1. **Describe the process of glycogen synthesis and glycogenolysis (E-Sep 2002)**

Glycogen metabolism includes both glycogen synthesis and its breakdown

Glycogenesis

Site: glycogen synthesis occurs mainly in liver (up to 6%) and muscle (up to 1%). Muscle contains about three to four times than liver because of greater mass

Importance:

Glycogen is the major storage carbohydrate in animals; it is a branched polymer of α-D-glucose. The excess carbohydrates are converted into glycogen for feature energy expenditure. Muscle glycogen is a readily available source of glucose for glycolysis within the muscle itself. Liver glycogen functions to store and export glucose to maintain blood glucose between meals. After 12–18 hours of fasting, the liver glycogen is almost totally depleted.

Reactions proper:

- Glucose is phosphorylated to glucose-6-phosphate, catalysed by hexokinase in muscle and glucokinasein liver.
- Glucose 6-phosphate is isomerized to glucose 1-phosphate by phosphoglucomutase.
- Glucose 1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide uridinediphosphate glucose (UDPGlc)* and pyrophosphate, catalyzed by UDPGlcpyrophosphorylase.
- Glycogen synthase catalyzes the formation of a glycoside bond between C1 of the activated glucose of UDPGlc and C4 of a terminal glucose residue of preexisting glycogen/glycogen, liberating uridinediphosphate (UDP).
- Further glucose residues are attached in the 1→4 position to make a short chain that is a substrate for glycogen synthase.

Branching

When the chain has been lengthened to at least 11 glucose residues, branching enzyme transfers a part of the 1→4 chain (at least six glucose residues) to a neighbouring chain to form a 1→6 linkage, establishing a branch point. The branches grow by further additions of 1→4-glucosyl units and further branching.

Glycogenolysis

- Glycogen phosphorylasecatalyses the phosphorylytic cleavage by inorganic phosphate (phosphorylysis) of the 1→4 linkages of glycogen to yield glucose 1-phosphate. The terminal glucosyl residues from the outermost chains of the

glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a 1→6 branch

- Another enzyme glucan transferase transfers a trisaccharide unit from one branch to the other, exposing the 1→6 branch point. Hydrolysis of the 1→6 linkages requires the debranching enzyme. Hydrolysis of the 1→6 linkages requires the debranching enzyme. Further phosphorylase action can then proceed. The combined action of phosphorylase and these other enzymes leads to the complete breakdown of glycogen.
- The reaction catalyzed by phosphoglucomutase is reversible, so that glucose 6-phosphate can be formed from glucose 1-phosphate. In liver and kidney, but not in muscle, there is a specific enzyme, glucose-6-phosphatase, that hydrolyzes glucose 6-phosphate, yielding glucose that is exported, leading to an increase in the blood glucose concentration.

2. What is normal serum cholesterol level? Describe the process of synthesis of cholesterol (E-Aug 2004, Feb 11)

Normal blood cholesterol levels = 150 - 200 mg/dL

CHOLESTEROL:

- It is a 27 carbon steroid molecule.
- It is structural component of cell membrane and It gives flexibility to cell membrane.
- Intermediates of cholesterol biosynthesis converted to vitamin – D, and steroid hormones.
- Cholesterol decomposed to bile salts, which are helpful in fat absorption.
- It is high resistant to electrical conductance so it helpful as insulator for nervous tissue.

Synthesis of cholesterol:

Half of the cholesterol in the body derives from biosynthesis. Liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day.

Site: cholesterol is mainly synthesized in liver and adipose tissue.

Precursors:

Acetyl CoA is the precursor for cholesterol synthesis.

Reactions:

The process has nine major steps:

1. Acetyl-CoAs are converted to 3-hydroxy- 3-methyl glutaryl-CoA (HMG oA).

Two moles of acetyl-CoA are condensed in a reversal of the thiolase reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to HMG-CoA by the action of HMG-CoA synthase.

2. HMG-CoA is converted to mevalonate by HMG-CoA reductase. HMGCoA reductase absolutely requires NADPH as a cofactor and two moles of NADPH are consumed during the conversion of HMG-CoA to mevalonate.

The reaction catalyzed by HMG-CoA reductase is the rate limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls.

