Role of Micronutrient in Human Body

Introduction

World Health Organization (WHO) data show that deficiency of micronutrients is far more severe. Approximately 1.7 million (2.8%) of deaths worldwide are attributed to micronutrients deficiency caused by lower consumption of fruits and vegetables that is regarded as top 10 selected risk factors for global mortality

According to WHO, iron (Fe), zinc (Zn), vitamin A (beta-carotene), selenium (Se), and iodine (I) are the most prevalent trace element deficiencies in living being. Population affected by their deficiencies showed low productivity, decreased cognition, learning disability among children, lower worker productivity, increased morbidity and mortality rates, impact on immune system, and high health-care costs

Micronutrients are essential elements needed by life in small quantities. They include microminerals and **Vitamins**. Microminerals or trace elements include at least iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, and molybdenum.

They are dietary minerals needed by the human body in very small quantities (generally less than 100mg/day) as opposed to macrominerals which are required in larger quantities.

The use of the term "mineral" here is distinct from the usage in the geological sciences.

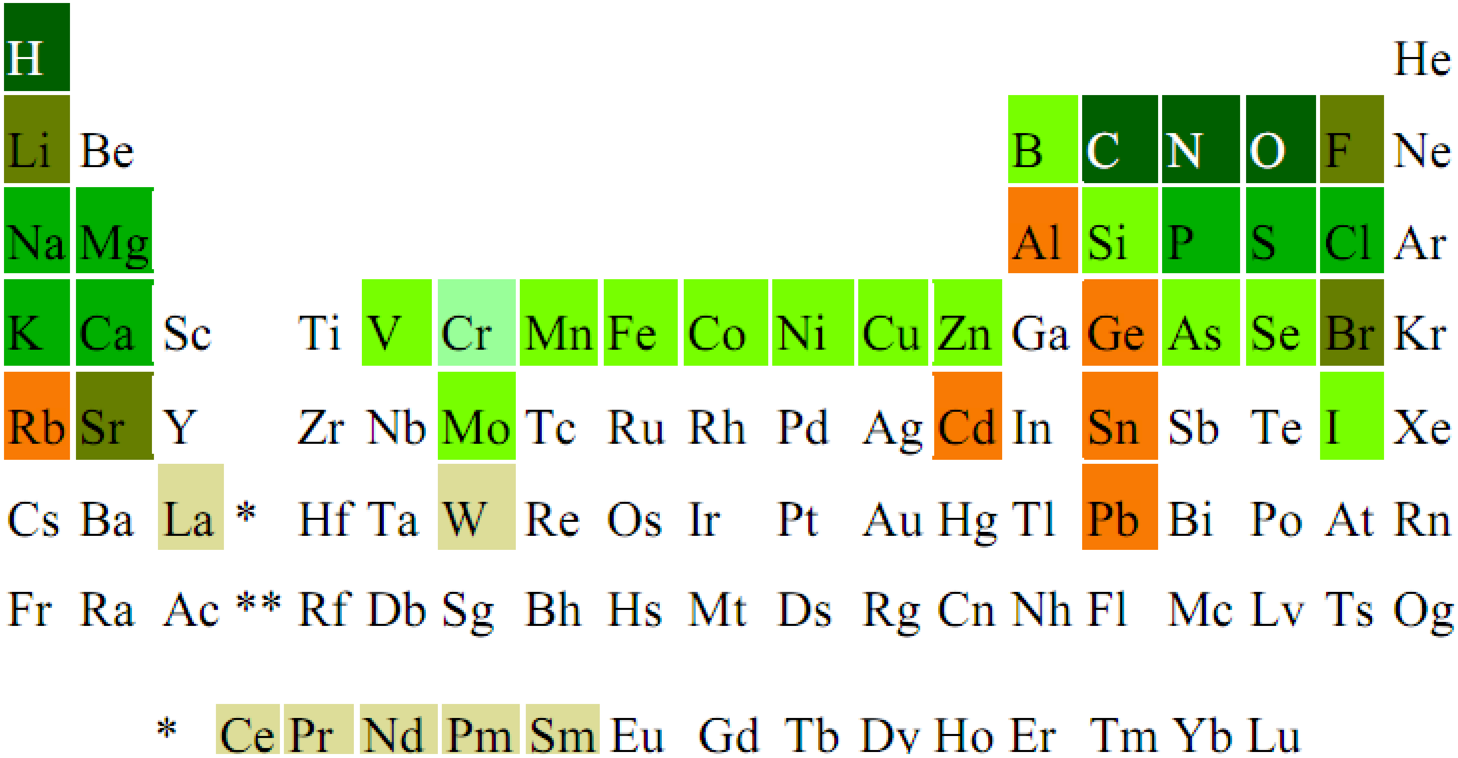
Vitamins are organic chemicals that a given living organism requires in trace quantities for good health, but which the organism cannot synthesize, and therefore must obtain from its diet.

Essential inorganic elements for humans

At least 20 chemical elements (minerals) are known to be required by human to support biochemical processes by serving functional and structural roles as well as being electrolytes (Zoroddu *et al.,* 2019). Oxygen, hydrogen, carbon and nitrogen (O, H, C, and N) are the most abundant elements in human body by weight and make up around 96% of the weight of the human body. Calcium makes up 920 g to 1200 g of the body weight of an adult, with 99% of it contained in the bones and teeth. This is around 1.5% of body weight (Berdanier *et al.,* 2013). Phosphorus occurs in amounts of around 2/3 of calcium, and makes up around 1% of an individual's body weight (National Library of Medicine, 2016). The other major minerals (sodium, chlorine, sulfur, magnesium, and potassium) make up only approximately 0.85% of the weight of human body. Together these 11 chemical elements (O, Ca, P, K, Na, H, C, N, Cl, Mg, S) make up 99.85% of the human body. The remaining ~18 ultratrace minerals consist of just 0.15% of the human body, or about 1 g in total for the average individual.

Different opinions exist regarding the essential nature of several ultratrace elements in humans (and other mammals too), even based on same data. For instance, there is no scientific agreement on whether chromium is considered an essential trace element in human. The US and Japan designate chromium as essential nutrient (Institute of Medicine (US) Panel on Micronutrients, 2001), but the EFSA (European Food Safety Authority), representing the EU, reviewed the scientific question in 2014 and does not approve (European Food Safety Authority, 2014).

Most of the suggested and known mineral nutrients are of low atomic weight relatively, and are reasonably common on soil (land), or for iodine and sodium, in the ocean:



H																		He
Li	Be													B	C	N	O	F	Ne
Na	Mg													Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	**	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Nh	Fl	Mc	Lv	Ts	Og
		*	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		

Legend:

🟩 The 4 basic organic elements

🟩 The quantity elements

🟩 The essential trace elements

🟩 Deemed essential trace element by the U.S., not by the EU

🟫 Suggested function from the deprivation effects or the active metabolic handling, but unclearly biochemical function in the humans

🟧 Limited circumstantial evidence for the trace benefits or biological action in the mammals

Table 4: Roles of minerals in biological processes

Dietary element	RDA (US) [mg]	UL (US and EU) [mg]	High nutrient density dietary sources	Term for deficiency	Term for excess	Category
Calcium	1200	2500; 2500	Dairy products, canned fish with bones (salmon, sardines), green leafy vegetables, eggs, nuts, tofu, thyme, oregano, dill, cinnamon, seeds.	hypocalcaemia	hypercalcaemia	Needed for heart, muscle, and digestive system health, builds bone, supports synthesis and function of blood cells
Chlorine	2300	3600; NE	Table salt (NaCl) is the main dietary source.	hypochloremia	hyperchloremia	Required for production of hydrochloric acid in stomach and in cellular pump functions
Chromium	0.035	NE; NE	Broccoli, grape juice (mostly red), meat, whole grain products	Chromium deficiency	Chromium toxicity	Involved in glucose and lipid metabolism, though its action mechanisms in the body and the amounts required for optimal health are inadequately defined
Cobalt	none	NE; NE	Essential in the synthesis of vitamin B_{12}, but as bacteria are required to synthesize the vitamin, it is commonly considered part of vitamin B_{12} that comes from consumption of animals as well as animal-sourced foods (eggs...)		Cobalt poisoning	

Copper	0.9	10; 5	Liver, seafood, nuts, oysters, seeds; some: whole grains, legumes	copper deficiency	copper toxicity	Required component of various redox enzymes, including the cytochrome c oxidase
Iodine	0.150	1.1; 0.6	Seaweed (kelp or kombu)*, eggs, iodized salt, grains	iodine deficiency	iodism Hyperthyroidism	Required for the synthesis of thyroid hormones, triiodothyronine and thyroxine and to prevent goiter: Iodine in biology
Iron	18	45; NE	Meat, seafood, nuts, beans, dark chocolate	iron deficiency	iron overload disorder	Required for many enzymes and proteins, notably the hemoglobin to prevent anemia
Magnesium	420	350; 250	Spinach, legumes, seeds, whole grains, nuts, peanut butter, avocado	hypomagnesemia, magnesium deficiency	hypermagnesemia	Required for processing ATP and for bones
Manganese	2.3	11; NE	Grains, seeds, nuts, legumes, leafy vegetables, tea, coffee	manganese deficiency	manganism	A cofactor in enzyme functions
Molybdenum	0.045	2; 0.6	Legumes, whole grains, nuts	molybdenum deficiency	molybdenum toxicity	The oxidases xanthine oxidase, sulfite oxidase, and aldehyde oxidase

Phosphorus	700	4000; 4000	Red meat, dairy foods, bread, rice, oats, fish, poultry. In biological contexts, usually seen as phosphate	hypophosphatemia	hyperphosphatemia	A component of bones, cells, in energy processing, in DNA & ATP (as phosphate) and various other functions
Potassium	4700	NE; NE	Sweet potato, tomato, potato, beans, seafood, banana, prune, lentils, dairy products, carrot, orange	hypokalemia	hyperkalemia	A systemic electrolyte and is also essential in coregulating ATP with sodium
Selenium	0.055	0.4; 0.3	Brazil nuts, seafoods, meats, grains, dairy products, eggs, organ meats	selenium deficiency	selenosis	Essential to activity of the antioxidant enzymes such as glutathione peroxidase
Sodium	1500	2300; NE	Table salt (sodium chloride, NaCl, the main source), milk, sea vegetables, and spinach.	hyponatremia	hypernatremia	A systemic electrolyte and is also essential in coregulating ATP with potassium
Zinc	11	40; 25	Oysters*, red meat, nuts, whole grains, poultry, dairy products	zinc deficiency	zinc toxicity	Pervasive and required for many enzymes such as carboxypeptidase, carbonic anhydrase, and liver alcohol dehydrogenase

Elements considered likely essential but unconfirmed

Element	Description	Excess
Arsenic	Essential in rat, goat, chicken, and hamster models, however no biochemical mechanism known in human.	arsenic poisoning
Boron	Boron is essential plant nutrient, required mostly for maintaining integrity of cell walls (Mahler, 2009). Boron has been indicated to be essential to complete life cycle in representatives of all the phylogenetic kingdoms, including the model species *Xenopus* laevis (African clawed frog) and *Danio rerio* (zebrafish). In animals, supplemental boron was shown to activate vitamin D and reduce calcium excretion.	Nontoxic
Bromine	Likely important to tissue development and basement membrane architecture, as a required catalyst to make collagen IV (Scott *et al.*, 2014).	bromism
Fluorine	Fluorine (in fluoride form) is not considered as an essential element because human does not require it to sustain life or for growth. Research shows that the primary dental benefits from fluoride occur at the surface from the topical exposure. Of the minerals presented in this table, fluoride is only one which the US Institute of Medicine established an Adequate Intake.	Fluoride poisoning

