# SECTION 1

# Basic Chemistry in Biology and Methods

Qualitative and quantitative estimation of biomolecules is common practice in biochemistry labs.

Understanding the fundamental aspects of chemistry are essential to get familiar with biochemistry. Ionization, concentration calculation, energy calculations are some basic aspects which are needed almost always while understanding biochemistry. Water is one of the most abundant molecules and universal solvent for all biochemical systems is known to influence almost all the biological phenomenon. This section is therefore focused on understanding the fundamental aspects of chemistry and consist of five different chapters. The first chapter is focused on understanding the biochemical milieu of cells, their spatial and temporal diversity. The second chapter is on water, pH, and concept of buffering in biological systems. The third chapter is more elaborate and describes the fundamental thermodynamics of cells. The fourth chapter is an extension of the third chapter and explains the phosphorylation or ATP generation systems, i.e. photosynthesis, substrate level phosphorylation, and oxidative phosphorylation. A final chapter in this section is dedicated to some fundamental chemical methods involved in the analysis of biomolecules. Some common techniques such as isolation of biomolecules, their quantification and quality assessment using, chromatography, enzyme assays, spectrophotometry, ELISA, etc. have been explained in brief to get an insight before we move on to next section on structure and function of molecules.

CHAPTER 1

# Introduction to Biochemistry

## Learning Objectives

- Understanding the basis of life
- Historical foundations of biochemistry
- The atomic composition of life
- Water as a solvent and biochemical milieu

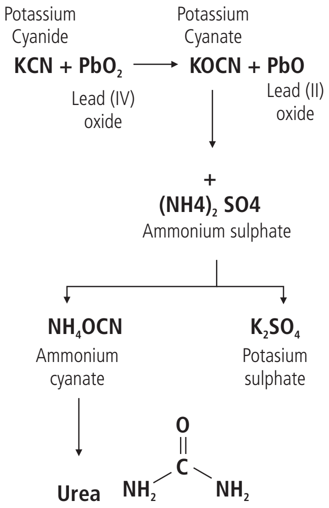
## Interesting Fact

By the beginning of the nineteenth century, it had become clear to chemists that the composition of living matter is strikingly different from that of the inanimate world. Antoine Lavoisier (1743-1794) noted the relative chemical simplicity of the "mineral world," and contrasted it with the complexity of the "plant and animal worlds"; the latter, he knew, were composed of compounds rich in the elements carbon, oxygen, nitrogen, and phosphorus. The development of organic chemistry preceded, and provided invaluable insights for, the development of biochemistry.

## 1.1 LEGACY OF BIOCHEMISTRY

Biochemistry consists of two words, bio pertaining to life and chemistry relating to the chemical behaviour and thus it depicts chemistry of life. Biochemistry focuses on the processes that occur at the molecular level in relation to the structure of a molecule and connects them with their function. Biochemistry is an integral part of science that not only interlocks biology and chemistry but also provides highly valuable connecting links between living and non-living material. It explains how life process operates at the chemical level and how fundamental principles of chemistry govern life. At the sub-atomic level, we are no different than a rock and a piece of metal, then how does life operates, and how non-living things do not share common features with us! Historically, when biochemistry was not born officially, was a world of vitalism. Vitalism was a commonly believed hypothesis about life, that living things are equipped with exclusive properties by virtue of vital forces such as spirit, and that makes them different from non-living things and it was very challenging for the early scientists to disprove this belief. In the early days when Chemistry predominated as science to study the chemical basis of life, Antoine Lavoisier proposed that combustion of a candle is similar to the respiration of animals, as both of these need oxygen. This hypothesis could be considered as one of the first ideas of biochemistry that originated from chemical sciences. However, the first important landmark in the birth of modern organic chemistry, as well as biochemistry, was Wohler's synthesis of Urea, that shattered the idea of vitalism and established that molecules of life can be synthesised from material from the non-living world. In fact, Friedrich Wöhler in 1828 synthesised urea using ammonium cyanate. Later, in 1845 Kolbe reported another inorganic-organic conversion of carbon disulfide to acetic acid before vitalism started to lose support. Fall of vitalism hypothesis and rise of mechanistic hypothesis supported the advent of biochemistry from chemical sciences as the quest for answering the mechanism of biochemical processes intensified. During the latter part of the nineteenth century, eminent scientists contributed a great deal to the elucidation of the chemistry of fats, proteins and carbohydrates.

**Wöhler's Urea Synthesis**



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### 1.1.1 The Quest for Catalysis

Discovery of an active principle named diastase in diastasis process during brewing by Anselme Payen and Jean-François Persoz, at a French sugar factory in 1833, was another important addition to the process of catalysis in biological systems. This was probably the first use of the suffix –ase for biological catalysts even when the term enzyme was not coined. The term catalysis was later coined by Berzelius in the year 1835. The basic idea of catalysis was connected to its cause by Cagniard de Latour in 1838 and later in 1898 by Louis Pasteur when they proposed that yeast contained active principles called ferments which are the ultimate factors for causing catalysis or fermentation process. In 1850 Louis Pasteur observed that fermentation of sugar into alcohol by yeast is catalyzed by substances, which he referred to as 'ferments' (later named as an enzyme). He found that these ferments are always associated with yeast cells. As he was not successful in dissociating the ferments from the yeast cells, he finally concluded that the ferments are an inherent property of yeast cells and are inseparable from them. Similar observations were made by W. F. Kühne in 1876 and coined the word "enzyme" (in Greek), which means, "in yeast" (en = in; zyme = yeast). Liebig was an opponent of Pasture, who believed that fermentation is an attribute of decay or death while pasture believed that it's the process of living cells. In 1897 another German scientist, Hans Buchner, discovered by accident that fermentation actually does not require the presence of living yeast cells. Buchner made an extract of yeast cells by grinding them and filtering off the remaining cell debris. Then he added a preservative, sugar to the resulting cell-free solution to preserve it for future study. He observed that fermentation, the formation of alcohol from sugar, occurred. Buchner then realized that living cells were not required for carrying out metabolic processes such as fermentation. Instead, there must be some small entities capable of converting sugar to alcohol. These entities were enzymes. Buchner's accidental discovery won him the 1907 Nobel Prize in chemistry. The next breakthrough in enzymology came in 1926 when J. B. Sumner from Cornell University, U.S.A., isolated, purified and also successfully crystallized the enzyme urease from jack bean. (Urease breaks down urea and produces ammonia and carbon dioxide). He found that the urease crystals are purely made of proteins and hence he reported that "enzymes are nothing but proteins". But his conclusion was vehemently opposed by the well-known German biochemist Richard Willstäter, (Nobel laureate), who insisted that enzymes are nothing but 'low molecular weight organic compounds' and the protein crystals that were found in the urease preparation could be 'impurities'. For their seminal contributions to enzymology, Sumner and Northrop were awarded Nobel Prize in 1935.

