Bioinorganic Chemistry
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Lecture - 03
Metals in Biology: Nature's Selection [Part-2 of 4]

So, today I will continue my lectures on Metals in Biology.

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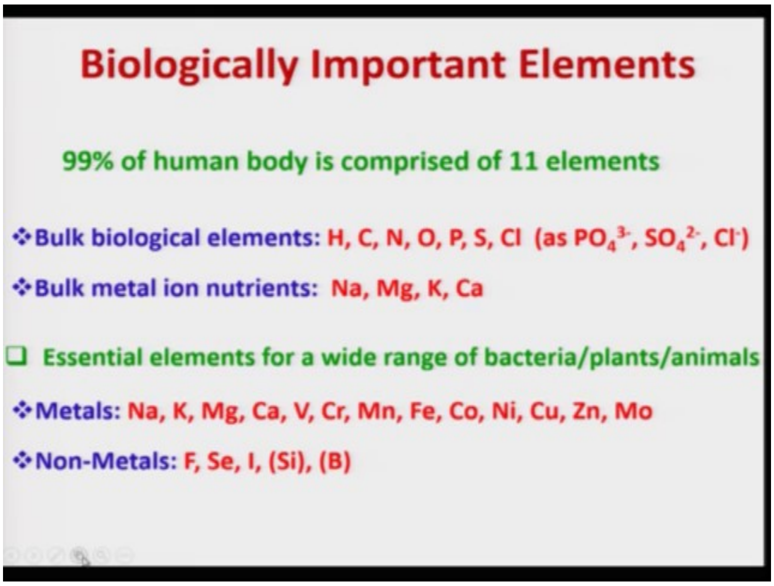

Biologically Important Elements

99% of human body is comprised of 11 elements

- **Bulk biological elements:** H, C, N, O, P, S, Cl (as PO_4^{3-}, SO_4^{2-}, Cl^-)
- **Bulk metal ion nutrients:** Na, Mg, K, Ca

☐ Essential elements for a wide range of bacteria/plants/animals

- **Metals:** Na, K, Mg, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo
- **Non-Metals:** F, Se, I, (Si), (B)

In my last lecture, we have discussed about the biologically important elements and as I have said that 99 percent of human body is comprised of 11 elements, and we also discussed biologically essential and non-essential elements.

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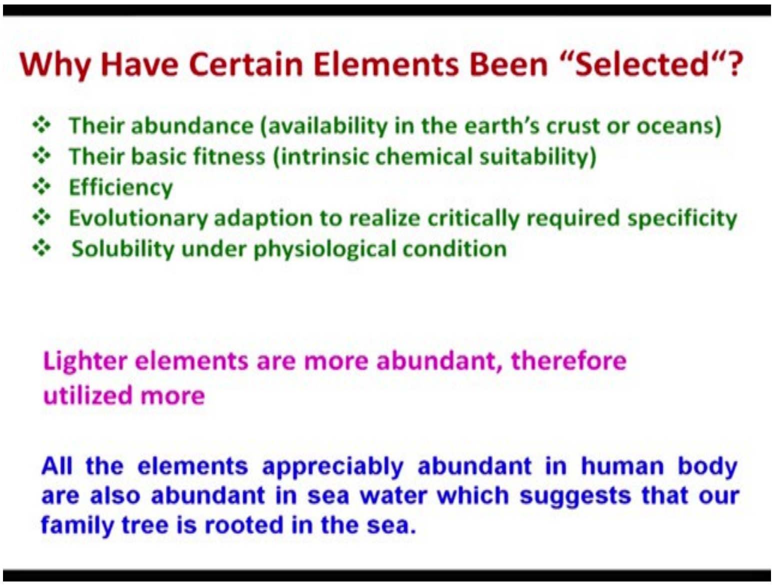

Why Have Certain Elements Been "Selected"?

- Their abundance (availability in the earth's crust or oceans)
- Their basic fitness (intrinsic chemical suitability)
- Efficiency
- Evolutionary adaption to realize critically required specificity
- Solubility under physiological condition

Lighter elements are more abundant, therefore utilized more

All the elements appreciably abundant in human body are also abundant in sea water which suggests that our family tree is rooted in the sea.

We also discussed in my last lecture that why certain elements are selected out of so many elements present in the periodic table. There are some guiding principles which nature has followed in order to select certain elements for their function and role. So, some of this we have already discussed that their abundance, their basic fitness, efficiency, evolutionary adoption to realize critically required, specificity, solubility under physiological condition. And what we have noted that lighter elements are abundant, therefore utilized more. All the elements appreciably abundant in human body are also abundant in sea water which suggests that our family tree is rooted in the sea.

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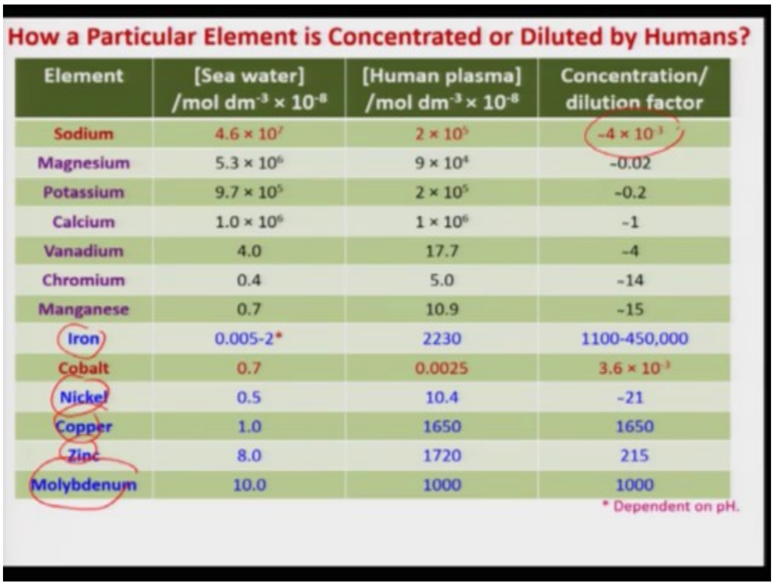

How a Particular Element is Concentrated or Diluted by Humans?

Element	[Sea water] /mol dm^{-3} × 10^{-8}	[Human plasma] /mol dm^{-3} × 10^{-8}	Concentration/ dilution factor
Sodium	4.6×10^7	2×10^5	-4×10^{-3}
Magnesium	5.3×10^6	9×10^4	-0.02
Potassium	9.7×10^5	2×10^5	-0.2
Calcium	1.0×10^6	1×10^6	-1
Vanadium	4.0	17.7	-4
Chromium	0.4	5.0	-14
Manganese	0.7	10.9	-15
Iron	0.005-2*	2230	1100-450,000
Cobalt	0.7	0.0025	3.6×10^{-3}
Nickel	0.5	10.4	-21
Copper	1.0	1650	1650
Zinc	8.0	1720	215
Molybdenum	10.0	1000	1000

* Dependent on pH.

We have shown in my last lecture that certain elements are either concentrated or diluted in human body. For example the sodium which is diluted while several such elements like iron, nickel, copper, zinc, molybdenum, they are concentrated in our body by many thousand (Refer Time: 02:18).

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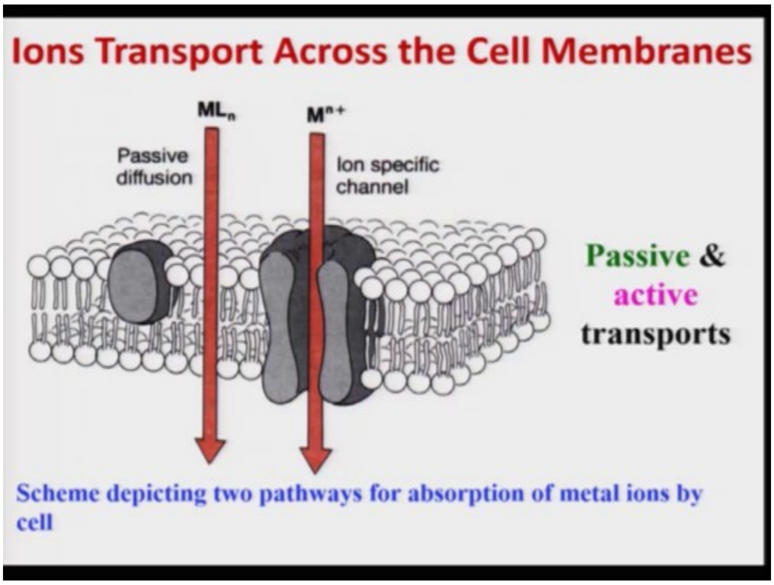

Ions Transport Across the Cell Membranes

Passive & active transports

Scheme depicting two pathways for absorption of metal ions by cell

And we also discussed that how these elements are transported across the cell membrane. They can be passive or active transport. And there can also be ion specific channels through which ion can transport across the membrane. We have also discussed that how the metal ion which are less abundant can also be increased their concentration in the body.