Lithium	It is unknown whether lithium has physiological role in any species, however nutritional studies involving mammals have shown its importance to health, resulting in a suggestion that it can be classed as essential trace element.	Lithium toxicity
Nickel	Nickel is an essential constituent of many enzymes, including hydrogenase and urease (Berdanier *et al.*, 2016). Although not required by humans, many are thought to be needed by gut bacteria, such as urease needed by some varieties of Bifidobacterium. In human, nickel may be cofactor or structural component of some metalloenzymes involved in hydrolysis, gene expression, and redox reactions. Nickel deficiency depressed growth in pigs, sheep, and goats, as well as diminished circulating concentration of thyroid hormone in rats.	Nickel toxicity
Strontium	Strontium has been indicated to be involved in calcium utilization in the body. It promotes calcium uptake into the bone at moderate dietary strontium level, but a rickets-producing (rachitogenic) action at higher dietary level.	Rachitogenic (causing Rickets)
Other	Vanadium and silicon have established, though specialized, biochemical roles as functional or structural cofactors in other organisms, and are likely, even possibly, used by mammals (including human). In contrast, tungsten, early lanthanides, and cadmium all have specialized biochemical usages in some lower organisms, but the elements seem not to be used by humans. Other elements considered to be likely essential include rubidium, aluminium, germanium, and tin (Insel *et al.*, 2004).	Multiple

Group of elements playing a role in the human body

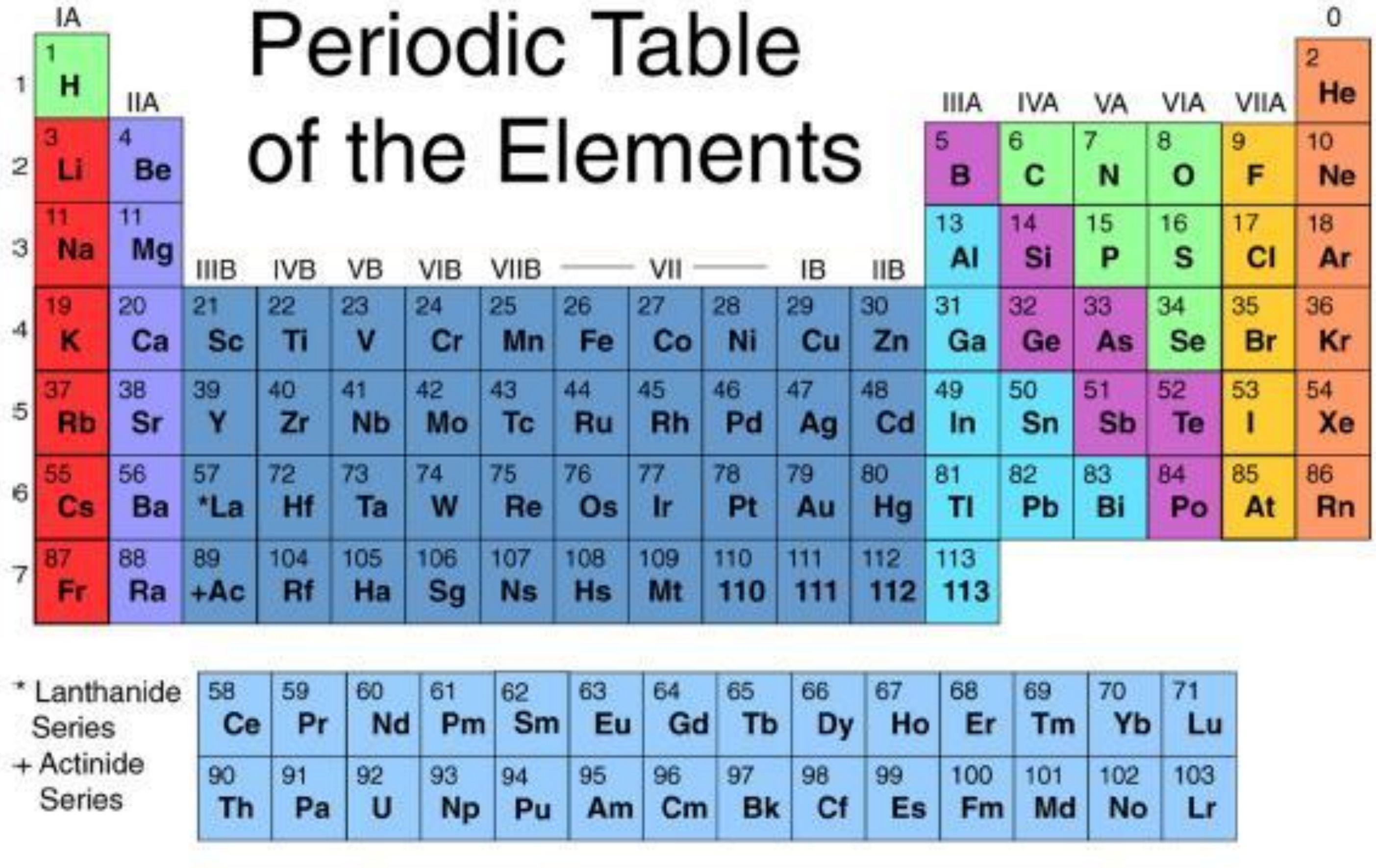
(a) Essential (18 Elements, e.g., Na, K, Mg, Ca, Mo, Mn, Fe, *Cu, and Zn*)
(b) Beneficial (8 Elements, e.g., Si, V, Cr, Ni, Se, Br, Sn and F.)
(c) Contaminating (20-30 trace elements)
(d) Polluting elements.

Requirement for Essential Elements

(i) It should be present in all healthy tissues of the body;
(ii) Should have a fairly constant concentration range; and
(iii) Its absence from the body should cause reproducible physiological **abnormalities which can be restored upon the readmittance of the same element.**

Toxicity of Elements

The most toxic effects are exerted by those elements of the periodic table which are adjacent to essential and beneficial elements such as Pb, Cd, Hg.



Periodic Table of the Elements

	IA	IIA	IIIB	IVB	VB	VIB	VIIB	VII			IB	IIB	IIIA	IVA	VA	VIA	VIIA	0
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	11 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	57 *La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	89 +Ac	104 Rf	105 Ha	106 Sg	107 Ns	108 Hs	109 Mt	110 110	111 111	112 112	113 113					

* Lanthanide Series	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
+ Actinide Series	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

- The toxic effects of elements such as Cd, Pb and Hg are exerted by virtue of their being potent enzyme inhibitors and this is due to the occurrence of phosphate and chloride ions in vivo which causes, the precipitation of insoluble phosphates and chlorides of these metals.

- The effects of toxic metals on a species are related to the rates of its absorption, distribution, and assimilation into an enzyme and excretion.

- Inorganic lead causes blood disorders by combining with sulphydryl group of enzymes involved in biosynthesis of Haeme and cytochromes.

- Soluble mercury salts are extremely toxic mainly because of their corrosive action on intestines, kidneys and brain.
- Alkyl mercury is more toxic because it is rapidly absorbed by red blood cells and then traverses the blood brain barrier, causing damage.

The mercury compounds get bound to proteins in cell membranes and thus influence the distribution of ionic electric potentials and passage of nutrients across these membranes causing permanent damage to brain cells.

- The alkyl mercury compounds can also cross placental barrier thus causing serious damage to developing fetus.
- Cd occurs to the extent of 0.5% in all zinc ores and thus impure Zinc plated vessels if used become a cause of renal damage, gastro-intestinal disorders and Cardiovascular malfunctions.

Role of Inorganic Species in Vivo

The elements of organic matter H, C, N, 0, P and S constitute the greatest proportion of body's atoms as shown below:
H = 63%; 0 = 25.5%; C = 9.5%; N = 1.4%

Main Group Ions

(a) Sodium and Potassium:

- Both these metal ions are responsible, for balancing electrical charges associated with negatively charged organic macromolecules in body; to maintain proper potential gradient across cell membranes which is required for the transmission of nerve impulses and to keep the osmotic pressure inside the Cell in balance so that the cell does not collapse.
- The process in which Na^+ ions are readily expelled out from cells and K^+ ions taken in its place is called ion transport or a *sodium pump* and is required for maintaining a balance between the fluids inside and outside the cells. The two concentrations in the body fluid being Na = 0.01 M and K = 0.15M. This difference in concentration in the two fluids produces an electrical potential gradient across the cell membranes which is essential for transfer of nerve impulses.

- Sodium has few specific functions also. Its extrusion from cell is associated with active transport of amino acid and glucose as both these enter the cell together. Potassium has a low charge density and so has the possibility of diffusing through hydrophobic solutions e.g. liquid - protein cell membrane.
- K^+ acts as an important cofactor for some internal enzymes such as pyruvate kinase essential for glycolysis. The most critical life process of protein synthesis by ribosomes requires high
concentration of K^+ ions.

(b) Calcium and Magnesium:

Like Na^+ and K^+ these ions also help to maintain membrane potential and transmit nerve signals. These two metal ions bridge two carboxyllate groups of lipoprotein and this bridging serves to stiffen the cell membranes as it is found that in its absence cell membranes tend to become porous. Mg^{2+} ions are concentrated in cells while Ca^{2+} are present in body fluids outside cells.

(i) Calcium: ***As mentioned above Ca^{2+} ions are mainly extra cellular*** **and act as cofactor for extra cellular enzymes. The most important role of Ca^{2+} is in the formation of bones where it is present as apatite $Ca_3(P0_3)_2$ and in enamel of teeth as florapatite $3(Ca)_3(PO_3)_2 CaF_2$.**

There is a very fine control over the precipitation of calcium salts in order that bone material can be transferred through the blood stream, to be deposited in a new region. Ca^{2+} is also important for blood clotting, hormone release, contraction of muscles, regular beating of heart and the formation of intra cellular cement.

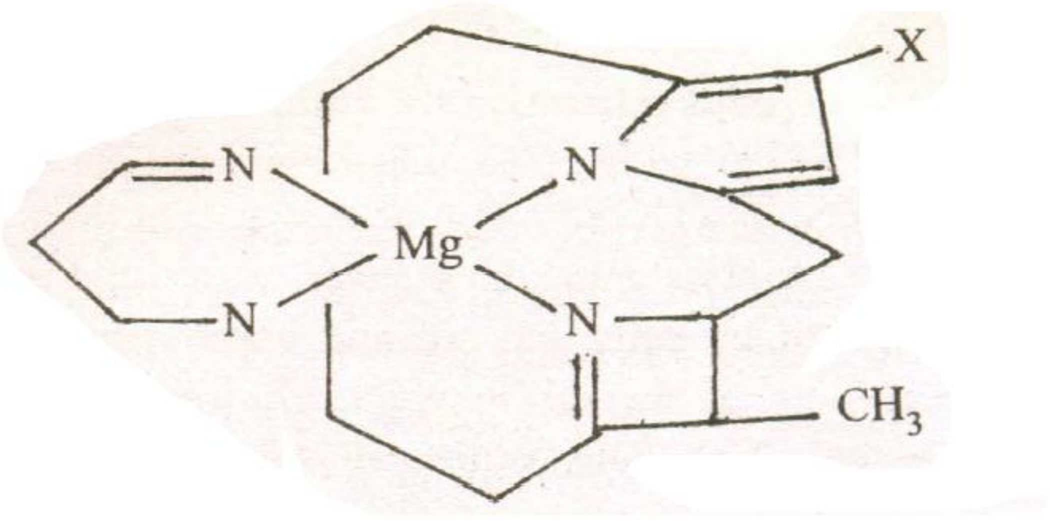
Calcium Pump: ***The Calcium pump is similar to sodium pump and*** **functions through a membrane bound Ca^{2+} Mg^{2+} Adenosine triphosphase and uptake of Ca^{2+} is associated with hydrolysis of ATP. It is found that muscles contract when concentration of Ca^{2+} in myoplasma reaches 10^{-6} mol cm-3 and relax as Ca^{2+} is released back in system. The released Ca^{2+} binds at active sites on muscle fibres and initiates contraction. The excess Ca^{2+} is immediately taken up by the system through membrane and is stored on calcium binding proteins. The molecular weight of Ca^{2+}, Mg^{2+} ATP are is in the range 100,000-120,000 daltons and its activity is promoted by proteins and phospholipids such as glycoprotein – which initiates ATP activity which regulates the activity of calcium pump. Two moles of Ca^{2+} bind per active phosphorylation site of protein and binding of first Ca2+ causes some conformational change which is followed by binding of second Ca^{2+}**

Vandium is found to inhibit the hydrolysis of ATPase by binding at active site originally occupied by phosphate thus retarding the binding of Ca^{2+}. Uptake of Ca^{2+}is very rapid and its levels are controlled by uptake and release of Ca^{2+}from mitochondria which take up Ca^{2+}by an energy dependent process which may either be respiration or hydrolysis of ATP. Ca^{2+}enters in response to the negative potential developed inside membrane during respiration, and its uptake is balanced by release of 2H^{+} ions from matrix. If respiration is some what inhibited Ca^{2+}can still be taken up by hydrolysis which can also generate a potential.

