REVIEW ARTICLE

TOTAL PARENTERAL NUTRITION (TPN): ROLE OF RIBOFLAVIN (VITAMIN B₂) AND CYANOCOBALAMIN (VITAMIN B₁₂)

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ABSTRACT

Total parenteral nutrition (TPN) replaces and maintains essential nutrients in patients in whom oral or tube feedings are contraindicated or inadequate. A nutritional assessment must be carried out before initiating TPN in order to determine nutritional needs and any metabolic changes due to the patient’s underlying condition, medications or concurrent therapies. In addition to carbohydrates, proteins and fats, certain amounts of micronutrients are also added to TPN solutions. These micronutrients include electrolytes, vitamins, and trace minerals. This review highlights some basic concepts regarding the use and formulation of TPNs along with their advantages and disadvantages and the importance of water soluble vitamins B₂ and B₁₂ in human nutrition.

Keywords: TPN, nutrients, riboflavin, cyanocobalamin.

1. INTRODUCTION

1.1. Total Parenteral Nutrition

Total Parenteral Nutrition (TPN) provides nutrients to patients who are considered as “unfed able.” The insertion of a catheter into a large central vein permits one to concentrate hypertonic dextrose calories in normal daily fluid requirements¹. TPN has great clinical importance to prevent and treat starvation often related to high morbidity and mortality². The principle of nutrition support guides us to use the least invasive and most physiologic method of feeding³. Intravenous infusion of central parenteral nutrition (CPN) or peripheral parenteral nutrition (PPN) is the only viable means to provide substrates for metabolism. However, the inherent risks are associated with their use such as thrombosis and infection due to which the TPN should precede placement of a central venous catheter³. The clinical application of TPN solutions requires inclusion of commonly used solutions such as dextrose, soybean oil emulsion, and synthetic crystalline L-amino acid solutions. Newer solutions containing glycerides and special purpose amino acid solutions are also used. Over the past 10 years, additional information has also been available leading to the rational use of vitamins and trace elements in parenteral nutrition solutions⁵.

2. HISTORICAL BACKGROUND

The parenteral nutrition therapy was born 35 to 40 years ago when the first step was taken to perform protein nutrition by the intravenous supply of amino acids in man⁶. Since that time, many efforts have been made to supply adequate amounts of energy intravenously. These efforts resulted in the development of two systems for parenteral nutrition: the lipid-carbohydrate system and the glucose system. The lipid-carbohydrate system, which corresponds to the nutrient contents of normal food, may be given either in a peripheral vein or through a central vein catheter. The glucose system is administered through a central venous catheter. However, many problems concerning the parenteral nutrition need to be solved and further elucidated. However, the present knowledge and technique in this field are far advanced over earlier methods. Now all patients who cannot take food in adequate amount orally or enterally may be kept in good nutritional status by TPN. In this way it is possible to prevent starvation and its complications in such patients⁷.

3. TPN ASSESSMENT

A nutritional assessment must be performed to determine nutrient needs, as well as any metabolic changes due to patient’s underlying condition, medications or concurrent therapies before initiating
TPN. Table 1 provides a list of factors to consider when assessing a patient’s nutritional status. The energy and protein requirement in the severely malnourished patients under physical stress, often ventilator-dependent with little mobility, is a difficult task. Some cases of critical illnesses also bring further challenges in determining the appropriate calorie level to be provided to the patients as the balance between the caloric expenditure and caloric provision must be maintained properly and that the lower calorie levels should be carefully monitored. The caloric requirements often increase in relation to stress, fever, and seizures, while a decrease in need may be seen in cases of sedation or reduced mobility. The nutrition formulation and other medication requirements are interrelated to each other and therefore need to be approached in a unified manner.

