Subject	Chemistry
Paper No and Title	15. Bioinorganic Chemistry
Module No and Title	22. Structure and function of hemoglobin
Module Tag	CHE_P15_M22_e-Text

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1. Learning Outcomes

After studying this module, you shall be able to understand:

- Why oxygen needs to be transported efficiently in living organisms?
- Role of hemoglobin in carrying out the vital function of oxygen transport.
- Historical evolution of hemoglobin- Who discovered this magnificent iron containing metalloprotein? Who elucidated its function and finally how was its structure discovered?
- Description of the structure of hemoglobin.
- A brief elucidation of functions of hemoglobin in transport of oxygen, carbon dioxide and nitric oxide.
- Biosynthetic pathway involved in the hemoglobin synthesis.
- Hemoglobin disorders that occur due to inadequate supply of oxygen to vital organs.

2. Introduction

Oxygen is the most essential element responsible for sustaining almost all forms of life on earth. If an adequate supply of oxygen is not circulated throughout the body to vital organs and tissues, then it may lead to severe health disorders such as brain damage, organ failure and even death. This respiratory demand for oxygen cannot be satisfied simply by the diffusion of oxygen from the environment to the respiring tissues; in fact special transport systems are required to carry out this vital function of oxygen transport in living organisms. It is here where the role of hemoglobin (the most intensively studied protein) comes into picture.

3. What is hemoglobin?

Hemoglobin (Hb) is an iron containing oxygen-transport globular metalloprotein belonging to a family of hemeproteins found inside the red blood cells (erythrocytes) of nearly all vertebrates as well as plasma of some invertebrates. Each red blood cell contains more than 300 million hemoglobin proteins that are responsible for carrying oxygen from the respiratory organs such as lungs or gills to the rest of the body i.e. the tissues **(Figure 1)**. The transported oxygen provides energy to power important biological functions (like oxidative phosphorylation) of an organism. Studies reveal that the average life span of Hb is same as that of red blood cells i.e. 120 days and approximately 6.25 gm of Hb are synthesized and destroyed every day in an adult person. Besides, the normal hemoglobin levels differ between males and females, ranging from 12-16 g/dL in women and 13-18 g/dL in men.

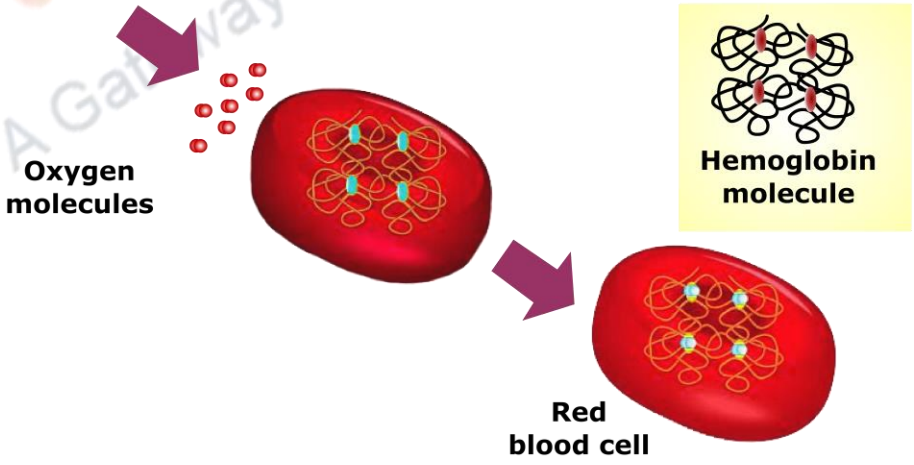

Figure 1: Transport of oxygen by human haemoglobin

4. Historical evolution of hemoglobin

In 1840, a German scientist named **Friedrich Ludwig Hunefeld** discovered the oxygen-carrying protein "hemoglobin" by viewing the blood of an earthworm. During his examination, Sir Hunefeld reported:
"I have occasionally seen in almost dried blood, placed between glass plates in a desiccator, rectangular crystalline structures, which under the microscope had sharp edges and were bright red." These red crystalline structures were called hemoglobin.
Thereafter, a series of articles describing the growth of hemoglobin crystals through successive dilution of red blood cells with solvent such as pure water, alchohol or ether followed by slow evaporation of the solvent from the resulting protein solution were published by a German physiologist "**Otto Funke**" during the year 1851. A few years later, the mechanism involving reversible oxygenation of hemoglobin was revealed by **Felix Hoppe-Seyler**, while the function of hemoglobin in the blood was elucidated by French physiologist Claude Bernard. However, like many other proteins, the structure of hemoglobin was too complex to be determined until X-Ray crystallography made it possible. **Max Perutz**, an Austrian-British molecular biologist, established the molecular structure of hemoglobin which was indeed a great discovery as it helped him win the Nobel Prize for Chemistry in 1962.