(ii) Magnesium: Magnesium is a stabiliser of internal structure and a cofactor for intracellular enzymes. Nucleotids exist inside cells as their Mg^{2+}complexes. Since Mg^{2+}binds preferentially to phosphates hence it is necessary for DNA replication and protein bio synthesis. A very important function of Mg^{2+}is in photosynthesis. Solar energy is the ultimate source of biological energy and photosynthesis is the process by which this energy is converted to chemical energy as shown below:

$$nH_2O + nCO_2 \xrightarrow{\text{Light}} (CH_2O)n + nO_2$$

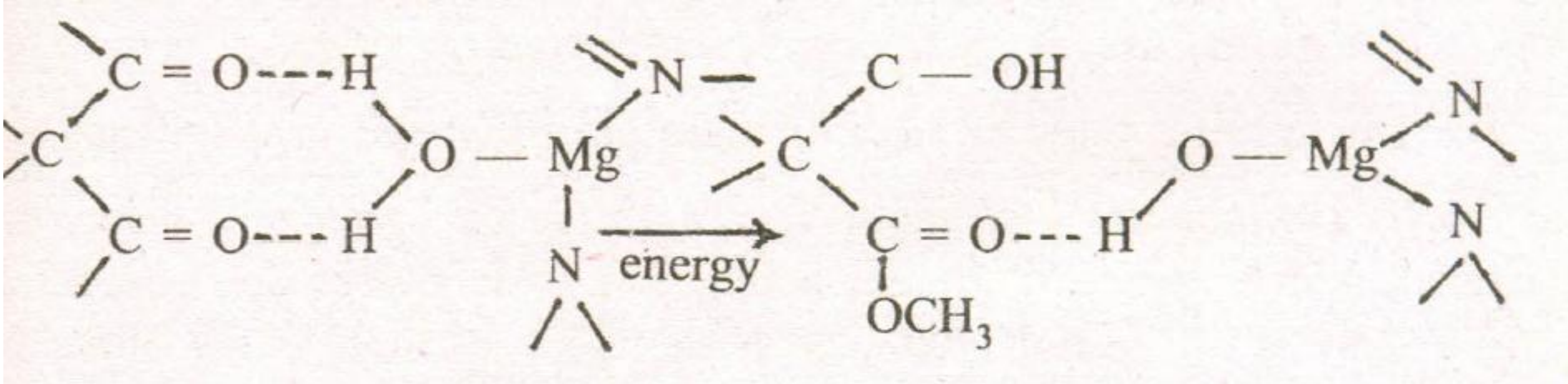
Photosynthesis is dependent upon a green pigment called chlorophyll which contains Mg^{2+} as the central metal ion and is also called magnesium porphyrin. The skeleton structure of chlorophyll is:



Skeleton Structure of Chlorophylls

if X = CH_3 it is chlorophyll a and if X = -CHO it is chlorophyll b.

In these porphrin compounds Mg^{2+} is normally four coordinate but further action with water or other solvent molecules may also occur. Thus five coordination can also occur giving a structure similar to magnesium tetraphenyl porphyrin hydrate where Mg^{2+} is out of the plane of nitrogen atoms and is square pyramidal. In chlorophyll hydrogen bonding interactions can occur and these result in polymeric structures. The role of chlorophyll in photosynthetic reduction of CO_2 is to provide electrons and to form chlorophyll radical. The electrons are transmitted through these to other intermediates of process.



Trace Elements

(1) General Roles

As mentioned before a total of thirty elements are essential for biological processes taking part in living organisms and out of these 19 are trace elements. The exact roles of these trace elements are still unknown though elements like Fe, Zn, Cu, Mn, Mo, Co, Ni and Se serve as key components of essential enzymes or other proteins which perform vital functions, while elements like Y, Cr, Cd, Pb, Sn, Li, F, Si, As & B -are essential for survival but their roles are undefined.

The most important group of trace elements is transition and post transition metals. The reason probably being that these contain incompletely filled d and f orbitals and are also, capable of forming complexes with S, N and donors, which are side chain constituents of proteins. Fe is very important as it is a vital constituent of hemoglobin and other heme proteins.

Except Ti, all other 1st series transition metals are important such as V, Cr, Mn, Fe, Co while post transition like Zn, Cu, Ni are also important. Titanium can be important as titanate but there is no evidence that it is needed. Molybdenum is definitely essential while remaining metals are absorbed by cells and are non toxic. Tungsten is known to decrease the activity of several enzymes requiring molybdenum and is itself a component of enzyme formate dehydrogenase of bacteria.

These trace elements probably, exhibit their vital roles in metabolism in several ways:

(a) As metallo, enzymes in which the metal ion is firmly bound to **and is an integral part of enzyme protein molecule,**

(b) As metal ion activated enzymes in which the metal ion is weakly **bound. Several different metals may be required to activate one particular enzyme.**

(c) May lock the geometry of enzyme protein so that only a specific **substrate may be attached and**

(d) May bind the substrate to enzyme thereby acting as a template **e.g., the use of Ni for cyclisation of corrin ring.**

The specific roles of trace Elements

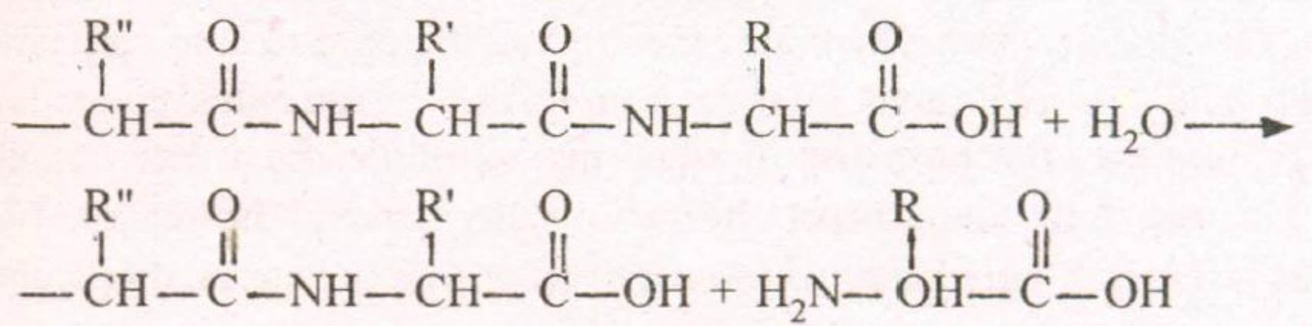
(ii) Zinc: *Which was shown in 1934 to be essential for the normal* growth is present in human body to the extent of 1.4-2.3 g and this is the second largest amount of a metal in humans. The reason why the studies on the importance of Zn have been slow is the fact that due to a $3d^{10}$ shell it is colourless and non magnetic and can thus be traced in body with difficulty. But is a strong Lewis acid and forms complexes similar to those of transition metals.

Zinc complexes form good pH buffering systems in vitro and are used in highly' important pH control mechanism in vivo. The blood plasma of man has a normal pH and if this falls below 7 or rises above 7.8 irreparable damage may occur and hence a very sensitive pH control system is needed for which Zn is essential.

Approximately 18 zinc metallo enzymes have been recognized.

Zinc metallo enzymes

(a) Carboxy Peptidase A: is present in pancreatic juice and helps in the digestion of proteins in animals and protein metabolism in plants. It has a molecular weight of 34,600 daltons and catalyses the hydrolysis of terminal peptide bond at the carboxyl end of protein. The enzyme acts better if the side chain of terminal residue is aromatic.



$$-\overset{\overset{\mathrm{R''}}{|}}{\mathrm{CH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{NH}-\overset{\overset{\mathrm{R'}}{|}}{\mathrm{CH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{NH}-\overset{\overset{\mathrm{R}}{|}}{\mathrm{CH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{OH} + \mathrm{H_2O} \longrightarrow$$

$$-\overset{\overset{\mathrm{R''}}{|}}{\mathrm{CH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{NH}-\overset{\overset{\mathrm{R'}}{|}}{\mathrm{CH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{OH} + \mathrm{H_2N}-\overset{\overset{\mathrm{R}}{|}}{\mathrm{OH}}-\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}-\mathrm{OH}$$

The structure of the enzyme determined by x-ray shows that zinc ion is coordinated to two histidine nitrogen atoms and an oxygen from the carboxyl side chain of glutamase residue and one water molecule completing a tetrahedral structure.

***(b) Carbonic Anhydrase*: is present in red blood cells and is involved** **in respiration. It accelerates the absorption of *CO_2 by red blood* cells in muscles and tissues and the reverse reaction involving the release of CO_2 to lungs. It also regulates the pH due to reaction.**

$$CO_2 + OH^- \longrightarrow HC0^{3-}$$

The enzyme has a molecular weight of 30,000 daltons and is found in two similar but not identical forms. The structure determined by x-ray shows zinc ion coordinated to three histidine imidazole nitrogens. The enzyme regulates pH around 7 by deprotonation and giving form E which is required for hydration of *CO_2* while the protonated form EH^+ is required *for reverse reaction.*

(c) alkaline phosphatese: For energy release

(d) Dehydrogenese and aldose: For sugar metabolism

(e) Alcohol dehydrogenase: For metabolism of alcohol.

(f) Metallothioneins: ***These are low molecular weight proteins rich*** **in cysteine content which can bind zinc and because of this serve as detoxifying agent. In their structure these contain 61 amino acids, an α-residue containing Cysteine and β-residue containing 9 cysteines and each Zn atom is coordinated to four cystein sulphur atoms in a tetrahedral coordination.**

<u>(iii) Copper: </u>Copper is essential for life and is the third most abundant **element in human body (approximately 100 mg) but larger amounts are toxic. About 4-5 mg. of the element is required in diet daily and its deficiency impaires the use of iron from liver, causing anaemia. It is bound to various oxidases and proteins either as metals to proteins or as blue proteins.**

Most copper biomolecules contain normal blue form but many others such as ascorbate oxidase contains one normal, three blue and two binuclear sites. In the normal sites Cu^{2+} is coordinated to a square set of ligands and the additional ligands occupy the vacant sites above and below the plane. For blue copper sites, the coordination, is unsymmetrical, with one long Cu-S bond and other short bonds. The intense blue colour is due to S-Cu charge transfer transition. For the coupled binuclear sites no structural data are available.

Copper is necessary for release of iron from storage proteins and is also an important constituent of following metalloenzymes.

