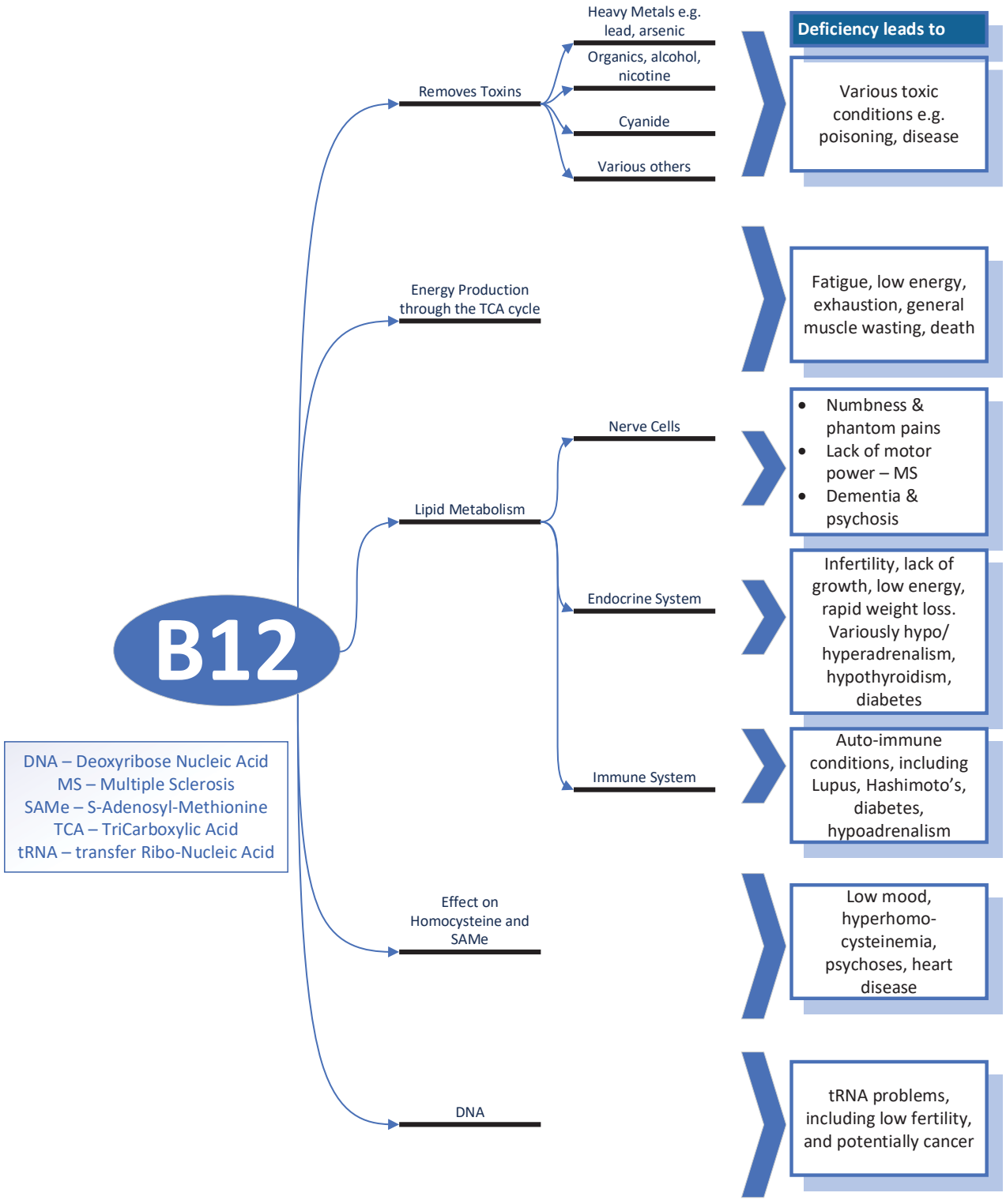
Chapter 1 Vitamin B12: a profile

"the word of the Lord was addressed to me, saying,
'before I formed you in the womb I knew you;
before you came to birth I consecrated you;
I have appointed you as a prophet to the nations.
So now brace yourself for action.
Stand up and tell them
all I command you.
Do not be dismayed at their presence,
or in their presence I will make you dismayed.
I, for my part, today will make you
into a fortified city,
a pillar of iron,
and a wall of bronze
to confront this land:
the kings of Judah, its princes,
its priests and the country people.
They will fight against you,
it shall not overcome you,
for I am with you to deliver you –
it is the Lord who speaks.'"

Jeremiah 1:4–5, 17–19

Figure 1-1 The role of vitamin B12 in the human body systems



Based on clinical experience and Banerjee and Ragsdale (2003); Dowling et al. (2016); Green (2017); Koury and Ponka (2004); Stubbe (1994)

1.1 Vitamin B12 characteristics

Vitamin B12 is the generic name for a group of compounds based on the cobalamin molecule which has the trace mineral cobalt at its centre. Cobalamin is a highly active complex organometallic molecule. It is the largest and most chemically complex of all the 13 known vitamins (with an intricate chemical formula $C_{62}H_{88}CoN_{13}O_{14}P^{+}$) and is generally characterised as red in colour. Cobalamin, like the other B vitamins and vitamin C, is a water-soluble vitamin, a characteristic which influences how it is absorbed, excreted and stored by the human body. The other vitamins (A, D, E and K) are fat-soluble.

