Keeping a clear head with vitamin B\textsubscript{12}

Vitamins are vital organic micronutrients that are required in our diet because they provide essential enzyme cofactors, and animals have dispensed with the ability to synthesize them. Vitamin B\textsubscript{12}, or cobalamin, is the most complex of the vitamins, and the elucidation of its physiological role, its structure and its biosynthetic pathways have been the subject of impressive scientific endeavours over the years. Vitamin B\textsubscript{12} deficiency leads to increased risk of cardiovascular disease, neurological symptoms and, in the most serious cases, pernicious anaemia. Cobalamin is synthesized only by prokaryotes, so we obtain it second-hand by eating other organisms that have accumulated the vitamin in their tissues. The richest dietary sources are liver, dairy products and also algae, many of which are like animals in that they require an exogenous supply of the vitamin for growth.

Discovering vitamin B\textsubscript{12}

It is hard to imagine that, not so very long ago, pernicious anaemia was as fatal a disease as leukaemia, with a reported 10,000 lives lost in the USA in 1 year alone. This quickly changed with the discovery by George Minot and William Murphy in the late 1920s that it was possible to treat the disease with what they termed a ‘liver diet’. To cure a patient by administering food was a strange concept, remote from the customary view of the time. In order to achieve palpable results from this treatment, however, the patient was required to eat 250 g of raw liver or drink the equivalent of raw liver juice every 24 hours. In 1934, Murphy and Minot were awarded the Nobel Prize in Medicine for their discovery, although, at the time, the aetiology of the disease and the substance responsible for the healing properties of the liver, were still a mystery. The active ingredient, referred to as extrinsic factor, stayed unknown until 1948, when it was isolated by two chemists, Karl Folkers of the USA, and Alexander Todd of the UK. It was found to be a cobalamin, a cobalt-containing tetrapyrrrole, which Folkers and Todd called vitamin B\textsubscript{12} (Box 1), and doses of a few milligrams daily were sufficient to treat pernicious anaemia.

In the 80 years since its discovery, we have come a long way in our understanding of vitamin B\textsubscript{12}. Advances in structural and molecular biology have helped to determine the nature of its various forms, its function in metabolism and the biosynthetic pathways by which it is made\textsuperscript{2}. Aside from its importance as a treatment for pernicious anaemia, vitamin B\textsubscript{12} is now understood to play a vital role in maintaining healthy nervous, circulatory and digestive systems. To this day, new reports of the clinical benefits of vitamin B\textsubscript{12} are being described. A recent noteworthy study reported that vitamin B\textsubscript{12} supplementation (alongside other B-group vitamins) could lower the rate of brain atrophy in the elderly with mild

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Key words: algae, cobalamin (vitamin B\textsubscript{12}), dietary deficiency, symbiosis, vitamin auxotrophy

Box 1. When is cobalamin vitamin B\textsubscript{12}?

The word vitamin is derived from the term ‘vital amine’. It is used to describe an organic compound that is required in small quantities by an organism that cannot synthesize it, and so must obtain it from its diet or the environment. Not all vitamins are amines, although the first vitamin to be described was vitamin B\textsubscript{1}, thiamine, and the name stayed. Vitamins are classified according to their biological and chemical activity, not their structure. For example, vitamin E includes the compounds tocopherol and tocotrienol, antioxidants required during fat metabolism. The B-complex vitamins (the largest group of vitamins) are extremely varied chemically, but are all water-soluble, and function as precursors for enzyme cofactors. The individual compounds are known as vitamers.

Vitamin B\textsubscript{12} is a generic term used for cobalamins that are (i) essential to an organism’s metabolism, and (ii) cannot be synthesized by that organism. Certain bacterial species require cobalamin for survival, but since they are also able to synthesize it, to them, it is not a vitamin.
cognitive impairment, thus offering a potential means to delay the onset of Alzheimer’s disease.