(a) Lysine Oxidase: *Which affects the elasticity of aortic walls.*

(b) Dopamine Hydroxylase: *Which affects brain functions.*

(c) Cerulo Plasmin: *Which plays a role in metabolism of iron and* is found in blood of animals, birds, reptiles and amphibians and has a molecular weight of 1,50,000 daltons. Its shortage causes wilson's disease which results in accumulation of copper in liver, kidneys and brain. This can be treated by administering EDTA which forms a soluble complex with copper which is excreted out.

(d) Plasto Cyanin and Azurin: *These are blue proteins which act as* electron transfer agents by the use of Cu^{2+}ICu^{+} *couple. Former occurs in* chloroplasts of green plants, contains one copper atom and is required in photosynthesis as an electron carrier, while later is found in bacteria and also contains one Cu atom.

(e) Superoxide Dismutase: *Amongst the several enzymes which* catalyze the reaction

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

One which is very important contains two identical sub units with a molecular weight of 16000 daltons and contain one Cu and one Zn atom. It is clear that the Cu atom of the enzyme is the active center while Zinc simply supports the role by maintaining the overall structure in place and can be replaced by other metals while the replacement of copper destroys the enzyme.

***(f) Haemocyanin: is in reality a non heme but Cu containing protein* which is an important oxygen carrier in respiration and is also called a respiratory protein. The molecular weight is one million and contains two subunits. The colourless deoxy form contains Cu^{1+} while the blue oxy form contains Cu^{2+}. The two copper atoms are 3.8± 0.4A° apart and each is coordinated with three amino acid residues. A similar structure has also been reported for tyrosinase which catalyses oxidation of phenol to O-phenyldiols and affects skin pigmentation.**

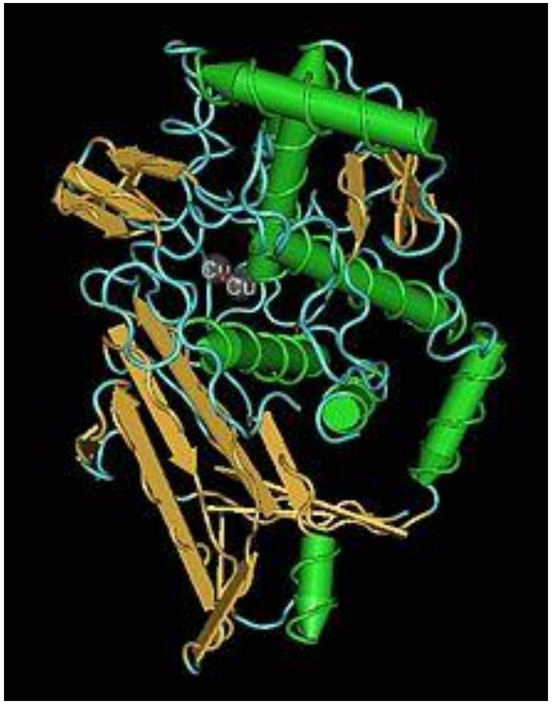


Figure: ***Haemocyanin***

(g) Oxidases: Are structurally very complicated since these contain **copper sites of all the four types interrelated by redox mechanism. Example is Laccase with a molecular weight of 1,10,000 daltons contains one normal copper site, one blue copper site and one coupled Cu^{2+} site and in total four copper atoms.**

(iv) Chromium Molybdenum and Tungsten:

The necessity of trace amounts Cr and Mo in diet was shown in 1955 and until the discovery of certain enzymes containing tungsten these were the only metals below 1st transition series which were known to be essential for living systems. The most important *role of Cr is in glucose tolerance and in its deficiency* glucose is removed from the blood slowly, causing diabetes. It works in conjunction with insulin as a molecule called *glucose tolerance factor where it is present as Cr(III) along with cysteine, glycine and glutamic* acids but the exact structure of molecule is unknown. It is also known to be present in nucleic acids where it is bound to phosphate groupings and influences the synthesis of nucleic acids, lipids and cholesterol. Physiological amounts of metal are transported in blood attached to plasma proteins such as transferrin on which Cr (III) competes for binding sites with Fe (III). Larger amounts of Cr (III) either ingested or on the skin are carcinogenic.

Molybdenum is known for its role in biological nitrogen chemistry and is present as catalysts of nitrogen fixing bacteria such as Rhizobium in the form of enzyme nitrogenase. These enzymes are found in blue green Algae and in free living bacteria which convert atmospheric nitrogen into ammonia and contain two proteins molybdoferredoxin and azoferodoxin. The former is a large protein with a molecular weight of 2,20,000 daltons is brown in colour, sensitive to air and contains two Mo, 24-36 Fe and S atoms together believed to be arranged as four [4Fe-4S] and two Mo-Fe-S clusters.

Azoferredoxin is yellow air sensitive and is a derivative of $Fe_4S_4(SR_4)_4$ and has a molecular weight of 60,000 daltons. There is only one 4Fe - 4S cluster shared equally by two subunits. Its exact role in nitrogen fixation is not known but it is believed that N_2 binds to molybdoferredoxin, Fe is reduced simultaneously and an electron is transferred *via* Fe to N_2. Protons are finally added to form ammonia and the required energy for the process is supplied by ATP. Mo is also necessary for the activity of flavoproteins, xanthene oxidase and aldehyde dehydrogenase.

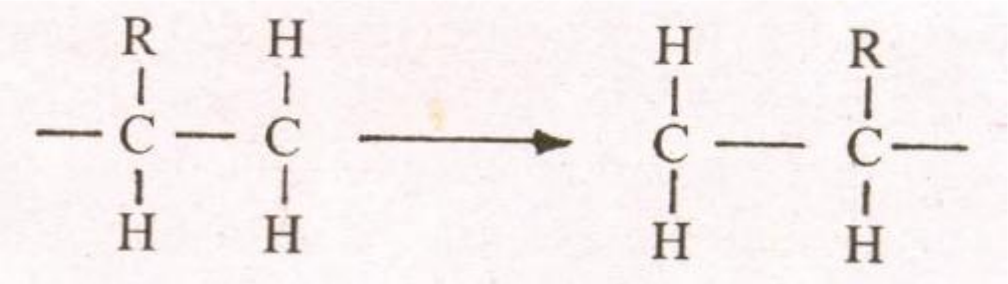
(v) Vanadium: Although the element has been shown to be beneficial to man but its exact function is not known, but is required in extremely small amount in diet. There is some evidence that it is involved in lipid metabolism and may prevent dental caries. In some animals it is used in oxygen transport, the transporting molecules being haemovanadium and is involved in building cellulose. In the nitrogenases of some free living bacteria vanadium may replace molybdenum but the rest of the structure remains the same.

(vi) Manganese: ***It is known in eight different oxidation states in*** **vitro but in vivo only +3 and +4 states are known which are important for both plants and animals. It is present as manganese transporting protein transmanganin. It is necessary for the function of mitochondria and is proposed to be a cofactor of the respiratory enzymes. It is an important constituent of enzyme** ***argninase which is produced by liver and converts*** **nitrogeneous waste products into urea, which is carried through blood to kidneys from where it is excreted out in urine. It is also essential for plant growth and has to be added to fertilizers if there is a deficiency of it in the soil.**

(vii) Nickel: **The bio inorganic chemistry of Nickel has been recognized only recently and its role in vivo is yet unknown. However its deficiency does impair iron absorption and leads to reduced iron content and haemoglobin level. It is also present in some enzymes one e.g. urease - which was believed to be a pure polypeptide for many years but in 1975 it was shown to contain Nickel which is essential for its activity. The enzyme catalyses the hydrolysis of urea to ammonia and carbonic acid. The mechanism believed to be involved isto bind urea molecules to Ni but it is yet to be proved. It is a large enzyme with six subunits each containing two Ni atoms.**

In several bacteria the enzymes hydrogenase which catalyses the reaction $2H_2 + 0_2 \longrightarrow 2H20$ contain nickel (III) as an important constituent, but the definite role of species Ni (III) is yet developing.

(viii) Cobalt: *The metal has been known to be an essential element* since 1930 and the one and the most important biochemical role is that it is an essential component of Vit B 12 which is a cofactor for a number of enzymes catalyzing the reactions of type



The adult human body contains 2 x 10^{-4} ug of Vit. B12 and is stored in liver. The ultimate source of Vit B12 is synthesis by micro organisms. The microphlora of human intestinal tract also performs the syntheses but the vitamin so formed cannot be absorbed into the body and so humans are entirely dependent on their diet as the ultimate source of this vitamin.

It is a porphyrin complex of Co(II) and the structure is similar to haemoglobin. The vitamin is commonly isolated in the form *cyanocobalamin* in which cyanide occupies position 5. The skeleton of the porphyrin ring system comprising of a tetradenate ring or corrin ring is present but the main difference is in the bridging units between different pyrole units. The corrin system has a greater flexibility which is helpful in initiating catalytic cycle. The vitamin and its derivatives are involved in many biological reactions mainly DNA and Haemoglobin synthesis, the metabolism of amino acids and hydride or methyl group transfer.

It is an essential cofactor for many enzymes and its role is to bring about Co – C homolytic bond cleavage and to form Co(III) and adenosyl radicals. When once cleavage has occurred, the adenosyl radical $RCH_2°$ accepts one hydrogen from the substrate SH to form the substrate radical S°. The role of the enzyme is to rearrange to produce radical P°, which takes up one hydrogen from RCH_3 to give product PH and the enzyme is recovered as such and starts another cycle.

One other aspect of cobalt chemistry is that it can be substituted for Zn^{2+} in many enzymes without much change in activity and examples of such enzymes are the carboxy peptidase and carbonic anhydrase.

(ix) Silicon: Silicon plays an important structural role in vivo influencing the bio synthesis of collagen and bony tissues. It is essential for the normal functioning of epithelial and connective tissues to which it imparts strength and elasticity by holding the protein molecules together Via sulphur cross bridges. Because of this the skin becomes more stable chemically and mechanically and also impermeable to liquids.

(x) Selenium: *The biological roles of selenium are totally dependent* upon concentration for e.g. it has been found that 0.1 ppm is essential while 10 pm is highly carcinogenic. Although its exact metabolic role is not known it is thought to be a part of enzyme glutathione peroxidase which helps to protect haemoglobin against the oxidation using H_20_2. The biologically active compound is R_2Se which is a part of a non haeme iron protein which helps in electron transfer.

***(*xi) Tin:** The functions of this trace element are very limited. It helps in maintaining DNA/RNA transportation and protein synthesis.

(xii) Iodine: Of all the trace elements iodine is heaviest and is a necessary constituent of thyroid harmones such as thyroxine and trido thyromine but its precise role is not yet known.

