VITAMIN $B_{12}$

*Syn* – *Extrinsic factor of Castle*

*Anti-pernecious anaemia factor*
1849: Addison described a familial type of anaemia – now known as ‘Addisonian Pernicious anaemia’

1934: William Murphy & George Minot showed that liver therapy was effective in treating pernicious anaemia
- received Nobel Prize
- Animal proteins contained ‘growth factor’, not present in vegetable foods & termed it as ‘Animal protein factor’
History

- 1948: George Minot isolated & crystallised Vitamin $\text{B}_{12}$
- 1949: Dorothy Hodgkin elucidated the structure of Vitamin $\text{B}_{12}$ by X-ray diffraction studies
- Robert Woodward synthesised Vitamin $\text{B}_{12}$ & proved the structure
Vitamin B12 has an empirical formula 

\[ \text{C}_{63} \text{H}_{88} \text{N}_{14} \text{O}_{14} \text{P}_{1} \text{Co}_{1} \]

- It contains 4.35% Cobalt by weight
- Its structure may be summarised as follows:
  - Corrin ring: Central structure made up of 4 reduced & extensively substituted ‘Pyrrole rings’, co-ordinated with a cobalt atom
  - DBI ring: 5\textsuperscript{th} Valency of Cobalt is covalently linked at right angle to 5,6 –dimethylbenzimidazole ring
    - The other end is attached to Pyrrole ring IV of Corrin ring system, by a ribose & phosphate moiety
  - R group: 6\textsuperscript{th} Valency of Cobalt is satisfied by the attachment of – Cyanide/Hydroxyl/Adenosyl/Methyl group
Sources

- Rich Sources: Liver, Meat, Fish, Eggs & Curds
- Poor Source: Vegetables & Milk
Recommended Dietary Allowance

- Adults: 1 μg/day (0.5 – 1.5 μg/day)
- Pregnancy, Lactation: 2 - 4 μg /day
Absorption, Storage, Transport & Excretion

- In diet, Vitamin $B_{12}$ is in a complex form attached to proteins.
- Vitamin $B_{12}$ is released by the action of HCl & proteiolytic enzymes in the stomach.
- In the stomach, $B_{12}$ first combines with ‘Intrinsic Factor of Castle’, a glycoprotein secreted by the parietal cells of the stomach.
- One intrinsic factor can bind two molecules of Vitamin $B_{12}$.
Absorption, Storage, Transport & Excretion

- IF – $\text{B}_{12}$ complex is transported to the terminal ileum, where it gets attached to mucosal cells
- IF – $\text{B}_{12}$ complex is internalised by endocytosis, with intrinsic factor being digested inside the mucosal cell
- $\text{B}_{12}$ is carried in blood to the liver cells by attachment to a specific glycoprotein ‘transcobalamin II’
- It is stored in the liver in combination with another glycoprotein ‘transcobalamin I’
- Normal serum levels of $\text{B}_{12}$: 0.008 to 0.042 $\mu$g/dl
- Methylcobalamin is the predominant form in circulation; very little amount of $\text{B}_{12}$ is excreted in urine
Functions

Biologically active co-enzyme forms B12 are called ‘Cobamide’ coenzymes.

Cobamide co-enzymes do not contain ‘Cyano’ group attached to cobalt.

Instead they contain either ‘5’-deoxyadenosine or methyl group.

DBI ring may have methyl substituted at 5,6 positions or may be methyl-free benzimidazole.
Functions (Contd)

Metabolic role:

- Carbon-carbon bond cleavage
  - Conversion of L-methylmalonyl CoA to Succinyl CoA, catalysed by the enzyme ‘L-methylmalonyl CoA Isomerase’

- Methyl activation reaction
  - Methylation of homocystine to form methionine catalysed by the enzyme ‘Homocystine-methyl Transferase’

- Carbon-oxygen bond cleavage
  - Metabolism of ‘Diols’ in bacteria
  - Ethylene glycol is converted to Acetaldehyde & water by the enzyme ‘Diol Dehydratase’
Functions (Contd)

- Carbon – Nitrogen cleavage
  - Lysine metabolism

- Methylation of pyrimidine ring to form thymine

- Conversion of ribonucleotides to deoxyribonucleotides by the action of enzyme ‘ribonucleotidase reductase’
**Metabolic outcome of B_{12} Deficiency**

- Methylmalonic aciduria
  - Deficiency of Vitamin B_{12} results in accumulation & excretion of methylmalonyl CoA resulting in ‘Methylmalonic aciduria’
  - Accumulation of methylmalonyl Coa in tissues results in production of abnormal Odd Chain Fatty Acids
  - These are then incorporated into myelin sheath causing breakdown of myelin sheaths → interruption in nerve transmission → neurological manifestations
Metabolic outcome of $B_{12}$ Deficiency

- Deficiency of Vitamin $B_{12}$ results in nonconversion of homocysteine to methionine resulting in accumulation & excretion of homocysteine causing ‘Homocystinuria’
- Nonavailability of methionine → defect in the formation of myelin sheaths demyelination of neurons & neurological lesions
Metabolic outcome of $B_{12}$ Deficiency

- **Folate trap**
  - In one carbon metabolism, production of methyl THFA is an irreversible step
  - THFA is regenerated only by the transfer of methyl group to cobalamin
  - In $B_{12}$ deficiency, THFA is not regenerated & folic acid is trapped as methyl THFA → ‘Folate trap’
  - This accounts for deficiency of folic acid associated with $B_{12}$ deficiency
Deficiency Manifestations

- B₁₂ deficiency is very common in INDIA, especially in vegetarians & low socio-economic group
- B₁₂ deficiency results due to –
  - Nutritional causes – lack of B₁₂ in the diet
  - Decreased absorption – Gastrectomy, Resection of Ileum
  - Intrinsic factor deficiency – Gastric atrophy, Achyla gastrica, Gastric Carcinoma
  - Fish tapeworm infestations
  - Pregnancy & Lactation
Deficiency Manifestations

- B12 deficiency leads to ‘Pernicious anaemia’ & ‘Subacute combined degeneration’ of neurons
- Signs & Symptoms includes – Weakness, easy fatigability, symmetrical parasthesia of extremities, areflexia, unsteadiness in gait, loss of position sense, loss of memory, confusion, delusion & psychosis in late stages
  - ‘Rhomberg’s sign’ – falling when eyes are closed
  - ‘Babinski’s sign’ – extensor plantar reflex
Deficiency Manifestations

Assessment -

- B12 estimation in serum by RIA
- Schilling’s test – Radiolabelled $1 \mu g$ Cobalt 60
  B12 is given orally & radioactivity is noted in liver & in faeces
  - Normally radioactivity is maximum in the liver & minimum in faeces
- FIGLU excretion test
- Detection of Methylmalonic acid & Homocystine in urine
Deficiency Manifestations

- Peripheral blood smear & bone marrow studies
- Gastric function tests
- Upper GI endoscopy
- Prompt administration of Vitamin B\textsubscript{12} along with Folic acid will reverse the symptoms