It is classed as a vitamin because it is an essential nutrient for humans which must be obtained regularly from food. Like other vitamins its role is to catalyse or regulate metabolic reactions in the human body. It plays a crucial role in many body processes. As described by the US PubChem Compound Database: "It is needed for hematopoiesis (the production of all types of blood cells), neural metabolism, DNA and RNA production, and carbohydrate, fat, and protein metabolism. It improves iron functions in the metabolic cycle and assists folic acid in choline synthesis."[8]

Vitamin B12 can only be made by microorganisms, such as bacteria and algae, providing the cobalt mineral is available to them from soil or water. The principal source of vitamin B12 for humans is animal products: the vitamin is made by microbes in the digestive tract of animals from where it is absorbed and deposited in their tissues. The main dietary sources for humans are therefore meat, fish, milk, eggs and cheese. There are virtually no plant sources of this vitamin, although some species of seaweed have been found to contain it (Watanabe et al., 2014). Vitamin B12 can also now be obtained from synthetic sources.

The absorption mechanism from the human gastrointestinal tract to circulation for crucial utilisation by the body's entire 100 trillion cells is a multi-step, delicate and complex process (Nielsen et al., 2012). This sensitivity means that absorption can be easily disrupted, for example by surgery, abnormal bacterial growth in the small intestine, intestinal disease or some medications that inhibit absorption. Prime causes are atrophic gastritis and lack of Intrinsic Factor (IF), a glycoprotein produced by the stomach that is necessary for absorption of vitamin B12. In addition to poor dietary intake, lack of vitamin B12 can arise from genetic conditions affecting the absorption pathway, or from many acquired conditions such as:

- Atrophic gastritis
- Pernicious anaemia (automimune destruction of the gastric parietal cells)
- Crohn's disease
- Intestinal infections
- Gastrointestinal surgery (especially if affecting the terminal ileum)
- Coeliac disease
- Treatment with antacids (acid is required to release vitamin B12 from food)
- Treatment with proton-pump inhibitors
- Use of some other medications and nitrous oxide (from anaesthetic or recreational use)

[8] Source: "Cobalamin", The NCI Thesaurus (NCIt), reproduced in the US National Center for Biotechnology Information (NCBI), (PubChem Compound Database) Compound ID 56840966 https://pubchem.ncbi.nlm.nih.gov/compound/56840966 (accessed 7 January 2019).

- Cyanide poisoning (for example, from smoke inhalation)

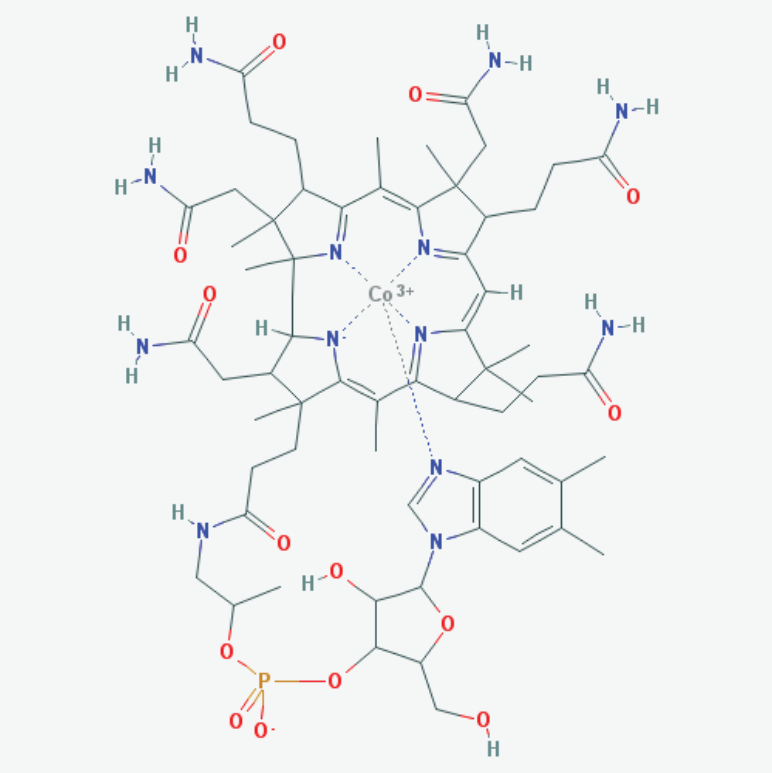
In the US National Library of Medicine's (NLM's) Medical Subject Headings (MeSH) vitamin B12 is described as "a cobalt-containing coordination compound produced by intestinal micro-organisms and found also in soil and water. Higher plants do not concentrate vitamin B12 from the soil and so are a poor source of the substance as compared with animal tissues. Intrinsic Factor is important for the assimilation of vitamin B12."[9]

Insufficient intake (from a diet lacking vitamin B12) or disrupted absorption of vitamin B12 results in a deficiency in all humans, including a pregnant woman, foetus and neonate, causing DNA damage.