3. Mevalonate is then activated by two successive phosphorylations, yielding 5-pyrophosphomevalonate.

4. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO2 .Isopentenyl pyrophosphate is in equilibrium with its isomer, dimethylallyl pyrophosphate, (DMPP).

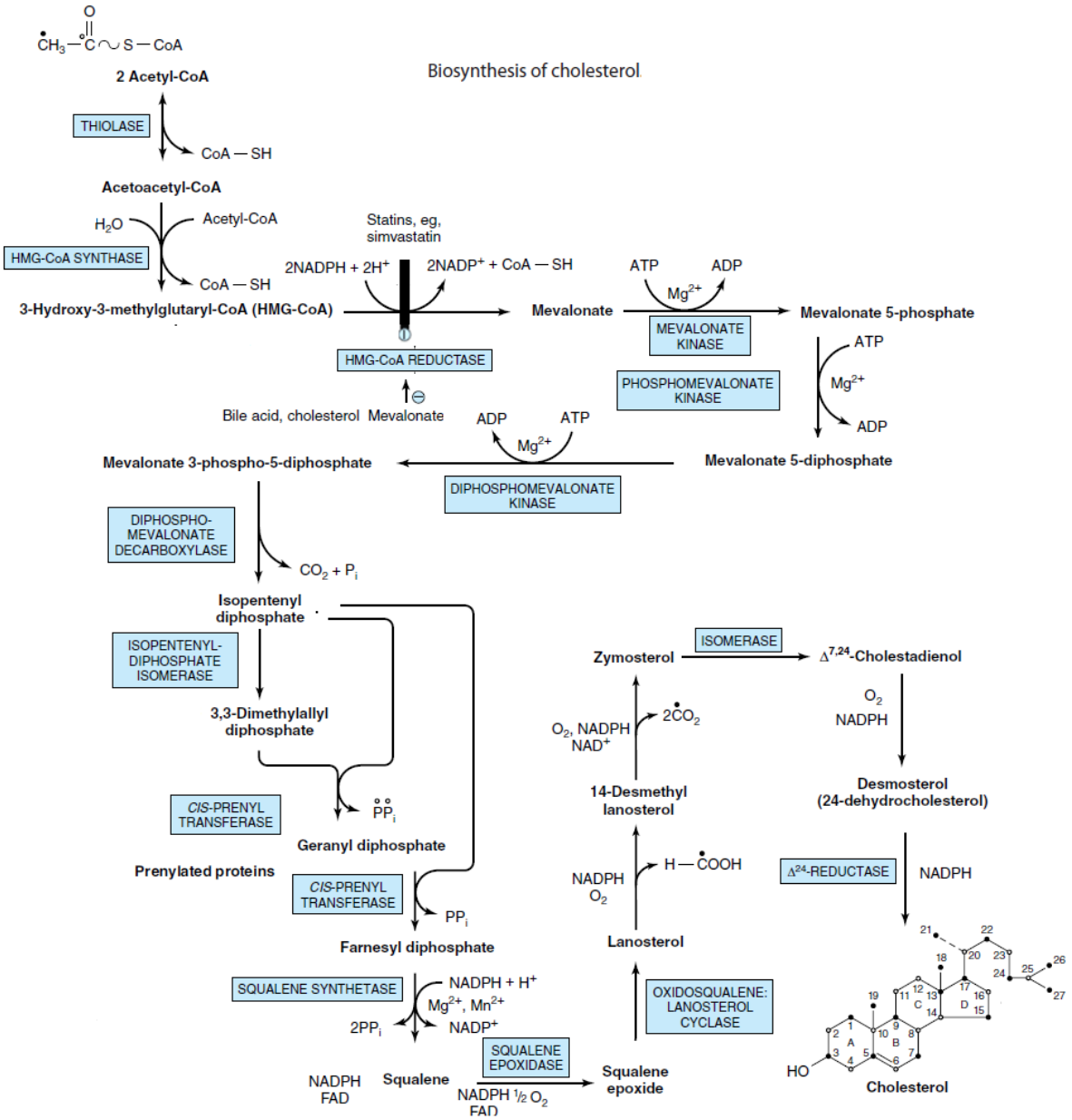
5. One molecule of IPP condenses with one molecule of DMPP to generate geranyl pyrophosphate, (GPP).