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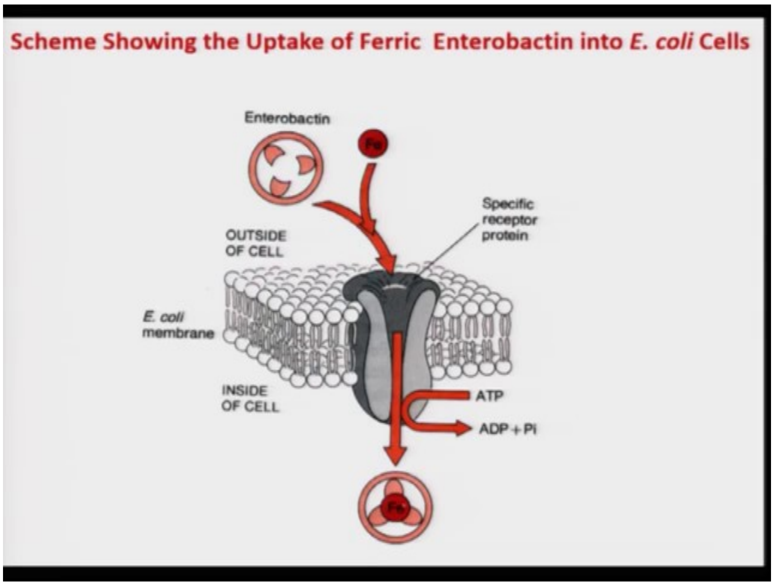

Scheme Showing the Uptake of Ferric Enterobactin into *E. coli* Cells

For example how iron is getting inside the cell by using Enterobactin, we have discussed this in details in my last lecture.

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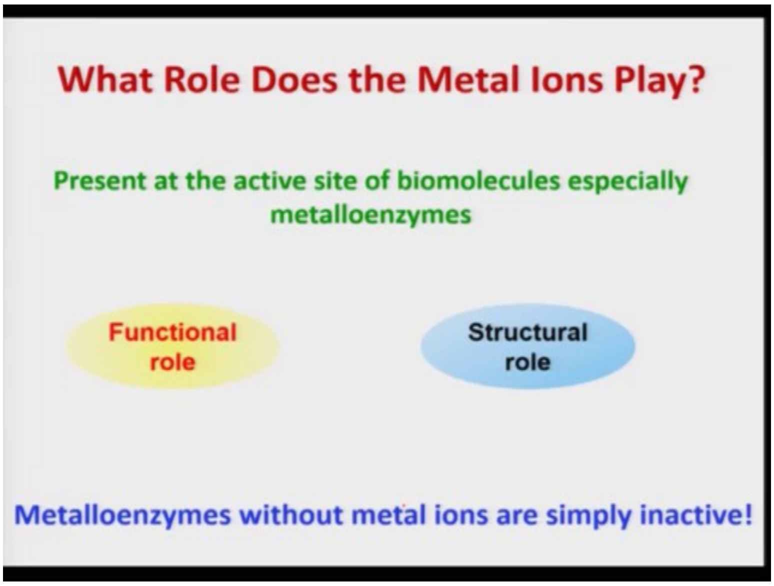

What Role Does the Metal Ions Play?

Present at the active site of biomolecules especially metalloenzymes

Functional role

Structural role

Metalloenzymes without metal ions are simply inactive!

Now, what role does the metal ions play? The metal ion is present in the active site of the metalloenzymes, and they have either functional role and or structural role. However, metalloenzymes without metal ions are simply inactive. So, you can see that how metal ion is important. Now, this being so there have to have a concentration range without that it also can act as a poison.

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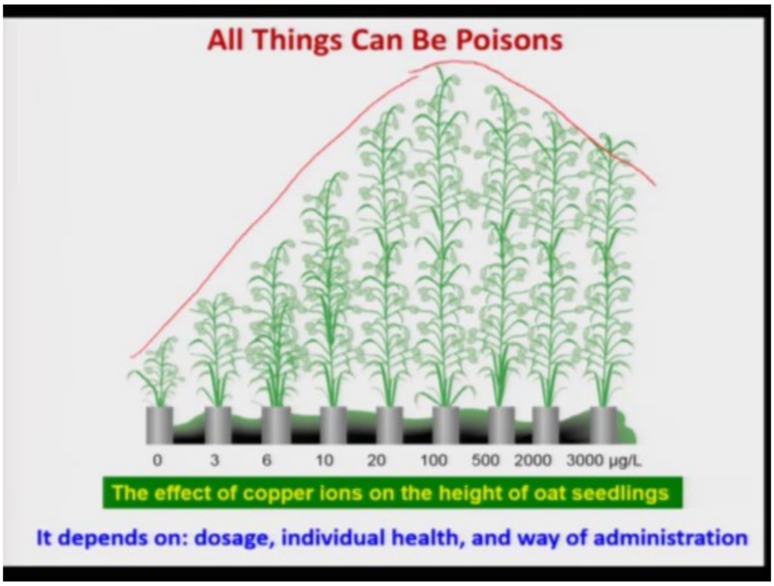

All Things Can Be Poisons

The effect of copper ions on the height of oat seedlings

It depends on: dosage, individual health, and way of administration

For example, I have shown here the effect of copper ions on the height of oat seedlings as you can see as the concentration of copper ion increases, then the length of the seedlings increases and after that it also decreases. So, it depends on dosage, indivual health health and the way of administration.

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Metal Ion Concentrations Must Be Maintained Within Certain Ranges in Cell

What we learned is that metal ion concentrations must be maintained within certain range inside the cell.

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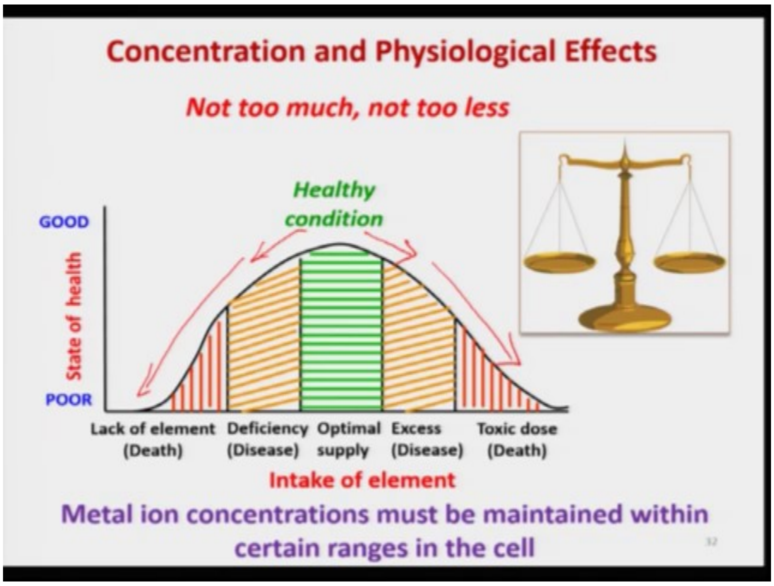

Concentration and Physiological Effects

Not too much, not too less

Metal ion concentrations must be maintained within certain ranges in the cell

So, schematically it has been shown over here, state of the health and intake of element has been plotted as you can see that for a healthy condition, you need to have a concentration level when the health would be very good. However, if you have either excess concentration or deficiency, both are bad, and both of this create variety of diseases for our body. And if the concentration increases to a value where it is highly toxic, it will cause a death. Similarly, without that element also the system would die. So, this is the physiological effect. So, we do not need too much not too less we have to maintain a healthy condition. So, metal concentration must be maintained within certain ranges in the cell.

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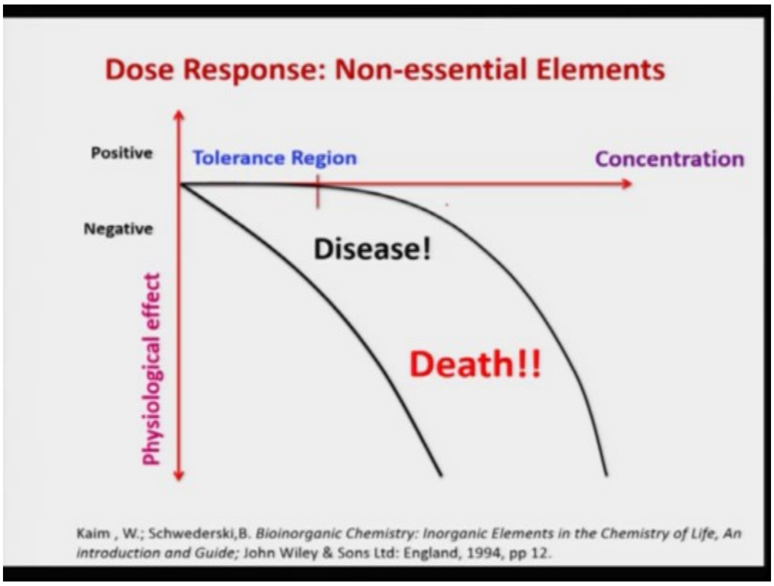

Dose Response: Non-essential Elements

Kaim , W.; Schwederski,B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life, An introduction and Guide;* John Wiley & Sons Ltd: England, 1994, pp 12.