1.1.1 Basic chemistry: B12 structure

Vitamin B12 is the largest vitamin with a molecular weight of 1355.388 grams per mole (g/mol), compared with: Vitamin A – 286.459 g/mol; Vitamin B3 – 123.111 g/mol; Vitamin B6 – 205.638 g/mol; Vitamin B9 (folate) – 441.404 g/mol; Vitamin C – 176.124 g/mol; Vitamin D – 384.648 g/mol; Vitamin E – 430.717 g/mol; Vitamin K – 450.707 g/mol.[10]

Figure 1-2 Cobalamin molecule



[9] Source: Medical Subject Headings (MeSH), US National Library of Medicine, Record Name: Vitamin B12, URL: https://www.ncbi.nlm.nih.gov/mesh/68014805 (MeSH).

[10] All figures from National Centre for Biotechnology Information, PubChem Compound Database, US. Accessed 29 January 2018. See also Kim et al. (2016).

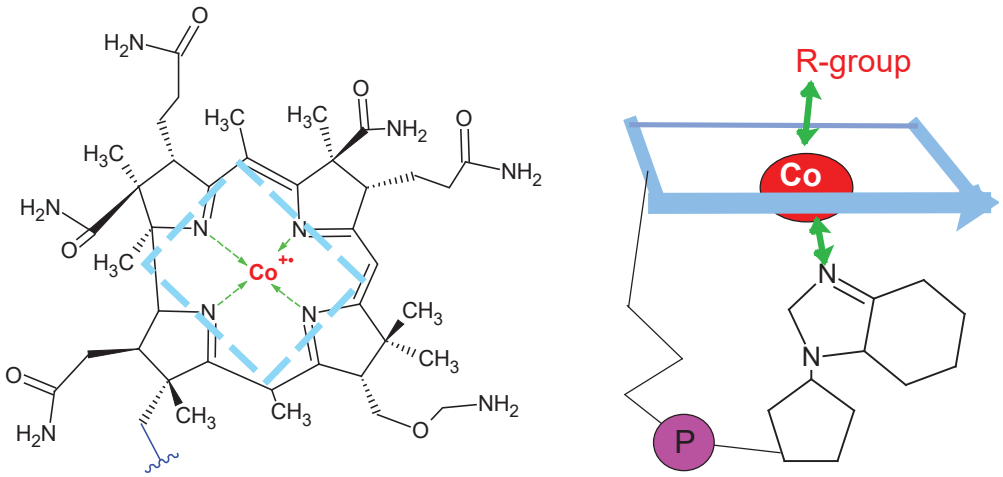
Source: National Center for Biotechnology Information. PubChem Compound Database: CID=56840966, https://pubchem.ncbi.nlm.nih.gov/compound/56840966 (accessed 7 January 2019) (PubChem Compound Database).

Ions (charged molecules) are very important in biochemistry. They make a substance soluble or not soluble in water or fat, and help it to be selective in what it binds to.

The cobalamin molecule has a cobalt ion in the centre surrounded by a corrin ring structure with the four pyrrole[11] nitrogens coordinated to the cobalt (Shane, 2008). This combination means that B12 has a highly attractive centre, and then a protective layer around it. This gives vitamin B12 its main properties:

- as a binding site for toxins such as heavy metals and strongly radical ions such as cyanide and oxygen radicals (which bind to the centre and are then protected from releasing the heavy metal whilst the body takes the combined molecule to the kidney to get rid of it);
- as a carrier for highly reactive ions such as H+ which would otherwise react with the nearest molecule, both damaging the molecule and losing the energy stored within the highly reactive ion. This is why vitamin B12 is so important in the energy production cycles such as the TriCarboxylic Acid Cycle (TCA);
- as a catalytic site where normally stable molecules are brought together and reconfigure in a highly energetic state, protected by the amine rings from reacting with other nearby (but random) molecules and losing their energetic state (for a series of catalytic reactions, including lipid metabolism and conversion of homocysteine to methionine which is then converted to S-adenosyl-methionione (SAMe));
- as a donor for methyl groups which can bind to and release from vitamin B12 under specific conditions – for example, vitamin B12 takes a methyl group from 5-methylene tetrahydrofolate, thereby reducing it to tetrahydrofolate – a form of folate needed for further metabolic reactions which eventually lead to products required for DNA synthesis.

Figure 1-3 Detail of cobalamin molecule



Graphics by Hugo Minney. The diagram on the left shows the cobalt atom positioned in the centre of a corrin ring by bonds of nitrogen from four pyrrole rings, illustrated as a square (tail foreshortened in blue, bottom

[11] A pyrrole ring is an organic compound characterised by a ring structure composed of four carbon atoms and one nitrogen atom.

left).The diagram on the right, a "side view", shows the molecular tail which allows the cobalt molecule to move relative to the pyrrole rings (up and down) so cobalt can bind to different-sized and different-strength R-groups, for example cyanyl, methyl, hydroxyl, adenosyl and heavy metals.

1.1.2 The B vitamins

Vitamin B12 is arbitrarily included amongst the B vitamins. We say "arbitrarily" because it has little in common with the chemical structure of other B vitamins. These vitamins are grouped together because they are water-soluble and have inter-related, cellular coenzyme functions (Kennedy, 2016). They act together in many human biochemical processes, for example the metabolism of homocysteine (Kennedy, 2016). Vitamin B12 has a particularly important interrelationship with folic acid (vitamin B9) in the folate cycle which leads to the synthesis of DNA and concurrently affects the methionine cycle as described in Chapters 4 and 9.

