

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These vitamins cannot be synthesized by the higher organisms, including man, and therefore they have to be supplied in small amounts in the diet.

Microorganisms are capable of synthesizing the vitamins. In fact, the bacteria in the gut of humans can produce some of the vitamins, which if appropriately absorbed can partially meet the body's requirements. It is an accepted fact that after administration of strong antibiotics to humans (which kill bacteria in gut), additional consumption of vitamins is recommended.

Microorganisms can be successfully used for the commercial production of many of the vitamins e.g. thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamin B12, ascorbic acid, P-carotene (pro-vitamin A), ergosterol (pro-vitamin D). However, from economic point of view, it is feasible to produce vitamin B12, riboflavin, ascorbic acid and p-carotene by microorganisms. For the production of ascorbic acid (vitamin C), the reader must.

Vitamin B12:

The disease, pernicious anemia, characterized by low levels of hemoglobin, decreased number of erythrocytes and neurological manifestations, has been known for several decades. It was in 1926 some workers reported the liver extracts could cure pernicious anemia. The active principle was later identified as vitamin B12, a water soluble B-complex vitamin.

Occurrence:

Vitamin B12 is present in animal tissue at a very low concentration (e.g. 1 ppm in the liver). It occurs mostly in the coenzyme forms- methylcobalamin and deoxyadenosylcobalamin. Isolation of vitamin B12 from animal tissues is very expensive and tedious.

Chemistry:

Vitamin B12 (cyanocobalamin) is a water soluble vitamin with complex structure. The empirical formula of cyanocobalamin is $C_{63}H_{90}N_{14}O_{14}PCO$. The structure of vitamin B12 consists of a corrin ring with a central cobalt atom. The corrin ring is almost similar to the tetrapyrrole ring structure found in other porphyrin compounds e.g. heme (with Fe) and chlorophyll (with Mg).

The corrin ring has four pyrrole units. Cobalt present at the centre of the corrin ring is bonded to the four pyrrole nitrogen's. Cobalt also binds to dimethylbenzimidazole and amino isopropanol. Thus, cobalt atom present in vitamin B12 is in a coordination state of six.

Biosynthesis:

Vitamin B12 is exclusively synthesized in nature by microorganisms. An outline of the pathway is depicted in Fig. 27.1. The biosynthesis of B12 is comparable with that of chlorophyll and hemoglobin. Many of the reactions in the synthesis of vitamin B12 are not yet fully understood.

Commercial Production of Vitamin B12:

Vitamin B12 is commercially produced by fermentation. It was first obtained as a byproduct of *Streptomyces* fermentation in the production of certain antibiotics (streptomycin, chloramphenicol, or neomycin). But the yield was very low. Later, high-yielding strains were developed. And at present, vitamin B12 is entirely produced by fermentation. It is estimated that the world's annual production of vitamin B12 is around 15,000 kg.

High concentrations of vitamin B12 are detected in sewage-sludge solids. This is produced by microorganisms. Recovery of vitamin B12 from sewage-sludge was carried out in some parts of United States. Unlike most other vitamins, the chemical synthesis of vitamin B12 is not practicable, since about 20 complicated reaction steps need to be carried out. Fermentation of vitamin B12 is the only choice.