6. GPP further condenses with another IPP molecule to yield farnesyl pyrophosphate, (FPP).

Finally, the NADPH-requiring enzyme, squalene synthase catalyzes the head-to-tail condensation of two molecules of FPP, yielding squalene (squalene synthase also is tightly associated with the endoplasmic reticulum).

8. Squalene undergoes a two-step cyclization to yield lanosterol. The first reaction is catalysed by squalenemonooxygenase. This enzyme uses NADPH as a cofactor to introduce molecular oxygen as an epoxide at the 2, 3 position of squalene.

9. Through a series of 19 additional reactions, lanosterol is converted to cholesterol.


Biosynthesis of cholesterol
2 Acetyl-CoA
THIOLASE
CoA — SH
Acetoacetyl-CoA
H2O
Acetyl-CoA
HMG-CoA SYNTHASE
CoA — SH
3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)
Statins, eg, simvastatin
2NADPH + 2H+
2NADP+ + CoA — SH
HMG-CoA REDUCTASE
Bile acid, cholesterol
Mevalonate
ATP
ADP
Mg2+
MEVALONATE KINASE
Mevalonate 5-phosphate
PHOSPHOMEVALONATE KINASE
ATP
Mg2+
ADP
Mevalonate 5-diphosphate
ADP
ATP
Mg2+
DIPHOSPHOMEVALONATE KINASE
Mevalonate 3-phospho-5-diphosphate
DIPHOSPHO-MEVALONATE DECARBOXYLASE
CO2 + Pi
Isopentenyl diphosphate
ISOPENTENYL-DIPHOSPHATE ISOMERASE
3,3-Dimethylallyl diphosphate
CIS-PRENYL TRANSFERASE
PPi
Geranyl diphosphate
Prenylated proteins
CIS-PRENYL TRANSFERASE
PPi
Farnesyl diphosphate
SQUALENE SYNTHETASE
NADPH + H+
Mg2+, Mn2+
2PPi
NADP+
NADPH
FAD
Squalene
SQUALENE EPOXIDASE
NADPH ½ O2
FAD
Squalene epoxide
OXIDOSQUALENE: LANOSTEROL CYCLASE
Lanosterol
NADPH
O2
H—COOH
14-Desmethyl lanosterol
O2, NADPH
NAD+
2CO2
Zymosterol
ISOMERASE
Δ7,24-Cholestadienol
O2
NADPH
Desmosterol (24-dehydrocholesterol)
Δ24-REDUCTASE
NADPH
HO
Cholesterol

3. **Write short notes on**
   a. **Mutarotation**

Definition: mutarotation is defined as the change in the specific optical rotation representing the interconversion of α and β form of the D-glucose to an equilibrium mixture. Cyclic sugars show mutarotation as α and β anomeric forms interconvert.

The specific opticalrotation of a freshly prepared glucose (a-anomer) solution in water is $+11.2^{o}$

Measurement of Optical rotation:

The α and β anomers are diastereomers of each other and usually have different specific rotations. A solution or liquid sample of a pure α anomer will rotate plane polarised light by a different amount and/or in the opposite direction than the pure β anomer of that compound. The optical rotation of the solution depends on the optical rotation of each anomer and their ratio in the solution.

For example if a solution of β-D-glucopyranose is dissolved in water, its specific optical rotation will be +18.7. Over time, some of the β-D-glucopyranose will undergo mutarotation to become α-D-glucopyranose, which has an optical rotation of +112.2. Thus the rotation of the solution will increase from +18.7 to an equilibrium value of +52.5 as some of the β form is converted to the α form. The equilibrium mixture is actually about 64% of β-D-glucopyranose and about 36% of α-D-glucopyranose, though there are also with traces of the other forms including furanoses and open chained form

b. Factors regulation the enzyme action

Enzyme concentration: rate of enzyme velocity is directly proportional to enzyme concentration when all other kept optimal

Substrate concentration: the rate of enzyme velocity is very high during the initial reaction and after subsequent addition of substrate leads to loss of enzyme activity and velocity

The velocity is expressed in µM of substrate converted per minute

The maximum velocity obtained at substrate saturation level is called as Vmax

Km-Michaelis menten constant:

According to Michaelis theory, the formation of enzyme substrate complex is a reversible while the breakdown of complex to enzyme and product is irreversible