### 1.1.2 Biochemistry as Independent Discipline

With a number of key discoveries in the later 19th century, biochemistry was already established as an independent discipline. Although the term "biochemistry" itself is derived from the combining form bio-, meaning "life", and chemistry first recorded in the English dictionary in 1848, while in 1877, Felix Hoppe-Seyler used the term (Biochemie in German) in the foreword to the first issue of Zeitschrift für Physiologische Chemie as a synonym for physiological chemistry and argued for the setting up of institutes dedicated to its studies. Yet the rise of biochemistry as a new disciple is often acknowledged to German chemist Carl Neuberg for coining Biochemistry in the year1903. The transition from the pre-war period of biochemistry to the colloid period was illuminated by the history of Otto Warburg's Atmungsferment.' Between 1913 and about 1929, Warburg believed that respiration was catalyzed by iron atoms bound to active colloidal "structure" in the cell. Warburg's ideas on respiration were quite different from the biochemists' ideas. Although Warburg did not accept the cytochromes as true respiratory enzymes until 1933, until the influence of Keilin. Keilin was a parasitologist in 1924, and observed the peculiar spectrum of the cytochromes in the blood-engorged larvae of horse parasites and found the same pigment in insects, animals, and bacteria.

### 1.1.3 Unravelling the Biochemical Cycles

Most of the biochemical pathways were deciphered once biochemistry was established as an independent domain in early 1900. In early 1900, Archibald Garrod elucidated some of the inborn errors of metabolism such as alkaptonuria and the metabolic aspects of amino acids were understood in the first decade of the 20th century. Glycolysis was among the first biochemical pathways deciphered in biochemistry, although it took almost 100 years for its complete elucidation. In the 1920s Otto Meyerhof was able to link together some of the many individual pieces of glycolysis discovered by Buchner, Harden, and Young. Meyerhof and his team were able to extract different glycolytic enzymes from muscle tissue and combine them to artificially create the pathway from glycogen to lactic acid. With all of these pieces available by the 1930s, Gustav Embden proposed a detailed, step-by-step outline of that pathway we now know as glycolysis. Carl Ferdinand Cori together with his wife Gerty Cori Worked on muscle glycogen metabolism during the 1930s and elucidated the glycogen metabolic pathways for which they received a Nobel Prize in 1947. Almost, at similar times, Hans Krebs and Kurt Henseleit in 1932 elucidated the Urea cycle involved in the excretion of nitrogenous waste in mammals. This cycle was described in more detail later on by Ratner and Cohen. Almost in the same period, what we know today as the citric acid cycle was being studied, and most of the components and reactions of the citric acid cycle were established in the year 1930s by the research of Albert Szent-Györgyi, who received the Nobel Prize in Physiology or Medicine in 1937 specifically for his discoveries pertaining to fumaric acid, a key component of the cycle. In the same year when Gyorgyi received Nobel Prize, Hans Adolf Krebs provided the complete description and reactions of the citric acid cycle for which he also received a Nobel Prize for Physiology or Medicine in 1953. Closely related to this work on metabolic cycles was the elucidation of the structure of adenosine triphosphate and coenzyme A by Lipman and mechanism of energy production by electron transport chain, which had led to a conception of the biochemical unity of the cell as a system of coupled metabolic cycles, with ATP as a universal medium of energy exchange.

**Note**

One of the most important features of the biochemistry of the 1930's was the introduction of new technologies in physical chemistry: X-ray crystallography, the ultracentrifuge, and isotopic labeling techniques. The isotopic labeling tracer method was as crucial for metabolic studies as spectroscopy was for the study of respiration, and has attracted the most historical interest.

### 1.1.4 Biochemistry Beyond Chemistry

The year 1953 was a year of great achievement for Biochemistry, Watson and Crick published their findings in a one-page paper, with the understated title "A Structure for Deoxyribose Nucleic Acid," in the British scientific weekly Nature on April 25, 1953, illustrated with a schematic drawing of the double helix. During the same period, proteins structures were also being studied and the first structure of protein myoglobin was deciphered by X-ray crystallography by John Cowdery Kendrew and Max Perutz in the year 1956, Later in 1961, they revealed the structure of haemoglobin. In the year 1962, Francis Crick, James Watson and Maurice Wilkins Received Nobel Prize in Physiology for the discovery of DNA structure while Nobel Prize in chemistry was shared by John Kendrew and Max Perutz from Cavendish Laboratory in London. Almost during the similar period, Prof. GN Ramachandran at Madras University solved the structure of collagen and proposed plot of torsion angles which later became famous as Ramachandran plot. Study of nucleic acid is central to the knowledge of life but its fusion with biochemistry started with works of Fredrick Sanger and Har Gobind Khurana who developed the method of DNA sequencing and deciphered genetic code respectively. Their experiments involved a subtle bland of enzymology and chemistry that few would have thought possible to combine. The scientists were busy removing the mist that was mitigating the light of knowledge but they still lacked insight into the cell. In 1990s research turned to find the structural details of the cell. The field of molecular biochemistry was also progressing at an almost unstoppable speed having expanded its horizons beyond human imagination with the introduction of PCR, creating waves of appreciation from every field of medicine and then coming out of the lab to help establish better therapies for various diseases by the introduction of gene therapy. More recently, the biochemistry has now mingled well with all other domains of science such as molecular biology, microbiology, computational biology and several huge projects have been completed. The year 2003 marked the completion of the first human genome sequence. This was followed by the development of the first draft of the human metabolome database in 2007, and later in 2015, human organ-wise protein map was also prepared. The emergence of systems biology, bioinformatics and computational biology is now bringing biochemistry at the pivotal juncture of various advanced subjects which are expected to bring a much understanding of global biochemical systems and perhaps coming century would witness the development of virtual simulations of complex biochemical milieu with extensive inclusion of thermodynamic and kinetic parameters.