**Nature’s most complex primary metabolite**

The molecular complexity of vitamin $\text{B}_{12}$ meant that it took Dorothy Hodgkin and her team over 10 years to determine its structure through X-ray crystallography, earning her the Nobel Prize in Chemistry in 1964 (Box 2). The molecule (shown in Figure 1) comprises a ring-contracted porphinoid with a cobalt ion ligated at the centre of the tetrapyrrole-derived macrocycle. The cobalt is liganded further by a lower axial base (a dimethylbenzimidazole) and an upper axial ligand (labelled X in Figure 1). In biological systems, two different upper axial ligands are found, either a 5′-deoxyadenosyl group in adenosylcobalamin or a methyl group in methylcobalamin.

Vitamin $\text{B}_{12}$, with a cyano group as the upper axial ligand has been synthesized *ab initio*, but the first attempt took 11 years, and involved more than 90 separate reactions performed by over 100 co-workers. The effort was not unrewarded: the stereochemical puzzles involved in the synthesis led to the Woodward–Hoffman rules, and a further two Nobel Prizes. However, bacteria still win the race! They are able to synthesize the vitamin within hours, in a process involving over 20 enzyme-catalysed steps, encoded by 30 different genes. So far there is no genetic evidence that eukaryotes are capable of this biological feat, and it is believed that only archaea and some bacteria carry out the production of this complex metabolite.

Cobalamin is synthesized from uroporphyrinogen III, the common precursor of all biological tetrapyrroles, including haem and chlorophyll. There are two routes by which cobalamin can be made, which differ principally in the timing of the cobalt insertion. The early insertion pathway is so called because cobalt is chelated before ring contraction, and the majority of the intermediates are in the metallated form. In the alternative ‘late insertion’ pathway, cobalt is inserted after ring contraction. In addition, oxygen is required for this latter step, and so it is also referred to as the aerobic pathway. Despite these differences, the two biosynthetic routes are remarkably similar in the nature and order of many of the biochemical reactions, even though the enzymes probably have different origins. The elucidation of the biosynthetic pathway has been another heroic effort, involving such approaches as sophisticated NMR and other analytical techniques to determine the structure of intermediates, use of mutants to establish the order of steps, and the reconstitution *in vitro* and *in vivo* of subsets of the pathway to verify the function of gene products.

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**Box 2. Dorothy Hodgkin**

Dorothy Mary Hodgkin (1910–1994), was a British chemist and pioneer of protein crystallography. She was the first to confirm the structure of penicillin and decipher the structure of vitamin $\text{B}_{12}$, for which she was awarded the Nobel Prize.

In 1969, 5 years after winning the Nobel Prize, she was able to determine the structure of insulin.

Dorothy Hodgkin was one of five ‘Women of Achievement’ selected for a set of British stamps issued in August 1996.

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**Who needs vitamin $\text{B}_{12}$?**

Cobalamin is necessary because it provides important enzyme cofactors. However, it is not universally required as a vitamin – in other words, a nutritional supplement. An organism can only be said to be dependent on vitamin $\text{B}_{12}$ if (i) it has an essential requirement for cobalamin for growth, and (ii) it is not able to synthesize it *de novo* (Box 1); this is the case for animals, including humans. Bacteria that use cobalamin in metabolism either synthesize it, or have alternative enzymes that do not need it as a cofactor, so that, in the absence of cobalamin, they can grow normally. In some species, both cobalamin-dependent and -independent forms are present, as is the case for *Escherichia coli*, which uses cobalamin if it is available, but does not require it. Land plants and fungi have dispensed with cobalamin altogether since they do not have any cobalamin-dependent enzymes. Algae – simple photosynthetic eukaryotes – are an interesting group; half of all algal species have an obligate requirement for the vitamin, so in that respect, they are like animals. The other algal species are either
Bacteria

Archaea

Protists

Algae

Land plants

Fungi

Animals

Many bacteria and archaebacteria synthesise cobalamin, to provide cofactors for a range of B₁₂-dependent enzymes. Those that don’t make it have alternative B₁₂-independent enzymes, but some species have both and will use B₁₂ if available.

Over half of all microalgae require cobalamin for growth, and many protists too, but they are unable to synthesise B₁₂ so must obtain it from their environment. Like prokaryotes, the rest have B₁₂-independent enzymes, but some organisms have both and will use B₁₂ if available.

