ubiquitous water and other inorganic compounds. Major organic molecules present in the living beings are: carbohydrates, proteins (of course, including enzymes), lipids and nucleic acids. Table 1–1 lists some details of these compounds.

Table 1–1. Major compounds of living beings

Organic compound	*Building block*	*Some major functions*	*Examples*
Carbohydrate :			
Monosaccharide	—	Energy storage; physical structure	Glucose, fructose, galactose
Disaccharide	Monosaccharides	Energy storage; physical structure	Lactose, maltose, sucrose
Polysaccharide	Monosaccharides	Energy storage; physical structure	Starch, cellulose, chitin, inulin, pectin
Protein	Amino acids	Enzymes; toxins; physical structures	Antibodies; viral surface; flagella; pili
Lipid :			
Triglycerides	Fatty acids and glycerol	Energy storage; thermal insulation; shock absorption	Fat, oil
Phospholipids	Fatty acids, glycerol, phosphate, and an R group*	Foundation for cell membranes	Plasma (cell membranes)
Steroids	Four-ringed structure†	Membrane stability	Cholesterol
Nucleic acid	Ribonucleotides; Deoxyri\bonucleotides	Inheritance; instructions for protein synthesis	DNA, RNA

* R group = a variable portion of a molecule.

† Technically, steroids are neither polymers nor macromolecules.

THE SCIENTIFIC METHOD OF APPROACH

Although biologists employ a variety of approaches in conducting research, the experimentally - oriented biologists such as biochemists and microbiologists often use the general approach known as the **scientific method.** They first gather **observations** of the process to be studied and then develop a tentative hypothesis—an educated guess—to explain the observations (Fig. 1-5). Thus, the **hypothesis** is simply a tentative explanation to account for observed phenomena. This step often is inductive and creative because there is no detailed, automatic technique for generating hypotheses. Next, they decide what information is required to test the hypothesis and collect this information through observation or carefully designed experiments. Then, they decide whether the hypothesis has been supported or falsified. If it has failed to pass the test, the hypothesis is rejected, and a new explanation or hypothesis is constructed. If the hypothesis passes the test, it is subjected to more severe testing. The procedure often is made more efficient by constructing and testing alternative hypotheses and then refining the hypothesis that survives testing. This general approach is often called the **hypothetico-deductive method.** One deduces predictions from the currently-accepted hypothesis and tests them. In *deduction,* the conclusion about specific cases follows logically from a general premise ("if . . ., then . . . ," reasoning). *Induction* is the opposite. A general conclusion is reached after considering many specific examples. Both types of reasoning are used by scientists.

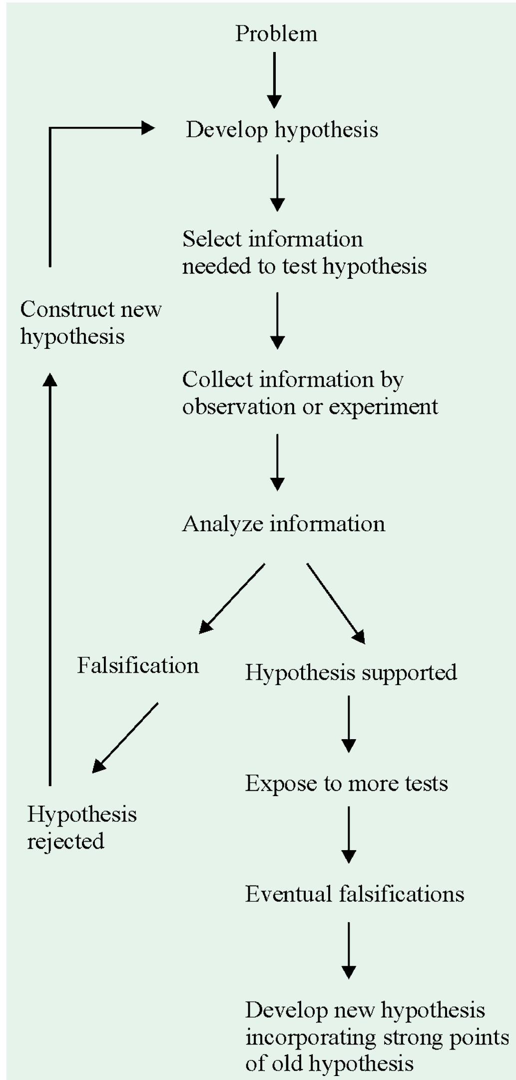


Fig. 1-5. The hypothetico-deductive method
This approach is most often used in scientific research.

When carrying out an experiment, it is essential to use a control group as well as an experimental group. The *control group* is treated precisely the same as the *experimental group* except that the experimental manipulation is not performed on it. In this way, one can be sure that any changes in the experimental group are due to the experimental manipulation rather than to some other factor not taken into account.

If a hypothesis continues to survive testing, it may be accepted as a valid theory. The term **theory** is applied to a hypothesis that has been extensively tested and that ties together and arranges the results of a number of observations and experiments. It provides a reliable, systematic, and rigorous account of an aspect of nature. It is important to note that *hypotheses and theories are never absolutely proven.* Scientists simply gain more and more confidence in their accuracy as they continue to survive testing, fit with new observations and experiments, and satisfactorily explain the observed phenomena.