Table 1. Important factors to consider when assessing a patient for TPN.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Assessment</th>
</tr>
</thead>
</table>
| Anthropometric data          | Recent weight changes  
                              | Current height and weight                                                 |
| Lab values                   | Comprehensive metabolic panel  
                              | Serum magnesium level  
                              | Serum phosphorus level  
                              | Serum triglycerides                                                |
| Medical / surgical history   | Anatomy (resections) / ostomies  
                              | Pre-existing conditions such as diabetes, renal failure, etc.  
                              | Liver disease                                                          |
| Diet / medication history    | Food / drug allergies  
                              | Diet intake prior to admission  
                              | Special diets  
                              | Herbal / supplement use  
                              | Home and current medications                                       |

4. DESIGNING THE FORMULATION

4.1. Carbohydrates
The carbohydrates are generally provided in amounts up to 60% of total kcal/day. In the hospitalized patients, the initial dextrose in TPN solutions should not exceed 7.2 g/kg/day (5 mg/kg/min) to minimize the occurrence of fatty liver and hyperglycemia.

4.2. Proteins
The protein is supplied in the range of 1.5 g/kg/day. The critical care, post-surgical, burn and dialysis patients require protein administration in the range of 1.2-2 g/kg/day but the protein requirement may be as high as 2-2.5 g/kg/day in severe catabolic states. In acute renal failure protein supplementation from 1.5-1.6 g/kg/day is necessary due to the protein loss through the glomeruli, dialysis or catabolism.

4.3. Fats
The intravenous fat emulsions (IVFE) are generally used to provide 20-30% of daily kcal unless conditions exist which complicate lipid administration of this amount, i.e. hypertriglyceridemia or propofol infusion syndrome. This should be carefully monitored as vitamin K typically increases proportionally with an increase in lipid concentrations (vitamin K content doubles with an increase from 10-20% of fats).

4.4. Micronutrients
The electrolytes in TPN solutions are added according
to the patient requirements, metabolic response to medications and recommended daily intakes. The typical ranges for parenteral electrolyte content are show in Table 2. The current recommendations for parenteral multivitamin injections by the Food and Drug Administration (FDA)\textsuperscript{15} are shown in Table 3. The short term (<1 week) TPN patient rarely needs supplementation of vitamin K, while the long term TPN patient require 2-4 mg/week of parenteral vitamin K for several weeks to months\textsuperscript{16,17}. A general trace element dosing guideline is listed in Table 4.

There is some concern regarding the addition of recommended manganese dose for long term TPN patients. Therefore, it should be periodically monitored to ensure that whole blood manganese levels must remain within safe limits for patients receiving TPN\textsuperscript{18}. When considering the dosing of multivitamins and trace elements, some adjustments may need to be made in certain settings. For example, in the presence of cholestasis, reduction in an amount of manganese and copper is often necessary\textsuperscript{19,20}.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Normal Serum Range</th>
<th>Parenteral Intake Range</th>
<th>Adult Enteral Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>135-145 mM/L</td>
<td>0-200 mEq/L</td>
<td>100-150 mEq/day</td>
</tr>
<tr>
<td>As needed to maintain acid-base balance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>3.5-5.1 mM/L</td>
<td>0-240 mEq/day</td>
<td>60-120 mEq/day</td>
</tr>
<tr>
<td>Acetate</td>
<td>As needed to maintain acid-base balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.3-4.7 mg/dl</td>
<td>0-60 mM/day</td>
<td>15-30 mM/day</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.7-2.5 mEq/L</td>
<td>0-48 mEq/day</td>
<td>8-24 mEq/day</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.2-11.0 mg/dl (ionized calcium 0.8-1.2 mEq/L)</td>
<td>0-25 mEq/day</td>
<td>9-22 mEq/day</td>
</tr>
</tbody>
</table>

Table 2. Normal serum electrolyte values and parenteral ranges.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Recommended amounts / day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin (B\textsubscript{1})</td>
<td>6 mg</td>
</tr>
<tr>
<td>Riboflavin (B\textsubscript{2})</td>
<td>3.6 mg</td>
</tr>
<tr>
<td>Pyridoxine (B\textsubscript{6})</td>
<td>6 mg</td>
</tr>
<tr>
<td>Cyanocobalamin (B\textsubscript{12})</td>
<td>5 mcg</td>
</tr>
<tr>
<td>Niacin</td>
<td>40 mg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>600 mcg</td>
</tr>
<tr>
<td>Pantothentic acid</td>
<td>15 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>60 mcg</td>
</tr>
<tr>
<td>Ascorbic acid (C)</td>
<td>200 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3300 IU</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>5 mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>10 IU</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>150 mcg</td>
</tr>
</tbody>
</table>

Table 3. Recommended daily intake of intravenous vitamins.
Table 4. Recommended adult daily intake of intravenous trace minerals.