Km value is substrate concentration at half maximal velocity, means 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration

Km is independent of enzyme concentration. If enzyme concentration is double, the Vmax will be double, but ½ Vmax will remain same. In other words irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration

Significance of Km:

Km is constant and characteristic feature of a particular enzyme for a specific substrate

Km denotes the affinity of enzyme for substrate. The lesser the km the more affinity for enzyme to substrate and vice versa

Kd dissociation constant

The affinity of an enzyme towards its substrate is inversely related to the dissociation constant

Kd = E+S ES complex E+P

Kd = k2/k1 and km = k2+k3/k1

Therefore, the smaller the tendency for the dissociation of the complex, the greater is the affinity of the enzyme for the substrate

Concentration of products

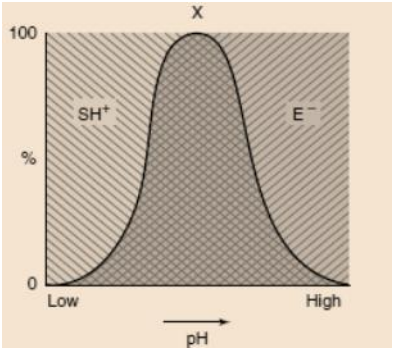
The rate of enzyme velocity is indirectly proportional to product concentration

Effect of temperature

The velocity of enzyme catalysed reaction is increases parallel upto a particular temperature due to supplying of activation energy and slowly falls in higher temperature due to degradation of enzyme molecule. The temp at which maximum amount of the substrate is converted to the product per unit is called the optimal temperature. Most of the enzymes works at optimal temperatures ranges betwee 37-50^{o}C , except some eg. Thermobacillus (Taq pol II). When we draw the plot a graph for velocity vs temp, we will get the bell shaped curve

Effect of pH

Each enzyme has a unique pH range for maximal activity, beyond these ranges enzyme velocity will slowdown. The optimal pH for most of the enzymes is pH 5-8.except pepsin with optimum pH of 1-2 and ALP has pH of 9-10 and acid phosphatase has pH 4-5 etc.



c. Anaplerotic reactions (SN-Apr 2001)

Anaplerosis of TCA Cycle:

TCA is a considered as major metabolic cycle in living organism. The several essential metabolites formed from intermediates of TCA cycle, which causes the deficiency of those intermediates and leads to cease of cycle. Therefore replenishment of deficient intermediates is termed as Anaplerosis

The various intermediates of TCA cycle used and refilled as follows

- Oxaloacetate and a-KG serve as Oxaloacetate and a-KG serve as precursor for the synthesis of aspartate and glutamate respectively. In order to replenish these intermediates the Anaplerotic reactions as follows –

Pyruvate + CO2 + ATP → oxaloacetate + ADP + Pi catalysed by pyruvate carboxylase

Glutamate + NADP + H2O → a-KG + NADPH + H + NH4 by glutamate dehydrogenase

- Another Anaplerotic reaction for malate

Pyruvate +CO2+NADPH +H → Malate + NADP + H2O by malate dehydrogenase (malic enzyme)

d. What are ketone bodies? Describe the formation of ketone bodies (SN-Feb 07, Aug 07, 08, Feb 11)

The ketone bodies are mainly three, namely Aceteacetate, B-Hyroxybutyrate and acetone. The later one is volatile in nature.Ketone bodies are water soluble and on oxidation the produce energy

Synthesis of Ketone bodies (Ketogenesis):

