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Vitamin B12

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***Abstract:* Vitamin B_{12} is an essential water soluble vitamin found in a variety of foods such as milk, meat, oysters, fish and calms. It is a member of a family of related molecules called corrin-oids. Its coenzyme form is deoxyadenosylcobalamin was first isolated by Barker of California.**

Coenzyme B_{12} has been called as biological Grignard reagent. A unique feature of this vitamin is the presence in its molecule of a heavy atom cobalt in trivalent state.NO other cobalt containing organic compound has been found in nature. Vitamin B_{12} is called as red vitamin because it exists as a dark red crystalline compound.

Gymnodinium , a marine dinoflagellate associated with red tides needs for growth Vitamin B_{12} suggesting that red tides may be due to occurrence of Vitamin B_{12} in sea water.

Keywords: Vitamin B12, Coenzyme B12, Red Vitamin, Cobalt

I. INTRODUCTION

A. Accepted Names

Cyanocobalamin
Hydroxocobalamin
Antipernicious Anemia factor
Lactobacillus lactis dorner factor

B. Obsolete Names

Animal protein factor
Factor X
Zoopherin
Physin
Erythrotin

Vitamin B_{12} is used in combination with other B vitamins in vitamin B complex formation. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of human body. It helps to maintain healthy nerve cells and RBCs needed to make DNA. Neither fungi, plants nor animals are capable of producing vitamin B_{12}.Only bacteria and archaea have the enzymes needed for its synthesis. It is the largest and most structurally complicated vitamin and can be produced industrially only through a bacterial fermentation process.

II. HISTORY

The discovery of vitamin B_{12} stemmed from the medical necessity to seek a cure for a mysterious and ultimately fatal disease first described in 1855 by Thomas Addison as"a very remarkable form of general anemia without any discoverable cause whatsoever".

In 1926, George Minot and William Murphy discovered that patients suffering from pernicious anemia could be cured by feeding them with half a pound of liver a day. In 1929, Castle suggested that gastric juice contained a factor (Intrinsic factor) together with a factor present in food (extrinsic factor), responsible for cure of pernicious anemia.

Moreover, since patients with pernicious anemia have lost the capacity to absorb vitamin B_{12} via the physiologic route, the efficacy of the liver fed to pernicious anemia patients was likely a function of two serendipitous circumstances.

A. The large amount of B_{12} present in a pound of liver, permitting absorption of B_{12} through a passive diffusion mechanism.
B. Liver is a rich source of folate, which would be destroyed by gentle heat required to prepare Minot and Murphy's therapeutic dietary concoction.

So, folate can replace the need for B_{12} in its role in DNA synthesis. Lateron, in 1948, this antipernicious anemia (APA) factor was isolated in crystalline form by Smith in England and by Edward Rickes and Carl Folkers in the united states.

III. OCCURRENCE

Vitamin B_{12} has been found only in animals; the chief source is liver(Hog liver and Calf liver).It is also present in milk, meat, eggs, oysters, and calms. Though required by eukaryotes, B_{12} is synthesized solely by prokaryotic micro-organisms.

Ruminants obtain Vitamin B_{12} from the resident flora of their foregut. In some species, B_{12} is obtained through coprophagial or fecal contamination of the diet, but for humans and other omnivores, the only source of B_{12} is foods of animal origin.

The highest amount of vitamin B_{12} are found in liver and kidney. Vegetables, fruits and other foods of non-animal origin are free from B_{12} unless contaminated by bacteria. B_{12} in food is resistant to destruction by cooking.

Source of vitamin B_{12} for marine animals such as clams, oysters, bony fish etc. is Blue green algae (BGA).

IV. PHYSICAL AND CHEMICAL PROPERTIES

Vitamin B_{12} is a dark red crystalline compound, which darkens to black at 212^0 C and does not melt upto 320^0 C.

It is soluble in water, alcohol and acetone but not in chloroform.

It is optically active and laevorotatory.

It is stable to heat in neutral solution but is destroyed by heat in acidic or alkaline solutions.

Magnetic susceptibility measurements of Vitamin B_{12} indicated that it is diamagnetic and it is a trivalent cobalt complex with octahedral d^2sp^3 bonding.