<table>
<thead>
<tr>
<th>Trace Elements</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>10–15 mcg</td>
</tr>
<tr>
<td>Copper</td>
<td>0.3–0.5 mg</td>
</tr>
<tr>
<td>Manganese</td>
<td>60–100 mcg</td>
</tr>
<tr>
<td>Selenium</td>
<td>20–60 mcg</td>
</tr>
<tr>
<td>Zinc*</td>
<td>2.5–5.0 mg</td>
</tr>
</tbody>
</table>

*Recommended zinc requirement per liter of ostomy or stool output lost: 12.2 mg / liter small bowel fluid; 17.1 mg / kg stool / ileostomy

5. ADVANTAGES AND DISADVANTAGES OF TPN

Barber et al. have discussed the following advantages and disadvantages of TPN admixtures.

5.1. Advantages

All components of a TPN are aseptically compounded in the pharmacy. It poses less risk of infection during administration, less nursing time needed i.e. 1 bag/day (no piggy bag to administer), less supply and equipment expenses such as only one pump and intravenous tubing is often required. Similarly, the storage is more convenient with fewer supplies and easy administration in home care settings. The glucose and venous access tolerance may be better in some situations and has possible applications in fluid restricted patients. It is also overall more cost effective in certain settings.

5.2. Disadvantages

The larger particle size of admixed lipid emulsions rule out the use of 0.22 μm membrane filters thus eliminating the chance of bacterial removal, if present due to any contamination. An admixture is more sensitive to destabilization with certain electrolyte concentrations or the addition of iron. It is difficult to visualize precipitate or particulate material in the opaque admixture. The admixed lipid emulsions are also less stable and more prone to separation of lipid components. Certain medications are incompatible with lipid emulsion portion of admixture while catheter occlusion is more common with daily lipid administration. It is also less attractive in pediatric settings due to pH and compatibility considerations.

6. WATER SOLUBLE COMPONENTS

As discussed earlier in Section 4.4, certain amounts of micronutrients are also added to TPN solutions (Table 2-4). Among most of the others, the role of two widely used light sensitive water-soluble vitamins i.e. riboflavin (vitamin B2) and cyanocobalamin (vitamin B12) in TPN solutions has been extensively studied. A brief account of these vitamins is given in the following sections.

6.1. Riboflavin

Vitamins are a group of complex organic compounds which are present in little amounts in natural food that are essential for normal metabolism. The first growth factor to be characterized from the B-complex vitamins was riboflavin (RF), in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Many of the B vitamins in their metabolically active forms are present as coenzyme derivatives. The formation of such compounds is regulated by the nutritional state, hormones, drugs, and other stimuli. The role of RF in health, regulation of metabolism and inborn errors in metabolism with neurological disorders has been comprehensively reviewed.

6.1.1. Historical background

The historical aspects of RF have been reviewed by Wagner and Folkers, Scott et al., and Loosli. However, the prevention of a deficiency state by using RF and other factors has been shown earlier by scientific studies of McCollum and Kennedy. After purification of yeast extract, the heat stable fraction contained a yellow growth factor that was able to fluoresce and was named RF. Warburg and Christian later discovered the physiological role of RF whereas it was synthesized by Kuhn et al. and Karrer et al.
6.1.2. Dietary reference intakes
RF current recommended dietary allowance (RDA) for adult men and women are 1.1-1.3 mg/day and 0.9-1.1 mg/day, respectively. In pregnancy and lactation, daily RF intake increases to 1.4 mg and 1.6 mg, respectively. The RDA is 0.3-0.4 mg/day for infants and it is 0.6-0.9 mg/day for children.\(^\text{35}\).