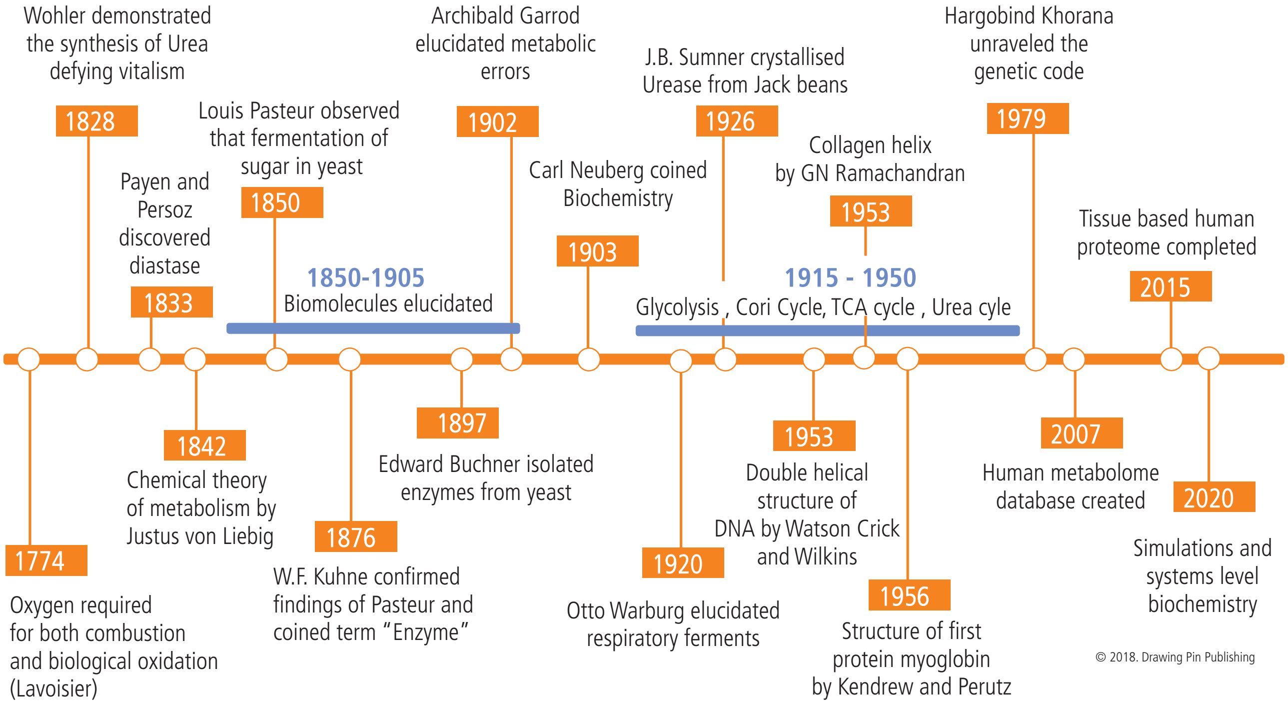

**Figure 1.1** Timeline of history of Biochemistry

## 1.2 ATOMIC COMPOSITION OF LIFE

Today we know 118 elements from atomic numbers 1 (Hydrogen) through 118 (Oganneson) which are often presented in the form of the periodic table. The first 94 elements, hydrogen through plutonium, all occur naturally, though some are found only in trace amounts and a few were discovered in nature only after having first been synthesized. Elements 95 to 118 have only been synthesized in laboratories or nuclear reactors. With the recently available information about the Universe, nearly 5% of the universe is made of visible matter rest is mostly dark matter and dark energy. The most abundant element in the visible matter of the universe is Hydrogen occupying nearly 75% of the proportion followed by the next 24% helium and the remaining 1% are other elements. However, the planet Earth that sustains life has a more diverse composition of its crust. The most abundant element on the Earth crust is oxygen (47%) followed by silicon (27%). Although the most abundant element in living organisms is also oxygen, yet they differ significantly from the composition of the Universe or Earth’s crust in preference for only a very few elements. Only about 30 of the 94 naturally occurring chemical elements are essential to living organisms. Most of the elements in living matter have relatively low atomic numbers; only five have atomic numbers above that of selenium, 34. Predominantly, living organisms contain only four elements in significant amount namely, carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) constituting about 96.5% of an organism’s weight. Few more elements such as phosphorous, sulphur, calcium, magnesium, iodine, copper, iron etc. are present in trace amounts. This composition differs markedly from that of the non-living inorganic environment and is evidence of a distinctive type of chemistry. The survey here excludes mineralized tissues such as bone and teeth, as they contain large amounts of inorganic salts of calcium and phosphorus. The relative abundance of elements is similar in all living organisms.

### 1.2.1 Carbon as the Scaffold of Biomolecules

Carbon is the key elemental building block for all known terrestrial life. It’s commonly assumed in astrobiology that it will also provide the basis for most life elsewhere in the universe. If we disregard water, most of the molecules present in living organisms essentially contain carbon, especially four key biomolecules are based on the carbon backbone. It may, therefore, be considered that carbon is an omniscient atom

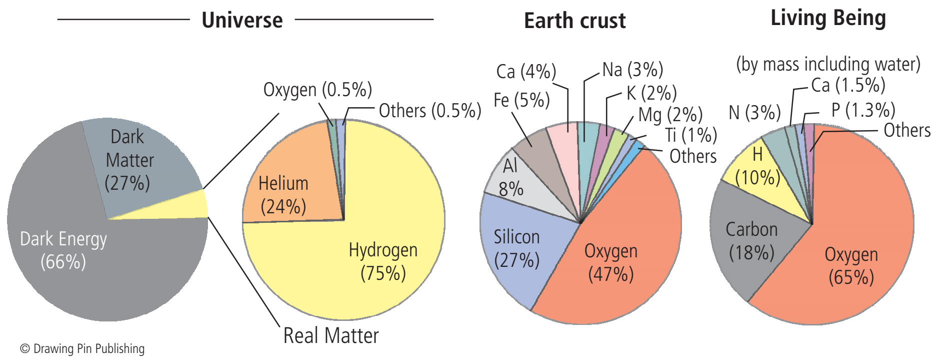

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**Figure 1.2** Atomic composition of Universe, Earth Crust and The living organisms.

in life forms and forms the scaffold of most biomolecules. There are several reasons why carbon is most suitable as an atom for supporting life. First is the ability of carbon to form a vast range of large, complicated molecules with itself and other elements, especially hydrogen, oxygen, and nitrogen by virtue of its tetravalency and catenation, which can give rise to the enormous diversity of molecules in life forms. In fact, there are nearly 10 million carbon-based compounds in living things! Although, tetravalency and catenation are also known in other members of the same group, where silicon is a prominent candidate, additional features such as maintaining the right balance of stability and flexibility in molecular transformations underlie the choice of carbon in life forms. Additionally, in aqueous systems at temperatures common on Earth, carbon is much superior to any other atom as a polymeric unit. Physiologically, the oxidised form of carbon is carbon dioxide which can easily be excreted by living organisms, representing another merit of carbon over other tetravalent atoms in the periodic table. Certain combinations of atoms, such as the methyl ($-CH_3$), hydroxyl (-OH), carboxyl ($-COOH$), carbonyl ($-C=O$), phosphate ($-PO_3^{2-}$), and amino (-$NH_2$) groups, occur repeatedly in organic molecules. Each such chemical group has distinct chemical and physical properties that influence the behaviour of the molecule in which the group occurs and they provide enormous chemical diversity to the biomolecules.

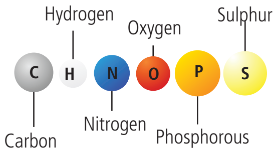
**Note**

It may be that we find it hard to see viable alternatives to carbon biochemistry because we have no experience of such alternatives. Being carbon-based life-forms ourselves, we may suffer from what's been called carbon chauvinism. On the other hand, scientists have so far discovered nothing in the chemistry of other elements to remotely compare with the millions of organic compounds to which carbon gives rise.