Empirical formula: $C_{63}H_{84}N_{14}O_{14}PCo$

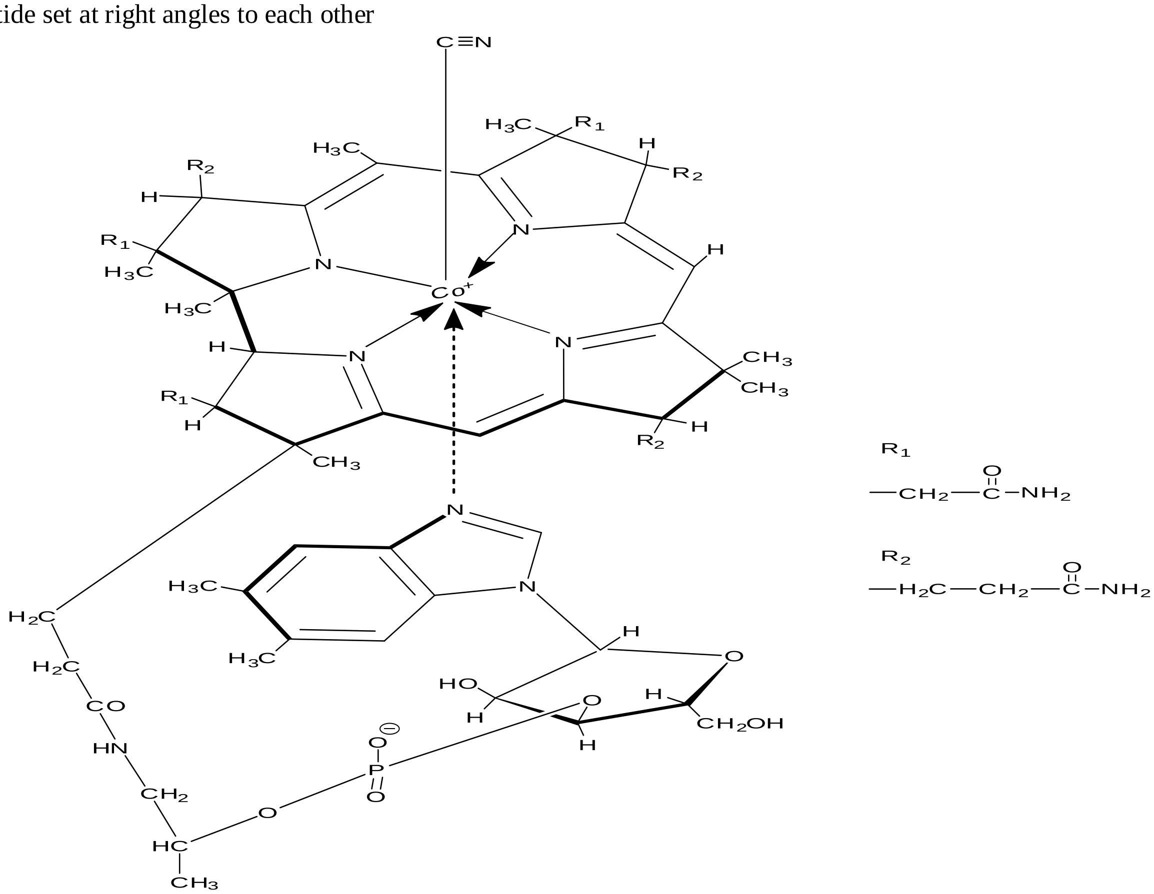
V. STRUCTURE OF VITAMIN B_{12}

Structure of Vitamin B_{12} has been established by Dorothy Crowfoot Hodgkin in 1957.

Vitamin B_{12} is an organometallic compound that has the unusual property of possessing a carbon-metal bond.

The molecule consists of two halves:

1) A planar group
2) Nucleotide set at right angles to each other



Structure of Vitamin B_{12} (Cyanocobalmin)

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A. Planar Group

The core planar group is a corrin ring with a single cobalt atom coordinated in the centre of the ring. The corrin ring like porphyrin is comprised of four pyrroles each of which is linked on either side to its two neighbouring pyrroles by carbon-methyl or carbon-hydrogen methylene bridges, with one exception.

In this exception, two neighbouring pyrroles are joined directly to each other. The fifth ligand of the cobalt projecting above the plane of the molecule is covalently bonded i.e R.