5. Structure of hemoglobin

Hemoglobin is a tetramer with a molecular weight of 65,000 consisting of four homologous globulin chains (oligomeric proteins possessing a three dimensional structure) that are connected together by disulphide bridges. The normal adult hemoglobin molecule contains two alpha-globulin chains comprising of 141 amino acids and two beta-globulin chains comprising of 146 amino acids. It is the arrangement and interactions of these amino-acid residues present within the protein that not only governs the protein's shape but also contributes substantially to its function. In infants, the hemoglobin molecule is generally made up of two alpha chains and two gamma chains while in adults, the gamma chains are gradually replaced by beta chains. Attached to each globulin chain is an iron porphyrin complex known as "**heme**" which plays a vital role in transporting oxygen and carbon dioxide in our blood **(Figure 2)**.
The heme group contains a single embedded iron atom, held in the centre of a square described by four nitrogen atoms and exists at the heart of an array of organic rings called a porphyrin. Each hemoglobin molecule binds four oxygen molecules as it contains four iron atoms, each capable of binding one oxygen. It is the iron contained in hemoglobin that accounts for the distinctive red colouration of our blood.
Further, details of the structure of hemoglobin (i.e. conformation changes on dioxygen binding) shall be discussed in the other modules.

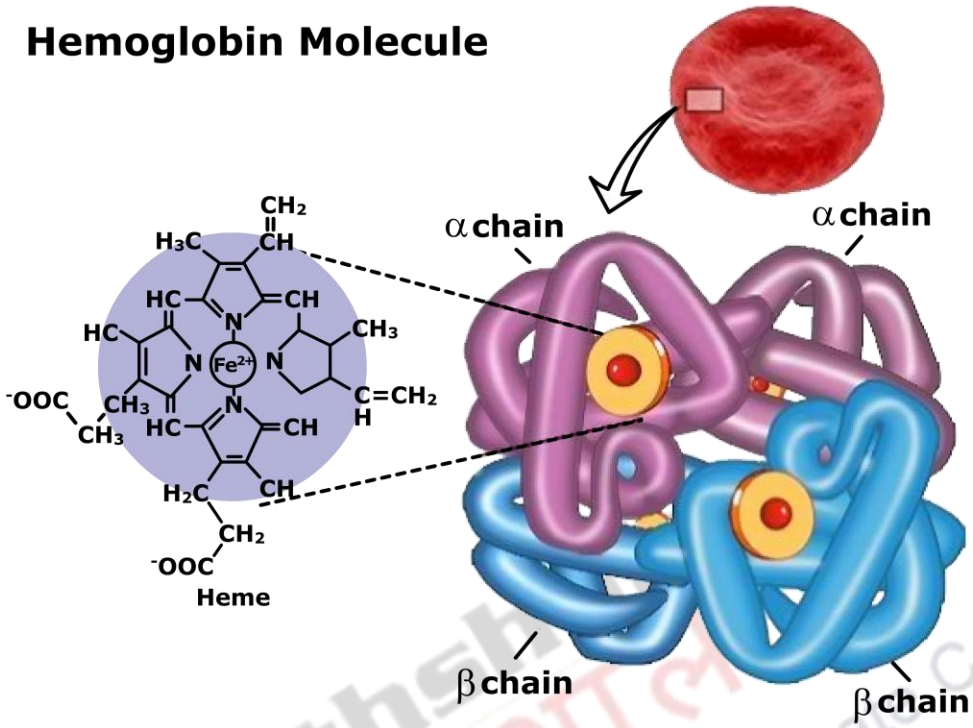

Figure 2: Structure of hemoglobin

6. Functions of hemoglobin

Due to low solubility of oxygen, only a small fraction of total oxygen gets dissolved in the circulatory system. However, a large amount of oxygen is needed for conversion of foodstuffs such as lipids and carbohydrates into available energy in the form of ATP. Therefore, oxygen carrier is required for increasing oxygen carrying capacity of the circulatory fluid. The protein hemoglobin serves as the oxygen carrier in the blood of vertebrates and has an oxygen binding capacity of 1.34 mL O_2 per gram. In an oxygen rich environment, hemoglobin binds oxygen with high affinity and subsequently releases oxygen in the tissues where oxygen content is not enough. In the tissues, hemoglobin picks up carbon dioxide for its transport to the lungs in a form of complex known as carboxyhemoglobin. Besides this, hemoglobin also plays important role in regulating blood pressure as it can efficiently transport nitric oxide which can relax the blood vessel walls allowing greater blood flow.