Now, here is a plot with non-essential element those elements which presents are not essential for our body. To see so there is a tolerance level and once that tolerance region crossed, then there will be a disease and eventually it will cause death.

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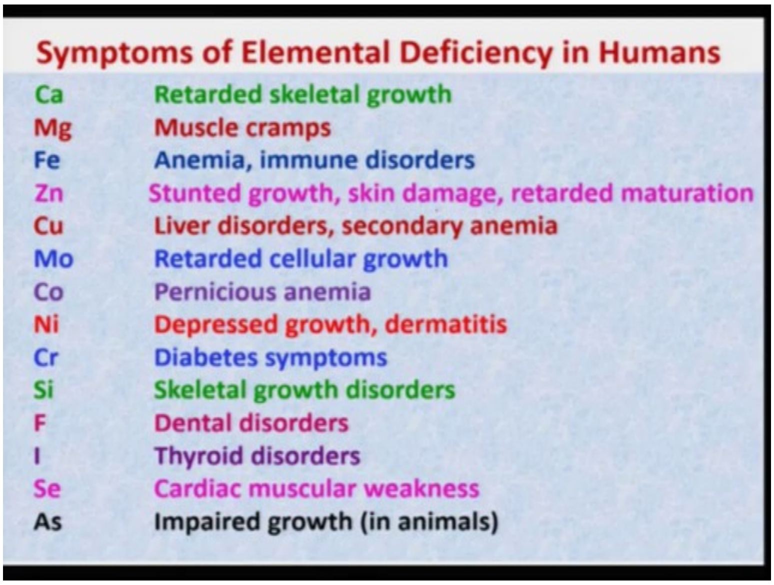

Symptoms of Elemental Deficiency in Humans

Ca	Retarded skeletal growth
Mg	Muscle cramps
Fe	Anemia, immune disorders
Zn	Stunted growth, skin damage, retarded maturation
Cu	Liver disorders, secondary anemia
Mo	Retarded cellular growth
Co	Pernicious anemia
Ni	Depressed growth, dermatitis
Cr	Diabetes symptoms
Si	Skeletal growth disorders
F	Dental disorders
I	Thyroid disorders
Se	Cardiac muscular weakness
As	Impaired growth (in animals)

I have shown here a table where the symptoms of elemental deficiency are shown. For example, calcium - retarded skeletal growth; magnesium - muscle cramps, iron - anemia; zinc - stunted growth, skin damage; copper - liver disorder; molybdenum - retarded cellular growth; cobalt - pernicious anemia; nickel - depressed growth; chromium -

diabetic symptoms; silicons - skeletal growth disorder; chlorine - dental disorder; iodine - thyroid disorder; selenium - cardiotic muscular weakness; arsenic - impaired growth, of course, in animals,

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So, what we need it that we need to have this element to a concentration which is necessary for our growth. If we do not take those foods carrying, for example, here I have shown that calcium supplements there are lots of food items is shown over here including milk. If we do not take enough food having calcium, then we have to take medicines.

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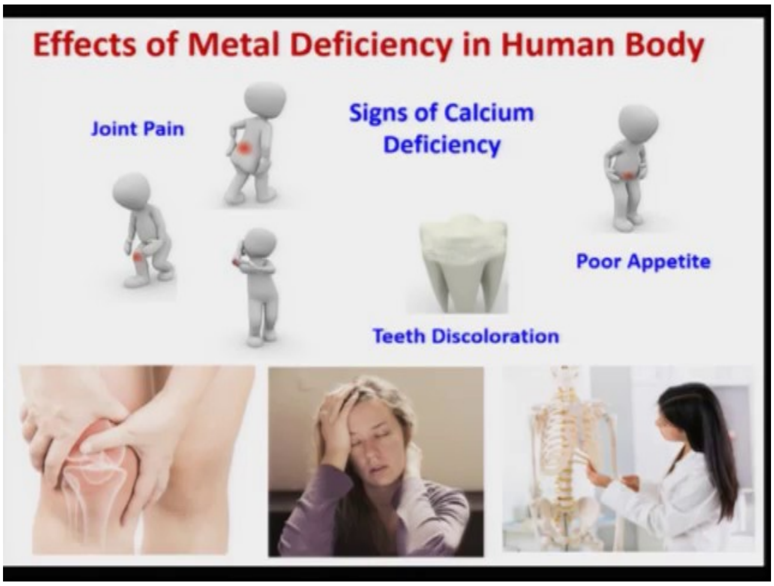

Effects of Metal Deficiency in Human Body

Now, the effect of metal deficiency particularly calcium deficiencies shown over here. So, you will have, because calcium you know forms these bones, so you have joint pains, teeth discoloration, poor appetite, lots of other things.

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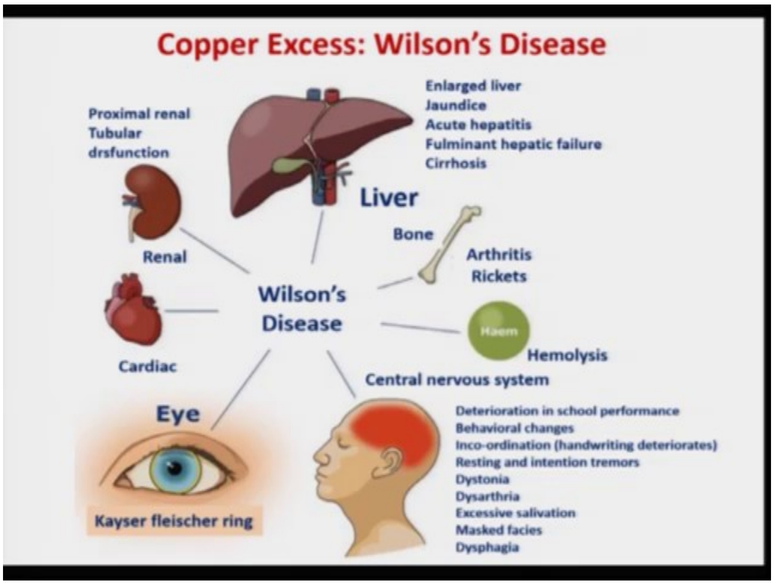

Copper Excess: Wilson's Disease

Copper, if you have excess, then it causes Wilson's disease. And Wilson's disease creates lots of problem in our body, particularly liver. We see enlarged liver, jaundice, acute hepatitis, lots of disease it creates. Arthritis rickets and in central nervous system also it effects, and there are also cardiac problem, renal problem. So, Wilson disease is very bad for us, and it is basically due to the copper ion if it is present in excess.

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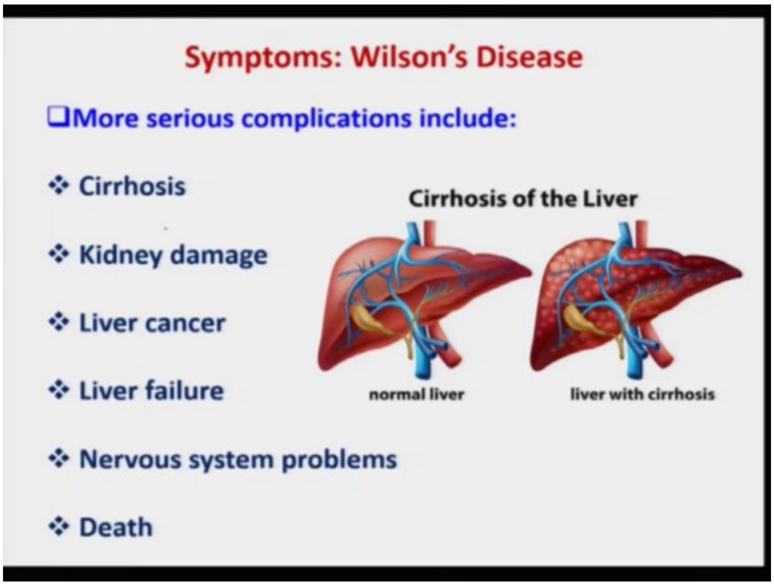

Symptoms: Wilson's Disease

☐ More serious complications include:

- Cirrhosis
- Kidney damage
- Liver cancer
- Liver failure
- Nervous system problems
- Death

So, some of these symptoms are shown over here. Cirrhosis kidney damage, liver cancer, liver failure, nervous system problem, and it can causes death. So, be careful with that.