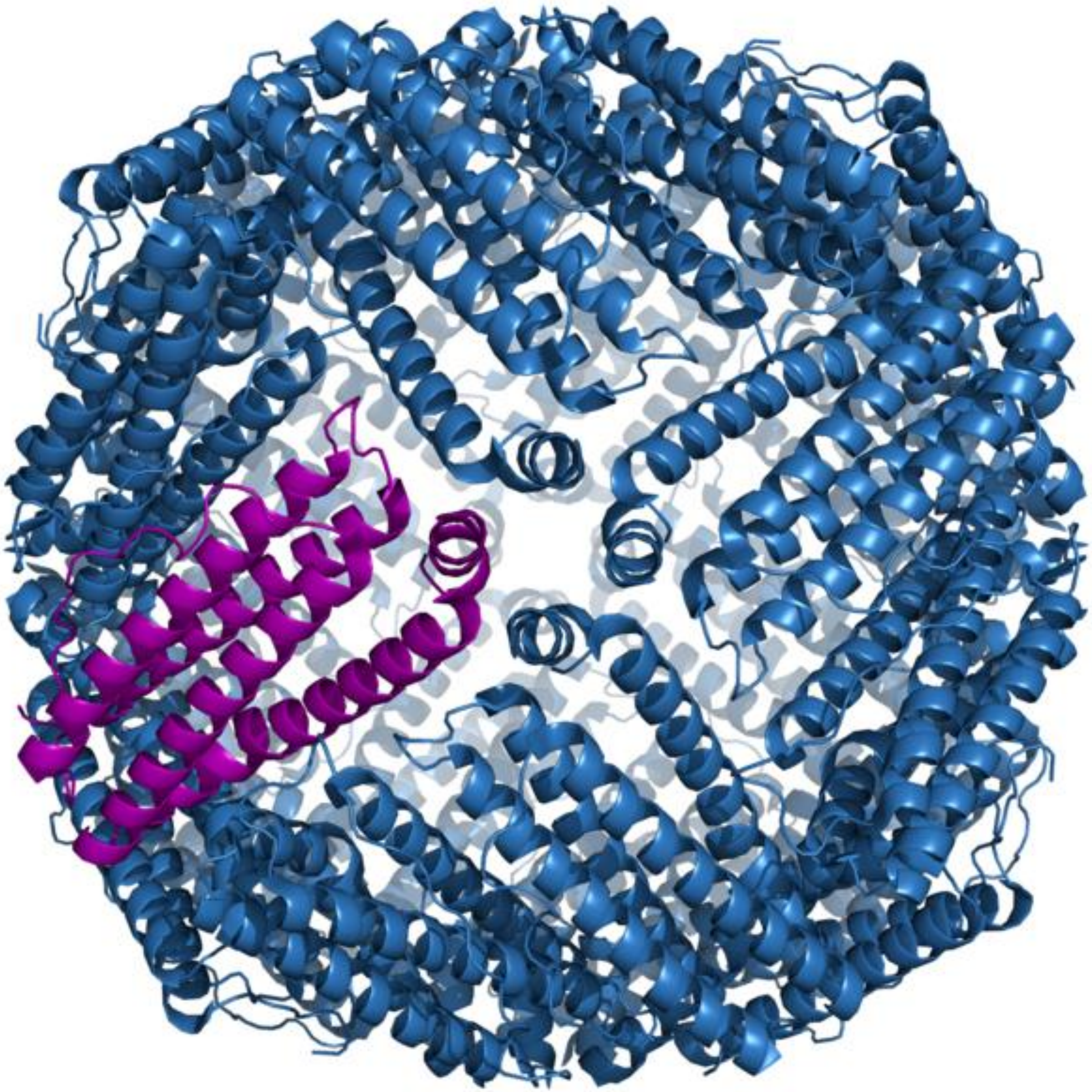
(xiii) Iron: Biologically iron is the most important transition metal and like Cu and Se it is toxic in higher concentration. It is extremely abundant in earths crest and has two oxidation states +2 and +3 which are easily inter convertable. It is involved in body in storage and function processes of which the following are important.

(a) As an oxygen carrier it is present at active centre of molecules
like haemoglobin.
(b) As myoglobin for oxygen storage in muscle tissues.
(c) As electron carrier (Cytochromes)
(d) For electron transfer in plants and bacteria (ferredoxins)
(e) As enzyme for different processes such as oxidation of aldehyde
(aldehyde oxidase) Decomposition of H202 (catalase and peroxidase) and Aerobic oxidation of Carbohydrates (succinic dehydrogenase)

Storage and Transport of Iron

A. Polynuclear Iron Containing Proteins

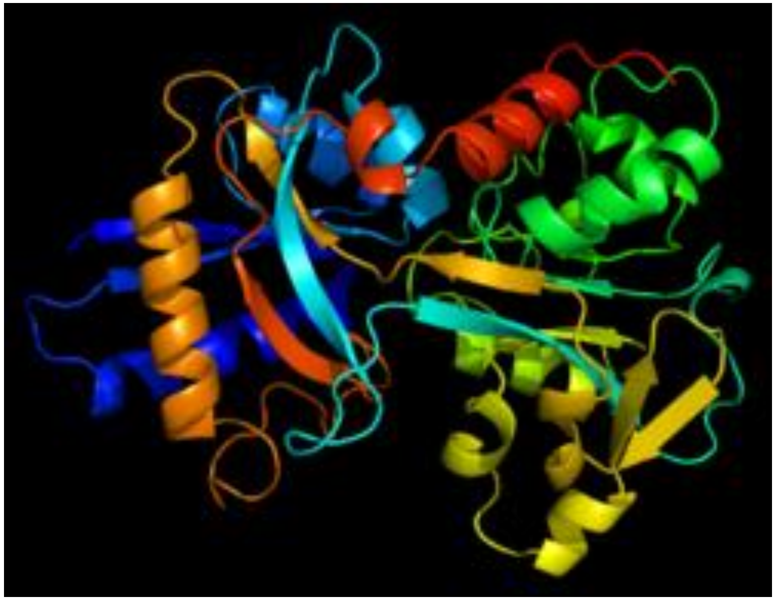
(i) Ferritin: Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. It is found in liver, spleen and bone marrow and serves to store excess iron so as to prevent its toxic build up and can be made available to body when ever required. It contains approximately 23% Fe in non toxic form. Structurally ferritin contains a spherical shell of protein called apoferitin made up of oxohydroxophosphatoiron(Ill) complex surrounding a micelle, which may contain 2000-5000 atoms of Fe. The manner in which iron is stored and released from ferritin is yet not known with certainty.



Structure of Ferritin

(ii) Transferrin: **Transferrins** are iron-binding blood plasma glycoprotein that control the level of free iron in biological fluids . ***It is a glycoprotein with a very large molecular*** **weight of 80,000 daltons. The main role is to carry ingested iron from the stomach in the form of Fe(III) to blood. Its structure is similar to globulin with two similar Fe^{3+} binding sites. The uptake of iron involves the oxidation of Fe^{2+} to Fe^{3+} which is catalyzed by a copper containing protein called Ceruloplasmin and this is the reason why copper deficiency disrupts iron metabolism causing anemia. The mechanism of release of transferring iron to the cells is uncertain.**

(iii) Phosvitin: ***It is a phosphoglycero protein with a molecular weight*** **of 30,000 daltons. It is synthesized in liver and contains one green and one brown Fe^{3+} metallo proteins, both of which contain poly nuclear Fe^{3+} in different coordination geometries.**

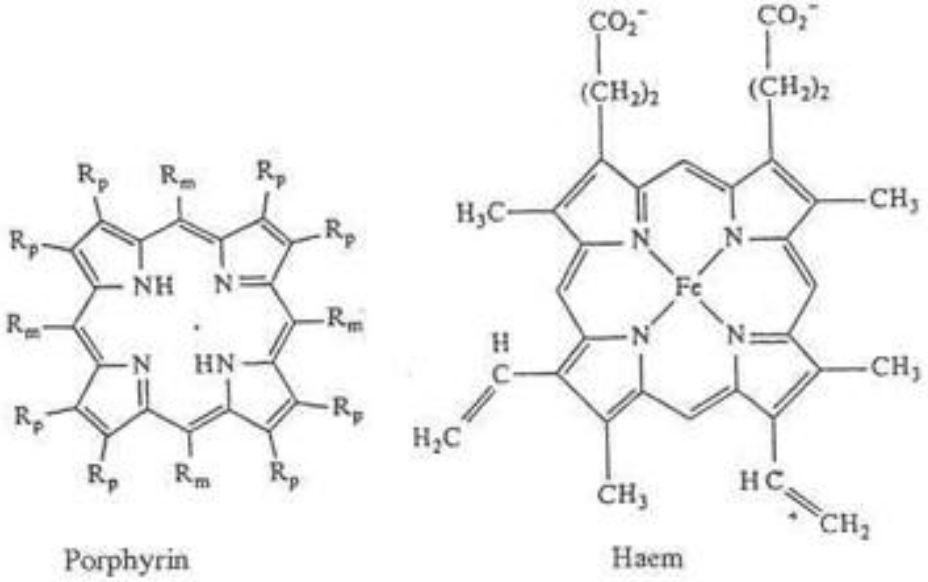


Structure of Transferrin

B. Oxygen Carrying Proteins

There are in all four oxygen carrying proteins for transport and storage of iron in biological moieties, out of which three are Fe (II) haeme proteins which are very well known and are Haemoglobin, Myoglobin and Cytochromes, while hemerytherin is a non heme Fe (II) protein.

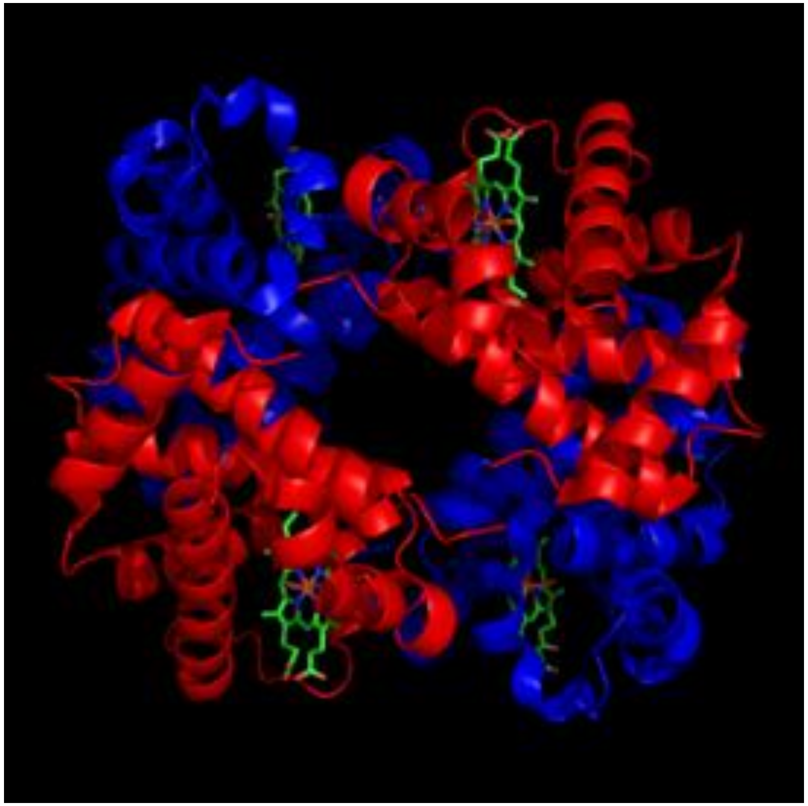
(i) Haeomoglobin: **the total iron content of human body is 4g out of which 70% is present as Haeomoglobin in the red blood corpuscles. It is responsible for transport of oxygen from lungs to muscles where it is converted into myoglobin for respiration, i.e., mitochendrial oxidation in which energy is released from the break down of sugar. It has a molecular weight of 65,000 daltons and contains four subunits each comprising of a porphyrin complex containing Fe^{2+} bound to four nitrogen atoms, the 5th position is coordinated by the midazole nitrogen of the histidine moity of a protein known as globin and this is called deoxyhaemoglobin. The 6th position is normally vacant but can be bonded by molecules H_20, 0_2 and $CO_{2.}$**



Porphyrin

Haem

Figure 2 The porphyrin ring and the structure of haem



Being five coordinated Fe(II) is high spin and, has a square pyramidal geometry. The 6th vacant position as mentioned above can be bonded to oxygen and if this happens an octahedral structure is formed in which high spin Fe(II) is changed to low spin and is called oxyhaemoglobin. When 0_2 is removed the position is replaced by H_20 molecule. Simultaneously the protein part absorbs H^+ and removes CO_2 from lungs as $HC0_3^-$. The blood removes the $HC0_3^-$ ions and reduced haemoglobin removes H^+, as the blood is returned to heart through veins and then pumped back to lungs, where $HC0_3^-$ is reconverted to CO_2 and this is exhaled out. The blood takes up oxygen again and the process is repeated.