Site: mitochondrial matrix of liver cells

Precursor: acetyl CoA

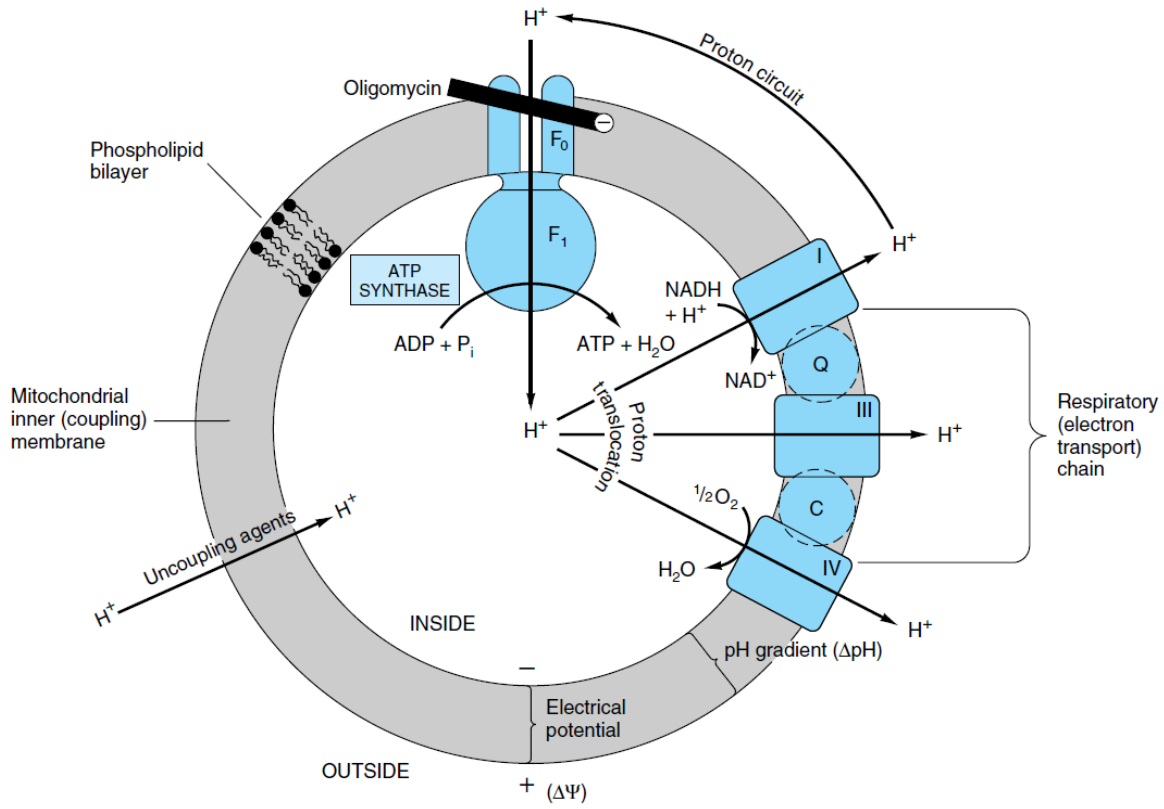
Reactions:

- Two molecules of acetyl CoA condense to form acetoacetyl CoA, this reaction is catabolized by thiolase
- Acetoacetyl Co A condence with another Acetyl CoA to produce b-hydroxy b-methyl glutaryl CoA (HMG CoA) synthase. This reaction is catalysed by HMG CoA synthase, it is key regulatory enzyme of ketogenesis
- HMG CoA is cleaved to acetoacetate and acetyl CoA by the action of HMG CoA lyase
- Acetoacetate can undergo spontaneous decarboxylation to for acetone
- Acetoacetate can be reduced by dehydrogenase to b-hydroxybutyrate

e. **Chemiosmotic theory** (SN-apr 01, sep02, feb 07, 08,09,Aug 11)

The coupling of oxidation with phosphorylation is termed oxidative phosphorylation is called as oxidative phosphorylation. Peter Mitchell in 1961 proposed a theory to explain oxidative phosphorylation.

Generation of proton gradient and synthesis of ATP: the complexes I, III and IV expel proton form inside to outside of the mitochondria membrane. This causes the electrochemical potential difference across the membrane, once established it inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the ATP synthase. This in turn depends on availability of ADP and P_i.



f. Justify the statement that vitamin D is an hormone (E-Apr 2001, Mar 2002, Sep 2002, SN-oct 2003)

Active form of vitamin D (calcitriol) is considered as calcitropic hormone, while cholecalciferol is the prohormone. The following characteristic features will demonstrate the vitamin D is a hormone –

- Cholecalciferol (prohormone) is synthesized in skin from 7-dehydrocholesterol by the action of UV light
- The biologically active form (Calcitriol) is synthesized in kidney
- Calcitriol is acted on specific target organs such as Bone, Intestine and kidney
- Calcitriol exactly acts as steroid hormones for instance it induces the synthesis of Calcium binding protein (Calbindin/calmodulin) from intestinal cells at mRNA level
- Actinomycin D inhibits the action of calcitriol. This supports the view that calcitriol exerts its effect on DNA leading to the synthesis of RNA
- Calcitriol synthesis is self-regulated by feedback mechanism

g. Thiamine (E-Oct 2003)

Common name: anti beri-beri vitamin.