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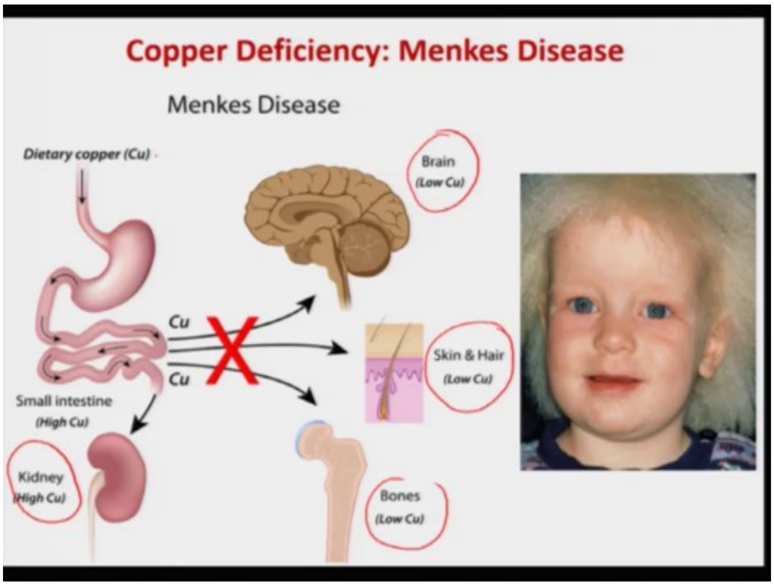

Copper Deficiency: Menkes Disease

Copper deficiency also creates Menkes disease and Menkes disease also I am not going to discuss all in details. And it creates problem in brain, skin and hair. Low copper contains creates the lot of problem bones, kidney and dietary problem also.

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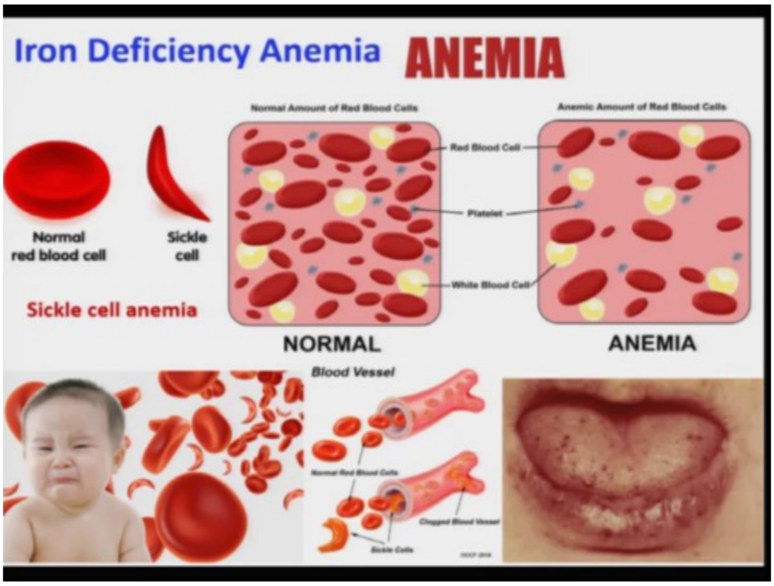

Iron deficiency causes an anemia. You all are familiar with anemia. So, we need to have proper iron concentrations in our body that we take from the diet.

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So, then the foods which are rich in iron are shown over here meat, egg and lots of vegetables. And if we do not have sufficient iron concentration in our body, then you have to have medicines to supplement the iron deficiency. And some of these medicines are shown over here which doctors usually prescribe.

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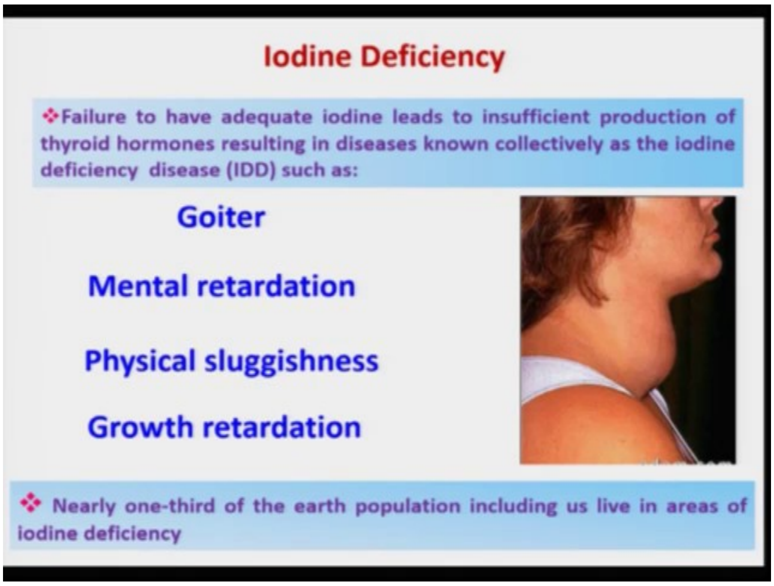

Iodine Deficiency

- Failure to have adequate iodine leads to insufficient production of thyroid hormones resulting in diseases known collectively as the iodine deficiency disease (IDD) such as:

Goiter

Mental retardation

Physical sluggishness

Growth retardation

- Nearly one-third of the earth population including us live in areas of iodine deficiency

Iodine deficiency failure to have adequate iodine leads to insufficient production of thyroid hormones which I have shown earlier resulting in disease known collectively as growth iodine deficiency disease IDD, such as Goiter, mental retardation, physical sluggishness, growth retardation. And nearly one-third of this earth population including us live in the area of iodine deficiency.

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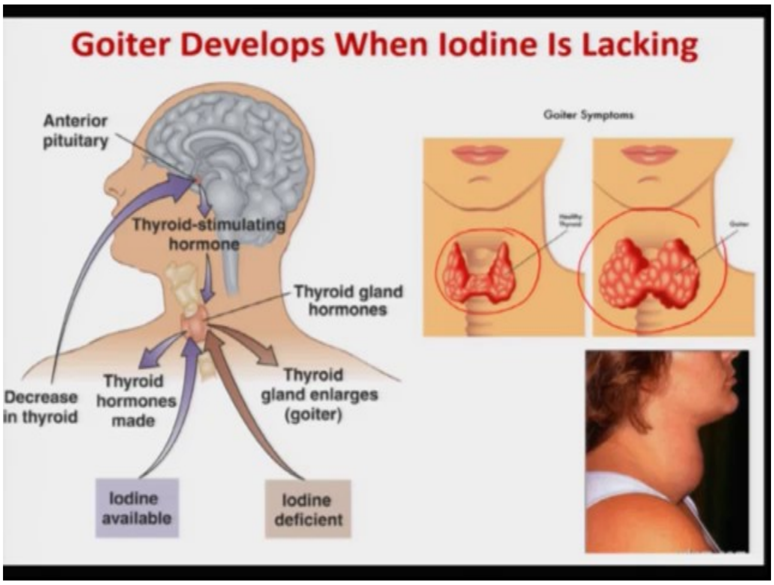

Goiter Develops When Iodine Is Lacking

And as you can see that this thyroid gland which is present in the neck as I have shown. And if iodine is lacking then what would happen the thyroid gland will swell causing goiter and other disease as shown earlier.

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Fluoride

Deficiency: Tooth cavities, tooth decay & loss of teeth

Excess: Fluoride causes dental fluorosis

Fluoride, another important element. So, if you have deficiency, then you have tooth cavities, tooth decay and loss of teeth. And if you have excess, fluoride causes dental fluorosis, you see there are all depositions. So, a deficiency also bad and excess also bad. So, you need to have proper concentration for our healthy life.

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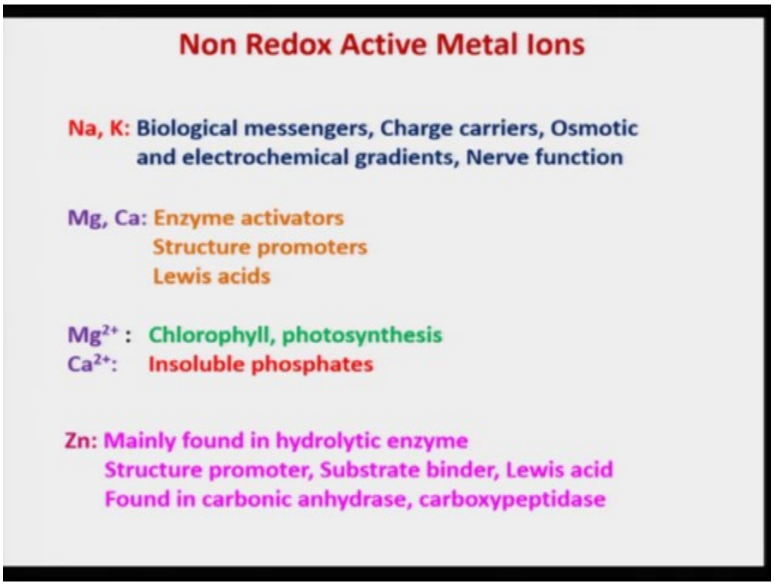

Non Redox Active Metal Ions

Na, K: Biological messengers, Charge carriers, Osmotic and electrochemical gradients, Nerve function

Mg, Ca: Enzyme activators
Structure promoters
Lewis acids

Mg^{2+} : Chlorophyll, photosynthesis
Ca^{2+}: Insoluble phosphates

Zn: Mainly found in hydrolytic enzyme
Structure promoter, Substrate binder, Lewis acid
Found in carbonic anhydrase, carboxypeptidase

Now, coming back to the metal ions, say for example, non redox active metal ions. Say sodium potassium's, what they do they, are actually biological messenger, charge carrier, osmotic and electrochemical gradients, nerve function, the responsible for that. Magnesium calcium lots of enzyme like enzyme activators, structure promoter, they are also Lewis acids, we will see some of this activity. Magnesium 2+ calcium 2+. You know that chlorophyll photosynthesis. Zinc mainly found in hydrolytic enzymes, structural promoter, substrate binder, Lewis acid, found in large number of enzymatic system such as carbonic anhydrase, carboxypeptidase etcetera.