6.1.3. Chemical structure and chemistry
The chemical structures of RF, FMN and FAD are shown in Fig. 1. RF contains a tricyclic isalloxazine nucleus to which a ribose side chain is attached at N-9 position. RF possess a highly conjugated system resulting in strong absorption bands in the UV and visible region (223, 267, 374, 444 nm)\(^\text{36}\) along with an intense yellowish green fluorescence. It acts as respiratory enzyme and is involved in biological redox reactions.

RF is sensitive to light\(^\text{36}\) and is degraded to several products on exposure to light\(^\text{37-42}\). The photodegradation of RF involves several mechanisms including intramolecular photo-reduction, intramolecular photoalkylation and intramolecular photoaddition. In addition to this, the molecule also undergoes intermolecular photoreduction and intermolecular photoaddition. These reactions occur under aerobic and anaerobic conditions. The photoaddition reactions occur in the presence of divalent ions such as \(\text{HPO}_4^{2-}\) and \(\text{SO}_4^{2-}\) in a concentration above 0.2 M and at a pH value above 6.0\(^\text{43-46}\). The kinetics of photodegradation reactions of RF and its major photoprodut, formylmethylflavin (FMF), in aqueous and organic solvents has been studied by Ahmad et al.\(^\text{41,42}\). The effect of phosphate\(^\text{47}\), borate\(^\text{48}\), citrate\(^\text{49}\), acetate\(^\text{50}\) and carbonate buffers\(^\text{50}\) have also been studied. The complexation of RF with caffeine in the presence and absence of phosphate buffer has been reported\(^\text{51,52}\).

A recent study on the effect of solvents on the photolysis of RF has been carried out\(^\text{53}\). It has been found that the values of apparent first-order rate constants \((k_{\text{obs}})\) are directly related to the dielectric constant and inversely proportional to the viscosity of the medium\(^\text{53}\). The various photoproducts of RF

![Fig. 1. Structures of RF, FAD and FMN.](image1)

![Fig. 2. The chemical structures of various photoproducts of RF.](image2)
identified in intramolecular photoreduction are FMF, carboxymethylflavin (CMF), lumichrome (LC) and lumiflavin (LF). The major product of intramolecular photoaddition is cyclodehydroriboflavin (CDRF). The chemical structures of these products are shown in Fig. 2.

### 6.1.4. Physicochemical characteristics of RF

The various physicochemical characteristics of RF are reported in the literature\textsuperscript{36,54,55} which are summarized as follows in Table 5:

<table>
<thead>
<tr>
<th>Table 5. Physicochemical characteristics of RF.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
</tr>
<tr>
<td><strong>Molar mass</strong></td>
</tr>
<tr>
<td><strong>Crystalline form</strong></td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
</tr>
<tr>
<td>([\alpha]_D^{25})</td>
</tr>
<tr>
<td><strong>pH of saturated solution</strong></td>
</tr>
<tr>
<td><strong>pK\textsubscript{a}</strong></td>
</tr>
<tr>
<td><strong>Redox potential (RF / dihydriboflavin), pH 7</strong></td>
</tr>
<tr>
<td><strong>Solubility (mg / 100 ml)</strong></td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Absolute ethanol</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Ether</td>
</tr>
<tr>
<td>Benzene</td>
</tr>
<tr>
<td><strong>Absorption maxima (pH 7.0)</strong></td>
</tr>
<tr>
<td><strong>Fluorescence emission (pH 7.0)</strong></td>
</tr>
<tr>
<td><strong>Principal infrared peaks (KBr disc)</strong></td>
</tr>
</tbody>
</table>