**Mnemonic**

CHONPS, a mnemonic acronym for the order of the most common elements in living organisms: carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur

**CHNOPS: Most abundant atoms**



## 1.3 WATER: THE ELEXIR OF LIFE

Water is an inorganic, transparent, tasteless, odourless, and nearly colourless chemical substance, that accounts for about 70% of a cell's weight, and 60% weight of a human being and therefore most intracellular reactions occur in an aqueous environment. About 71% surface of the Earth is also covered with water. Life on Earth began in the ocean, and the conditions in that primaeval environment put a permanent stamp on the chemistry of living things. Life, therefore, hinges on the properties of water. In each water molecule ($H_2O$) the two H atoms are linked to the O atom by covalent bonds. The two bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive. Consequently, there is an unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and of negative charge on the O. When a positively charged region of one water molecule (that is, one of its H atoms) comes close to a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can result in a weak bond called a hydrogen bond. These bonds are much weaker than covalent bonds and are easily broken by the random thermal motions due to the heat energy of the molecules, so each bond lasts only an exceedingly short time. But the combined effect of many weak bonds is far from trivial. Each water molecule can form a maximum of **four hydrogen bonds** through its two H atoms and two lone pair electrons to other water molecules arranged in a tetrahedral orientation, producing a network in which hydrogen bonds are being continually broken and formed and at an average, each water molecule has 3.5 hydrogen bonds at a given time in the liquid state. It is only because of the hydrogen bonds that link water molecules together that water is a liquid at room temperature, with a high boiling point and high surface tension rather than a gas. On freezing all four hydrogen bonds give rise to a cage-like structure that traps air spaces resulting in a decreased density of water due to which ice floats on the surface of the water.

**Note**

The word water comes from Old English wæter, from Proto-Germanic *watar (source also of Old Saxon watar, Old Frisian wetir, Dutch water, Old High German wazzar, German Wasser, Old Norse vatn, Gothic wato), from Proto-Indo-European *wod-or, suffixed form of root *wed- ("water"; "wet"). [5] Also cognate, through the Indo-European root, with Greek ύδωρ (ýdor), Russian вода́ (vodá), Irish uisce, Albanian ujë.

### 1.3.1 Life-Supporting Properties of Water

Water is called the **universal solvent** because it is capable of dissolving more substances than any other liquid. It means water is capable of transporting valuable chemicals, minerals, and nutrients inside or outside living world maintaining a constant supply of nutrients. The viscosity of water is about $10^{-3}$ Pa·s or 0.01 poise at 20°C (68 °F), making it a freely flowing material at physiological temperatures. Due to their polar nature, water molecules cannot only form hydrogen bonds with neighbouring water molecules but also with several other polar molecules, as well as interacting with positively or negatively charged ions. We will understand in the following chapters that most of the biomolecules except lipids are hydrophilic and therefore interact well with water and hence remain mostly soluble in keeping the cytoplasm in its dynamic state. In contrast, nonpolar molecules, which cannot interact with water, are poorly soluble in an aqueous environment and hence help in creating cell boundaries like plasma membranes. Consequently, the interactions between water and the other constituents of cells are of central importance in biological chemistry. Besides polar nature, a high dielectric constant of water (79.8). Liquid water can be split into the elements hydrogen and oxygen by passing an electric current through it by a process called electrolysis. The decomposition requires more energy input than the heat released by the inverse process (285.8 kJ $mol^{-1}$, or 15.9 MJ $kg^{-1}$), this property enables several biochemical methods like gel electrophoresis possible in aqueous solutions. Not only water provides a medium for biochemical reactions but also participates itself in many fundamental reactions of life. Photosynthetic cells use the sun's energy to split off water's hydrogen from oxygen. Hydrogen is combined with $CO_2$ (absorbed from air or water) to form glucose and release oxygen. All living cells use such fuels and oxidize the hydrogen and carbon to capture the sun's energy and reform water and $CO_2$ in the process (cellular respiration). Water is also central to acid-base neutrality and enzyme function. An acid, a hydrogen ion ($H^+$, that is, a proton) donor, can be neutralized by a base, a proton acceptor such as a hydroxide ion ($OH^-$) to form water, which is possible due to very small value of the equilibrium constant of water ($1.8 \times 10^{-16}$) and ionic product of $10^{-14}$ accounting for neutral pH at physiological conditions. Furthermore, the specific heat of water is 1 calorie $g^{-1}$ °C (4.186 Joules $g^{-1}$ °C) and latent heat of water is 540 calorie $g^{-1}$ which are much higher than any other common substance. As a result, water acts as heat sink and plays a very important role in temperature regulation. Thermoregulation in primates by virtue of sweating is one such implication of high latent heat of the water. The density of water is maximum at 4°C (i.e. 1 mg per $cm^3$), and both ice, as well as heated water, has low density. This makes colder water to float on the surface, so aquatic life can survive in the water below even during winter. The surface tension of the water is high (73 dynes $cm^{-1}$) compared to any other solvent, thus water can form drops and allows the transport of sap by capillary action. Also, water has good heat conduction ability making it an important heat transfer material in cells.

**Note**

Pure water is usually described as tasteless and odourless, although humans have specific sensors that can feel the presence of water in their mouths, and frogs are known to be able to smell it. However, water from ordinary sources (including bottled mineral water) usually has many dissolved substances, that may give it varying tastes and odours.

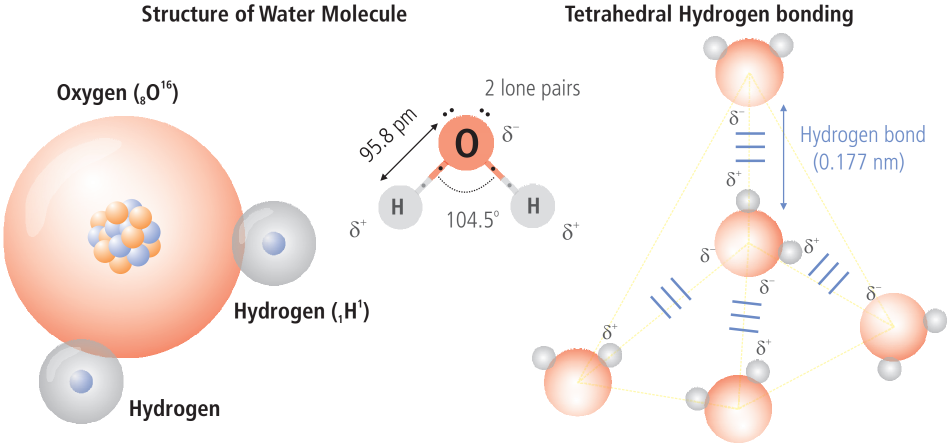


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**Figure 1.3** Structure of water and its basic properties.

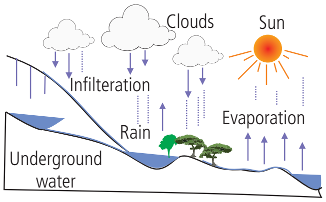
**Table 1.1** Summary of various life supporting properties of water.

| Feature | Value | Impact on biochemical systems |
|---|---|---|
| Polarity | High | Hydrogen bonding and biomolecular interactions |
| Dielectric constant | 79.8 | Weak ionic interactions and hence high solubility of biomolecules |
| Viscocity | $10^{-3}$ Pa·s | Easy flow of water in biological systems, cyclosis |
| Density | 1 g $cm^{-3}$ | Maximum at 4ºC, helps marine life to survive in winter |
| Latent Heat | 2257 J $g^{-1}$ °C | Thermoregulation during sweating and evaporation |
| Specific Heat | 4.186 J $g^{-1}$ °C | Heat sink and thermoregulation |
| Surface tension | 73 dynes $cm^{-1}$ | Capillary action and movement of water in microchannel |
| Equilibrium constant | $1.8 \times 10^{-16}$ | High propensity to combine $H^+$ and $OH^-$ to form water |
| Ionic Product | $10^{-16}$ | Equal number $H^+$ and $OH^-$ hence neutral pH |
| Electrolysis | 15.9 MJ $kg^{-1}$ | Ability to carry out electrophoresis and other biochemical methods |