The most important factor in the process is its reversibility and that it involves only Fe^{+2} and not +3. Many groups such as CN^-, CO can also bind to oxygen site and if this happens no O_2 can be bonded as these ligands are not replaceable arid this may cause death.

As soon as one subunit of haemoglobin picks up 0_2 the high spin configuration in which radius of Fe^{+2} is 0.92 A° changes to low spin configuration in which radius is 0.75A°. Due to this contraction Fe(II) moves into the plane of porphyrin ring and in doing so it causes, the histidine molecules attached to it to move towards the porphyrin plane. This process brings about conformational changes in the peptide chain and ruptures some or all $-COO^-$ NH_3 bridges. The constrained haemoglobin molecule then relaxes by exposing the 6th position of remaining heme groups to oxygenation and this phenomenon is called *cooperative interaction. The advantage of this process is that once one subunit is* oxygenated other sub units are predisposed to take up oxygen more easily and process continues.

The end product of glucose metabolism is CO_2 which is converted. into the soluble HCO_3^- ion by water.

$$CO_2 + H_2O \longrightarrow H \; + HCO_3^-$$

The protons produced in the process are picked up by the terminal amine groups of deoxyhaemoglobin which acts as a buffer. The reverse process occurs in lungs.

The ability of haeme unit of haemoglobin to bind oxygen is annulled if iron is oxidised to +3 state. Because the structure of Fe (III). subunits is similar to oxygenated subunit, the ability of other subunits to release their O_2 is greatly impaired and the entire molecule becomes non functional and a disease *methemoglobinemia develops.*

(ii) Myoglobin: ***It is a monomeric protein with a molecular weight* of 17,000 daltons having a single polypeptide chain. This is used to store iron in muscles. The active site in myoglobin is also a haeme moity in which Fe(II) is coordinated to four pyrrole nitrogens of a porphyrin ring and is in equitorial plane. The function of myoglobin like hemoglobin is to bind oxygen which is kept in store until required for metabolic action. It is very good binder of oxygen even at low pressures and since the need oxygen is needed in those tissues which have already consumed oxygen and produced CO_2 and are at lower pressure, the 0_2 from haemoglobin is passed on to myoglobin. It is thus clear that both these are essential for the effective transport of oxygen.**

(iii) Cytochrome: ***These are also a group of proteins which function*** as electron carriers in oxidation releasing energy. These are small molecules with molecular weight of 12000 and bring about reversible oxidation by changing iron from +2 to +3 state. There are mainly three types of cytochromes, a, b & c according to the type of porphyrin rings these contain. Their skeletal structure consists of an iron atom bonded to four pyrrole N atoms in each porphyrin ring and 5th and 6th sites are occupied by the N and S atoms of associated proteins. The main difference when compared to haemoglobin is that the 5th position of cytochrome C is occupied by a imidizole N atom of histidine and 6th position is coordinated to thioether ‘s’ atoms of methionine. Electron transfer occurs though the haeme edge and involves the reaction.

$$\mathrm{Fe(III)} + e \rightleftharpoons \mathrm{Fe\ (II)}$$

Cytochromes a, band c have slightly different reduction potentials and reactions involve all three one after the other in the order b, c, a and the release of energy from oxidizing glucose is gradual. This evolved energy is stored in the cell in the form of ATP and is used when required by the cell.

(c) **Other iron - Porphyrin Molecules**

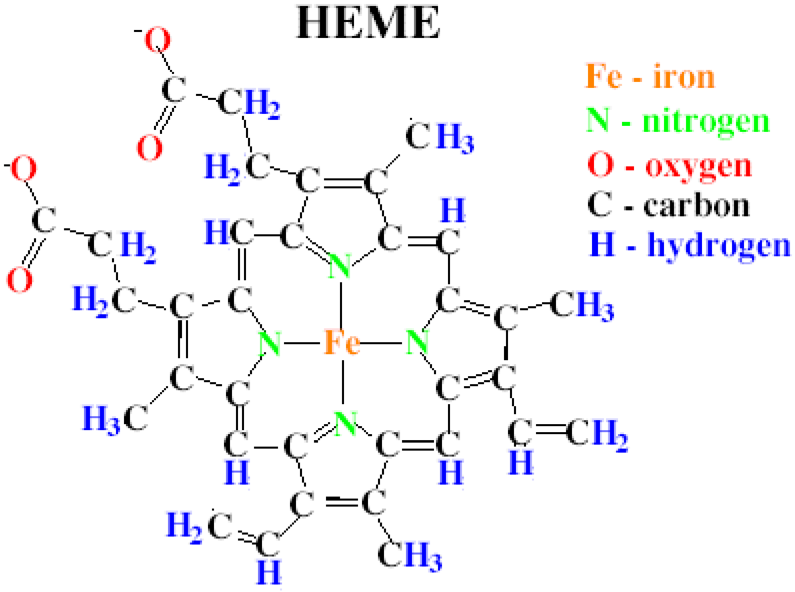
Two other Fe(III) haeme proteins are known which can serve as oxygen carrier and as electron transfer agents and contain Fe^{2+}/Fe^{3+} redox couple in the porphyrin moity, which is responsible for transferring electrons to molecular oxygen in respiratory chain. These suppress the accumulation of poisonous H_20_2 and other peroxy compounds resulting from various oxidative processes in biological system.

(i) Catalase: This enzyme catalyses the decomposition of H_20_2 *to* H_20 and 0_2 which is brought about either by disproportionation or by reduction through alcohols as shown and is called the peroxidative activity of the enzyme.

$$2H_2O_2 \xrightarrow{\text{Catalyse}} 2H_2O + O_2$$

$$2H_2O_2 + RCH_2OH \xrightarrow{\text{Catalyse}} 2H_2O + RCHO$$

Catalase has a molecular weight of 2,50,000 daltons and is comprised of four subunits each containing haeme Fe(III) in the active site coordinated equatorially to four pyrrole N atoms of porphyrin ring, one axial position is occupied by H_20 while on the other axial position is placed an amino acid of a protein chain. Kinetic investigations on decomposition show that both the atoms of liberated oxygen molecule come from the same molecule of H_20_2. The reaction is pH independent and it is believed that three intermediates are formed in the reaction` of catalase with H_20_2.



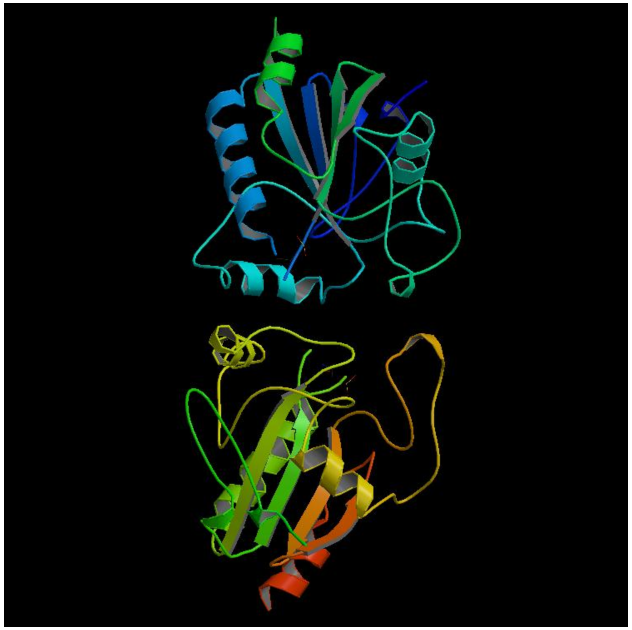
$$P + H_2O_2 \longrightarrow \text{Compunnd I} + H_2O$$

$$\text{Compunnd I} + AH \longrightarrow \text{Compunnd II} + A^-$$

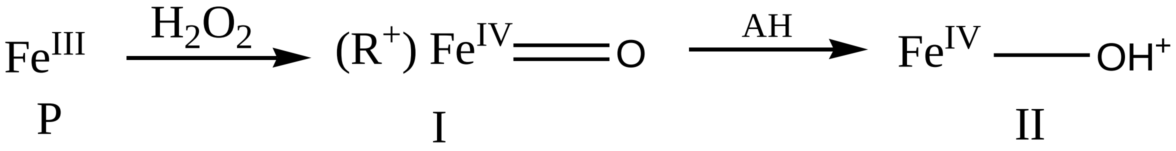
$$\text{Compunnd II} + AH \longrightarrow P + A^*$$

(ii) Peroxidase: These are water soluble monomeric Fe(III) haeme **proteins and occur in most plants, animals and bacteria. The Fe (III), which is the active site of the enzyme can be reduced to Fe(II) by many reducing agents. Just as in catalase in this enzyme also Fe (III) is coordinated to four pyrole nitrogens, 5th position is coordinated by histidine N and 6th by H_20 or any other ligand. The enzyme activity is inhibited by CN^- + N_3 which have a tendency to replace H_20 and get coordinated at 6th position. The reduction of H_20_2 takes place in two steps each being one electron in nature. The catalytic activity is shown by following steps where P is the peroxidase and AH a reducing substrate**

$$\text{P} + H_2O_2 \longrightarrow \text{Compunnd I} + H_2O$$
$$\text{Compunnd I} + \text{AH} \longrightarrow \text{Compunnd II} + \text{A}^-$$
$$\text{Compunnd II} + \text{AH} \longrightarrow \text{P} + \text{A}^*$$



The compound I ad II can be formulated as:



$$\underset{\text{P}}{Fe^{III}} \xrightarrow{H_2O_2} \underset{\text{I}}{(R^+)\,Fe^{IV}{=}O} \xrightarrow{\text{AH}} \underset{\text{II}}{Fe^{IV}{-}OH^+}$$

where R^+ is a cation radical formed on porphyrin ligand.

(D) **Iron-Sulphur Proteins**

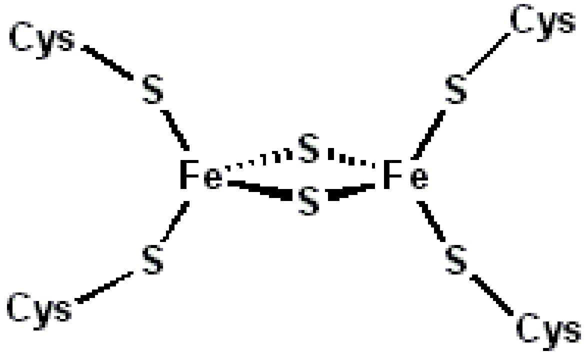
These are low molecular weight compounds which function as electron carriers in biological redox reactions. These consist of long peptide chains coordinated through cysteine sulphur atom to a non haeme iron center consisting of either one single iron atom or a cluster of iron and sulphur atoms along with acid labile sulphide sulphur. These are commonly represented as n –(Fe - S') centers where n is the number of iron cations per protein and S' is the number of sulphide ions. If these proteins are acidified, S^{2-} which is labile goes out as H_2S. Iron is tetrahedrally coordinated to four S atoms of which one must belong to a cysteine molecule. There are two important compounds of this category.

(i) Rubredoxin: This is the simplest iron-sulphur, protein, with a **molecular weight of 6000 and, containing only one Fe atom and no labile S^{2-} ion. The protein containing Fe(III) is red in colour while that containing Fe(Il) is colourless. The redox potential of the Fe(III)/Fe (II)system is 0.057 volts which is responsible for rapid electron transfer in the system:**

$$[\mathrm{Fe}^{\mathrm{III}}(\text{S-Cyst})_4]^{-} + e \rightleftharpoons [\mathrm{Fe}^{\mathrm{II}}(\text{S-Cyst})_4]^{2-}$$

Rubredoxin is found in anaerobic bacteria and is involved in redox reactions taking place in biological systems.