Plants and fungi have no cobalamin dependent enzymes and therefore have dispensed with B₁₂ altogether.

Animals require vitamin B₁₂ in their diets. Insects may be an exception to this.

Figure 2. Simplified schematic representation of the tree of life illustrating the distribution of cobalamin biosynthesis and utilization in different organisms

like plants, or use cobalamin facultatively when present (Figure 2).4

The role of vitamin B₁₂ in metabolism

In prokaryotes, there are over 20 enzymes for which cobalamin is a cofactor. These enzymes can be grouped into three broad classes. The cobalamin-dependent isomerases, such as methylmalonyl-CoA mutase and cobalamin-dependent ribonucleotide reductase (type II) utilize adenosylcobalamin in rearrangement/reductase reactions, whereas methyl transferases, such as those involved in methanogenesis, use methylcobalamin (Figure 1). There are also the reductive dehalogenases, although the role of cobalamin in the dehalogenation process has not yet been fully deduced.

In humans, vitamin B₁₂ is the cofactor for just two enzymes. Methylmalonyl-CoA mutase is involved in odd-chain fatty acid metabolism, where it converts methylmalonyl-CoA into succinyl-CoA, which can then be respired further in the tricarboxylic acid cycle. The enzyme uses adenosylcobalamin, where homolytic cleavage of the weak cobalt–carbon bond generates a 5’-deoxyadenosyl radical that promotes the complex 1,2 rearrangement. The other enzyme is B₁₂-dependent methionine synthase (METH), which catalyses the transfer of a methyl group from methyltetrahydrofolate to homocysteine to form methionine. In this reaction, vitamin B₁₂ acts as an intermediary methyl carrier. Although methionine is an essential amino acid, the vast majority of the free methionine in the cell is used for the formation of S-adenosylmethionine, the universal methyl donor. This compound is involved in many biosynthetic processes, as well as in gene regulation (via methylation of DNA), and protein regulation and repair.

Bypassing the need for vitamin B₁₂

As mentioned previously, many organisms do not require cobalamin. In eukaryotes, this is because they possess an alternative isomeric methionine synthase, METE, which can catalyse the direct transfer of a methyl group from methyltetrahydrofolate to homocysteine. Plants and fungi have just METE, whereas some bacteria, for example E. coli (which does not make cobalamin), have genes for both isomeres. In the absence of cobalamin, expression of the metE gene is up-regulated so that this form of methionine metabolism comprises 3% of cellular protein. Such high levels are necessary because the enzyme has a k₅₀ 100-fold lower than METH. Likewise, some algal species, such as the model green alga Chlamydomonas reinhardtii, have both isomeres, whereas others have just one or the other (K.E. Helliwell, G.L. Wheeler, K.C. Lepotos, R.E. Goldstein and A.G. Smith, unpublished work). Interestingly, from inspection of sequenced genomes, insects do not appear to encode either METH or METE, which suggests that they may have an as yet undescribed form of methionine metabolism.

Sources of vitamin B₁₂

A varied diet

Humans, like many other animals, require vitamin B₁₂ in the diet, but in very small amounts (a recommended daily allowance of 2 µg per day). Indeed, adults can get by with eating no vitamin B₁₂ at all for 3–5 years, if the liver is replete initially. However, a dietary deficiency of vita-
min B₁₂ affects the activity of both methylmalonyl-CoA mutase and methionine synthase, which in turn can lead to a number of pathologies. Vitamin B₁₂ is especially important for healthy nervous and circulatory systems. In the latter case, the vitamin is required for proper DNA synthesis during red blood cell production. Furthermore, the raised level of plasma homocysteine due to reduction in methionine synthase activity is a risk factor in both coronary and cardiovascular disease, and for dementia and Alzheimer’s disease³,⁸. Given the shared role in methionine metabolism, a folate deficiency can have the same signature symptoms as a deficiency in vitamin B₁₂. The converse is also true: it is possible to mask a deficiency in vitamin B₁₂ by taking folate supplements, and it is because of this risk that, whereas folate fortification of grains is mandatory in some countries, such as the USA, it is not universally practised.