The eight B vitamins are listed in Table 1-1. Deficiencies in most of these are now rare, due to nutritional improvements (which begs the question: why does the same not apply to B12? – Maybe because of modern changes in agricultural methods which deplete cobalt in the soil?).

Table 1-1 Physiologic roles and deficiency signs of B-complex vitamins

Vitamin	Physiologic roles	Deficiency effects
Thiamine (B_1)	Co-enzyme functions in metabolism of carbohydrates and branched-chain amino acids	Beri-beri, polyneuritis, and Wernicke-Korsakoff syndrome, weight loss, confusion, anorexia, muscle weakness, cardiovascular symptoms.
Riboflavin (B_2)	Co-enzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis (swollen, cracked lips), angular stomatitis (lesions at the corner of the mouth), and dermatitis, edema of the mouth and throat, hyperemia, hair loss, sore throat, reproductive problems, itchy or red eyes, degeneration of the liver and nervous system.
Niacin (nicotinic acid and nicotinamide)	Co-substrate/co-enzyme for hydrogen transfer with numerous dehydrogenases	Pellagra with diarrhoea, dermatitis, neurological symptoms such as apathy, headache, fatigue, loss of memory, and dementia.
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Co-enzyme functions in metabolism of amino acids, glycogen, and sphingoid bases	Microcytic anaemia, electroencephalographic abnormalities, Naso-lateral seborrhoea (red itchy rash), glossitis, dermatitis with cheilosis, depression and confusion, weakened immune function, and peripheral neuropathy (epileptiform convulsions in infants).
Pantothenic acid	Constituent of co-enzyme A and phosphopantetheine involved in fatty acid metabolism	Deficiency is rare but symptoms include fatigue, sleep disturbances, impaired coordination, and gastrointestinal disturbances with anorexia.

Vitamin	Physiologic roles	Deficiency effects
Biotin	Co-enzyme functions in bicarbonate-dependent carboxylations	Thinning hair, red rash, conjunctivitis, ketolactic acidosis, aciduria, seizures, skin infection, brittle nails, fatigue, depression, nausea, hallucinations and paraesthesias of the extremities.
Folate/folic acid (Vitamin B_9)	A precursor needed to make, repair, and methylate DNA; a cofactor in various reactions; especially important in aiding rapid cell division and growth, such as in infancy and pregnancy.	Megaloblastic anaemia, atrophic glossitis, depression, raised homocysteine, Neural Tube Defects (NTDs) as described in this book.
Vitamin B_{12} Various cobalamins; commonly cyanocobalamin or methylcobalaminin vitamin supplements	A co-enzyme involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism.	Neurological and neuropsychiatric impairment, megaloblastic anaemia, gastrointestinal disorders, autoimmune polyendocrine syndrome, as described in this book.
Compiled from: *Report of a joint FAO/WHO expert consultation* Table 5, page 27 (FAO & WHO, 2001) and National Institutes of Health Office of Dietary Supplements: *Thiamine, Riboflavin, Niacin, Pantothenic Acid, Biotin, Folate, Vitamin B12 Fact Sheets for Health Professionals* (NIH ODS, 2018a). Vitamin B12 information partly from our clinical experience.		

1.1.3 Forms of vitamin B12

Cobalamin is the chemically pure form but it is normally bound to other atoms, resulting in several types of B12 (see Table 1.2).

Table 1-2 The most commonly used forms of B12 attached to different ions

Form of Vitamin B12	Molecular weight	Biochemistry in humans and animals
Methylcobalamin	Cobalamin (MW 1329) with a methyl group (MW 15) = Total MW 1344 g/mol or 0.744 mol per kg	Methyl donor to DNA. Co-enzyme in the folate cycle leading to DNA synthesis, and in the interlinked homocysteine-methionine cycle. By extension, affects DNA methylation and supply of S-adenosyl methionine (SAM), impacting on nerve Schwann cell insulation, hormone management, allergy and immune system management.

Table 1-2 The most commonly used forms of B12 attached to different ions

Form of Vitamin B12	Molecular weight	Biochemistry in humans and animals
		Can be manufactured, and can be injected or be given in oral tablet form. All forms of vitamin B12 are water soluble.
Adenosylcobalamin	Cobalamin (MW 1329) with an adenosyl group (MW 267) = Total MW 1596 g/mol or 0.627 mol per kg	Used in Krebs cycle to generate energy, in the mitochondria. Appears to be the active form of B12 in "active B12" (holotranscobalamin) in blood serum. Can be manufactured, and can be injected or given in oral tablet form.
Hydroxocobalamin	Cobalamin (MW 1329) with a hydroxyl group (MW 17) = Total MW 1346 g/mol or 0.743 mol per kg	Stable form of commercially manufactured cobalamin which converts relatively easily in the human body into methylcobalamin and adenosylcobalamin. Can be injected.
Cyanocobalamin	Cobalamin (MW 1329) with a cyanide group (MW 26) = Total MW 1355 g/mol or 0.738 mol per kg	Highly stable form of commercially manufactured cobalamin. Whilst the majority of humans can convert cyanocobalamin into an active form of B12 (methylcobalamin or adenosylcobalamin – the level of cyanide released from this conversion is fairly small and should not cause symptoms), a proportion are not able to make use of cyanocobalamin because the molecule is too stable, and the molecule is removed rapidly from the body by the kidneys because it is a recognised "B12 + toxin" molecule.
Source: All figures from National Centre for Biotechnology Information PubChem Compound Database, US. Compound ID numbers: 10898559, 70678541, 5460373, 5311498. Accessed 29 January 2018 (NIH, 2017). *See also 3.3 on page 70*		
Note Figure 1-3 *on page 27 illustrates how the Cobalt atom can move to expose the bond and make the molecule more able to be used in the body (for less highly charged R-groups eg -CH3 and -adenosyl), or to hide the bond making it less able to be used (for highly charged and dense R-groups eg -CN)*		