Predominant form of B_{12} has R= 5-deoxyadenosyl (located in mitochondria)

5-Deoxyadenosylcobalamin is the natural form of Vitamin B_{12}. The other natural form of B_{12} is Methylcobalamin where $R=CH_3$ (located in plasma and cytosol).

Vitamin B_{12} is also present as Hydroxocobalamin in minor amounts. The most stable pharmacological form of the Vitamin B_{12} is Cyanocobalamin.

The Co atom in hydroxo and cyanocobalamin is fully oxidised in Co^{3+} state, whereas the cobalt exists as reduced Co^{3+} or Co^{2+} in the 5'-deoxyadenosylcobalamin and Methylcobalamin forms.

B. Nucleotide

The nucleotide consists of the base 5,6-dimethylbenzimidazole and a phosphorylated sugar ribose 3-phosphate.

The sixth ligand of the central cobalt atom is occupied by one of the nitrogen of the 5,6-dimethylbenzimidazole attaches to ribose, which connects to a phosphate linking the sugar to one of the seven amide groups of the corrin ring by aminopropyl residue.

C. Absorption and transport of Vitamin B_{12}

It is an active process,which occurs in the ileum. The active physiological processes of B_{12} absorption are complex and involve discrete anatomical areas of the gastrointestinal tract, as well as specific B_{12}-binding and chaperone molecules.

Dietary B_{12} is released from protein complexes by enzymes in gastric juice, aided by low p^H of stomach maintained by HCl secreted from parietal cells.

- B_{12} bound to protein in food

↓ DIET

- B_{12} released from food protein by gastric acid and pepsin
- B_{12} bound to salivary R binder
- Intrinsic factor produced by parietal cells.

STOMACH LUMEN

↓

- B_{12} released from R binder
- B_{12} bound to Intrinsic factor
- R binder becomes degraded

INTENSTINAL LUMEN

↓

- IF-B_{12} complex taken by receptor mediated endocytosis involving cubulin, RAP and megalin
- B_{12} released from IF
- B_{12} bound to TC

ENTEROCYTE (ILEUM)

Normal physiology of B_{12} absorption

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On release from proteins in food, B_{12} combines with a salivary R-binder (Cobalophilin), a binding protein secreted in saliva. Cobalophilins are a group of antigenically related, unspecific corrinoid binding proteins, formerly known as R-proteins because of their rapid mobility on electrophoresis. Salivary R-binder is digested by pancreatic trypsin in the duodenum. The B_{12} is thus released and then transferred to the gastric glycoprotein, IF produced by the same parietal cells responsible for gastric acid production.

Binding of B_{12} to IF is favoured by the less acidic medium of the upper small intestine than the stomach. IF is a glycoprotein with a molecular weight of 45000 Da. It is produced in the microsomes or endoplasmic reticulum of the gastric parietal cells in the fundus and body of the stomach. On binding the vitamin, protein undergoes a conformational change, resulting in dimerization and resistant to proteolysis. Vitamin B_{12} absorbed from the distal third end of the ileum by receptor mediated endocytosis. There are IF-Vitamin B_{12} binding sites on the brush border of the mucosal cells in this region.

Within the ileal mucosal cell, Vitamin is released by lysosomal proteolysis of IF and is then carried into blood by two main B_{12} transport proteins haptocorrin and transcobalamin .

Transcobalamins 1 and 3 are referred to as Haptocorrin and carries 70%-80% of total circulating B_{12}. The large differences in %age saturation and proportion of total circulating B_{12} bound between transcobalamin and haptocorrin are largely the function of their respective half lives.

The half lives for transcobalamin-B_{12} (Holotranscobalamin) and haptocorrin-B_{12} (holohaptocorrin) have been estimated to be < 2h and~10 days respectively.

VI. METABOLISM

Vitamin B_{12} is converted to coenzyme B_{12} (Deoxyadenosyl B_{12}) by a two step process. The process requires NADH and ATP for this conversion

A. Ist Step:

Vitamin B_{12} (Co^{3+}) is reduced to Vitamin B_{12} (Co^{2+}) in the reaction requiring FAD and NADH.

$$B_{12a}\,(Co^{3+}) \xrightarrow[\text{NADH} \quad \text{NAD}^+]{} B_{12r}\,(Co^{2+})$$

B. Second Step

The reduced form of B_{12} then undergoes a reaction with ATP that yields the B_{12} coenzyme , inorganic trimetaphosphate is released from the ATP in the course of this transformation.