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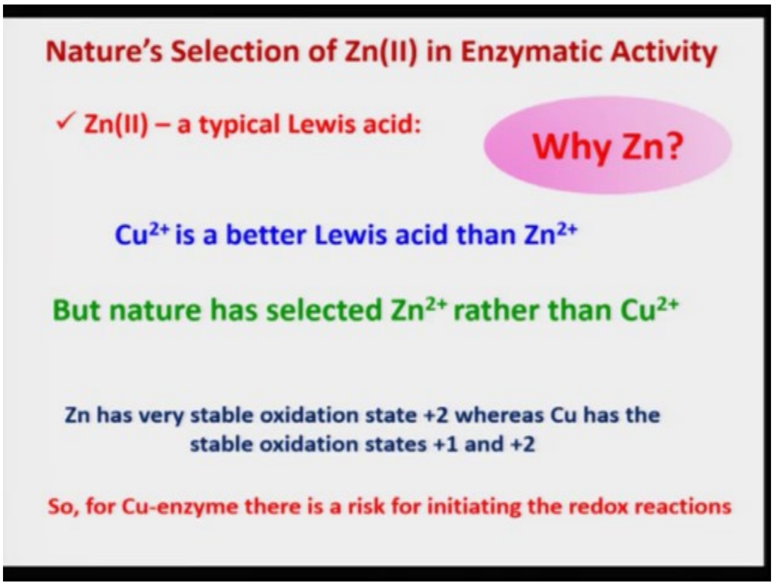

Nature's Selection of Zn(II) in Enzymatic Activity

✓ Zn(II) – a typical Lewis acid:

Why Zn?

Cu^{2+} is a better Lewis acid than Zn^{2+}

But nature has selected Zn^{2+} rather than Cu^{2+}

Zn has very stable oxidation state +2 whereas Cu has the stable oxidation states +1 and +2

So, for Cu-enzyme there is a risk for initiating the redox reactions

Now, why zinc is being selected in the enzymatic activity? Zinc, it is there in 2+ is a typical Lewis acid. However, if you talk about Lewis acidity, then copper two plus is a better Lewis acid than zinc 2 plus, but still nature selected zinc rather than copper. Zinc has however, very stable oxidation state plus 2 whereas, copper has the stable oxidation states of +1 and +2, of course, +3 is highly oxidizing. So, it will not be stable in the biological system.

So, for copper enzyme, there is a risk for initiating the redox reaction. So, nature wanted to avoid because it will be complicated and that is why zinc is being selected. Also zinc has several other characteristic property compared to other elements in the periodic table.

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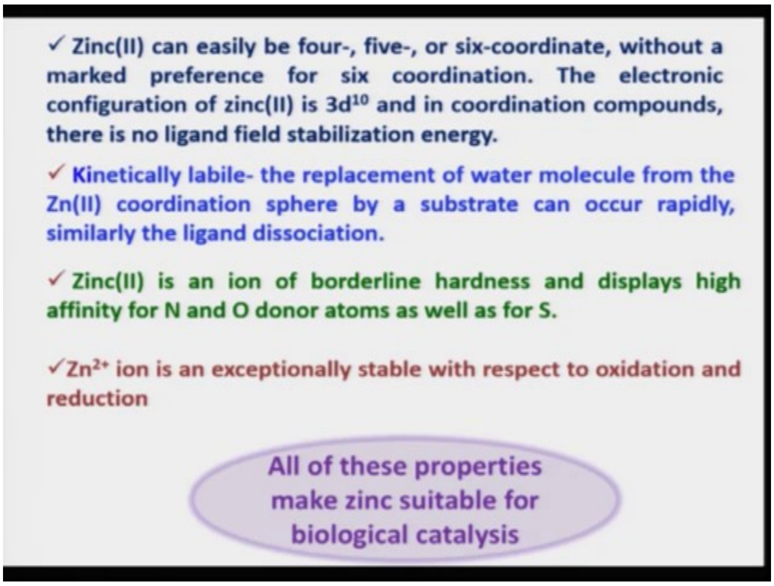

- ✓ Zinc(II) can easily be four-, five-, or six-coordinate, without a marked preference for six coordination. The electronic configuration of zinc(II) is $3d^{10}$ and in coordination compounds, there is no ligand field stabilization energy.
- ✓ Kinetically labile- the replacement of water molecule from the Zn(II) coordination sphere by a substrate can occur rapidly, similarly the ligand dissociation.
- ✓ Zinc(II) is an ion of borderline hardness and displays high affinity for N and O donor atoms as well as for S.
- ✓ Zn^{2+} ion is an exceptionally stable with respect to oxidation and reduction

All of these properties make zinc suitable for biological catalysis

For example, Zn(II) can easily be four, five or six-coordinate without a marked preference for six coordination. The electronic configuration of Zn(II)is $d^{10}$and so there is no ligand field stabilization energy. Kinetically labile- this enables the replacement of water molecules from the zinc coordination sphere by a substrate which can occur very rapidly, similarly ligand dissociations also. Zinc is an ion of borderline hardness, and thereby it can bind both hard and soft donor atoms like nitrogen, oxygen, sulfur. Zn^{2+}ion is an exceptionally stable with respect to oxidation and reduction. All these characteristic properties make zinc suitable for this biological catalysis.

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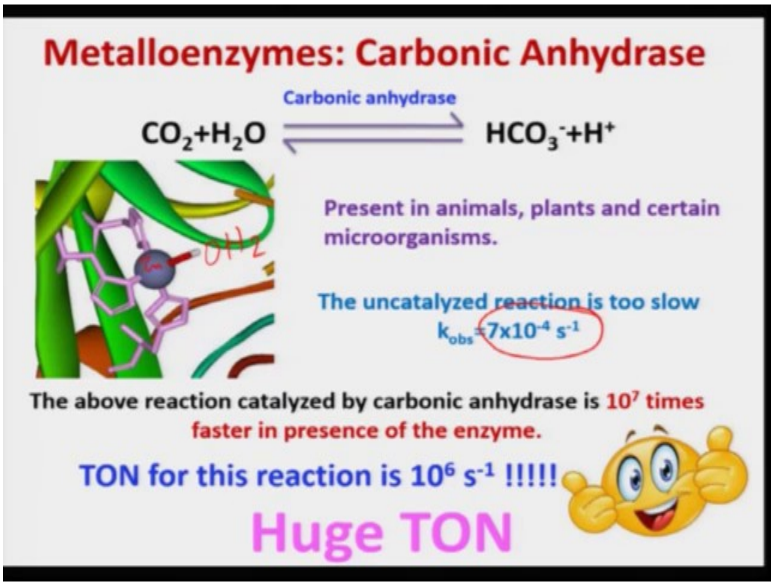

Metalloenzymes: Carbonic Anhydrase

$$CO_2+H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} HCO_3^-+H^+$$

Present in animals, plants and certain microorganisms.

The uncatalyzed reaction is too slow
$k_{obs} = 7\times10^{-4}\ s^{-1}$

The above reaction catalyzed by carbonic anhydrase is 10^7 times faster in presence of the enzyme.

TON for this reaction is $10^6\ s^{-1}$!!!!!

Huge TON

Let me explain little bit more on one zinc containing enzyme called carbonic anhydrase. What carbonic anhydrase is doing? The carbon dioxide plus water forms bicarbonate plus H+ which is catalyzed by carbonic anhydrase. Now, what would happen? So, this is the structure of carbonic anhydrase as you can see that there are 3 histidine ligated to zinc center, and one-fourth position is ligated with water. This carbonic anhydrase is present in animal's plants and certain microorganisms. However, if you do not use carbonic anhydrase, the same reaction is too slow, k observe is $7x10^{-4}$ per second very slow as you know our body produces carbon dioxide which have to be released from the body.

Thus this bicarbonate formation carbon dioxide is a gas so bicarbonate permission is very, very essential, and nature had to design an enzyme carbonic anhydrase which actually speed up this reaction at the order of 10^7 times, and turnover number for this reaction is 10^6 per second a huge turnover number.