## 1.3.2 The Water Cycle

Besides being the basis of all life forms, for humans water has few more applications in sustaining life and its social structure. It is also used conventionally to irrigate crops, to prepare food, to wash clothes, utensils, as a coolant and raw material in industries, and generation of hydroelectricity. The water in inland sources originates from the ocean, passing billions of times through the water cycle. The amount of water on earth has been constant for millions of years. A drop of water may spend 2 to 3 weeks in a river, 100 years in a lake or a 1000 years in a glacier. The water we drink today may have once flowed down the Indian Ocean or it may have washed the holy idol in a temple or watered the crops in some countryside. Interestingly, water is recycled at a constant rate in nature, however, all the water that is present on the Earth is not drinkable, hence its availability is limited by usage and therefore scarcity of drinking water remains an important socio-economic issue. The water cycle (known scientifically as the hydrologic cycle) refers to the continuous exchange of water within the hydrosphere, between the atmosphere, soil water, surface water, groundwater, and plants. Water moves perpetually through each of these regions in the water cycle consisting of evaporation from oceans and other water bodies into the air and transpiration from land plants and animals into the air, precipitation, from water vapour condensing from the air and falling to the earth or oceans and finally runoff from the land usually reaching the sea.

**Water Cycle**



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## 1.4 ORIGIN OF BIOMOLECULES AND LIFE

Much before the advent of biochemistry most of the theories of the origin of life were inspired by vitalism and thus special creation of living organism with religious faith were much popular. However, the experimental research in origin-of-life studies began in a basement laboratory in the chemistry department of the University of Chicago in 1953. Harold Urey, a Nobel laureate in chemistry, and Stanley Miller, then a graduate student, put together a tabletop apparatus designed to simulate the chemical processes that might have occurred on the planet soon after its birth.

## 1.4.1 Oparin-Haldane Conjecture

In the famous experiment, AI Oparin, Harold Urey and Stanly Miller used water ($H_2O$), methane ($CH_4$), ammonia ($NH_3$), and hydrogen ($H_2$) in sealed sterile 5-liter glass flask connected to another small 500 ml flask half-full of water. The water in the smaller flask was heated to produce vapours which were allowed to enter the larger flask. Continuous electrical sparks were fired between the electrodes to simulate lightning in the water vapours and gaseous mixture. This was to simulate the actual environmental conditions present at the Earth billions of years ago. This simulated environment was then cooled again so that the water condensed and trickled into a U-shaped trap at the bottom of the apparatus. After a day, the solution turned pink in colour and the end of one week of continuous operation, the boiling flask was removed, and mercuric chloride was added to prevent microbial contamination. The reaction was stopped by adding barium hydroxide and sulfuric acid and evaporated to remove impurities. Using paper chromatography, Miller identified five amino acids present in the solution: glycine, $\alpha$-alanine and $\beta$-alanine, aspartic acid and $\alpha$-aminobutyric acid (AABA). Scientists called this scenario the **Oparin-Haldane conjecture**, but it was given a provocative nickname **primordial soup** to endure the popular consciousness. After Miller's death in 2007, scientists examining sealed vials preserved from the original experiments were able to show that there were actually well over 20 different amino acids produced in Miller's original experiments. Miller had also performed more experiments, including one with conditions similar to those of volcanic eruptions. This experiment had a nozzle spraying a jet of steam at the spark discharge when these experiments were reproduced in 2017, researchers were able to produce most organic molecules, 22 amino acids, 5 amines and many hydroxylated molecules.

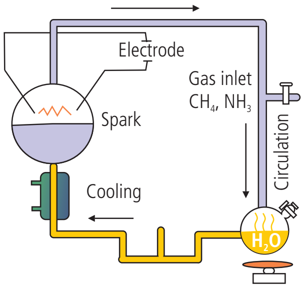
**Note**

$CO_2 \rightarrow CO + [O]$ (atomic oxygen)
$CH_4 + 2[O] \rightarrow CH_2O + H_2O$
$CO + NH_3 \rightarrow HCN + H_2O$
$CH_4 + NH_3 \rightarrow HCN + 3H_2$ (BMA process)
$CH_2O + HCN + NH_3 \rightarrow NH_2\text{-}CH_2\text{-}CN + H_2O$
$NH_2\text{-}CH_2\text{-}CN + 2H_2O \rightarrow NH_3 + NH_2\text{-}CH_2\text{-}COOH$

## 1.4.2 Biomolecules to Life

The aforementioned experiments on the origin of life and their recent validation strongly suggest that the first biomolecules were formed in this primordial soup of life, which later continued to have biochemical reactions unless enzymes emerged and they speeded the process of biochemical transformation. For a long time, the role of catalysis and transmission of genetic information remained obscure. A chicken an egg paradox exited that what emerged first, nucleic acids or proteins. As one was responsible for the transmission of genetic information while other for catalysis. The paradox was resolved in 1991, with the discovery of ribozymes, RNA molecules with catalytic activities and this established the **RNA world hypothesis** that RNA was the first macromolecule to emerge during origin and evolution of life. Initial biomolecules, begin to aggregate and collect into cellular structures called coacervates, which are mainly organic-rich droplets formed via liquid-liquid phase separation, mainly resulting from the association of oppositely charged molecules (macro-ions, polyelectrolytes, polysaccharides, proteins, etc. A. I. Oparin from Russia named coacervate to protobionts, which is a kind of coacervate but a steady globule that is apt to self-arrange if a suspension of proteins, polysaccharides and nucleic acids, if agitated. Besides RNA world hypothesis, **metabolism first hypothesis** is another well-argued logic for the beginning of life from the primordial soup. This suggests that the earliest steps toward life required neither DNA nor RNA, and may not even have involved spatial compartments like cells; the earliest reactions could have occurred in the voids of porous rock, perhaps filled with organic gels deposited as suggested in the Oparin-Haldane model and consisted of a series of simple chemical reactions running without the aid of complex enzymes, via the catalytic action of networks of small molecules, perhaps aided by naturally occurring minerals. It is believed that such a system arose and that much of that early core remains as the universal part of modern biochemistry, the reaction sequences shared by all living beings.

**Oparin experiment**



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**Note**

The earliest known life-forms on Earth are putative fossilized microorganisms, found in hydrothermal vent precipitates, that may have lived as early as 4.28 billion years ago, relatively soon after the oceans formed 4.41 billion years ago.

## 1.5 BIOCHEMICAL MILIEU OF CELLS

Biochemistry of cells is more or less universal with the context-specific difference in the organism or cell, yet they are provided with all common essential life-supporting chemical status. A comparison between a prokaryotic cell of bacteria and a eukaryotic cell provides some common features of all the cells such as aqueous environment and water as predominant molecule accounting for 70% or more of total cell mass. Consequently, the interactions between water and the other constituents of cells are of central importance in biological chemistry. Table 1.1 enlists and compares fractions of various components from the bacterial and mammalian cell and indicates almost similar proportions in various molecules.