Chemical name: Thiamine

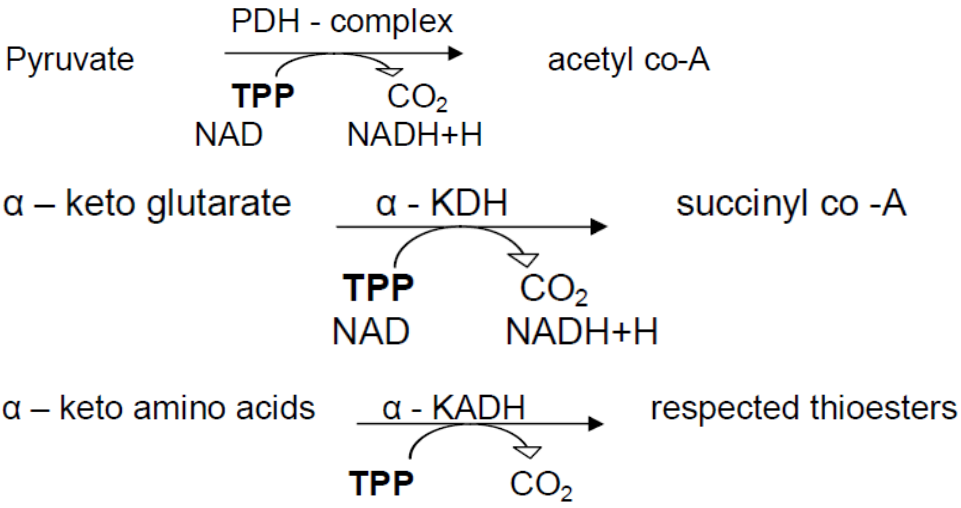
Structure: it is made up of pyrimidine and thiazole ring linked with Methylene Bridge.

Functions:

The vitamin functions are dependent on its active form. The active form of vitamin is TPP (thiamine pyrophosphate). It is synthesized by phosphorylation of thiamine.

Thiamine + ATP ⟶ TPP + AMP

The Thiamine pyrophosphate is acts as Co – carboxylase, and involved in oxidative decarboxylation reactions, &transketolase reactions.



Involved in HMP – shunt for the synthesis of pentoses, and NADPH.

Sources:

Plant sources like Cereals (outer layer), pulses, oil seeds, nut and yeast.

Animal sources like organ meats, pork, milk etc.

RDA:

Adults: 1 – 1.5 mg

Children: 0.7 – 1.2 mg.

Pregnancy & lactation – 2mg.

Deficiency:

The deficiency of thiamine leads to disease called beri – beri, its features are depending on its type as follows,

Wet beri-beri:

- It related to edema of face, trunk, and serous cavities.
- Breathless ness, palpitation, swollen calf muscles, elevated systolic pressure, fast and bouncing pulse is seen.
- The heart becomes weak.

Dry beri-beri:

- It is not related to edema, and mostly related to degeneration of nervous system (peripheral neuritis).
- Muscles are weak and unable to movement and patients are become bedridden.

Infantile beri-beri:

- The child has symptoms like sleeplessness, restlessness, vomiting, convulsions, and death.

h. Vitamin B12 (E-Feb 11)

Common name: anti – pernicious anemia vitamin

Chemical name – cyanocobalamin

Structure – it is made up of corrin ring, dimethylbenzimidazole, and substituent group it may be cyanide, hydroxyl, or nitrite.