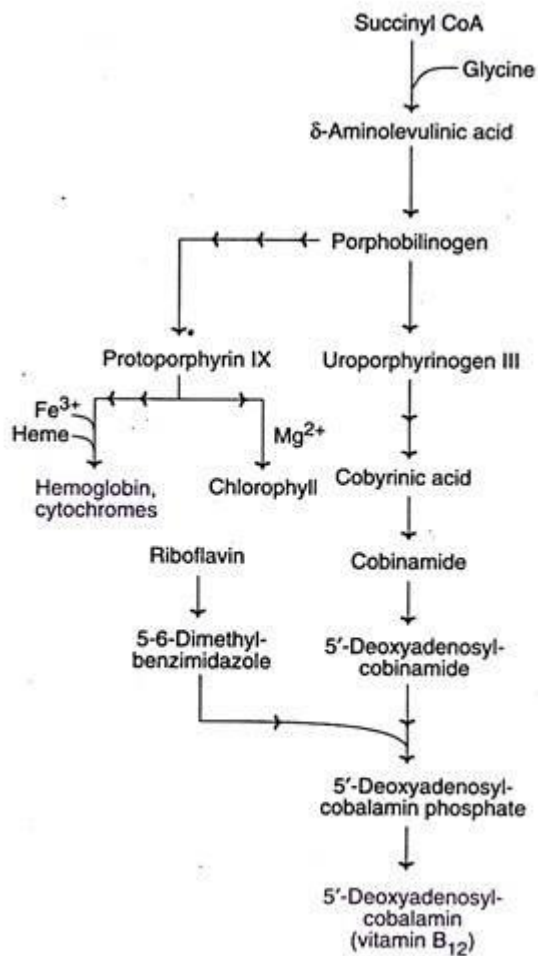


Fig. 27.1 : An outline of the biosynthesis of vitamin B₁₂

Microorganisms and Yields of Vitamin B12:

Several microorganisms can be employed for the production of vitamin B12, with varying yields. Glucose is the most commonly used carbon source. Some examples of microbes and their corresponding yields are given in Table 27.1. The most commonly used microorganisms are — *Propionibacterium freudenreichii*, *Pseudomonas denitrificans*, *Bacillus megaterium* and *Streptomyces olivaceus*.

Genetically engineered strains for vitamin B12 production:

By employing modern techniques of genetic engineering, vitamin B12 production can be enhanced. A protoplast fusion technique between *Protaminobacter ruber* and *Rhodopseudomonas spheroides* resulted in a hybrid strain called *Rhodopseudomonas protamicus*. This new strain can produce as high as 135 mg/l of vitamin B12 utilizing carbon source.

Production of Vitamin B12 Using *Propionibacterium* sp:

Propionibacterium freudenreichii and *P. shermanii*, and their mutant strains are commonly used for vitamin B12 production. The process is carried out by adding cobalt in two phases.

Anaerobic phase: This is a preliminary phase that may take 2-4 days. In the anaerobic phase 5'-deoxyadenosylcobinamide is predominantly produced.

Aerobic phase:

In this phase, 5, 6-dimethyl- Benz imidazole is produced from riboflavin which gets incorporated to finally form coenzyme of vitamin B-p namely 5'-deoxyadenosylcobalamin.

In recent years, some fermentation technologists have successfully clubbed both an anaerobic and aerobic phases to carry out the operation continuously in two reaction tanks.

The bulk production of vitamin B12 is mostly done by submerged bacterial fermentation with beet molasses medium supplemented with cobalt chloride. The specific details of the process are kept as a guarded secret by the companies.

Recovery of vitamin B12:

The cobalamins produced by fermentation are mostly bound to the cells. They can be solubilized by heat treatment at 80-120°C for about 30 minutes at pH 6.5-8.5. The solids and mycelium are filtered or centrifuged and the fermentation broth collected. The cobalamins can be converted to more stable cyanocobalamins. This vitamin B12 is around 80% purity and can be directly used as a feed additive. However, for medical use (particularly for treatment of pernicious anemia), vitamin B12 should be further purified (95-98% purity).

Production of Vitamin B12 using *Pseudomonas* sp:

Pseudomonas denitrificans is also used for large scale production of vitamin B12 in a cost-effective manner. Starting with a low yield (0.6 mg/l) two decades ago, several improvements have been made in the strains of *P. denitrificans* for a tremendous improvement in the yield (60 mg/l). Addition of cobalt and 5, 6-dimethyl Benz imidazole to the medium is essential. The yield of vitamin B12 increases when the medium is supplemented with betaine (usual source being sugar beet molasses).

Carbon Sources for Vitamin B12 Production: Glucose is the most commonly used carbon source for large scale manufacture of vitamin B12. Other carbon sources like alcohols (methanol, ethanol, isopropanol) and hydrocarbons (alkanes, decane, hexadecane) with varying yields can also be used. A yield of 42 mg/l of vitamin B12 was reported using methanol as the carbon source by the microorganism *Methanosarcina barkeri*, in fed- batch culture system.