(ii) Ferredoxin: ***These are also called high potential iron proteins*** and consist of a single peptide chain of 98 amino acids. Its active site is composed of two iron centers bridged by two labile S^{2-} sulphur and each iron is bound to two cysteine sulphur atoms of protein chain in such a manner that individual $(\text{cyst-S})_2\text{Fe}(\text{S}^{2-})_2$ units appear tetrahedral. Electron transport occurs with very small energy transfer as the redox potential is –0.2 to –0.4 volts.



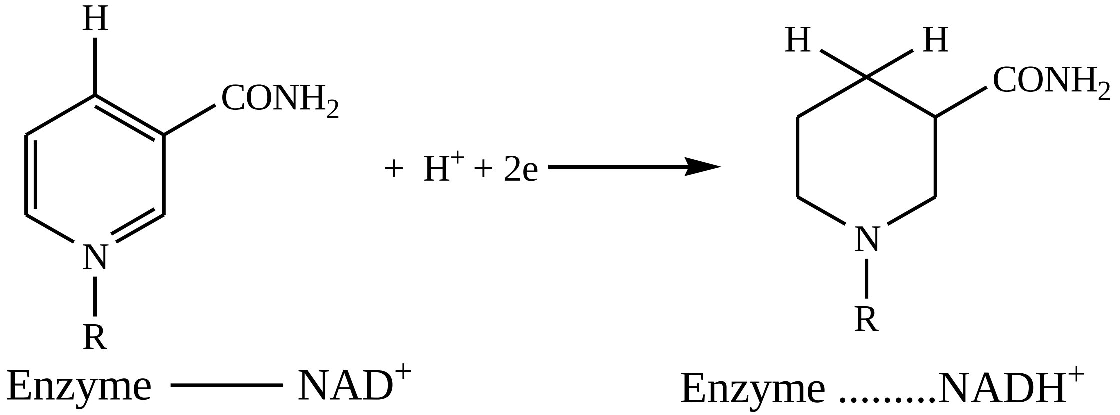
Metalloenzymes

Metalloenzymes which may actually be classified as a sub-class of metalloproteins are defined as those enzymes which contain a definite quantity of a functional metal ion which is present at an active site of enzyme throughout *i.e., even in resting stage. These are different from metal activated enzymes which do not bind the metal but the same is,*required for the activity of enzymes. Known metalloenzymes number several hundred. In many cases it is possible to remove the metal atom and, then restore it keeping the overall protein structure intact. The protein moity from which the metal has been removed is called *Apoprotein* and the original metalloprotein can be recovered back by adding the metal atom.

Metal ion	Metalloenzyme/Metallo protein
Zn^{2+}	Alcohol Dehydrogenase, Alkaline phosphatase, Carbonic anhydrase,Carboxy petidase
Cu^{+} or Cu^{2+}	Superoxide dismutase, Ascorbate oxidase, CytochromoCoxidase, Cerulo plasm
Co^{2+}	Vitamin B^{12}
Fe^{2+} or Fe^{3+}	Aldehyde oxidase, Catalse, Peroxidase, Cytochrome, Ferredoxin, Rubredoxin
Mn^{2+}	Arginase
Mo^{4+}	Xanthine oxidase, Nitrate Reductase
Ni^{2+}	Urease
Mg^{2+}	Kinase, Phosphoglucomutase, DNA Polymerase
Ca^{2+}	Thermolysin

Zinc Containing Metalloenzymes

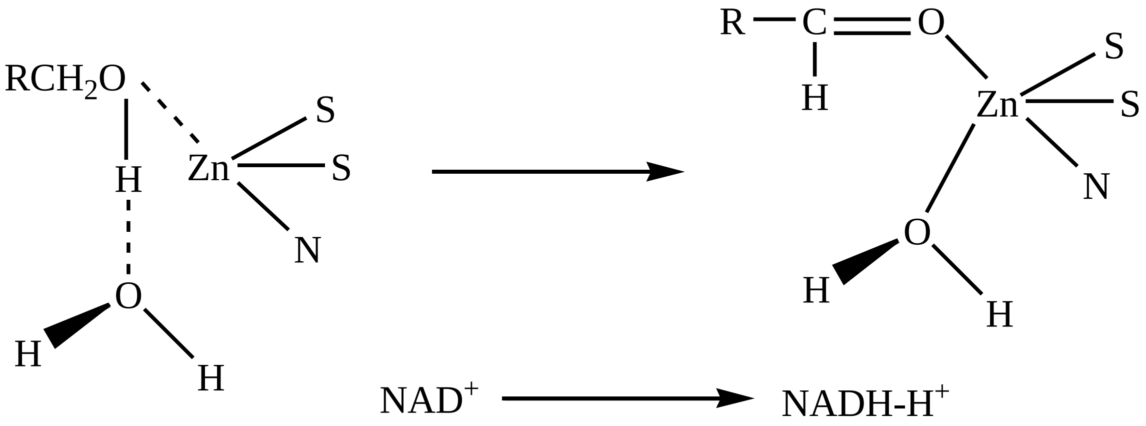
(i) Alcohol Dehydrogenase: ***These are pyridine nucleotide dependent*** **redox enzymes (NAD+) which bring about the reversible oxidation of an alcohol to a carbonyl moity as shown below:**



$$R'R^2CHOH + NAD^+ \rightleftharpoons R'R^2C{=}O + NADH + H^+$$

The most important enzyme of this category is liver *alcohol dehydrogenase* abbreviated as LADH which contains two NAD$^+$ moities and four atoms of zinc per molecule situated in different environment. The active Zn^{2+} site is coordinated to two cysteine sulphur atoms (Cys-46+Cys-17 4) an imidazole nitrogen of histidine (his-67) and a water molecule giving an approximately tetrahedral structure.

The oxidation of alcohol catalysed by *alcohol dehydrogenase* requires a neutral water molecule which is bound to metal providing a 5 coordinated species and the presence of NAD$^+$ is essential to cause release of protons by conformational changes. At the start of reaction metal bound alcohol is hydrogen bonded to metal bound water molecule. The proton involved in hydrogen bonding is transferred first to the coordinated water molecule and then to the solvent and NAD$^+$ present, while the active site abstracts the hydride ion as shown below:



Enzyme —— NAD$^+$ —— Alcohol Enzyme —— NADH —— Aldehyde *(ii) Alkaline phosphatase:* ***This catalyses the hydrolysis of orthophosphates*** **and possesses maximum activity at pH = 8. The reaction involve the formation of an enzyme-phosphate intermediate through the phosphorylation of hydroxy group, which then reacts with water to form inorganic phosphate.**

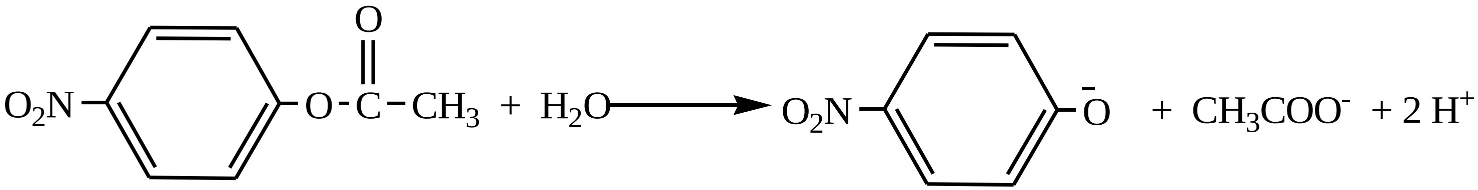
The most thoroughly studied enzyme has been isolated from E. Coli and contains four zinc atoms per mole of enzyme, which break into two subunits by reaction with acids. Two zinc ions are responsible for activity while other two for structural balance. The mechanism proposed suggests that only half the sites are active at a particular point and hence normally only one enzyme unit is phosphorylated while the other subunitserves to bind the substrate molecule which is then subsequently phosphorylated, as the first unit releases its phosphate group. It is found that the activity of the enzyme is enhanced by adding Mg^{2+} which may be due to the ability of Mg^{2+} to promote deprotonation of the water group of the substrate which then attacks the phosphoryl group to be transferred.

(iii) Carbonic Anhydrase: This was the first metalloenzyme to be **discovered and occurs in plants, animals and micro organisms. This catalyses the reversible hydration of CO_2 which is of physiological importance for transport of CO2 formed by oxidation of food, to lungs and thereafter its release to atmosphere;**

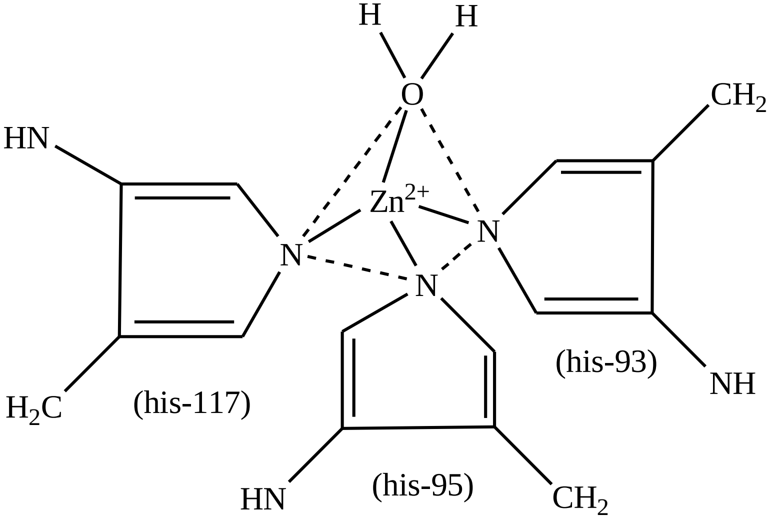
$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

$$CO_2 + OH^- \rightleftharpoons HCO_3^-$$

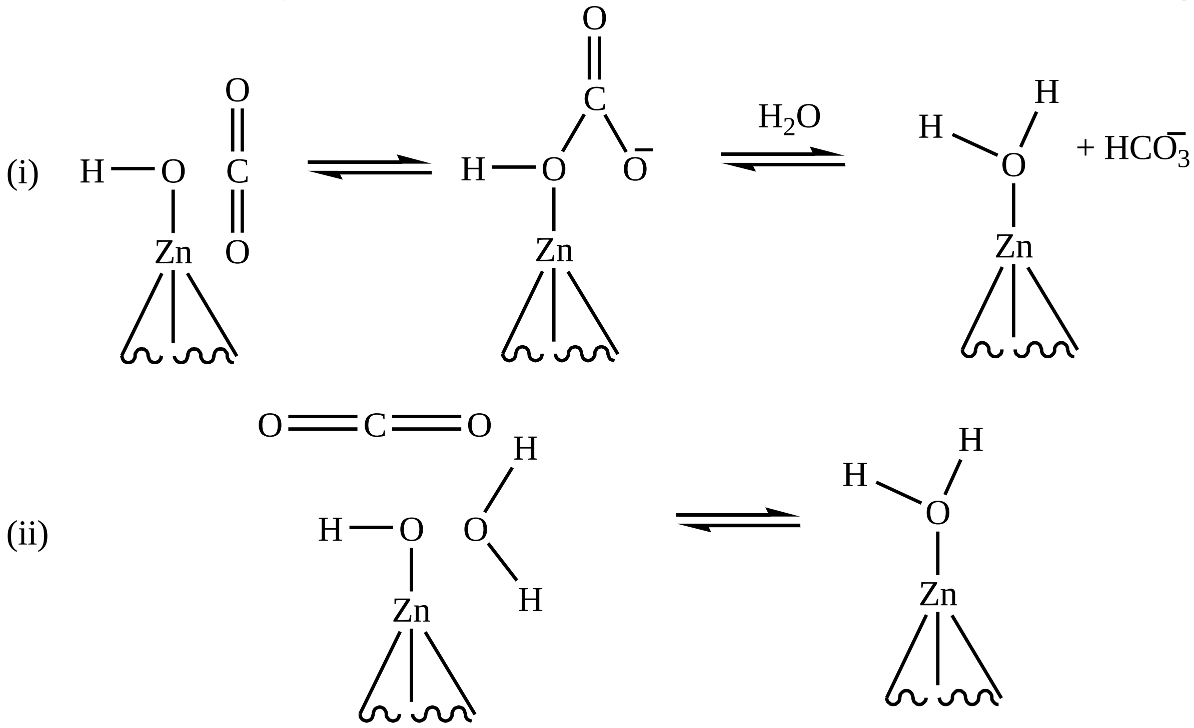
In addition, this also catalyses the hydration of carbonyl compounds and hydrolysis of esters



The enzyme occurs in three forms A, Band C out of which the most well characterized form is B which is a monomeric protein with molecular weight of 30,000 daltons, contains one Zn^{2+}per mole, bound to three histidine residues (his-93, 95 and 117) in a distorted tetrahedral environment and the fourth coordination site is occupied either by a water molecule or OH^- ion as shown below:



It is reported that Zn^{2+} is directly involved in the catalytic activity of enzyme although the exact mechanism is still uncertain and catalytic activity may be due to zinc bound water molecule or ionization of some protein group. It is suggested that some zinc-hydroxide mechanism takes place as illustrated below in the following two schemes.