7. Synthesis of hemoglobin

The synthesis of hemoglobin takes place during the proerythroblast stage of the RBC cycle by a series of biochemical reactions in the bone marrow. The heme part of hemoglobin is synthesized in the mitochondria and the cytosol of immature red blood cells while the globin protein parts are synthesized in the cytosol with the help of ribosomes. Similar to the other proteins, the synthesis of the globin chain is determined by the nucleotide sequences in the structural **genes** on chromosomes 16 and 11. The synthesis of heme begins with the condensation of glycine & succinyl CoA to form **δ**-aminolevulinic acid (ALA) in the mitochondrion of the cell **(Figure 3)**.

Thereafter, the ALA is transported to the cytosol where a series of reactions generate a ring structure called coproporphyrinogen III. This molecule then returns to the mitochondrion and undergoes an addition reaction to produce protoporhyrin IX. Protoporhyrin IX subsequently combines with iron to form heme with the help of enzyme known as ferrochelatase. Heme unit thus synthesized leaves the mitochondria and is joined to the globin chains in the cytoplasm. A disruption at any point in the production of hemoglobin may result in several disorders such as iron deficiency anaemia, lead poisoning, thalassemia and sideroblastic anaemia.

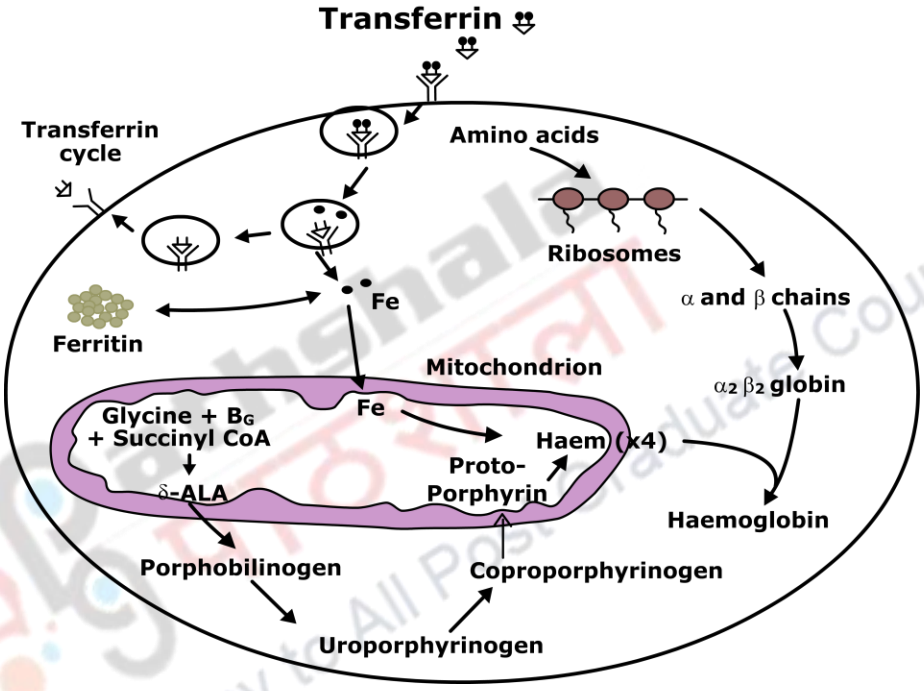

Figure 3: Bio-synthesis of the most intensively studied protein "Hemoglobin"

8. Hemoglobin disorders

Hemoglobin disorders are inherited blood diseases that could lead to inadequate supply of oxygen to the vital organs. Such disorders are passed down through families in similar way as blood type, hair colour and texture, eye colour and other physical traits. It has been estimated that approximately 300 000 babies are born each year with severe forms of these diseases. Generally, these disorders are most prevalent in tropical regions but population migration has spread them to several countries. Therefore, efforts must be applied worldwide to effectively reduce such type of disorders through a strategic balance of disease management and prevention programmes.