1.1.4 Cobalamin isomers/analogues

In the same way that carbon monoxide is so similar to oxygen at a molecular level that it binds to haemoglobin, there are a number of molecules that look sufficiently like vitamin B12 that the body can be deceived. Perhaps the most obvious of these are the phytocobalamins: plant-manufactured molecules with a cobalt ion in the middle that are very similar, but are of no use in animal biochemistry.

In some ways, this is what IF in the stomach is for: to identify the real vitamin B12 and exclude phytocobalamins in the diet from entering the bloodstream in any more than trace amounts.

1.2 Vitamin B12 - history of discovery

Medical journals first began to report symptoms that we can now compare with the symptoms of vitamin B12 deficiency nearly 200 years ago. We have identified three distinct periods over the last two centuries:

1.2.1 Period I: 1824 to 1926

In the nineteenth century James S. Combe (1796-1883) of Edinburgh described a “deadly wasting disease” (Combe, 1824). The condition may have been known earlier as he refers to earlier works (Lieutaud, 1816; Parr, 1819). This description sparked a spate of medical papers reporting similar wasting diseases among people not obviously suffering from starvation or nutritional deficiency (Addison, 1849; Barclay, 1851; Fenwick, 1870; Flint, 1860). Thomas Addison (1793-1860) made an association with neuropsychiatric disorder (Addison, 1849; Vaidya et al., 2009). In 1872, Anton Biermer of Switzerland (1827-1892) gave the illness the name "pernicious anaemia" because it was almost always fatal (Biermer, 1872; Huser, 1966). Symptoms identified included megaloblastic red blood cells, and plaques in the spinal column (post-mortem of course) (Biermer, 1872; Charcot, 1868; Ehrlich, 1880). In 1900, Russell gave the first clinical description of the condition of Subacute Combined Degeneration (SACD) of the spinal cord (Russell et al., 1900).

In 1910, the American physician Richard C. Cabot (1868-1939) presented a natural history of the disease for 1,200 patients. Only six were in remission. The remainder usually survived only between one and three years after developing the symptoms (Cabot, 1910).

By the 1920s, government sources reported 10,000 unexplained deaths each year in the US alone, with similar symptoms. However, the haematologists began to consider this to be a haematological condition, and progress to identify the cause and develop a treatment was held back. The depletion of red blood cells was considered the most important aspect of the illness until more modern methods of investigation led to a broader view of its manifestations.

1.2.2 Period II: 1926 to 1979

Until the accidental discovery of the liver diet by George R. Minot and William P. Murphy in 1926 the strong connection with diet had not been made. These researchers were developing a more “integrative multidimensional view” of disease (Wailoo, 1997, p. 97). Almost by accident, they discovered that the liver diet could cure this deadly disease. It appears that George H. Whipple, Minot and Murphy were studying a cure for anaemia in dogs, and had already found that a liver diet helped bleeding dogs (dogs deliberately bled to give an artificial anaemia effect) recover more quickly. They tried the liver diet with adults who presented with pernicious anaemia, and found a

similar recovery (Minot & Murphy, 1926). The disease, and death from the disease, must have been widespread at this time, because they were awarded the Nobel Prize for Physiology & Medicine in 1934 "for their discoveries concerning liver therapy in cases of anaemia".[12]

Even though the actual factor that cured the disease was not known, liver was readily accessible, and many people benefited. At one point, people would have an extremely painful injection of half a litre of liquefied liver monthly.

In 1929, the haematologist William Bosworth Castle (1897-1990) discovered that a gastric component, which he called “intrinsic factor” (IF), was missing in pernicious anaemia (Elrod & Karnad, 2003).

By the 1940s, vitamin B12 had been identified as the active factor in curing pernicious anaemia (Cohn & Surgenor, 1949; Smith, 1948). Then in 1948 the “extrinsic factor”, that is, vitamin B12, was isolated in crystalline form as cyanocobalamin from liver by two independent scientific teams: Edward L. Rickes, Norman G. Brink, Frank R. Koniuszy, Thomas R. Wood and Karl Folkers at Merck laboratories in the US (Rickes et al., 1948b) and E. Lester Smith and F. Parker at Glaxo laboratories in the UK (Smith, 1948). Further work on the crystal structure analysis was done by the British chemist Dorothy Crowfoot Hodgkin (Scott & Molloy, 2012). In 1956 she described the structure of this large molecule in work which won her the Nobel Prize for Chemistry in 1964 (Hodgkin, 1958; Hodgkin et al., 1956; Hodgkin et al., 1955; Kamper & Hodgkin, 1955).[13]

We see this period as the “golden age” of understanding of vitamin B12. B12 deficiency was associated with neurological problems, including multiple sclerosis-like presentations (Simson et al., 1950; Sobotka et al., 1958; Welch, 1957), and problems with absorption had been connected with the failure of the stomach to produce acid (Colombo et al., 1955; Haq et al., 1952; Ott et al., 1948; Rickes et al., 1948a). There are even hints that in these early days people understood the way that B12 in the blood is activated (Lorber & Shay, 1950, 1952).