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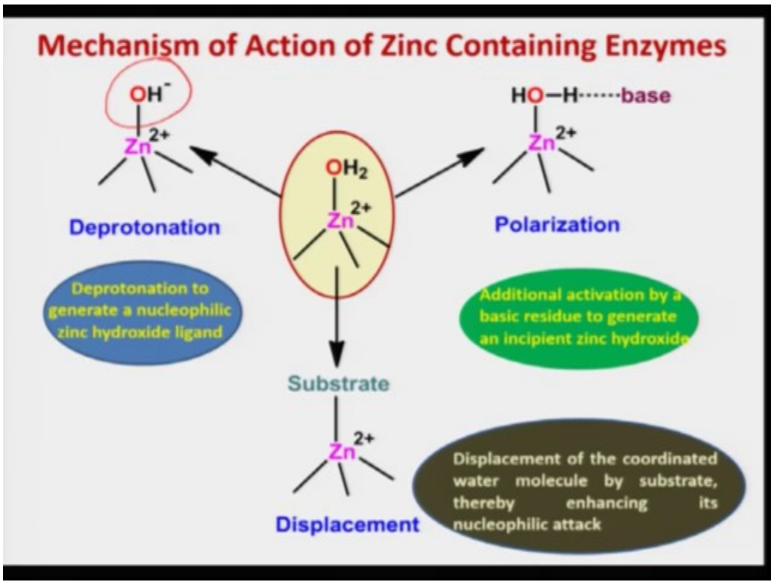

Mechanism of Action of Zinc Containing Enzymes

Deprotonation to generate a nucleophilic zinc hydroxide ligand

Additional activation by a basic residue to generate an incipient zinc hydroxide

Displacement of the coordinated water molecule by substrate, thereby enhancing its nucleophilic attack

The mechanism of action of zinc containing enzymes are shown over here. So, $Zn^{2+}$is basically and Lewis acid. Now, water the pk_a is around 14. Now, when it binds with zinc what would happen the pk_a of this water drastically becomes low is close to 7, that means, the proton is more acidic. And so then it leads to a deprotonation just like shown over here OH^- because protons are highly acidic. Even a base can polarize and even decreases the pk_a of water lower than this, it can be more acidic. So, additional activation by a basic residue to generate an incipient zinc hydroxide, substrate can replace water and thereby enhancing its nucleophilic attack. So, a poor nucleophile is increasing its nucleophilicity just by coordinating with zinc.

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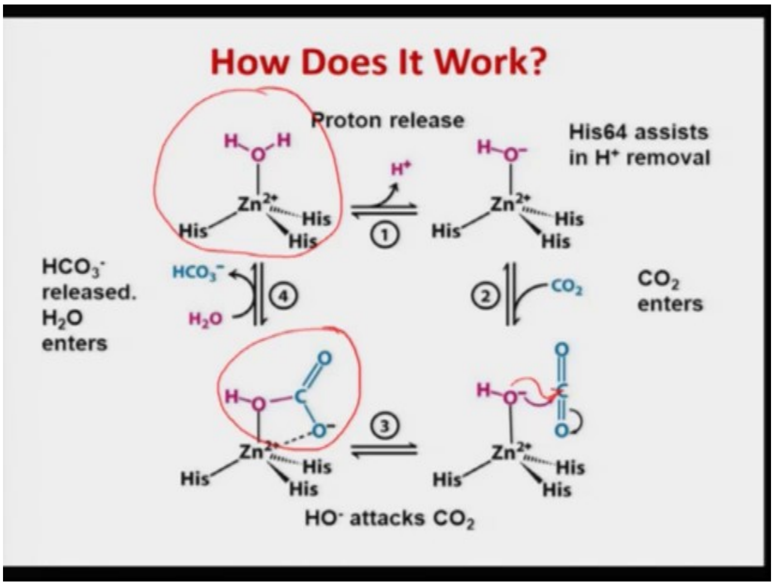

How Does It Work?

Proton release

His64 assists in H^+ removal

CO_2 enters

HO^- attacks CO_2

HCO_3^- released. H_2O enters

And this is what is happening as you can see this mechanism of its work. So, this is the resting state of this enzyme carbonic anhydrase, 3 histidine ligated with zinc ion, and the fourth position is water molecule. And as I have said the proton is acidic, so it s releases proton and histidine 64 assists in the H+ removal. So, it becomes OH minus and then carbon dioxide enters, and this negative you know there is a nucleophilic attack to carbon. And this forms a intermediate very unstable intermediate of this type as you can see this bicarbonate, then be released very easily, water replaced this bicarbonate, and it convert again the enzyme. So, this is the way it works and then again the catalytic cycle starts.

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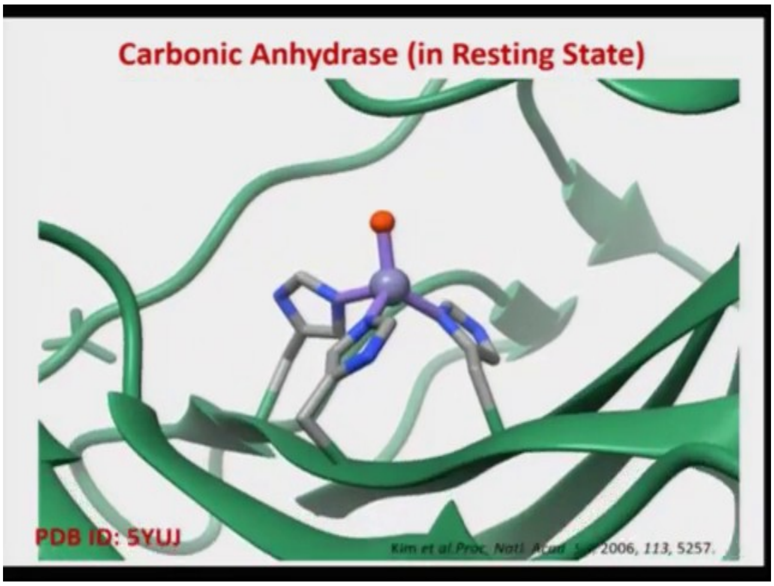

Carbonic Anhydrase (in Resting State)

PDB ID: 5YUJ

Kim et al Proc. Natl. Acad. Sci. 2006, *113*, 5257.

So, as I have shown in the mechanism, so you see that put in structure carbonic anhydrase in the resting state. You see that the huge proteins which are wrapping this, this complex. And as you can see that at the middle a zinc is sitting over here, and this is the water this is the 3 histidine.

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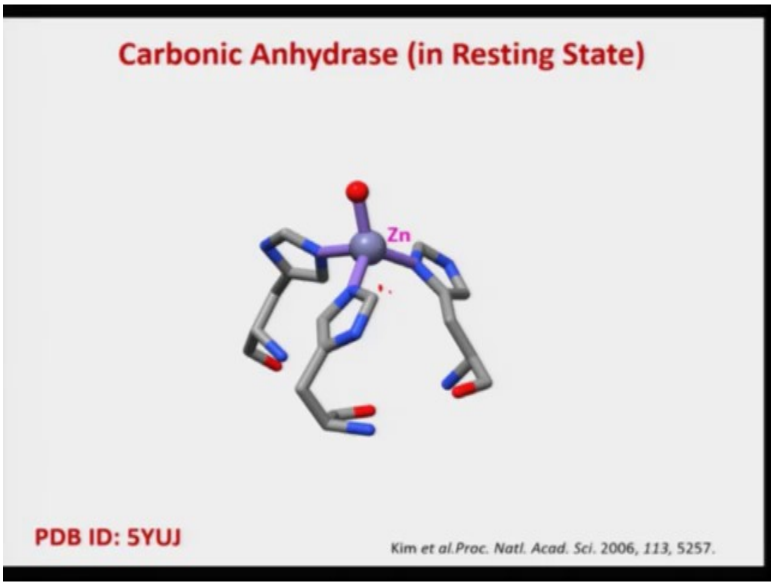

Carbonic Anhydrase (in Resting State)

PDB ID: 5YUJ

Kim *et al*. Proc. Natl. Acad. Sci. 2006, *113*, 5257.

Here I have removed it all this proteins, so you can see this in organic molecules (Refer Time: 21:10) and complex the zinc is sitting over here ligated with histidine and water.

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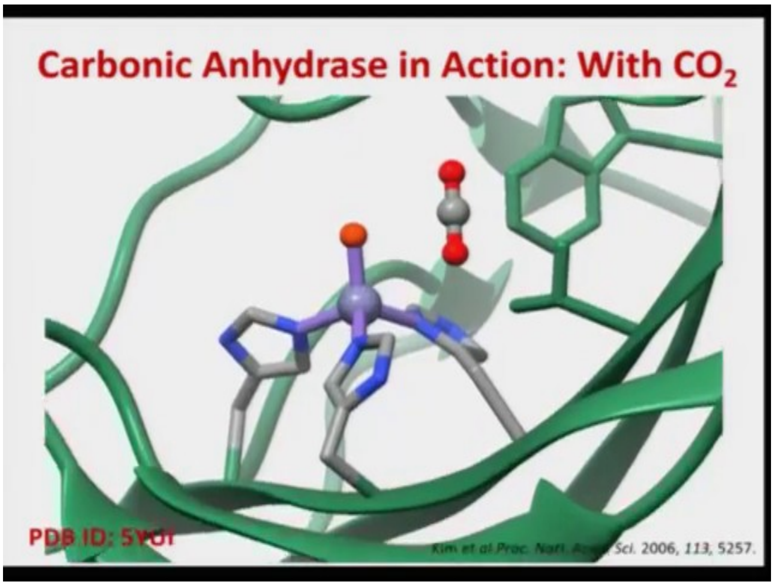

Carbonic Anhydrase in Action: With CO_2

PDB ID: 5YUI

Kim et al.Proc. Natl. Acad. Sci. 2006, *113*, 5257.