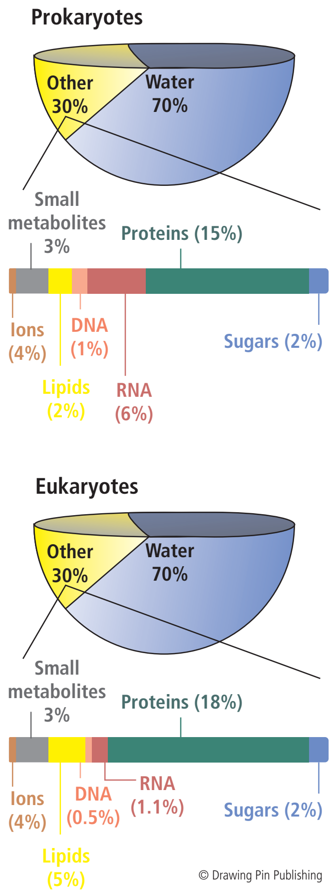
The remaining 30% of the chemical composition of a cell include various biomolecules and ions. Proteins occupy the most abundant fraction with almost 15% of the cell mass, while nucleic acids constituting 7% fraction with RNA almost 6 times more than the DNA content. Nearly 4% of the fraction of cell mass is contributed by ion and other small molecules. Both lipids and carbohydrates account for nearly 2% each towards the cell mass. Among ions and small molecules, predominant ions are sodium ($Na^+$), potassium ($K^+$), magnesium ($Mg^{2+}$), calcium ($Ca^{2+}$), phosphate ($HPO_4^{2-}$), chloride ($Cl^-$), and bicarbonate ($HCO_3^-$), which constitute 1% or less of the cell mass. These ions are involved in a number of aspects of

**Table 1.2** Comparision of various components of a bacterial and mammalian cell.

| | Percent wet weight in the cell | |
|---|---|---|
| **Component** | **Bacterial Cell** | **Mammalian cell** |
| $H_2O$ | 70 | 70 |
| Inorganic ions ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cl^-$, etc.) | 1 | 1 |
| Miscellaneous small metabolites | 3 | 3 |
| Proteins | 15 | 18 |
| RNA | 6 | 1.1 |
| DNA | 1 | 0.25 |
| Phospholipids | 2 | 3 |
| Other lipids | - | 2 |
| Polysaccharides | 2 | 2 |

Adapted from Bruice Albert, The cell

**Biochemical milieu of cell**



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cell metabolism, and thus play critical roles in cell function. If we exclude water, the remaining dry weight of cells is mostly contributed by four major biomolecules, carbohydrates, proteins, nucleic acids and lipids accounting for about 80-90% of the cell mass.

## 1.5.1 Life is Based on Four Key Biomolecules

As we discussed above the chemical composition of cells and life forms, most of the dry matter of cell is composed of four key biomolecules. These four biomolecules are carbohydrates, lipids, proteins and nucleic acids. Carbohydrates are by definition, polyhydroxy-aldehydes or polyhydroxy-ketones and often polymerise into large molecules such as starch and cellulose. In monomeric form, they account of most of the energy-generating fuel of the cell, while in the polymeric form they either represent storage fuel or structural components. Proteins are the most dynamic and versatile in terms of both structure and function. They are defined as polymers of amino acids. Proteins are available as enzymes to catalyse reactions, structural components of various cells and tissues, hormones for metabolic regulation and signalling molecules. Lipids are a collection of all hydrophobic molecules and mostly contain long hydrocarbon chains connected with functionally active groups. They too perform various functions such as the constitution of cell membranes, regulation of metabolism in the form of hormones and energy production by oxidation in vital organs such as heart and muscles. Nucleic acids are polymers of nucleotides, which in turn is made up of three components, a ribose sugar, a nitrogenous base and one or more phosphates. The primary role of nucleic acids is storage and transmission of genetic information and maintenance of genetic constitution within a population. In monomeric form, nucleotides are known to act as important energy transducers, such as adenosine triphosphate or ATP, popularly known as the energy currency of the cell. Among these four, only lipids are different in two aspects, first, they are not polymeric and second they are insoluble in water.

## 1.5.2 Scale of Length of Biomolecules

The scale of biological entities spans from angstrom sized atoms to several meters of complete organisms, even cells can span from few micrometres to several centimetres, approximately 15 orders of magnitude on the scale of length. All biochemical entities are made up of atoms which are in the Angstrom size range. An individual carbon atom is roughly 0.2 nm in diameter and it would take about 5 million of them, laid out in a straight line, to span a millimetre. Little larger than atoms are small molecules such as water in the size range of 0.3 nm. The sizes of ions inside a cell can are defined by their ionic radii, which are a fraction of a nanometre. Most commonly occurring ions, $Na^+$, $K^+$ $Mg^{2+}$ and $Cl^-$ have their ionic radii in the rage of 0.09 nm, 0.13 nm, 0.07 nm and 0.18 nm respectively. Slightly bigger are the monomeric units of key biomolecules. Amino acids, nucleotides, fatty acids and monosaccharides have the sizes approximately 0.5

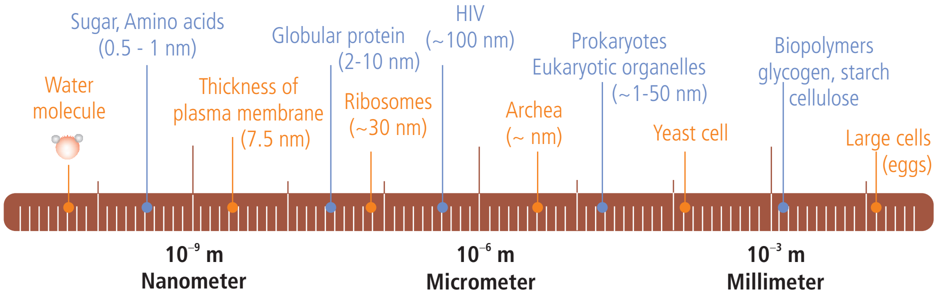

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**Figure 1.4** Scale of various biomolecules.

to 1 nm. High energy molecules such as ATP and NADH are approximately 1.5 nm and 2.5 nm respectively. As these small units of biomolecules begin to polymerize they give rise to relatively larger entities. Nucleic acids can be as large as several centimetres in length, globular proteins are in the size range of 2–10 nm while fibrous proteins may span several millimetres. Lipids when organise in bilayer can be several micrometres in length and approximately 7.5 nm in thickness. An average cell size is about 100–300 mm for eukaryotes while 5–30 mm for prokaryotes, with a few exceptions. Figure 1.2 illustrates the scale of biomolecules in living organisms. Besides these variations in the basic dimensions, biomolecules greatly differ in their geometries and arrangement of atoms in three-dimensional space giving rise to enormous diversity. The diversity of structures of various biomolecules shall be discussed in respective chapters in section 2.