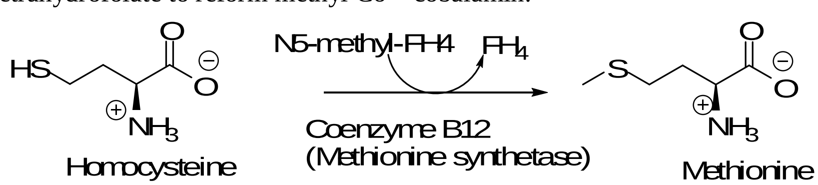
$$B_{12r}\,(Co^{2+}) \xrightarrow[\text{ATP} \quad \text{P.P.P}]{\text{Adenosyl transferase}} \text{Coenzyme } B_{12}\ \text{(Adenosylcobalamin)}$$

VII. ROLE OF VITAMIN B_{12} AS A COENZYME IN THE BODY

Vitamin B_{12} is required as a coenzyme for the following metabolic reactions:

A. Methylation of Homocysteine to Methionine

The overall rxn of methionine synthetase is the transfer of the methyl group from methyl-tetrahydrofolate to homocysteine. Coenzyme B_{12} serves as a carrier of methyl group. Cobalt accepts a methyl group from methyl-tetrahydrofolate, forming methyl Co^{3+}-cobalamin. Transfer of the methyl group onto homocysteine results in the formation of Co^{+}-cobalamin ,which can accept a methyl group from methyl-tetrahydrofolate to reform methyl Co^{3+}-cobalamin.



An absence of Vitamin B_{12} inhibits the reaction and leads to formation of N^5-methyltetrahydrofolate called as Tetrahydrofolate trap.

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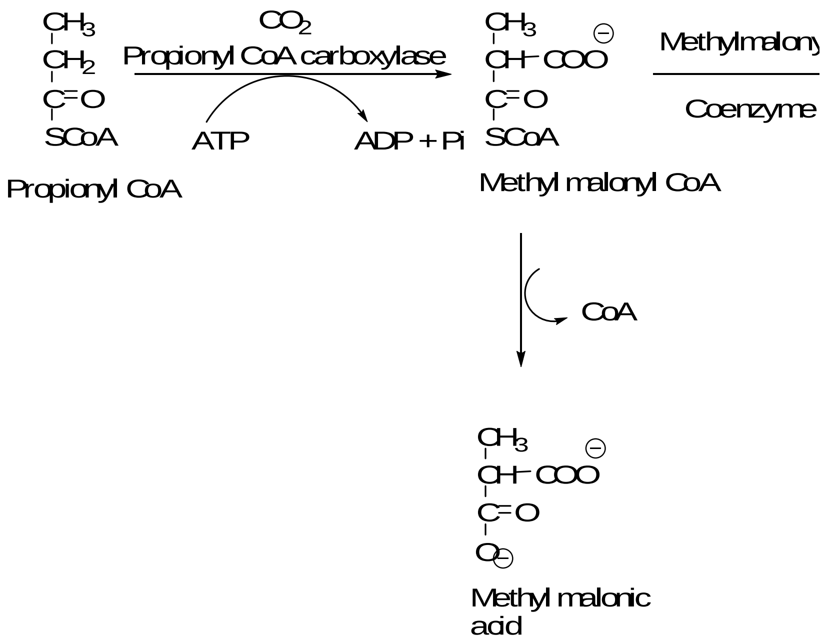
B. Isomerisation of L-Methylmalonyl CoA to Succinyl CoA

Methyl malonyl CoA arises directly as an intermediate in the catabolism of valine and is formed by the carboxylation of propionyl CoA.

Coenzyme B_{12} catalyses 1,2-shift of a hydrogen atom from one carbon atom of the substrate to the next with a concomitant 2,1 shift of some other group. Example: Hydroxyl, alkyl etc.