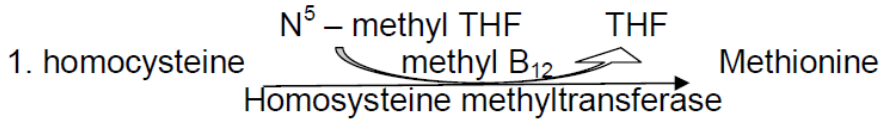
Metabolism:

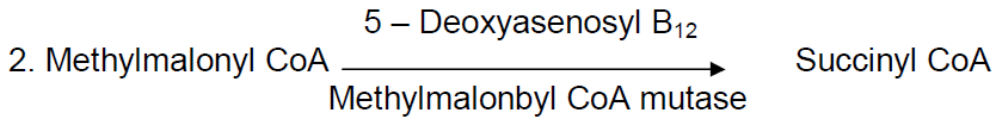
The vitamin in diet is called extrinsic factor of "CASTLE" which is absorbed by the help of intrinsic factor produced form stomach parietal cells. The 1 – 2 mol of B12 is banded to intrinsic factor this complex is stable in acid conditions as well as form proteolytic and bacterial decomposition.

Cobalamin – IF complex cross the epithelial cells through specific receptors. In the epithelial cells the Cobalamin is converted to methylcobalamin. Methyl cobalamin is transported to blood by the help of transcobalamins. The excess cobalamin is taken up by liver and stored in the form of deoxyadenosyl B12.

Functions:

The two main reactions in the body is dependent on B12.





RDA:

Adults - 3µg

children's – 0.5 – 1.5 µg

Pregnancy and lactation – 4 µg.

Sources:

Animal foods are rich sources of B12. This vitamin is also synthesized form

Intestinal bacteria.

Deficiency syndrome:

The following causes leads to vitamin deficiency –

- Autoimmune destruction of parietal cell which secrete intrinsic factor.
- Malabsorption of vitamin.
- Insufficient production of intrinsic factor and HCl in old age.
- Insufficient dietary vitamin was seen in strict vegetarians.

The above all causes leads to vitamin deficiency and finally pernicious anaemia; it is characterized by low hemoglobin, less number of RBC's, neurological manifestations (neuronal degeneration, demyelination).

In neurological disorders – numbness and tingling of fingers and toes are earlier symptoms, in later stages confusion, dementia, psychosis are seen. These all neurological symptoms are due to accumulation of Propionyl CoA and Methylmalonyl CoA, the first which inhibits acetyl CoA carboxylase, and the latter is competitive inhibitor for Malonyl CoA. The both these accumulated metabolites will stop the fatty acid synthesis and culprit myelination process. The improper myelination leads to neurological disorders.

i. **Acute Intermittent Porphyria** (AIP) (SN-Apr 2001)

- It is one of the major disorders in heme synthesis. It is an inherited autosomal dominant trait
- The enzyme PBG-deaminase involved in the conversion of porphobiliogen to uroporphyrinogen.
- The levels of ALA and PBG are elevated in blood and urine. Fresh urine is colourless, but on standing it become colour due to photooxidation of PBG to porphobilin. Because this reason urine sample should collected freshly and transported in dark colour bottle
- Patients have acute abdominal pain. In some cases neurological symptoms like sensory and motor disturbances, agitation ad confusion.
- Female have less severe manifestations than male, since female sex hormones have a stimulatory effect on ALA synthase

Treatment: starvation, carbohydrate rich diet, drugs like barbiturates

j. Physiological jaundice

Most infants develop visible jaundice due to elevation of unconjugated bilirubin concentration during their first week. This common condition is called physiological jaundice. This pattern of hyperbilirubinemia has been classified into two functionally distinct periods.

Phase one

1. Term infants - jaundice lasts for about 10 days with a rapid rise of serum bilirubin up to 12 mg/dL
2. Preterm infants - jaundice lasts for about two weeks, with a rapid rise of serum bilirubin up to 15 mg/dL

Phase two - bilirubin levels decline to about 34 umol/l (2 mg/dL) for two weeks, eventually mimicking adult values.

1. Preterm infants - phase two can last more than one month.
2. Exclusively breastfed infants - phase two can last more than one month.

Causes

Possible mechanisms involved in physiological jaundice

1. Increase bilirubin load on liver cells:

- Increased red blood cell (RBC) volume
- Increased circulation of bilirubin in the liver
- Decreased RBC survival

2. Defective hepatic uptake of bilirubin from blood plasma:

- Decreased ligadin (Y protein)
- Increased binding of Y proteins by other anions
- Decreased liver uptake especially in phase two

3. Defective billirubin conjugation:

- Decreased UDPG activity

4. Defective bilirubin excretion