8.1 Sickle cell anaemia

Sickle cell anaemia is a genetic disease of the red blood cells associated with episodes of acute illness and progressive organ damage due to abnormality in the oxygen-carrying protein hemoglobin. This disease develops as a result of mutation in a gene on chromosome 11

that codes for the beta subunit of the hemoglobin. This leads to improper formation of hemoglobin molecules causing red blood cells to be rigid and take a concave shape like a sickle used to cut wheat **(Figure 4)**. Because of their irregular shape, these sickle cells can't squeeze through small blood vessels as easily as the almost spherical shaped normal red blood cells. As a result, irregularly shaped cells get stuck in the blood vessels which then stop the oxygen transport to various tissues causing severe pain and damage to the organs.

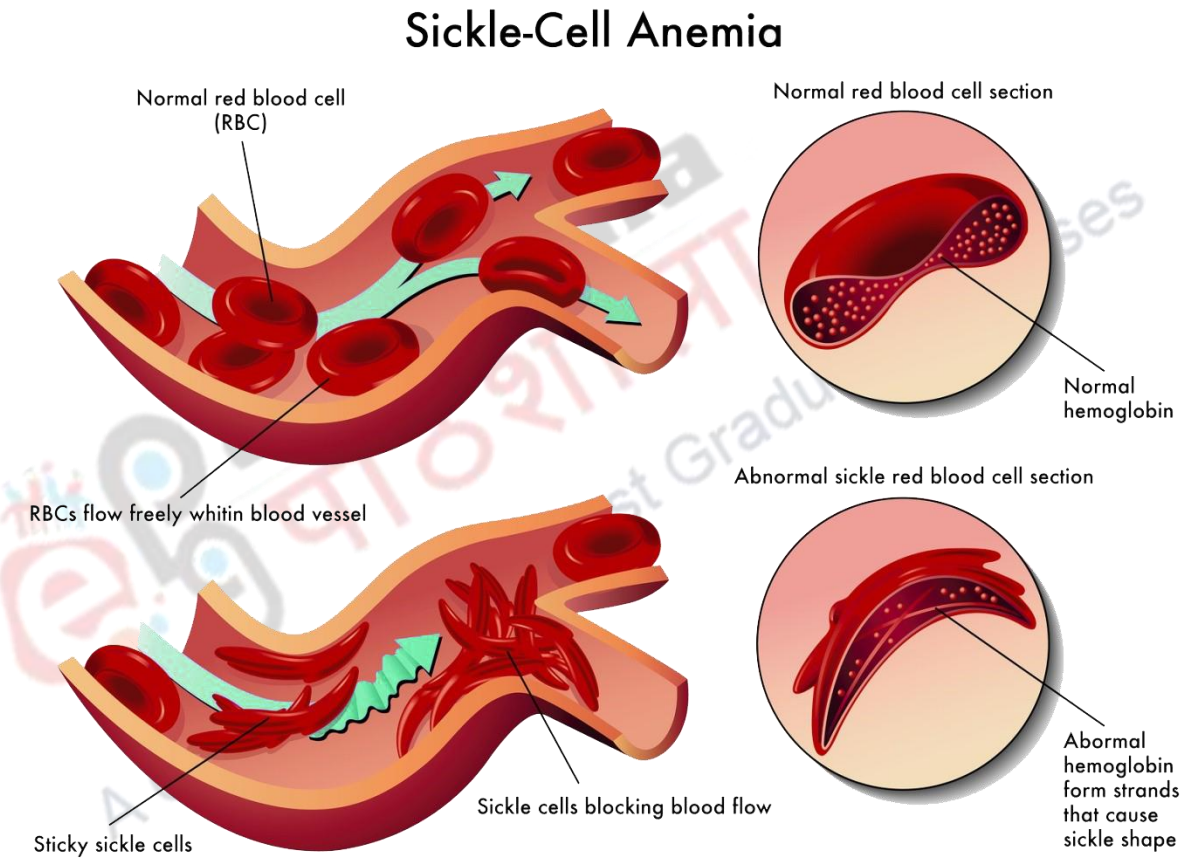

Figure 4: A comparison of the normal red blood cells with the sickle cells

8.2 Thalassemia

Thalassemia is an inherited autosomal recessive blood disorder that affects a person's ability to produce hemoglobin, resulting in mild or severe anaemia. It occurs when the production of the hemoglobin chains (mostly the alpha and the beta forms) is largely impaired, eventually causing the destruction of red blood cells **(Figure 5)**.