### 6.1.5. RF deficiency

RF deficiency is not caused only by dietary inadequacy. Certain endocrine abnormalities, specific drugs, and diseases may interfere significantly with the utilization of the vitamin. The abuse of alcohol, prolong use of drugs, mal absorption or other underlying illnesses may affect vitamin metabolism\textsuperscript{24,56,57}. In experimental animals, RF deficiency caused marked disrupted hepatic architecture. Mitochondria of mice increased greatly in size with an increase in the number and size of cristae\textsuperscript{58}. The villi decrease in the small intestine of rat while its length increases\textsuperscript{59}. These structural abnormalities decrease iron absorption and increase iron loss from the intestine\textsuperscript{60}. RF deficiency also plays an important role in intermediary metabolism, particularly in lipid, protein, and vitamin metabolism\textsuperscript{61}. Other effects include loss of hair, skin disturbances, degenerative changes in the nervous system, impaired reproduction and cataract formation\textsuperscript{62}. Anemia is also known to develop during late stages of deficiency with metabolic changes\textsuperscript{63}.

Clinical features of human RF deficiency include weakness, fatigue, mouth pain and tenderness, burning and itching of the eyes and possible personality changes. The advanced deficiency may include cheilosis, angular stomatitis, dermatitis, corneal vascularization, anemia, and brain dysfunction\textsuperscript{62}. Higher intake of RF can reduces cataract formation\textsuperscript{64}. In patients having keratoconus, RF administration as eye drops delays its progression\textsuperscript{65}. The activity of glutathione reductase is reduced by UVA light in the lens because of the light sensitivity of its FAD coenzyme\textsuperscript{66}. 
6.1.6. Food related issues
On exposure to UV light and during cooking and processing, appreciable amounts of RF may be lost. Prolonged storage of milk in clear bottles or containers may result in RF degradation while opaque plastic containers provide greater protection. Milk must be protected against light otherwise some significant amounts of RF and vitamin A would be lost and the flavor will deteriorate.

6.1.7. Absorption, transport and storage
The coenzyme derivatives of RF (FMN, FAD) are hydrolyzed before absorption. Little dietary RF is found as free in nature. Flavins are absorbed in the upper gastrointestinal tract by specialized transport rather than by passive diffusion. The upper limit of intestinal absorption of RF at any one time is approximately 25 mg. The rate of intestinal absorption of RF is increased by bile salts and the bioavailability of RF may be affected by a number of metals and drugs that form chelates or complexes with RF and FMF. The urinary excretion of RF greatly increases by accidental ingestion of boric acid.

6.1.8. Clinical uses
Both in experimental animals and in humans, RF deficiency may be protective against malaria. Malaria parasitemia is known to increase with iron and vitamin supplement that included RF. RF is involved in the pathogenesis of vascular diseases, including cardiovascular, cerebrovascular, and peripheral vascular disorders. RF also shares the property of stabilizing the enzyme variation. Homocysteine levels rise and serum concentrations of flavin cofactors fall as expected with the treatment of hyperthyroidism. Supplementation RF may be useful additive along with beta-blockers in the treatment of migraine headaches.

Other uses of RF include increasing energy levels, boosting immune system function, maintaining healthy hair, skin, mucous membranes, and nails, slowing aging, boosting athletic performance and promoting healthy reproductive function. It is also useful in the treatment of cancer sores, memory loss including Alzheimer's disease, ulcers, burns, alcoholism, liver disease, sickle cell anemia, and lactic acidosis caused by treatment with a class of AIDS medications called Nucleoside analog reverse transcriptase inhibitors drugs.

6.1.9. Pharmacology
There is a general agreement between the dietary RF intake and RDA with no demonstrable toxicity. Increase in melanin by topical administration of RF to the skin has been observed. Photosensitization of vinca alkaloids by RF may distort results of efficacy testing of cytotoxic drugs. RF forms a complex with chromate and then increase DNA breakdown because of a chromium induced free radical mechanism. In the presence of visible light, RF and its degradation products may enhance mutagenicity. Similarly, RF deficiency may also result in enhance carcinogenesis, particularly nitrosamines. It is also reported that the deficiency of RF along with shortage of other vitamins may possibly enhance development of precancerous lesions of the esophagus.