Dietary deficiency is especially common for strict vegetarians and vegans, because, unlike most vitamins, fruit and vegetables do not provide vitamin B₁₂, since it is absent from higher plants (Figure 2). Instead, the best dietary sources are from vitamin B₁₂-requirers, so animal products have high levels (Figure 3). In addition, many macroalgal (seaweed) species, such as Porphyra, known as nori when used to wrap sushi, are very good sources. An unlikely source of the vitamin is, in fact, soil bacteria – which are ingested from vegetables that have not been washed very thoroughly.

A lack of cobalamin in the diet is not the only cause of vitamin B₁₂ deficiency. Vitamin B₁₂ is bound in the digestive tract by a glycoprotein known as intrinsic factor (IF), which has a very high affinity for vitamin B₁₂. The IF–B₁₂ complex is internalized by interaction with a receptor on the mucosa of the ileum. Some forms of congenital pernicious anaemia are the result of defects in this uptake system. Problems with uptake are also common in the elderly, where an autoimmune disorder leads to reduced production of active IF⁸.

Whatever the cause, clinical vitamin B₁₂ deficiency can be treated with injections of vitamin B₁₂ or oral supplements, which are in the form of cyanocobalamin. This is rapidly converted into the active forms of the cofactor in the body, which raises the question: what happens to the cyanide?! Alternatively, natural supplements can be taken, and preparations from cyanobacteria such as Spirulina are often recommended because these are rich in several vitamins and iron, but, in fact, the major form of cobalamin in this organism, and indeed many other cyanobacteria, is pseudocobalamin⁹. This has a different lower axial ligand (an adenine rather than a dimethylbenzimidazole group; Figure 1), which is recognized much less efficiently by IF, and so is not biologically available for humans.

All (for one) at sea
In 2005, a study revealed that half of all algal species require vitamin B₁₂ for growth⁴, a somewhat surprising

Figure 3. Sources of vitamin B₁₂. Many vitamin preparations (a) contain B₁₂, but for dietary sources, the highest levels are found in liver (b), and red meat (f), as well as dairy products (d, e) and eggs (g). In addition, algae such as Porphyra (c; used to make nori) are also a rich source.
observation given the fact that these are photosynthetic and thus assumed to be completely autotrophic. From a survey of 326 algal species, 171 required exogenous vitamin B₁₂ as a growth supplement. Algae are a very diverse group of organisms that include red algae (Rhodophyta), green algae (Chlorophyta) and brown algae such as marine diatoms and dinoflagellates. The vitamin B₁₂ requirement shows no relationship to established algal lineages: all the phyla contain species that require the vitamin and species that do not, despite millions of years of evolutionary divergence. In fact, vitamin B₁₂ requirements vary within individual genera including Chlamydomonas in the Chlorophyta, and Thalassiosira (diatoms). This suggests that vitamin B₁₂ auxotrophy has arisen independently numerous times during evolution.

Levels of cobalamin in solution in natural aquatic environments are insufficient to support algal growth. Instead, algae that have an obligate requirement for vitamin B₁₂ have been shown to acquire it directly from cobalamin-synthesizing bacteria, which receive fixed carbon in return. The notion of obtaining essential nutrients through evolved symbiotic interactions is not uncommon to life. Think only of corals with their zooxanthellae symbionts, or lichens, which are a symbiotic combination of fungi with photosynthetic algae or cyanobacteria, or plants with nitrogen-fixing root nodules containing rhizobial bacteria. It is not surprising that vitamins – which can be incredibly complex molecules – often form the basis of such symbioses. They are difficult to produce, are required in small quantities and, by definition, are essential for growth and must be obtained from an external source.

Conclusions

The complex molecular nature, metabolic role and physiological significance of cobalamin (vitamin B₁₂) have challenged the minds of many scientists for almost a century. We have come to understand how it is synthesized by prokaryotes, its function in metabolism and the relative importance to different organisms, and its role as the basis for symbiosis. As humans, we rely on an external source of vitamin B₁₂ for healthy living. The association of cobalamin deficiency with coronary disease and dementia, coupled with the recent finding that high doses of the vitamin can improve cognitive impairment, suggests that a diet of liver and seaweed could help us all keep a clear head.

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