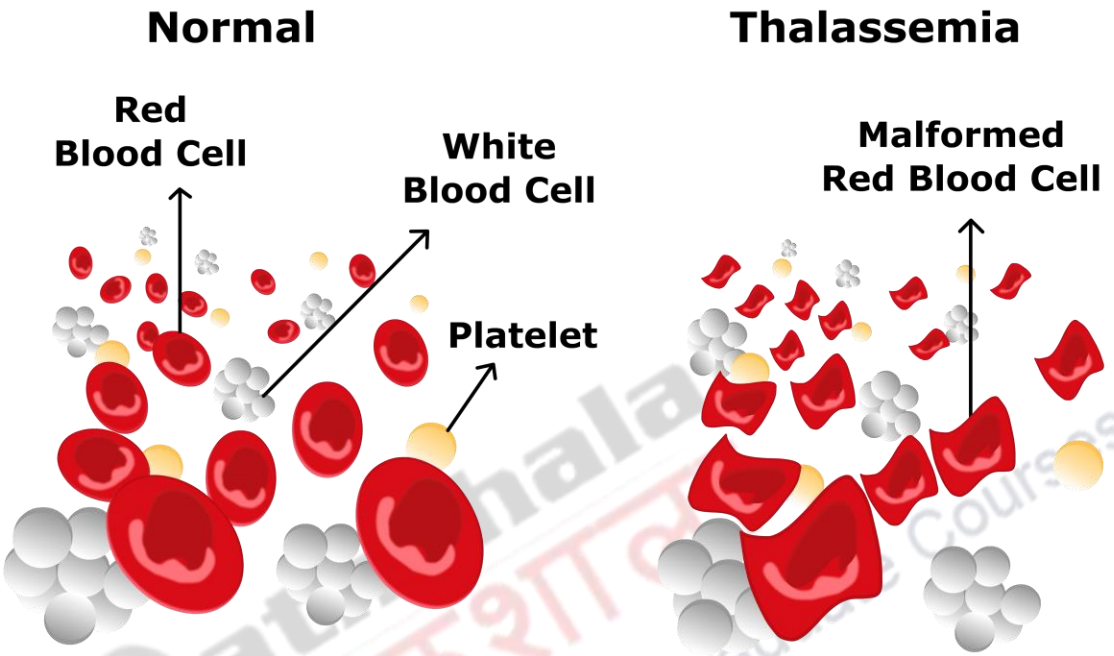

Figure 5: A comparison of the normal red blood cells with the malformed red blood cells (affected with thalassemia)

Primarily, there are two types of thalassemia:

a) Alpha Thalassemia (occurs when genes related to the alpha globin protein are missing or mutated). Normally, four genes are required for the synthesis of the alpha globin chain; moderate to severe anaemia can occur when more than two genes are affected. In such a case, the beta globin chains combine to generate abnormal beta tetramers that cannot bind oxygen.

b) Beta-Thalassemia (occurs when similar gene defects affect production of the beta globin protein). Beta globin chains usually need two genes for their synthesis, most severe form of the disease affecting both genes. In this case, no such alpha tetramers are formed; instead the alpha globin chains get degraded in the absence of beta globin chains.

8.3 Porphyria

Porphyrias-"derived from the ancient Greek word *porphura*, meaning purple" are a group of rare inherited metabolic blood disorders caused by altered activities of enzymes within the heme biosynthetic pathway involved in the production of porphyrins and heme (in other words, it occurs due to genetic mutation at any one of the enzymatic steps explained in **figure 6**). As the body lacks the enzymes required for completion of the heme synthesis, porphyrin tends to get accumulated in tissues and blood, thereby affecting either the skin (cutaneous porphyria) or the nervous sytem (acute porphyria). The symptoms of porphyria vary depending upon on which essential enzyme is missing. However, the most common symptoms of this disease include abdominal pain, light sensitivity (causing rashes or blisters), numbness, paralysis or mental disorders.