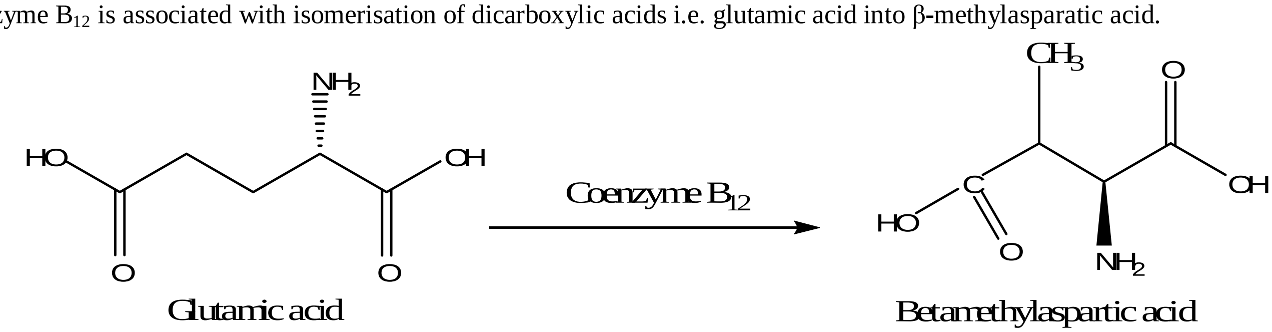
Conversion of L-Methylmalonyl CoA to Succinyl CoA is an example of 1, 2-shift.



1) In Vitamin B_{12} deficiency, as a result of reduced activity of mutase, there is an accumulation of methylmalonyl CoA, some of which is hydrolysed to methylmalonic acid, which is excreted in the urine. This can be exploited as a means of assessing Vitamin B_{12} nutritional status.
2) Methylmalonyl CoA inhibits the synthesis of fatty acids from acetyl CoA at concentrations of the order of those found in tissues of Vitamin B_{12}-deficient animals.

C. Isomerisation of Dicarboxylic Acids

Coenzyme B_{12} is associated with isomerisation of dicarboxylic acids i.e. glutamic acid into β-methylasparatic acid.



D. Dismutation of Vicinal Diols

Coenzyme B_{12} also catalyses dismutation of vicinal diols to the corresponding aldehydes eg. Propane-1,2-diol into propionaldehyde.

1) *Here, B_{12} Is An Important Cofactor In*
   a) Maintenance of normal DNA synthesis as evident under conditions of B_{12} deficiency,which lead to defective DNA synthesis and Megaloblastic anaemia.
   b) Regeneration of Methionine for the dual purposes of maintaining protein capacity and methylation capacity.
   c) Avoidance of Homocysteine accumulation, an amino acid metabolite implicated in vascular damage and several associated degenerative diseases including Alzheimer disease and Osteoporosis.

VIII. VITAMIN B_{12} REQUIREMENTS

Total body pool of vitamin B_{12} is of the order of 1.8µmol (2.5 mg), with a minimum desirable body pool of about 0.3µmol (1 mg).
Daily loss is about 0.1% of the body pool in subjects with normal intrinsic factor secretion and enterohepatic circulation of the vitamin.
Requirements are probably between 0.1 to 1 µg per day, reference intake ranges between 1 to 2.4 µg per day, which is lower than the average intake of 5 µg per day by non-vegetarians in most countries.

AGE	U.K. 1991	EU 1993	U.S./CANADA 1998	FAO 2001
0-3m	0.3	_	0.4	0.4
4-6m	0.3	_	0.4	0.4
7-9m	0.4	0.5	0.5	0.5
10-12m	0.4	0.5	0.5	0.5
1-3y	0.5	0.7	0.9	0.9
4-6y	0.8	0.9	1.2	1.2
7-8y	1.0	1.0	1.2	1.8
MALES				
9-6y	1.0	1.0	1.8	1.8
11-13y	1.2	1.3	1.8	2.4
14-15y	1.5	1.3	2.4	2.4
>16y	1.5	1.4	2.4	2.4
FEMALES				
9-10y	1.0	1.0	1.8	1.8
11-13y	1.2	1.3	1.8	2.4
14-15y	1.2	1.3	2.4	2.4
>16y	1.5	1.4	2.4	2.4
Pregnant	1.5	1.6	2.6	2.6
Lactating	2.0	1.9	2.8	2.8

Reference Intakes of Vitamin B_{12} (µg per day)

1) *EU:* European Union
2) *FAO:* Food and Agriculture Organisation
3) *WHO:* World Health Organisation