1.2.3 Period **III**: 1979 to date

More recently, there seems to be widespread refusal to accept that vitamin B12 deficiency exists. Chanarin laments in his book *The Megaloblastic Anaemias* (Chanarin, 1979):

> *“Nevertheless the investigation of these problems is increasingly a lost art. The pressures accompanying the management of patients with leukaemia has led to decreasing interest in other blood disorders. The simple elucidation of the cause of megaloblastic anaemia is poorly done, criteria on which diagnoses are made are often inadequate, and conclusions reached are often incorrect.”*

There could be many reasons for this refusal. Conspiracy theorists might argue that pharmaceutical companies have nothing to gain from people getting well, and a lot to gain by keeping them away

[12] Nobelprize.Org. (2016) *The Nobel Prize in Physiology or Medicine 1934* [Online]. Available from: https://www.nobelprize.org/nobel_prizes/medicine/laureates/1934/ [Accessed 12 November 2016]. (NobelPrize.org, 2016).

[13] The Nobel Prize in Chemistry 1964 was awarded to Dorothy Crowfoot Hodgkin *"for her determinations by X-ray techniques of the structures of important biochemical substances"*. "The Nobel Prize in Chemistry 1964". *Nobelprize.org.* Nobel Media AB 2014. Web. 31 Jan 2018. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1964/

from simple and low-cost (and effective) solutions such as vitamin supplements. Nationalists might argue that developed countries like the UK or US could not possibly have nutritional deficiency in their populations. Eminent scientists might argue that the cause of the symptoms is far more complex. The result is that patients fail the test for B12 deficiency in spite of presenting with obvious symptoms; physicians set criteria for diagnosis that guarantee that only the most extreme cases will be diagnosed; and they promote expensive solutions and restrict access to a simple, cheap, and effective supplement. This means misery for a great many people.

In 1981, we identified our first B12-deficient patient, diagnosed with neuropsychiatric symptoms, in keeping with GMC guidelines. As it happens, hospital laboratory haematologists had refused to measure the B12 level for the above patient on three occasions, because there was "no macrocytosis".

In 1996, the above patient's daughter presented, aged 26, with symptoms similar to those of her mother in 1981. After careful investigation to exclude other possible causes, we diagnosed vitamin B12 deficiency and started B12-replacement therapy. We (and the patient) observed massive improvement. This sparked the realisation that:

1) **Vitamin B12 deficiency may be far more widespread in the Caucasian population in north-east England than commonly thought. We estimate that as much as 18% of the population (see below) may both be genetically sensitive to low vitamin B12, and at the same time have limited B12 intake from the diet. The same ratio may apply everywhere else where there is a Caucasian population.**

2) **Vitamin B12 deficiency is not always accompanied by megaloblastic (immature, volume greater than 97 fL) red blood cells (also known as macrocytosis). Neurological and neuropsychiatric symptoms may be far more common as early presenting symptoms.**

3) **The disease may be passed from parent to child, i.e. it may be a genetic sensitivity to low B12 in the diet, rather than something infectious or occurring randomly.**

1.3 Deficiency prevalence and manifestation

1.3.1 Prevalence of B12 deficiency in the modern population

The 1926 figure of 10,000 deaths per year in the US suggests that B12 deficiency was a serious problem. It means that in a population of 117 million[14] at least 0.5% would ultimately be given B12 deficiency as the cause of death. However, the vast majority of people with problems of B12 deficiency do not develop the fatal symptoms of rapid muscle wastage and death within two months that would lead to a post-mortem diagnosis. Most die from other causes. Many deaths likely to be related to B12 deficiency would be attributed to other causes (e.g. falling asleep whilst working with machinery, autoimmune disease, starvation due to inability to work, anaemia, other neurological conditions).

The true present-day prevalence of vitamin B12 deficiency is not known. This is because most studies so far undertaken have been localised and focused on specific groups (such as vegetarians)

[14] According to the US Census Bureau, the population of the United States was 117.4 million at 1 July 1926 (US Population by Year [online] http://www.multpl.com/united-states-population/table.)

rather than the general population. A recent World Health Organisation (WHO) technical consultation on folate and vitamin B12 deficiencies noted that vitamin B12 deficiency had the potential to be a worldwide public health problem, affecting millions of people, but that more research was needed to establish its prevalence. It recommended that population-based studies designed specifically to assess folate and vitamin B12 status in the whole population should be encouraged (McLean et al., 2008).