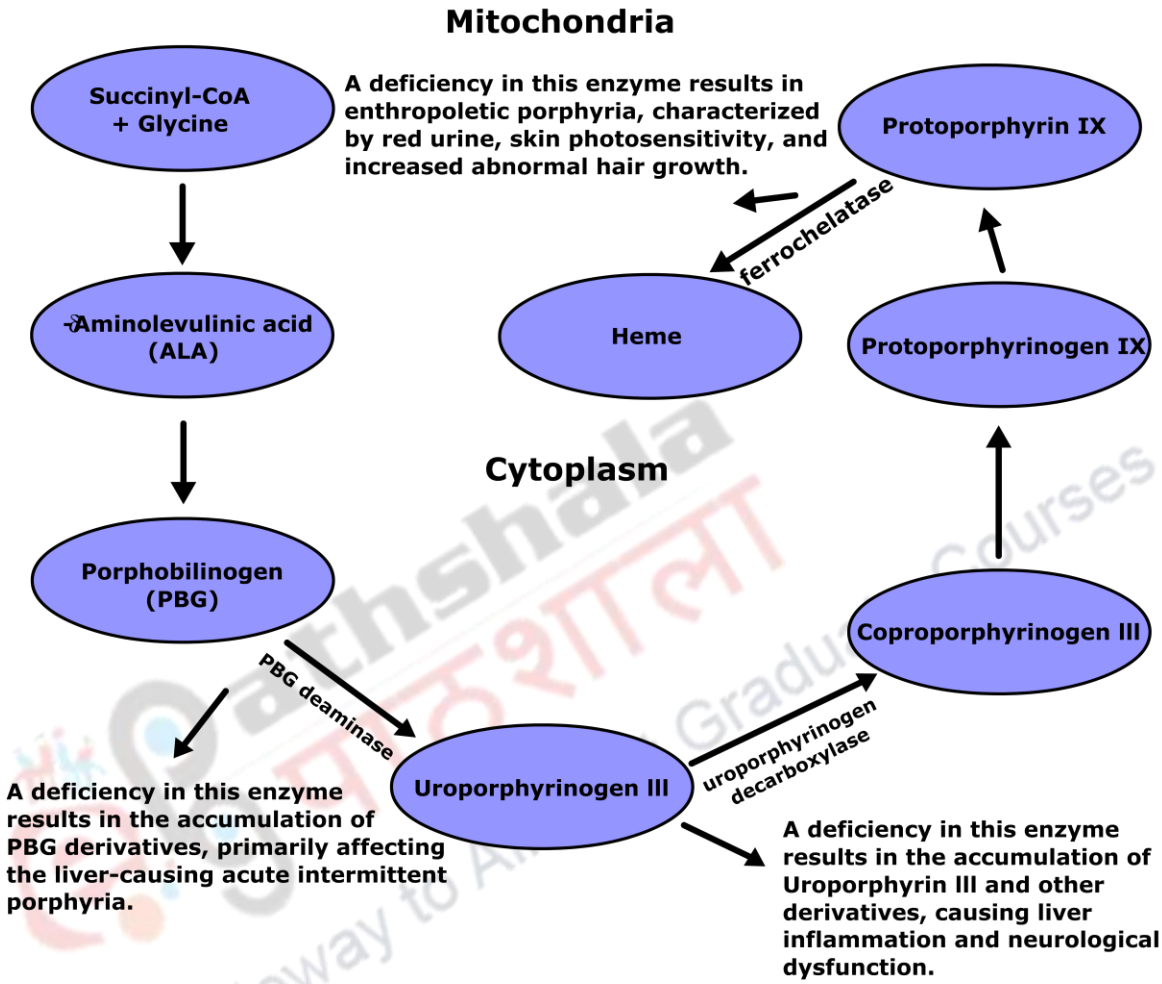

Figure 6: A simplified diagram of the Heme synthetic pathway and the enzymes commonly associated with porphyrias

8.4 CO poisoning

Carbon monoxide is a very dangerous gas and can cause fatal poisoning since it binds hemoglobin preferentially over oxygen when both are present in the lungs owing to its higher affinity towards hemoglobin in comparison to oxygen. Once carbon monoxide sticks to hemoglobin forming a very bright cherry red carboxyhemoglobin, it keeps riding around never giving their seats up to the oxygen. Eventually, blood loses all of its ability to transport oxygen and there is no way to get oxygen to your brain, heart, or other cells which eventually stops all the biochemical reactions **(Figure 7)**.

Carbon monoxide binding equation at equilibrium:

$Hb(aq) + 4CO(g) \rightleftharpoons Hb(CO)_4(aq)$

So, inhalation of even trace amount of carbon monoxide can cause headaches, fatigue, depression and dizziness. However, if exposure is chronic it can lead to more serious complications like heart disease and sometimes death.

