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

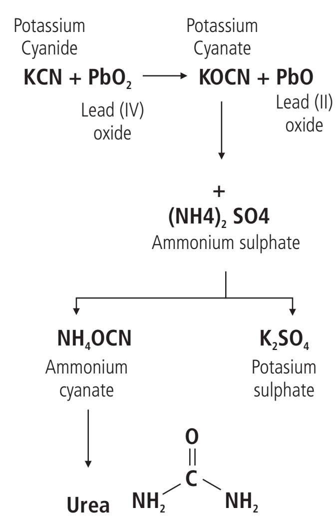
Interesting Fact

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



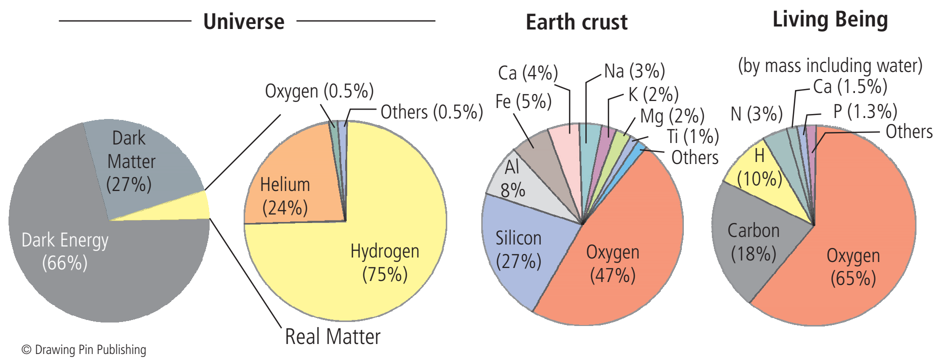


Figure 1.2 Atomic composition of Universe, Earth Crust and The living organisms.

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.

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.

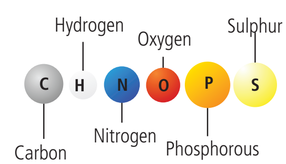
Note

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



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 ὕδωρ (ýdor), Russian вода́ (vodá), Irish uisce, Albanian ujë.

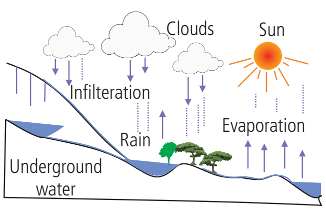
Table 1.1 Summary of various life supporting properties of water.

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	1.8×10^{-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



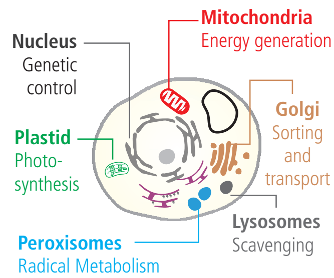
1.4 ORIGIN OF BIOMOLECULES AND LIFE

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 recell 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



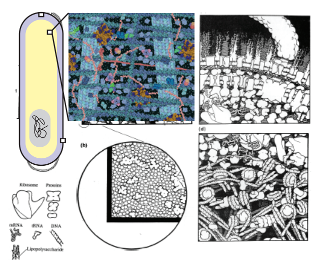
1.5.4 Cells are Highly Crowded

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.

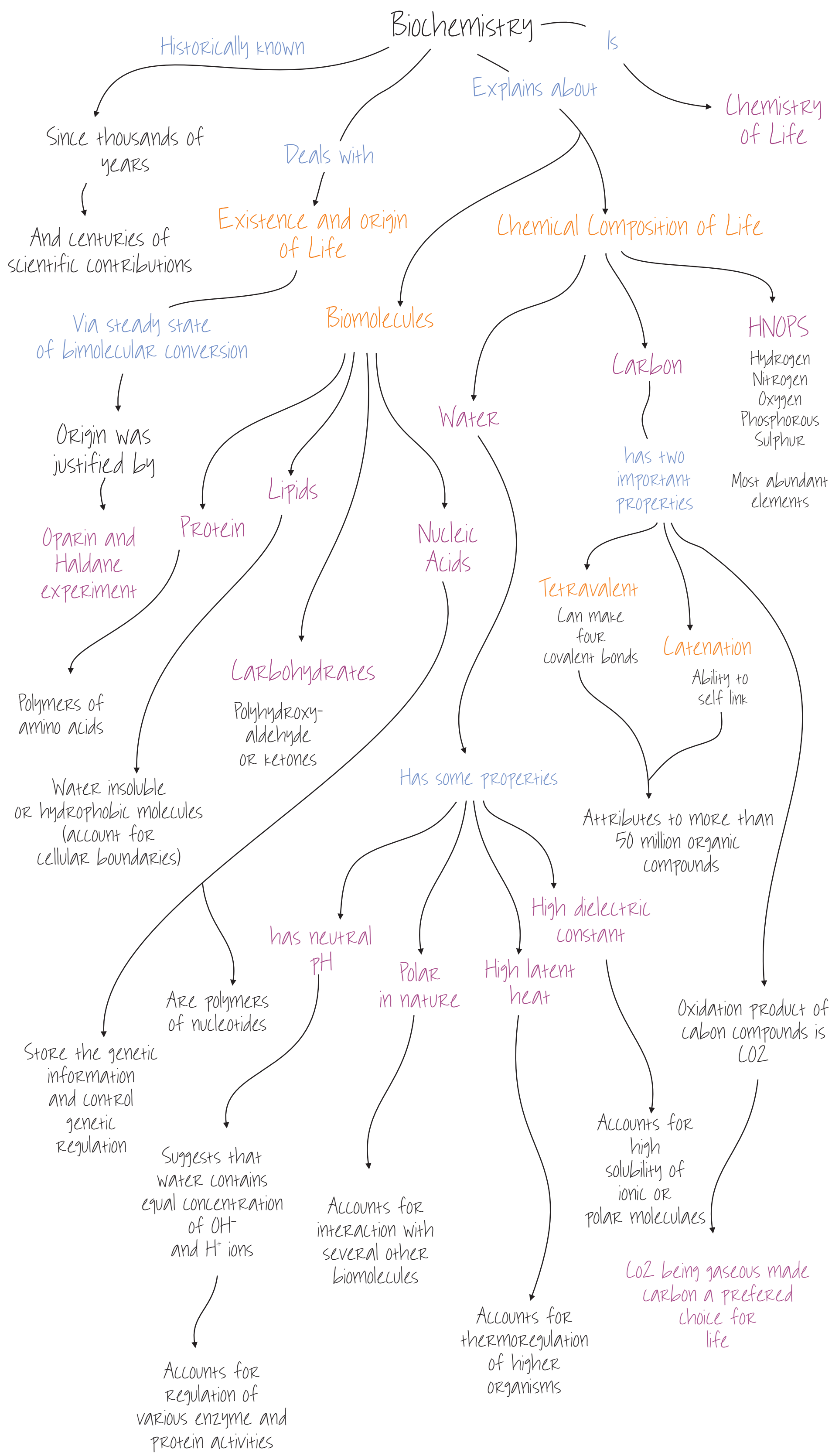
Add to Your **Knowledge**

- 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 μm^3), yeast cell (30 μm^3) and animal cell (3000 μm^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.