In addition, most studies use the serum B12 level as a marker of vitamin B12 deficiency but this is problematic for the following reasons:

- The serum B12 blood test itself has serious limitations (see Chapter 2 of this book);
- There is no consensus on deficiency cut-off points. In their report for the WHO, the authors noted that: "There is a need for international reference materials and more interaction and communication among laboratories regarding these analyses so that population prevalences of deficiency can be correctly determined and compared" (de Benoist, 2008).
- The threshold levels for deficiency are in any case questionable (Wong, 2015). For instance, people may have blood levels of B12 which appear "normal" but be suffering from functional B12 deficiency, due for example to failure of intracellular transport of B12 by transcobalamin II (Turner & Talbot, 2009).

It is our experience that vitamin B12 deficiency is very much under-recognised. At my medical practice in Horden, County Durham, in 2015, in a population of 5,760 patients, there were 1,036 patients (18%) diagnosed with vitamin B12 deficiency. This was confirmed by whether their symptoms were relieved by giving supplements of B12 by injection (Chandy, 2006a).

A major research project for the US Department of Agriculture in the Framingham (Massachusetts) Offspring Study led by nutritional epidemiologist Katherine L.Tucker found an even higher prevalence - 39% of those studied had low plasma B12 levels, suggesting that "Nearly two-fifths of the U.S. population may be flirting with marginal vitamin B12 status…" (McBride, 2000).

In the UK, the nutritional status of the population is assessed through the National Diet and Nutrition Survey (NDNS) rolling programme, begun in 2008, which is funded by Public Health England (PHE) and the UK Food Standards Agency (FSA). This relies, however, on a representative sample of just 1,000 people and what we would consider a very low cut-off point for serum B12 of 150 pmol/L (Public Health England and Food Standards Agency, 2018). A deficiency rate of 6% of the population aged under 60 in the UK is commonly quoted (Hunt et al., 2014).

According to National Health and Nutrition Examination Surveys in the US from 1999 to 2002, "the prevalence of deficiency (serum vitamin B-12 < 148 pmol/L) varied by age group and affected ≤3% of those aged 20–39 y, ≈4% of those aged 40–59 y, and ≈6% of persons aged ≥70 y. .. Marginal depletion (serum vitamin B-12: 148–221 pmol/L) was more common and occurred in ≈14–16% of those aged 20–59 y and >20% of those >60 y" (Allen, 2009).

In Latin America, Africa and Asia, vitamin B12 deficiency rates are even higher because of vegetarian diets or poor nutrition (Allen, 2009).

Vitamin B12 deficiency can occur at any age but is particularly prevalent in the elderly due mainly to malabsorption problems. Estimates of the prevalence of vitamin B12 deficiency among older people

range between 5% and 40% depending on the definition of vitamin B12 deficiency used (Andrès et al., 2004; Wong, 2015). A population-based cross-sectional analysis of 3,511 people in the UK aged 65 or over found that 1 in 20 aged 65-74 years and 1 in 10 aged over 75 years had significantly low B12 blood levels (Clarke et al., 2004). These are the ratios also quoted by the NHS (NHS, 2016c).

1.3.2 Categories of B12 deficiency

The absorption route of vitamin B12 in the body is complex and delicate (see Chapter 2.1.6 *Causes of B12 deficiency*). Disruption of the process at any stage can lead to deficiency. This partly explains why we have found deficiency to be so widespread. Genetic factors also appear to play an important part (see Chapter 5.10 *Inheriting the genes for vitamin B12 deficiency*).

The main categories of vitamin B12 deficiency that we encountered at the Shinwell Medical Practice are listed in Section 2.3.1.

1.3.3 Body systems where vitamin B12 is important

Vitamin B12 plays a key role in many body systems and organs and the list of these is increasing (Volkov, 2008). It is needed for energy production through the Krebs cycle, for the synthesis of DNA via the folate cycle which affects every one of the trillions of cells in the body, and for the expression (activity) of genes through epigenetic processes.[15] It affects the proper functioning of the peripheral and nervous systems, cognitive function and mood, formation of blood in the bone marrow, skin and mucous membranes, bones (Clemens, 2014), the glandular system, the digestive system, the immune system, fertility, pregnancy and development of the embryo and neonate.

Vitamin B12 deficiency consequently manifests as a huge range of different symptoms, some of which appear to be unrelated to each other and have been misdiagnosed (as other conditions) because of this. Many examples of misdiagnosis are given in this book; others are described in the classic work by Sally Pacholok and Jeffrey Stuart *Could it be B12 An Epidemic of Misdiagnoses*? (2011). Vitamin B12 is so fundamental to animal life and metabolism that the symptoms are also widespread. However, all of the observed symptoms of deficiency relate to one or more of the **six fundamental functions of B12 in the human body**, which are:

1. Manufacture and normal functioning of blood cells. It therefore affects all rapidly dividing cells, epithelial cells (skin and mucous membrane) and bone marrow cells.
2. Energy production through the Krebs Cycle (also known as TriCarboxylic Acid Cycle or Citric Acid Cycle);
3. Lipid metabolism, affecting:
    a. Metabolism of fats, carbohydrates and the synthesis of proteins, with deficiency leading to general dysfunction in many systems;
    b. Nerve cell conduction (integrity of the myelin sheath) and neurotransmitters, including effects on the brain;
    c. Endocrine (glandular) systems;
    d. Immune systems;
4. Conversion of homocysteine to methionine, then to SAMe and amino acids, with effects on many metabolic processes.