### Practice Question

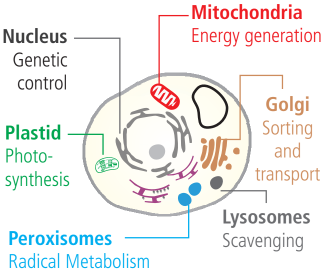
Q. What is burst size of a virus and bacteria? What governs this burst size?

Hint: The number of viruses or bacteria that are produced when an infected cell releases them is called burst size. Some viruses infect bacteria whereas others infect mammalian cells, but the sizes of both groups of viruses are relatively similar, whereas the hosts differ in size by a characteristic volume ratio of 1000. This helps explain the fact that burst sizes from bacteria are about 100 whereas in the case of mammalian cells the characteristic burst size is ≈100,000.

## 1.5.3 Biochemical Systems of Eukaryotes are Compartmentalised

During the initial period of origin of life, the cellular processes were less complex and all the phenomenon required a similar kind of environment. However, with the increasing complexity of biochemical processes and evolution of biochemical reactions, different thermodynamic and kinetic requirement developed. This has driven the compartmentalization of the cell. One of the prominent theories of compartmentalization is endosymbiosis. The compartments inside a cell, most of which are membrane-bound are called organelles and are characterized by segregation of biochemical reactions, biochemical environment etc. Many vital biochemical processes take place in or on membrane surfaces. Lipid metabolism, for example, is catalyzed mostly by membrane-bound enzymes, and oxidative phosphorylation and photosynthesis both require a membrane to couple the transport of $H^+$ to the synthesis of ATP. The nucleus is the control centre as it stores genome and is the principal site of DNA and RNA synthesis. The cytosol that constitutes a little more than half the total volume of the cell, is the site of protein synthesis and degradation. It also performs most of the cell's intermediary metabolism which mostly includes anaplerotic reactions, which degrade small molecules synthesized some new molecules to provide the building blocks for macromolecules. Endomembrane systems such as endoplasmic reticulum and Golgi bodies are mainly involved in protein and lipid trafficking. Lysosomes, are the organelles with reduced pH and contain digestive enzymes that degrade non-functional intracellular organelles, as well as macromolecules and particles taken in from outside the cell by endocytosis. A lysosome with some endocytosed material is called endosomes. Peroxisomes are small vesicular compartments that contain enzymes utilized in a variety of oxidative reactions. Most of the cellular environment is neutral with pH close to 7, and maintain the constant pH by buffering mechanisms (discussed in Chapter 3). The cytoplasm of most cells maintains a constant reducing environment, which is mainly regulated by special reducing enzymes such as Glutathione peroxidase, superoxide dismutase etc. These regulate the redox balance in the cytosol by keeping reactive oxygen species (ROS) at very low levels, yet some organelles such mitochondria, peroxisomes and endoplasmic reticulum maintain an oxidising environment.

**Compartmentalization**



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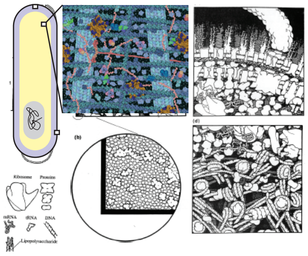
## 1.5.4 Cells are Highly Crowded

The biochemical environment of the cells is not as clean as it appears in most of the illustrations and pictures in text books. In fact the cell analogous a highly crowded environment tightly packed with molecules which are moving at a speed of a rifle bullet. Macromolecules can occupy 30% of the cellular volume with their concentrations reaching 300 grams per Litre. Goddshell in 1991 made an interesting calculation for E coli and mentioned that in a 100 nm$^3$ volume, there are on an average, of 30 ribosomes, over 100 protein factors, 30 amino acyl-tRNA synthetases, 340 tRNA molecules, 2-3 mRNA molecules and 6 RNA polymerase molecules. Besides this, if we include factors of bioprocesses, such as glycolysis the same part of cell would also contain nearly about 130 glycolytic enzyme molecules, 100 enzyme molecules from the citric acid cycle and a host of other anabolic and catabolic enzymes. Furthermore, if we include small molecules and ions, there would be approximately 30,000 small molecules, including precursors and cofactors. Also included are approximately 50,000 ions. In a cubic lattice, small molecules are about 3.2 nm apart, and ions at a distance of about 2.7 nm, suggesting a highly crowded state inside a cell. But this was just a static glimpse, environment of cell is highly dynamic. It is estimated that if an average 160 kDa protein were unhindered by surrounding molecules, it would travel at an average speed of about 500 cm s$^{-1}$ at 300 K in a confined or crowded environments, the rates of biochemical reactions can differ markedly from their dilute, well-mixed counterparts. Crowding reduces the diffusion coefficient of reactants, slowing diffusion-limited reactions and giving rise to fractal-like reaction kinetics. The same protein molecule of 160 kDa, that could move a distance of 10 nm (equivalent to its own size) in about 2 ns in non-crowded conditions, but the in real crowded conditions of the cell, direction and force the protein to perform a 'random walk' in space is greatly hindered and it would require almost 2 ms to traverse the 10 nm distance, almost a thousand times as long. This happens with almost every other molecule in the cells and indicate the real scenario. It has also been suggested that macromolecular crowding plays a role in cellular compartmentalization and phase separation. The stimulation of protein aggregation by crowding might account for the existence of molecular chaperones that combat this effect. Positive results of crowding include enhancing the collapse of polypeptide chains into functional proteins, the assembly of oligomeric structures and the efficiency of action of some molecular chaperones and metabolic pathways.

### Mnemonic

Compartmentalization of a cells is analogous to compartmentalization of our homes. Outfitting each room of your house with all the resources necessary to perform every household duty would be a waste of time, money and space. Cells compartmentalize their resources in the same way you do in your home, allowing each part of the cell to flourish in its own tiny environment.

**Molecular Crowding in Cell**



### Practice Question

Q. What is molecular crowding and how it makes cellular reaction different from biochemical assays performed in a test tube?

Hint: The phenomenon of macromolecular crowding alters the properties of molecules in a solution when high concentrations of macromolecules such as proteins are present. Crowding results in surprisingly large quantitative effects on both the rates and the equilibria of interactions involving macromolecules, but such interactions are commonly studied outside the cell in uncrowded buffers. The addition of high concentrations of natural and synthetic macromolecules to such buffers enables crowding to be mimicked in vitro and should be encouraged as a routine variable to study.