A. *Industrial preparation of Vitamin B_{12}*

1) Mother Liquors of Microbial Formation of Antibiotics like Sreptomycin, Aureomycin and Tetramycin after removal of antibiotic.
2) *Addition of Cobalt to Fermentation:* Vitamin B_{12} contains about 4% cobalt. Addition of small amounts of cobalt salts to broth increases the B_{12} production upto
3) *Fermentation processes Producing only B_{12}* without any other highly valuable product are economically justified only when they have definite advantages over fermentation producing antibiotics at same time.
4) *B_{12} from Activated Sludge:* Activated sludge from sewage disposal plants contains considerable amounts of B_{12}. It will have industrial success only when enormous amounts of solid raw material are disposed off as a paying product (used as fertiliser)

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IX. VITAMIN B_{12} DEFICIENCY

Also known as hypocobalaminemia. It refers to low blood levels of Vitamin B_{12}.

A. Causes

1) Inadequate dietary intake
2) Intestinal malabsorption
3) Increased requirements
4) Failure of utilization of absorbed Vitamins
5) Drug induced malabsorption

a) *Inadequate Dietary Intake:* Dietary B_{12} deficiency arises in adult vegans who shun all meat, fish, eggs, cheese and other animal products from their diet. Largest group of vegans in the world consists of Hindus. Not all vegans develop B_{12} deficiency, it may arise in non-vegetarian subjects who exist on inadequate diets because of poverty.

b) *Intestinal Malabsorption:* Since the terminal portion of the ileum is the site for physiologic absorption of B_{12} via the IF-mediated mechanism, diseases, abnormalities, and removal of this portion of intestine can

c) *Causes Include*

i) *Crohn's Disease:* It is a granulomatous disease most commonly affects terminal ileum and ascending colon. It is manifestated by malabsorption of nutrients from diet.

ii) *Ileum Resection or Ileostomy:* Involve removal of Vitamin B_{12} receptor.

iii) *Infestation of Gut With Fish Tapeworm:* Diphyllobothrium latum consume B_{12} both complexed with intrinsic factor and free, making it unavailable to the host.

iv) *Gastrointestinal Disease:* Surgical removal of source of IF or site of absorption of Vitamin B_{12} causes malabsorption of Vitamin B_{12}.

a. *Total Gastrectomy:* The anemia is developed after depletion of body stores, which usually occurs within 5 years.

b. *Partial Gastrectomy:* Partial removal of stomach and refashioning the junction with gut. The sterile duodenum part will colonized with bacteria, which will consume huge amount of the vitamin.

d) *Failure of Absorption of Absorbed Vitamins*: Congenital transcobalamin deficiency and haptocorrin deficiency which are the most important of the plasma B_{12} carrier proteins causes failure of utilization of absorbed Vitamins.

e) *Drug Induced Malabsorption:* A number of drugs impair Vitamin B_{12} absorption such as:

i) Antimicrobial, Neomycin
ii) Antigout, Colchicine
iii) Alcohal
iv) Anticonvulsant, phenytoin

B. Lack of Intrinsic Factor

It may be congenital or may be Tdue to gastrectomy. It causes Pernicious Anemia.

1) *Pernicious Anemia*

a) It is a type of anemia due to body's inability to absorb Vitamin B_{12} from gastrointestinal tract.

b) Because body has stores of Vitamin B_{12}, pernicious anemia takes years to establish and diagnosed in adults with an average age of 60.

c) The disease is more common in women than in men and is associated with blood group A

2) *Cause:* An autoimmune mechanism in which body's immune system attacks lining of stomach. Antibodies are produced against Intrinsic factor, a protein necessary for absorption of Vitamin B_{12}. So,it lowers IF levels.

3) *Diagnosis*

a) *By Schilling Test*: It is the gold standard method for detecting B_{12} malabsorption. The test requires the oral administration of Cobalt-57-labelled B_{12} ($^{57}Co\text{-}B_{12}$) to a fasting subject and complete collection of urine during the subsequent 2h after the oral radioactive dose. This saturates all available plasma B_{12}-binding proteins such that any of the $^{57}Co\text{-}B_{12}$ that is absorbed which is of low molecular weight is flushed into the urine. Low excretion of the radioactivity in urine indicates impairment of the ability to absorb Vitamin B_{12}. Then patients identified having malabsorption are given exogenous source of IF with 57Co-B_{12} to check for pernicious anemia. If the IF correct the malabsorption then patient does have pernicious anemia.

b) *Treatment:* It consists of intramuscular injections of 1000 mcg of Vitamin B_{12} at weekly intervals until B_{12} stores are replenished followed by monthly injections for life. Oral administration of partially purified preparations of intrinsic factor will restore the absorption of Vitamin B_{12}.