When carbon dioxide comes inside the protein, you see that x-ray structure is shown over. Here this is the carbon dioxide and this is they are coming close.

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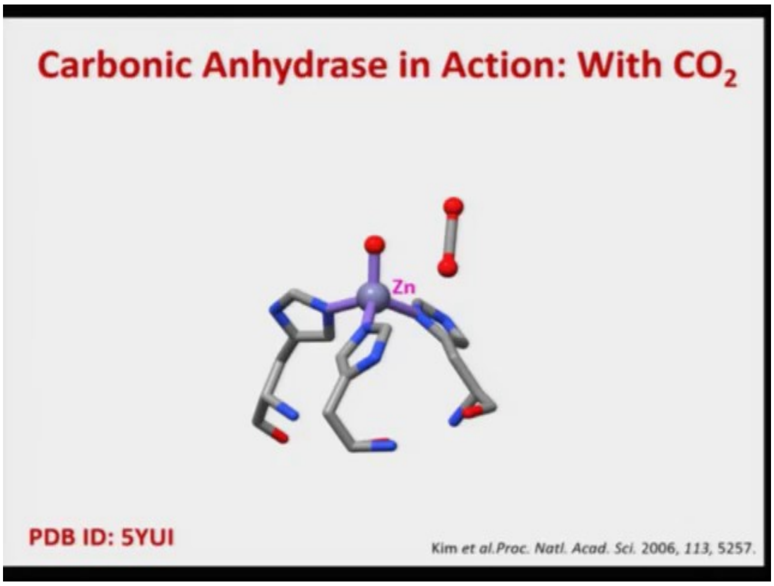

Carbonic Anhydrase in Action: With CO_2

PDB ID: 5YUI

Kim et al.Proc. Natl. Acad. Sci. 2006, *113*, 5257.

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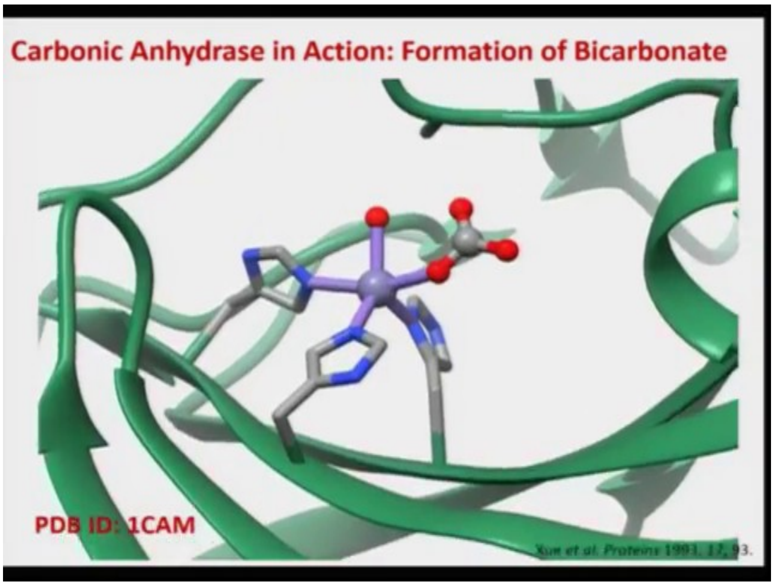

Carbonic Anhydrase in Action: Formation of Bicarbonate

PDB ID: 1CAM

Xue *et al. Proteins* 1993, *17*, 93.

And then there will be a nucleophilic attack which converts carbon dioxide to a bicarbonate. You also can see that the zinc is ligated with the bicarbonate the X-ray structure is shown over here.

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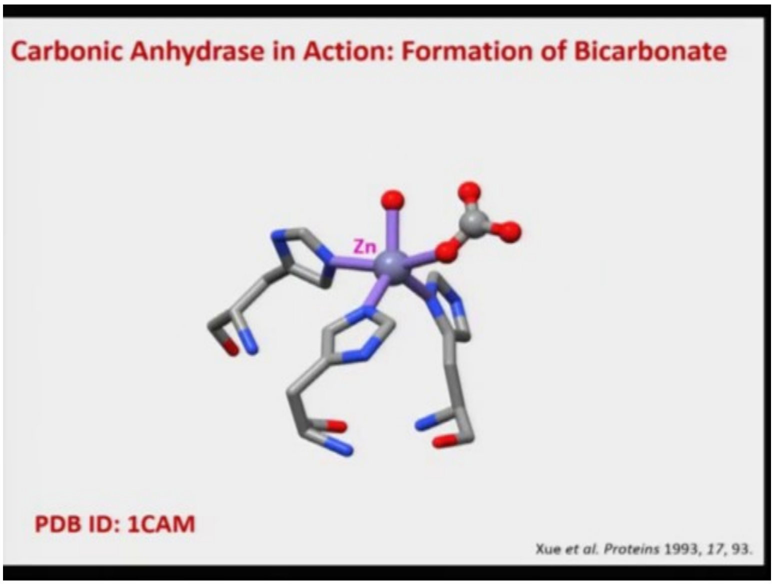

Carbonic Anhydrase in Action: Formation of Bicarbonate

PDB ID: 1CAM

Xue *et al. Proteins* 1993, *17*, 93.

And if you remove this protein, you can see the structure. As you can see that this bicarbonate will be released and gives rise to the resting state of the enzyme and another catalytic cycle starts. I now will talk about another hydrolytic metal enzyme urease.

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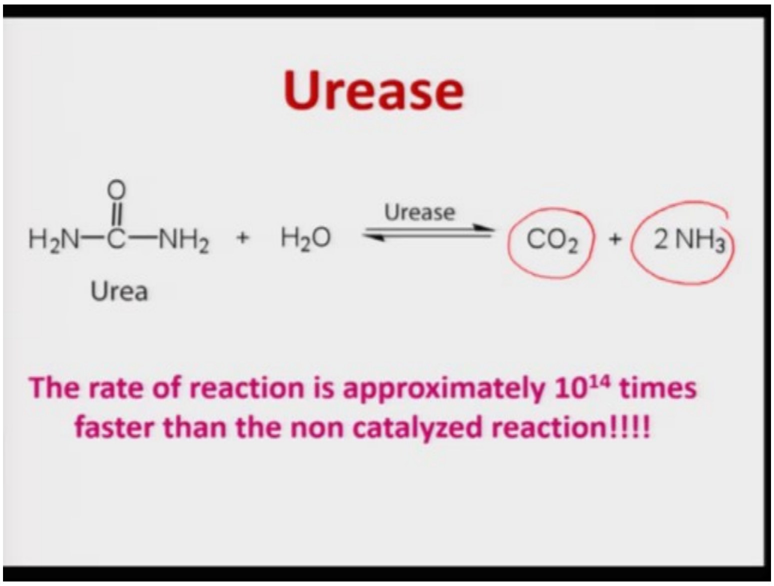

Urease

$$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 + H_2O \underset{}{\overset{\text{Urease}}{\rightleftharpoons}} CO_2 + 2\,NH_3$$

Urea

The rate of reaction is approximately 10^{14} times faster than the non catalyzed reaction!!!!

What urease is doing? It hydrolyze the urea into carbon dioxide and ammonia. And the rate of the reaction is approximately 10^{14} times faster than the non catalyzed reaction.

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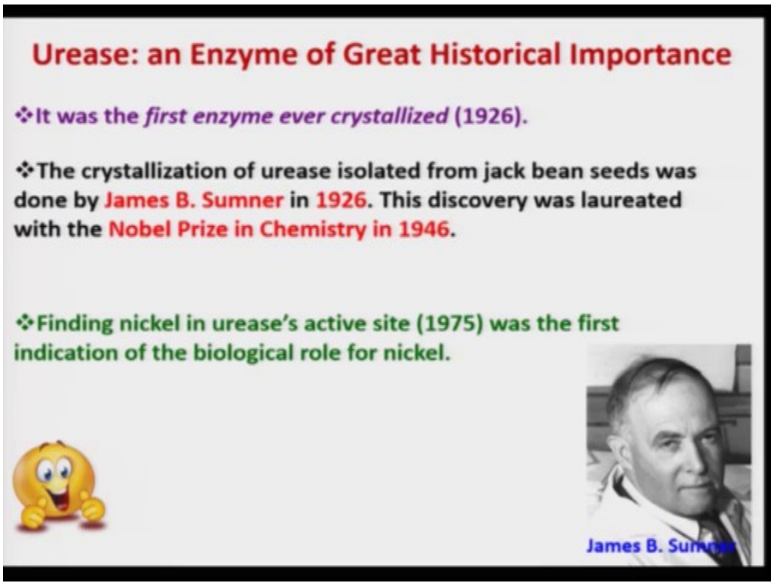

Urease: an Enzyme of Great Historical Importance

- It was the *first enzyme ever crystallized* (1926).
- The crystallization of urease isolated from jack bean seeds was done by James B. Sumner in 1926. This discovery was laureated with the Nobel Prize in Chemistry in 1946.
- Finding nickel in urease's active site (1975) was the first indication of the biological role for nickel.