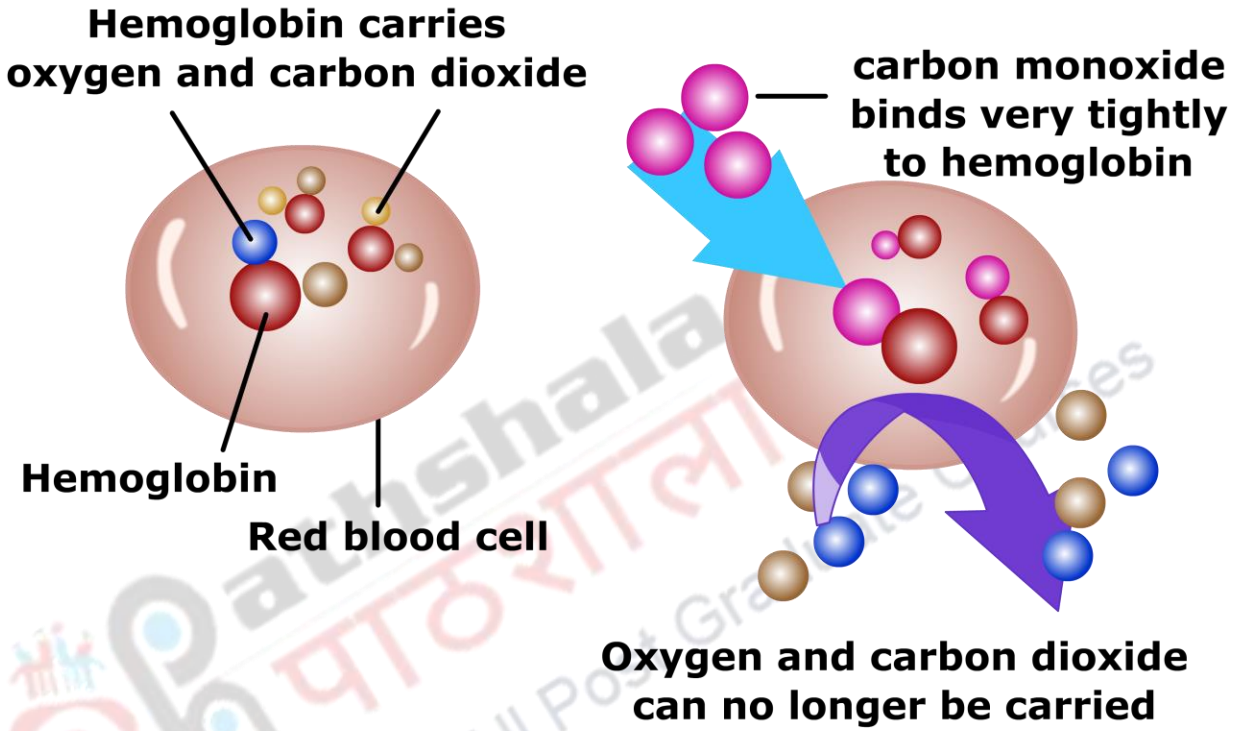

Figure 7: Illustration of CO poisoning

9. Summary

- Oxygen is the most essential element that sustains all forms of life on earth and if an adequate supply of oxygen is not circulated throughout the body to vital organs and tissues, then it may lead to severe health disorders such as brain damage, organ failure and even death.
- Special transport proteins such as hemoglobin (oxygen-transport globular metalloprotein found inside red blood cells of nearly all vertebrates as well as plasma of some invertebrates) are required for the efficient transport of oxygen across the body.
- This oxygen carrying protein was discovered by Hunefeld in 1840 and its final structure was determined by Max Perutz many years later with the help of X-Ray Crystallography.
- It is a tetrameric molecule consisting of globin chains that are attached to an iron-porphyrin complex called heme, responsible for binding oxygen.
- Apart from transporting oxygen from lungs to tissues, hemoglobin is also involved in the transport of carbon dioxide from the tissues to the lungs and in the transport of nitric oxide for regulating blood pressure.
- The synthesis of hemoglobin occurs during the proerythroblast stage of the RBC cycle by a series of biochemical reactions in the bone marrow.
- Abnormalities associated with the hemoglobin synthesis may lead to serious disorders such as sickle cell anaemia, thalessemia, porphyria and CO poisoning as a result of which oxygen cannot be transport efficiently to the vital organs.