C. *Megaloblastic Anemia*

It is a type of anemia caused by impaired DNA synthesis that results in macrocytic red blood cells, abnormalities in leukocytes and platelets and epithelial cells of the mouth and gastrointestinal tract.

1) *Cause:* Cobalamin (Vitamin B_{12}) and folate (Vitamin B_9) deficiency.
2) *Symptoms*
a) Weakness
b) Fatigue
c) Shortness of breath
d) Neurologic Abnormalities
3) *Treatment:* Doses of Cobalamin (1000 mg/weak hydroxocobalamin administrated intramuscularly over 30 days)1 mg of folic acid daily for 30 days

D. *Signs and Symptoms of Vitamin B_{12} Deficiency*

1) Vitamin B_{12} deficiency lead to anemia and neurologic dysfunction.
2) Anemia causes weakness, fatigue, light-headedness, rapid heartbeat, rapid breathing and colour of skin becomes pale yellow.
3) It may cause bleeding including bleeding gums.
4) Nerve cell damage
5) Tingling or numbness to fingers and toes.
6) Difficulty walking, mood changes, depression, memory loss, decreased fertility.
7) Inhibition of DNA synthesis (Purines and Thymidine) causes anemia with bone marrow premegablastosis (results from inhibition of DNA synthesis due to deficiency of Vitamin B_{12}.

E. *Health Benefits of Vitamin B_{12}*

1) *Cell Maintenance:* Vitamin B_{12} helps in maintaining different types of cells that exist in human body. eg. Important functions like formation, repair and maintenance of RBCs are largely dependent upon Vitamin B_{12}. Also, Vitamin B_{12} helps in maintaining a strong nervous system.
2) *DNA Formation:* Vitamin B_{12} is an important element for regular formation of DNA in human body. This practice is performed during cell division. In case of lack of Vitamin B_{12}, abnormal cell formations known as megaloblasts will develop. This causes anemia in anemia in human body.
3) *Fatigue:* Vitamin B_{12} is an appreciable source for relieving the human body from fatigue and weakness. Thus, it improves overall stamina of body.
4) *Cholestrol:* It is helpful in reducing cholesterol level in human body. So, it helps in controlling the level of triglycerides which helps in maintaining the proper functioning of human heart.
5) *Sickle cell Disorder:* Vitamin B_{12} save the patient’s body from endothelial damage so it is an important health component for treating the severe problem of sickle cell anemia.
6) *Alzheimer’s Disease:* Vitamin B_{12} is effective in treating Alzheimer’s disease which is accompanied by symptoms like confusion and congenetive degeneration.
7) *Anemia:* It helps in treatment for various types of anemia like pernicious anemia and megaloblastic anemia.
8) *Breast Cancer:* Women suffering from breast cancer are deficient in Vitamin B_{12}. Thus, a diet rich in this Vitamin is recommended for prevention of development of this terrible disease.

III. ACKNOWLEDGEMENT

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REFERENCES

[1] Zempleni J., Bucker Rucker B., McCornick Donald B., Suttie John W., “Handbook of VITAMINS”, **2007**,4, 414-445
[2] BENDER DAVID A., “Nutritional Biochemistry of the Vitamins”, **2003**, 2, 299-321
[3] SEBRELL.W.H.,“ THE VITAMINS Chemistry, Physiology, Pathology”, **1954**, 1, 396-481
[4] Beale John M., Block John H.,“Wilson and Gisvold’s Textbook of ORGANIC MEDICAL AND PHARMACEUTICAL CHEMISTRY”, 12, 951-954
[5] FINAR I.L.,“Stereochemistry and the Chemistry of Natural Products”, **2008**, 5,863-864
[6] JAIN J.L., JAIN SUNJAY, NITIN JAIN,“ Fundamentals of Biochemistry”, **2014**, 7, 1354-1358