6.2. Cyanocobalamin
Cyanocobalamin (CC) is the pure form of the chemical compound having activity. Vitamin B12 is also named as "generic descriptor" because the body can convert CC to any one of the active vitamin B12 compounds, so by definition this makes CC itself a form (or vitamer) of B12.

6.2.1. Historical background
A series of important contributions from diverse fields including human and animal nutrition, medicine, chemistry, microbiology, X-ray crystallography and pharmaceutical science are involved in the history of discovery of vitamin B12. To seek a cure for a mysterious and fatal disease is the original impetus that led ultimately to the discovery of B12 which was first described in 1855. Pernicious anemia cured by feeding a half pound of lightly cooked liver to patients in Thorndike Hospital in Boston made the epochal discovery of vitamin B12. The fact that liver is a
rich source of folate, which would not be destroyed by the gentle heat, folate can replace the need for B₁₂ in its role in DNA synthesis. For their pioneer observations, Minot, Murphy and Whipple were awarded the Nobel Prize in Physiology and Medicine in 1934. After 20 years of this discovery when Folkers and his group from Merck and GlaxoSmith, simultaneously announced successful purification and crystallization of reddish needle like crystals of a new vitamin designated as vitamin in B₁₂. In patients with pernicious anemia, this vitamin showed clinical and biological activity by the gold standard assay of demonstrating efficacy in inducing and maintaining remission. To identify the molecular structure of this compound, Smith gave some of his crystals to Dorothy Hodgkin, an X-ray crystallographer working at Oxford. This task was accomplished over 8 years, involving an estimated 10 million calculations. Hodgkin was awarded the Nobel Prize in Chemistry in 1964 for her work on the elucidation of the structure of B₁₂, as well as the structures of penicillin and insulin. The next step was the total chemical synthesis of B₁₂, which took 11 years to accomplish in 100 separate reactions. This work was led by Robert Woodward, who received the Nobel Prize for Chemistry in 1965.

6.2.2. Structure and chemistry
In addition of CC, methylcobalamin (Fig. 3) is the other major natural form of B₁₂ and predominantly present in human plasma and within the cytosol. The cobalt atom in hydroxocobalamin (Fig. 3) is fully oxidized in the Co (III) state, whereas the cobalt exists as reduced Co (I) or Co (II) in the 50-deoxyadenosylcobalamin and methylcobalamin.

Fig. 3. Chemical structures of CC, methylcobalamin and hydroxocobalamin.
forms. In the presence of light and a source of cyanide, all forms of cobalamin are converted to CC, glutathionylcobalamin, sulfitocobalamin, and nitritocobalamin. These all forms of cobalamin have also been identified in cell and tissue extracts. Techniques to separate and identify various forms of cobalamin include microbiological methods, thin layer chromatography (TLC), chromatobioautography and HPLC methods. The sixth ligand of the central cobalt atom is occupied by one of the nitrogens of the 5,6-dimethylbenzimidazole base and other nitrogen of the 5,6-dimethylbenzimidazole attaches to ribose, which connects to a phosphate, linking the lower axial ligand back to one of the seven amide groups of the corrin ring by an aminopropyl residue that serves as a molecular sling to attach it to the ring. It has been noted that compared with porphyrin rings, corrins are more flexible and less planar when viewed from the side. Biologically active forms of B₁₂ play varied roles in reactions involving different substrates. All of these may be classified into one of three categories:

1. Mutas, involving exchanges of hydrogen and some other group between two adjacent carbon atoms, which may or may not be followed by elimination of water or ammonia.
2. Ribonucleotide reductase involving the reduction of the ribose in a ribonucleotide to deoxyribose.
3. Methyl group transfer reactions, such as methane synthase, acetate synthase, and methionine synthase.

Mutas and ribonucleotide reductase involves a Co (II) intermediate oxidation state whereas the methyl group transfer reactions involve a Co (I) oxidation state. In all three types of reactions, the Co (III) is in the resting state. The key to the catalytic role of the cobalamin is somewhat weak cobalt-carbon bond and the sensitivity of the active coenzymes to free radical damage by oxygen.