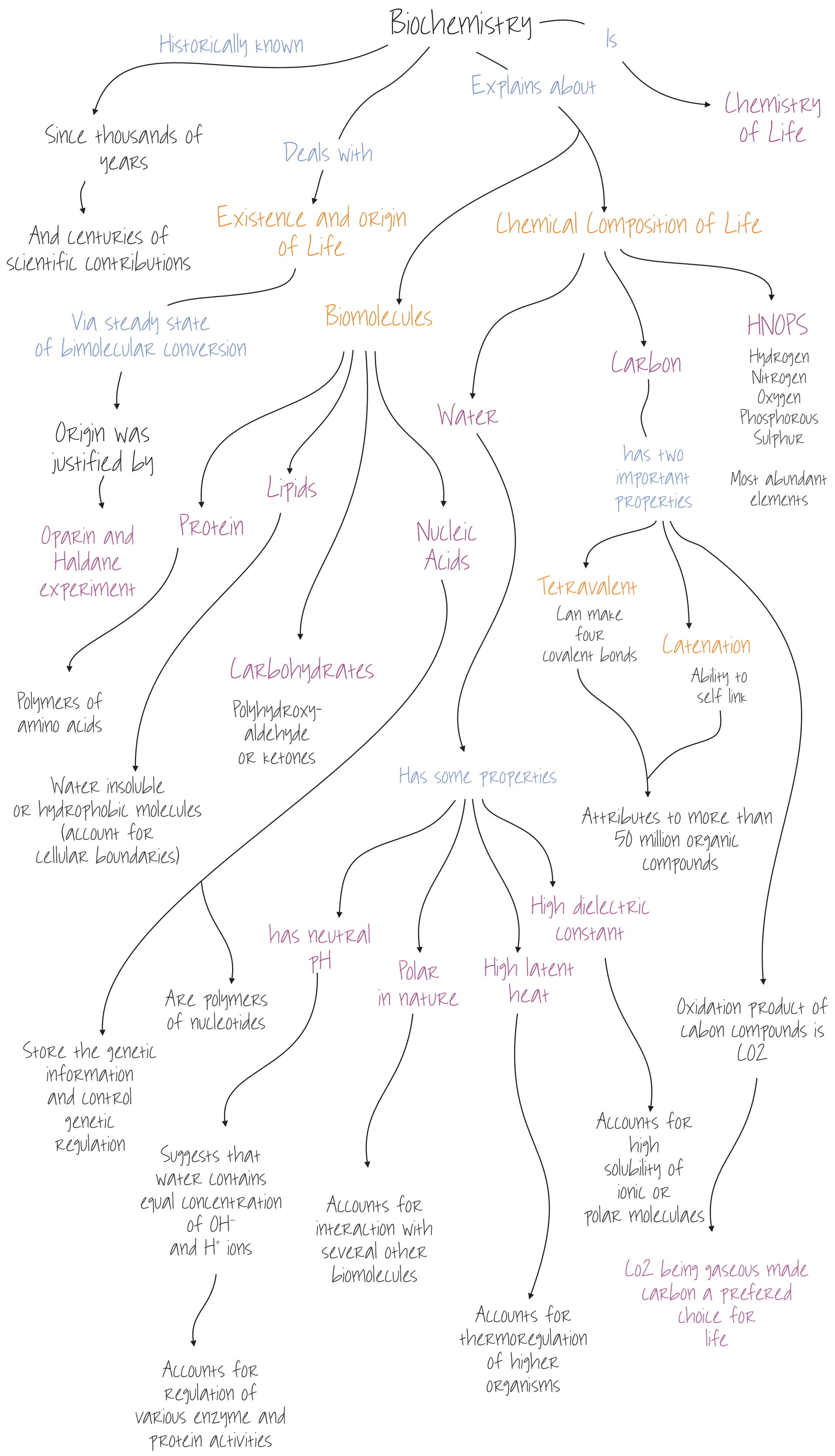
## Add to Your **Knowledge**

- Antoine Lavoisier in 1775, first proposed a mechanism for photosynthesis, a process wherein plants take in carbon dioxide and release oxygen. Lavoisier was also the first to investigate cell respiration in animals.
- In the period of 1773–1783, Chemistry became dominated by the phlogiston theory, or the hypothetical principle of fire. In this principle, burning (oxidation) was caused by liberating phlogiston, with ash as the dephlogistigated substance.
- In 1869, Friedrich Miescher first identified what he called "nuclein" inside the nuclei of human white blood cells
- In 1919, Phoebus Levene, discovered the order of the three major components of a single nucleotide (phosphate, pentose sugar, and nitrogenous base).
- In 1944 While working on bacterial samples, Oswald Avery first suggested in 1944 that the genetic material of the cell was possibly the deoxyribonucleic acid.
- In 1959, Erwin Chargaff began to challenge Levene's previous conclusions. He noted that the nucleotide composition of DNA differs among species and do not repeat in the same order reached two major conclusions and formulated famous Chargaff's parity rules.
- Frederick Sanger discovered the first and complete protein structure in 1958. The protein that was first identified is insulin.
- Sixteen years after the discovery of the triplets of the DNA, Fred Sanger had successfully sequenced the genome of a bacteriophage which contained more than 5000 nucleotides.
- Not long after, he was able to sequence the DNA of the human mitochondrial genome which consisted of more than 16 000 nucleotides.
- In 1960, the biochemist Robert K. Crane revealed his discovery of the sodium-glucose cotransport as the mechanism for intestinal glucose absorption. This was the very first proposal of a coupling between the fluxes of an ion and a substrate that has been seen as sparking a revolution in biology.
- The first molecules of life might have met on clay, according to an idea elaborated by organic chemist Alexander Graham Cairns-Smith at the University of Glasgow in Scotland. These surfaces might not only have concentrated these organic compounds together, but also helped organize them.
- The deep-sea vent theory suggests that life may have begun at submarine hydrothermal vents spewing key hydrogen-rich molecules. Their rocky nooks could then have concentrated these molecules together and provided mineral catalysts for critical reactions.
- The revelation last week that tiny eight-legged organisms (water bears) survived exposure to the harsh environment of space on an Earth-orbiting mission is further support for the idea that simple life forms could travel between planets.
- The incredible survival tale of the tiny tardigrades, also called water bears, is a dramatic reminder that life can survive space travel. The dot-sized invertebrate creatures endured 10 days of exposure, and upon return to Earth and supports Panspermia theory of the origin of life.

## Value Based **Questions**

1. What is life and what role does biochemistry play in differentiating a living organism from non-living?
2. What is vitalism? How this belief was shattered in the early developments of biochemistry?
3. Discuss the hypothetical presence of silicon-based life forms and why it was not preferred over carbon despite tetravalency and ability of self-linking?
4. What are the similarities between the composition of seawater and cells, how does it hint about the origin of life in oceans?
5. If an alien form was identified which has silicon-based structure, what differences would you expect in the biochemistry of those aliens? If we presume that life originated from extraterritorial life forms? How would you support with scientific evidence?
6. Q6. Calculate the number of protein molecules in a bacterial cell (1 $\mu m^3$), yeast cell (30 $\mu m^3$) and animal cell (3000 $\mu m^3$), provided that the protein mass per volume is about 0.2 mg/ml and an average number of amino acids per protein is 350?

# concept map



Term Biochemistry was coined by Carl Neuberg, however other prior use of the term is also reported.

Vitalism prevailed before biochemistry originated. A major blow to vitalism came from the experiment of Wohler, who demonstrated the synthesis of Urea.

Most of the early discoveries in Biochemistry were related to the process of catalysis and fermentation in living cells, by Pasteur, Buchner, Sumner and Leibig.

Oxygen is the most abundant element in Earth crust as well as living beings. However, on excluding water carbon is most abundant element of life forms

Water is elexir of life by virtue of its various life supporting properties such as high dielectric constant, polar nature and ability to make hydrogen bonds.

Almost 70% of the cell is water and remaining 30% is made of ions, metabolites, and four key biomolecules, i.e. nucleic acids, proteins, lipids and carbohydrates.