IMPORTANCE

Modern biochemistry is relatively a new branch and much of the work has been conducted during the present century. The enormous growth of biochemical literature has permitted in many cases the correlation of physical events in biological systems with the help of chemical processes. For a biochemist, therefore, a physiological process does not merely mean to elucidate the nature of the chemical substances involved in it but also to find out the physical relations among these substances and of these substances to the environment. Thus, the principal objective of biochemistry is to fill the wide gap between the highly integrated functions of the living cell and the various properties of its individual chemical constituents. A biochemist, therefore, has to perform an important arduous task of carrying the research work with utmost sincerity, patience and honesty. **Prof. Hopkins** (1931) has rightly remarked :

"He (biochemist) should be bold in experiment but cautious in his claims. His may not be the last word in the description of life, but without his help the last word will never be said."

The *American Society of Biological Chemists* (1965) has, indeed, worked out a definition of a *biochemist* as a guideline for eligibility for membership in that society. The definition reads as follows :

"A biochemist is an investigator who utilizes chemical, physical or biological technics to study the chemical nature and behaviour of living matter."

Ernest Baldwin (1937) entrusts the biochemist with the task of, "the study of physicochemical processes associated with the manifestations of what we call life—not the life of some particular animal or group of animals, but life in its most general sense."

LITERATURE

A number of review publications and research journals appear periodically on various topics of biochemistry from different regions of the globe. A few of them are mentioned below :

REVIEWS

1. Advances in Carbohydrate Chemistry
2. Advances in Enzymology
3. Advances in Protein Chemistry
4. Annual Review of Biochemistry
5. Annual Review of Microbiology
6. Annual Review of Physiology
7. Annual Review of Plant Physiology
8. Biochemical Journal
9. Biological Reviews
10. Biochemical Society Symposia
11. British Medical Bulletin
12. Essays in Biochemistry
13. Harvey Lectures
14. Physiological Reviews
15. Vitamins and Hormones

JOURNALS

1. American Journal of Physiology
2. Analytical Biochemistry
3. Archives of Biochemistry and Biophysics
4. Biochemical Journal
5. Biochemische Zeitschriff
6. Biopolymers
7. Canadian Journal of Biochemistry
8. Comparative Biochemistry and Physiology
9. Endocrinology
10. Enzymologia
11. Indian Journal of Biochemistry
12. Indian Journal of Medical Research
13. Journal of Bacteriology
14. Journal of Biological Chemistry
15. Journal of Cell Biology
16. Journal of Cellular Physiology
17. Journal of Chromatography
18. Journal of Endocrinology
19. Journal of Lipid Research
20. Journal of Molecular Biology
21. Journal of Nutrition
22. Journal of Physiology
23. Nature
24. Plant Physiology
25. Proceedings of the Natural Academy of Sciences
26. Trends in Biochemical Sciences

REFERENCES

1. **Allen GE :** Life Sciences in The Twentieth Century. *John Wiley. 1975.*
2. **Bayliss WM :** Principles of General Physiology. *Longmans Green and Co., London. 1924.*
3. **Browne CA :** A Source Book of Agricultural Chemistry. *Chronica Botanica Co., Waltham. 1944.*
4. **Chittenden RH :** The Development of Physiological Chemistry in the United States. *Amer. Chem. Soc. Monograph No. 54, New York. 1930.*
5. **Chittenden RH :** The First Twenty-five Years of the American Society of Biological Chemists. 1945.
6. **Fruton JS :** Molecules and Life. Historical Essays on the Interplay of Chemistry and Biology. *Wiley Interscience, New York. 1972.*
7. **Henderson LJ :** The Fitness of the Environment. *Beacon Press, Boston. 1958.*
8. **Ihde AJ :** The Development of Modern Chemistry. *Harper and Row, Publishers, Inc., New York. 1964.*
9. **Kornberg A :** The Two Cultures : Chemistry and Biology. *Biochem.* **26,** *6888-6891, 1987.*
10. **Lipmann F :** Wanderings of a Biochemist. *Wiley, New York. 1971.*
11. **McCollum EV :** A History of Nutrition. *Houghton Mifflin Company, Boston. 1957.*
12. **Needham J (editor) :** Chemistry of Life. Eight Lectures on the Histroy of Biochemistry. *Cambridge Univ., Press, New York. 1970.*
13. **Needham J, Baldwin E (editors) :** Hopkins and Biochemistry. *Heffer and Son Ltd., Cambridge. 1949.*
14. **Orten JM, Neuhaus OW :** Biochemistry. *8th ed. The C.V. Mosby Co., Saint Louis. 1970.*
15. **Partington JR :** A History of Chemistry. *Macmillan and Company Ltd., London. 1961, 1964.*
16. **Pirie NW, in Needham J, Green DE :** Perspectives in Biochemistry. *Cambridge Univ., Press, Cambridge. 1937.*
17. **Schrödinger E :** What is Life ? *Cambridge University Press, New York. 1944.* [Reprinted (1956) in What is Life ? and Other Scientific Essays. *Double-day Anchor Books, Garden City, New York.*]
18. **Solomons TWG :** Organic Chemistry. *2nd ed., John Wiley and Sons, New York. 1980.*
19. **Sumner JB :** The isolation and crystallization of the enzyme urease. *J. Biol. Chem. 69: 435-441, 1926.*
20. **van Niel CB :** Bacteriological Reviews. **13 :** *161, 1949.*
21. **Vogel HJ, Vogel RH :** Some chemical glimpses of evolution. *Chem. Eng. News. 45: 88, 1967.*
22. **Waksman SA :** Sergei N. Winogradsky. *Rutgers Univ., Press, New Brunswick. 1953.*
23. **Williams RJ, Lansford EM (editors) :** Encyclopedia of Biochemistry. *Reinhold Publishing Corp., New York. 1967.*
24. **Willstätter R :** From my life. *Benjamin. 1965.*