6.2.3. Physiochemical characteristics of B₁₂
The various physiochemical characteristics of vitamin B₁₂ are summarized in Table 6.

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>C₆₃H₈₈CoN₁₄O₁₄P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar mass</td>
<td>1355.37</td>
</tr>
<tr>
<td>Appearance</td>
<td>Dark red solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>&gt;210°C with decomposition</td>
</tr>
<tr>
<td>[α]D²⁵</td>
<td>−59°</td>
</tr>
<tr>
<td>pKₐ</td>
<td>3.3</td>
</tr>
<tr>
<td>Solubility (mg/100ml)</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1 in 80</td>
</tr>
<tr>
<td>Ethanol (98%)</td>
<td>1 in 180</td>
</tr>
<tr>
<td>Chloroform and Ether</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Absorption maxima [A (1%, 1cm)], water</td>
<td>278 nm [119], 361 nm [207], 550 nm [63]</td>
</tr>
<tr>
<td>Infrared absorption (KBr disc)</td>
<td>1660, 1497, 1575, 1220 cm⁻¹</td>
</tr>
</tbody>
</table>

6.2.4. Vitamin B₁₂ analogs
Many analogs of vitamin B₁₂, collectively called corrinoids, are known to exist in nature. These include two major sub-classifications such as cobamides, which contain substitutions in the place of ribose, for e.g., adenoside and cobinamides, which lack a nucleotide. The analogs of B₁₂ are distinguished microbiologically from the vitamin forms by organisms such as Euglena gracilis and Lactobacillus leichmannii, whose growth is sustained by the cobalamins but not by the cobamides or cobinamides. It is unclear whether B₁₂ analogs are inert or inhibit...
B₁₂-dependent reactions. The sources of B₁₂ analogs, whether from diet, gut bacteria, or endogenous breakdown of B₁₂ are unknown. The B₁₂ analogs have been found in fetal blood and tissues₁⁰⁴,₁⁰⁵.

6.2.5. Dietary sources
Vitamin B₁₂ is synthesized by prokaryotic microorganisms, although it is also required by eukaryotes. In some species, B₁₂ is obtained through coprophagia or fecal contamination of the diet but for humans and other omnivores, the only source of B₁₂ (other than supplements) is food of animal origin. The highest amounts of B₁₂ are found in liver and kidney (>10 mg =100 g wet weight), it is also present in shellfish, organ and muscle meats, fish, chicken, and dairy products (1-10 mg = 100 g wet weight)₁⁰⁶.

6.2.6. Daily requirements
The RDA for males and females, age 14 years and older, is 2.4 mg/day. The RDA ranges from 0.9 to 1.8 mg/day for children, age 1-13 years. Due to a lack of adequate data, no RDA has been established for infants <1 year of age. Instead, adequate intakes have been estimated of 0.4 mg/day for age 0-6 months and 0.5 mg/day for age 7-12 months respectively₁⁰⁷.

6.2.7. Absorption and intestinal transport
The distinct mechanisms for B₁₂ absorption are of two types i.e. active and passive. Active physiological processes of B₁₂ absorption involve discrete anatomical areas of the gastrointestinal tract with specific B₁₂ binding and chaperone molecules. Dietary B₁₂ is released from protein complexes primarily by enzymes in gastric juice, aided by the low pH of the stomach that is maintained by normal gastric output of hydrochloric acid from parietal cells. On release from proteins in food, B₁₂ combines rapidly with a salivary R binder, part of a family of B₁₂ binding proteins, known as haptocorrins. Subsequently, the salivary R binder is digested by pancreatic trypsin in the upper small intestine. The B₁₂ is thus released and then transferred to the gastric glycoprotein, intrinsic factor (IF), produced by the same parietal cells responsible for gastric acid production. Binding of B₁₂ to IF is favored by the less acidic milieu of the upper small intestine than the stomach. All forms of B₁₂ are absorbed by the same IF-dependent mechanism₁⁰⁸-₁¹¹. The IF-B₁₂ complex, in contrast to free IF, is resistant to enzyme digestion₁¹². The formation of the complex is believed to protect not only the IF but also the B₁₂, which is known to be susceptible to side-chain modification of the corrin ring as well as removal of the alpha (lower axial) ligand₁⁰⁴,₁⁰⁵. Because of the appreciable amount of B₁₂ undergoing enterohepatic recycling, B₁₂ deficiency develops more rapidly in individuals who mal-absorb the vitamin that is the case in vegans, who ingest none of the vitamin. The active mechanism for B₁₂ absorption is extremely efficient for small (a few mcg) oral doses of B₁₂. This is the mechanism by which the body acquires B₁₂ from normal dietary sources₁⁰⁴,₁⁰⁵.