The first is a two step *nucleophilic attack of Zn - OR on CO_2 and* second is a one step general base assisted attack of H_20 on CO_2.
Metallocarbonic anhydrases containing Mn (II), Fe (II), Co (II), Ni (II), Cu (II), Hg (II) have been prepared by adding appropriate metal ion to apoenzyme *(i.e., carbonic anhydrae from which Zn^{2+} has been removed)* and the structures and activities of these are similar to the original enzyme.

Enzyme Inhibition:

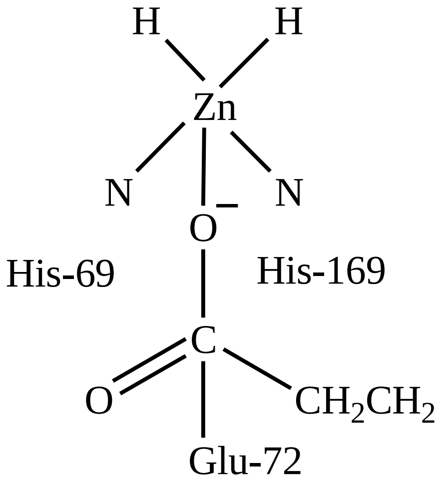
Several classes of compounds serve as inhibitors for the enzyme. These include sulphonamides, imidazoles, and simple anions like azide, cyanide, iodide, and their inhibitory action is due to the fact that these are bound to metal displacing the Zn bound water molecule. Imidazole forms a five coordinate complex and does not displace aquo group. This leads to a weak binding of CO2 in its appropriate position causing decrease in activity.

(iv) Carboxy Peptidase: *The most important carboxy peptidase is carboxypeptidase A which is a Zn (II) containing enzyme released in* pancreatic juice and digests the protein by hydrolysis of peptides. Structurally enzyme contains 307 amino acids and only one gram atom of zinc in a single polypeptide chain and has a molecular weight of 34,600 daltons.

The enzyme reacts with peptide bond situated in the protein molecule. If the enzyme hydrolyses the terminal bond it is called exo-peptidase, if it catalyses a non terminal bond it is called endo-peptidase and if hydrolyses a peptide bond associated with an amino terminal residue it is called Amino peptidase. The enzyme is very specific in activity and two essential requirements for its activity are the co terminal end must have a L-configuration and the carboxyl group must be free. The zinc may be removed by chelating agents giving inactive apoenzyme and activity is restored by adding $Zn^{2+.}$ Other metals which can substitute Zn^{2+} are Fe^{2+} ,Mn^{2+} , $C0^{2+}$ and Ni^{2+} but no activity is observed if substitution is done with Cd^{2+} and Hg^{2+}

Structure of *Carboxy Peptidase:*

The x-ray studies have shown the molecule to be ellipsoidal in shape, zinc is bound by two imidazole N of histidines 69 and 169, one carboxylate oxygen of glutamate residue 72 and the fourth position is occupied by water molecule giving an approximately tetrahedral structure.



Mode of action: The enzyme shows its activity by two possible mechanisms.

(i) The carboxylate oxygen attacks carbonyl C-atom of peptide bond nucleophilically and this releases the terminal amino acid forming a mixed anhydride which when hydrolyzed regenerates enzyme.

(ii) The coordinated Carbonyl group is polarized by Zn^{2+} in such a way that the water molecules is activated and makes nucleophilc attack on carbonyl C atom. The resulting intermediate on protonation decomposes to give amine and carboxylic functions and regenerates enzyme.

The main inhibitor of enzyme is β-phenyl propionate which has a tendency to get bound to enzyme and reduce its activity through a carboxylate-metal ion interaction.

Copper Containing Metalloenzymes

(i) ***Superoxide Dismutase**: **This enzyme is widespread in occurrence*** **and has a very important function causing disproportionation of superoxide ion O^{2-} which is a toxic radical produced in biological systems by incomplete or one electron reduction of molecular oxygen.**

$$O_2 + e \rightleftharpoons O_2^-$$

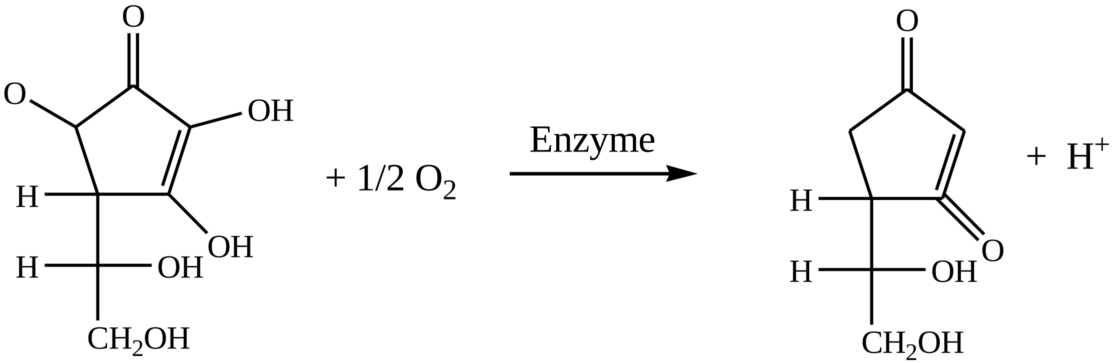
The enzyme catalyses the reaction of superoxide ion to dioxygen and peroxide and thus prevents its accumulation.

$$2O_2^- \rightleftharpoons O_2 + O_2^-$$

Structure of Superoxide dismutase: **The best studied enzyme has been isolated from Bovine erythrocytes.**

It has a molecular weight of 31,400 daltons and is a dimeric protein consisting of two Cu (II) and two Zn (II) ions sharing a common moiety of histidine-6 moity. The geometry around Cu (II) is square planar, three histidine residues his-44, 46 and 118 occupying three positions equatorially and axial position is occupied by water molecule. The Zn (II) ion has a tetrahedral environment with three nitrogen donors his 65, 69, 78 and one oxygen donor which is the carboxylate oxygen of asparate moity (Asp - 81).

(ii) Ascorbate Oxidase: ***The enzyme has a wide spread occurrence*** **in plants, is blue in colour and catalyses the oxidation of Ascorbic acid to dehydroascorbic acid by molecular oxygen. The oxidation occurs in two steps each of which is one electron in nature and a free radical is formed. After first step the enzyme, becomes colourless due to Cu (I) ion which reacts with O_2 to restore back the blue form containing Cu (II). Thus the copper present in the enzyme is reversibly reduced and oxidized.**



The enzyme occurs in plants, has a molecular weight of 1,50,000 daltons and contains 0.26% Cu (II) corresponding to 5 atoms of Cu (II) per molecule.

Each reaction shown above releases one H^+ and Cu(I) enzyme which reacts with molecular oxygen as shown below and Cu(II) enzyme is recovered.

$$4Cu'\text{-}Enz + 4H^+ + O_2 \rightleftharpoons 4Cu''\text{-}Enz + H_2O$$

(iii) Cytochrome C Oxidase: **This enzyme contains two types of cytochromes and two copper ions and is a terminal member of electron transport chain interacting with dioxygen and cytochrome-c. It has high molecular weight and is bound firmly to membrane protein. The magnetic susceptibility measurements correspond to low spin Fe (III)d^5, Cu(II)d^9 and high spin Fe (III) - Cu(II) model.**

The intermediates formed during the reaction between enzyme and dioxygen show a mixed oxidation state. Cytochrome C and electrons appear to enter through cytochrome a and are transferred to Cu (II) resulting in Cu(I) which then donates two electrons to dioxygen to form peroxide. The activity of the enzyme is inhibited by molecules as NO, CN^-, N_3^- and CO.

(iv) Galactose Oxidase: **This enzyme oxidises primary alcohols to aldehydes and reduces dioxygen to hydrogen peroxide. The molecular weight is 68,000 daltons and only one copper atom is present per mole and is therefore a very simple molecule.**

Structurally the Cu atom is bound by two imidazole nitrogens and one water ligand in a pseudo square planar environment but the 4th ligand may be either a histidine residue or an indole group of tryptophan. The most important inhibitory action is seen if the enzyme is oxidised by N-Bromo succinimide which may, be due to a change in the stereochemistry around Cu2+ from square planar to tetrahedral.

(v) Plastocyanin: **One of the blue copper proteins obtained from certain bacterial sources containing one copper ion per mole of protein is plastocyanin, which has a molecular weight of 16,000 daltons. It is found in chloro plasts of photosynthetic plants and its action cycles between the two oxidation states of copper i.e., Cu (I) and Cu (II).**

The geometry around copper atom is tetrahedral and the binding sites contain two Nitrogen atoms of the imidazole group of two histidines, his - 37 and - 87 and two sulphur atoms of cysteine - 84 and methionine - 92. The presence of cysline and methionine in the enzyme molecule has been confirmed by spectral measurements. The role of the enzyme is not well understood but plays some kind of part in Photosynthetic electron transfer chain.

(vi) Ceruloplasmin: Most of the copper circulating in human blood is present as the blue protein ceruloplasmin which stores and transports Cu. Smaller amount is also present in lymph, gastrointestinal secretions and spinal fluid. Copper atom forms an important part of its structure and remains attached to it throughout. Attempts to exchange copper with radioactive ^{64}Cu or ^{67}Cu have been unsuccessful but it can be removed by treating with diethyldithiocarbamate and the enzyme is restored backby adding Cu (II) to apoenzyme.

It is a glycoprotein having 7 or 8 copper atoms per mole and thehuman enzyme has a molecular weight of 1,40,000 daltons and contains four subunits. Its structure and physiological roles are not- well understood but is supposed to (i) catalyse the four electron reduction of dioxygen to water with corresponding oxidation of substrate *(ii) helps in oxidation* of Fe(II) and (iii) functions as copper transport protein. The first two functions operate together in a cyclic process called *ferroxidase activity.* It helps in copper transport by binding half of copper to a albumin which is the main carrier of blood between intestine and liver.

Cobalt Containing Metalloenzyme

(i) Cobalt B_{12} Coenzymes: ***The biological activity of cobalt is exhibited*** **by the important Coenzyme vitamin BI2 which is a macromolecular Cobalt (III) Complex and is known as cyanocobalmin and is required only in trace amounts. Its normal proportion in blood is 2 x 10^{-4} lu/mc and its deficiency causes *Pernicious Anemia. It is synthesized by certain* bacteria such as stryptomyces olivaceous. It is a water soluble red complex and is the only known naturally occurring organometallic compound.**