Now, this urease is an enzyme of great historical importance. It was the first enzyme ever crystallized in 1926. The crystallization of urease isolated from Jack Bean Seeds was done by James B. Sumner in 1926. The discovery was laureated the Nobel Prize in Chemistry in 1946. Finding nickel in urease's active site was the first indication of the biological role for a nickel.

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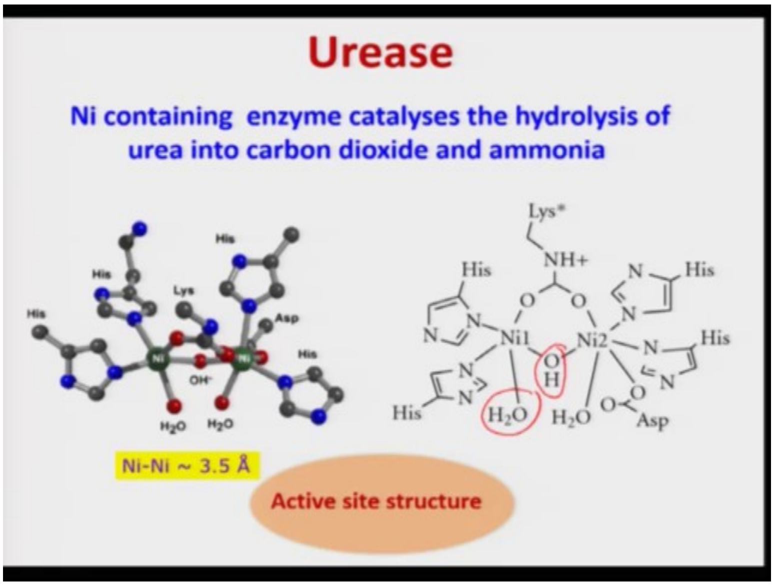

Urease

Ni containing enzyme catalyses the hydrolysis of urea into carbon dioxide and ammonia

Ni-Ni ~ 3.5 Å

Active site structure

The active site structure of this nickel containing urease enzyme is shown over here. As you can see that both the nickel centers are ligated to two histidine and one water molecule, and there is a bridging OH group which is actually bridged between two nickel center. And the distance between two nickel is around 3.5 Angstrom.

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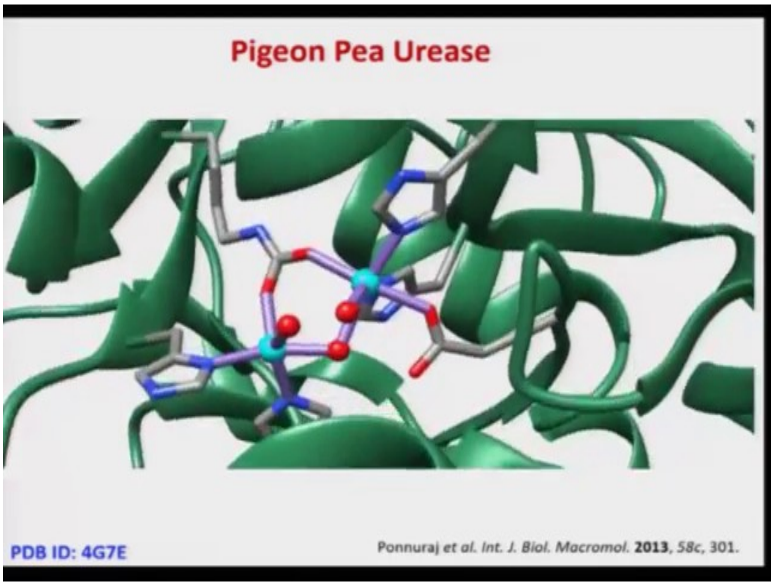

Pigeon Pea Urease

PDB ID: 4G7E

Ponnuraj *et al.* *Int. J. Biol. Macromol.* **2013**, *58c*, 301.

Now, I am showing here, the x-ray structure of urease is shown over here. As you can see that a huge amount of protein is surrounded around this metal center.

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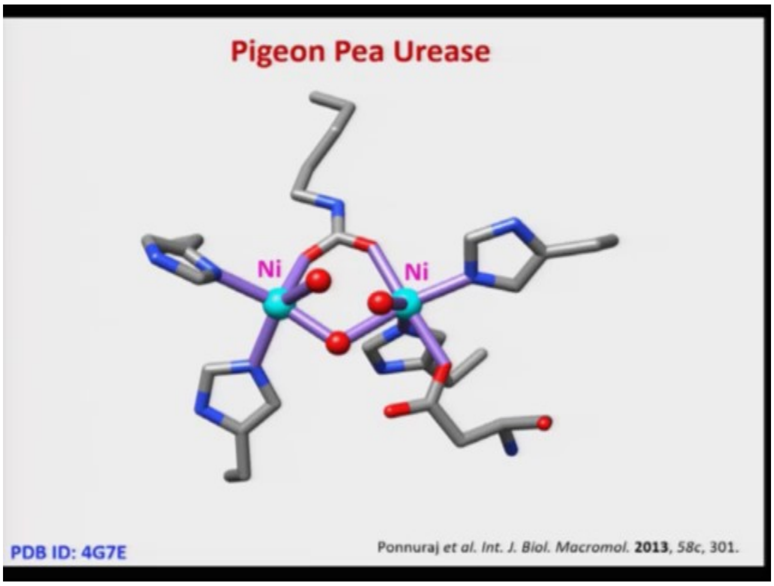

Pigeon Pea Urease

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And once you remove this protein, you see that there in organic molecules which is a di nickel center and which is bridged by a hydroxyl group, it can be hydroxo or water bridged between two nickel center.

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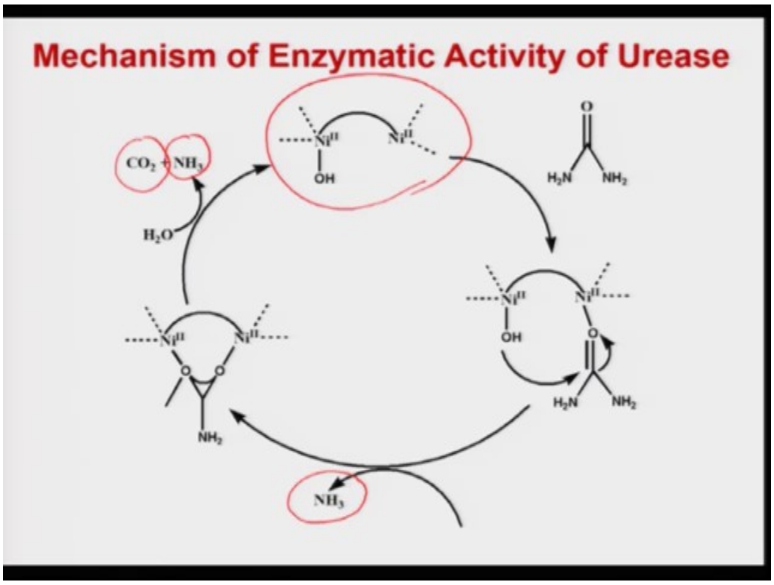

Mechanism of Enzymatic Activity of Urease

I will now discuss about the mechanistic action of this urease enzyme. So, as I have shown that this is di nickel center over here and then urea coordinates to one of these nickel through this carbonyl group, and then it increases the electrophilicity of this oxo group, so that this hydroxo of the other nickel center attack at this carbon. And after that it releases one molecule of ammonia, and then subsequently after hydrolysis releases another molecule of ammonia and carbon dioxide. Then this catalyst, urease is regenerated which again starts the cycle. So, this is the way this urease enzyme works.

So, in this lecture we have discussed so far the importance of metal ions in biological system. Metal ion concentration must be maintained within certain range within the cell; otherwise it causes various disease in our daily life. We also discussed today the role of non redox active metal ions in particular.

We have seen why nature has selected mostly zinc ion in the various metal enzymes that catalyze hydrolytic and related reaction. The metal acts mainly as a Lewis acid. And also does not change its oxidation state. In this regard, you also have discussed mechanistic action of two hydrolytic enzyme namely carbonic anhydrase and urease. In my next lecture, however, I will discuss the utilization of redox active metal ions in biological system.

Thank you.