6.2.8. Metabolism
Once within the cell, B₁₂ participates as a cofactor in two important metabolic reactions, i.e. mitochondrial and cytosolic. In the mitochondrial reaction, B₁₂ in the form of 50-deoxyadenosylcobalamin is required for the enzyme methylmalonyl CoA mutase. This enzyme catalyzes the conversion of methylmalonyl CoA to succinyl CoA, an intermediate step in the conversion of propionate to succinate during the oxidation of odd-chain fatty acids and the catabolism of ketogenic amino acids. In the cytosolic reaction, B₁₂ in the form of methylcobalamin is required in the folate-dependent methylation of the sulfur amino acid homocysteine to form methionine, which is catalyzed by methionine synthase. Methionine, apart from being necessary for adequate protein synthesis, is also a key precursor for the maintenance of methylation capacity through synthesis of the universal methyl donor S-adenosylmethionine. In addition, the methionine synthase reaction is ultimately necessary for normal DNA synthesis. B₁₂ is thus an important cofactor in the maintenance of normal DNA synthesis, as becomes evident under conditions of B₁₂ deficiency, which lead to defective DNA synthesis and megaloblastic anemia₁¹³.
6.2.9. Vitamin B₁₂ deficiency

Many investigators have reported a high prevalence of B₁₂ deficiency in elderly patients¹¹⁴-¹¹⁷, primarily on the basis of raised serum or urine methylmalonic acid or homocysteine levels with or without low serum B₁₂ concentrations. Some estimates suggested that the prevalence of B₁₂ deficiency may be as high as 30-40% among the elderly due to the condition of food and B₁₂ mal-absorption caused by chronic gastritis, gastric atrophy, and perhaps other unknown causes¹¹⁸. As a result there is a growing concern that the prevalence of B₁₂ deficiency may have been underestimated. The classic clinical manifestations of B₁₂ deficiency, notably megaloblastic anemia occur only in the most severely B₁₂ depleted individuals¹¹⁹. Metabolic abnormalities¹²⁰-¹²² often occur before serum B₁₂ concentrations reach a level that would be considered deficient by standard criteria. Results of surveys of B₁₂ status in the elderly patients indeed indicate that the prevalence of deficiency is much higher if based on serum or urine methylmalonic acid concentrations¹¹⁶. In recent years, several studies have reported an apparently high prevalence of low B₁₂ status and varying degrees of B₁₂ deficiency in both children and young adults in diverse locations, such as Guatemala, Mexico, India, and Israel¹²⁰-¹²³. The causes of B₁₂ deficiency in these populations are unclear, but may be related to a combination of low intake and unrecognized mal-absorption. It has recently been suggested that H. pylori may also initiate autoimmune destruction of the gastric mucosa leading to pernicious anemia¹²⁴,¹²⁵.

6.2.10. Dietary deficiency

Dietary B₁₂ deficiency arises in adult who use only vegetables¹²⁶. B₁₂ deficiency causes anemia or neuropathy¹²⁷ while in childhood, B₁₂ deficiency occurs in those infants who are born to severely B₁₂-deficient mothers¹²⁸-¹³⁰. These infants develop megaloblastic anemia at ~3-6 months of age, because they are born with low stores of B₁₂ and are fed breast milk of low B₁₂ content. B₁₂ deficiency has also been observed in children fed with macrobiotic diets¹³¹,¹³².

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