[15] Epigenetics is the study of the effect of chemical compounds added to single genes (for example, through diet, including intake of vitamin B12) which regulate the activity of genes.

5. Correct synthesis and transcription of DNA (through interaction with folate), the genetic material of every cell.
6. Removing toxins, e.g. pollutants and poisons such as cyanide from cigarette smoke, lead and arsenic.

1.3.4 Vitamin B12 metabolism

The metabolism of vitamin B12 in the human body is complex and not fully understood (Shane, 2008). It is beyond the scope of this book to describe its extensive biochemistry but in what follows we provide an overview of the metabolic action of vitamin B12 as so far known. This biochemistry has far-reaching effects on DNA synthesis and DNA methylation (an epigenetic modulation of DNA through the transfer of methyl groups – consisting of carbon with 3 hydrogen atoms, CH_3 - between molecules), and on synthesis of myelin (the protective sheath surrounding nerves).

It is generally understood that vitamin B12 participates in three metabolic pathways via two coenzyme forms activated in different parts of the human cell:

- In the cytosol of cells, vitamin B12, in the form of methylcobalamin, is a cofactor in the methionine synthase enzyme necessary for a chemical reaction that converts the amino acid homocysteine to another amino acid methionine. Methionine is then further metabolised to S-adenosylmethionine (SAMe) which is a "methyl donor in many reactions, including the methylation of DNA, histones and other proteins, neurotransmitters, and phospholipids, and the synthesis of creatine. These methylation reactions play important roles in development, gene expression, and genomic stability" (Shane, 2008). SAMe is particularly important for maintaining cell membranes (Bottiglieri, 2002).

- The above reaction catalysed by methionine synthase is interlinked with the folate (vitamin B9) cycle: methionine synthase takes a methyl group from the folate compound N5-methyltetrahydrofolate (5-methyl-THF) and donates it to homocysteine, a reaction which generates tetrahydrofolate and methionine. If vitamin B12 is deficient, this reaction cannot take place and cellular folate accumulates as 5-methylTHF which cannot be used for further reactions. This is called the "methyl-folate" trap hypothesis. It leads to impaired DNA synthesis because folate is needed to synthesise thymidylate, a nucleotide required in formation of DNA and RNA. The methyl-folate trap and its consequences in megaloblastic anaemia and the occurrence of birth defects is described in more detail in Chapters 4 and 5.

- In the mitochondria of cells, vitamin B12 (in the form of adenosylcobalamin) is a cofactor for methylmalonyl coA mutase, an enzyme which catalyses the conversion of methylmalonyl CoA to succinyl CoA which then enters the Krebs cycle and heme biosynthesis (Shane, 2008).

Much of the damage caused by vitamin B12 deficiency is attributed to reduced action of the methionine synthase enzyme (Scott, 1999 quoted in Smulders) which causes homocysteine to accumulate and impairment of the methylation cycle. Some researchers hypothesise that the neuropathy typical of vitamin B12 deficiency is most likely to result from hypomethylation of myelin basic protein through this route (Smulders et al., 2006).

In the other enzymatic reaction, vitamin B12 deficiency causes methylmalonic acid (MMA) to accumulate in the mitochondria which in turn leads to the accumulation of unusual fatty acids in myelin (nerve sheaths), resulting in altered myelin with reduced components of phospholipids, sphingomyelin and ethanolamine, which has been suggested as another way in which vitamin B12 may affect myelin (Gröber et al., 2013; Shane, 2008; Smith & Coman, 2014).

1.3.5 How vitamin B12 deficiency manifests

Vitamin B12 deficiency is more common in females – significantly, 80% of sufferers (four out of five) are female. We think this is because the female body has considerably more metabolic challenges than the male, with monthly hormone cycles and the associated build-up and breaking down of the uterus, and the changes in the body due to pregnancy and lactation.

In order to understand more about the symptoms, it is useful to consider how vitamin B12 is used in the body, and therefore what might stop working in the event of deficiency. Vitamin B12 is:

- Essential for the transport and storage of folate in cells and for conversion of folate to its active form;
- Essential for DNA synthesis and transcription and the growth and maturation of cells;
- Essential for the metabolism of fats, carbohydrates (carboxylic acid or Krebs cycle) and the synthesis of proteins (amino acid metabolism).

This means that all rapidly dividing cells, including epithelial cells and bone marrow cells, will have the greatest need for vitamin B12. These cells are particularly sensitive to nutritional deficiency and may malfunction, resulting in slower wound healing, disturbances in growth and development, and so on.

An example which is used as a diagnostic marker is the maturation of red blood cells (RBCs - erythrocytes). RBCs should mature into doughnut-shaped cells without a nucleus, with a high surface area-to-volume ratio for efficient oxygen and carbon dioxide transport. In B12 deficiency, the cells do not mature as far as they should; some still contain their nucleus and others have not progressed to the doughnut shape from spherical. This leads to the higher MCV (Mean Cell Volume) – not a larger diameter but a much lower surface area-to-volume ratio.

This would also indicate that vitamin B12 may be involved in the prevention (repair) of cancer. This is discussed in Chapter 9.

Vitamin B12 is also:

- Essential for lipid metabolism and the proper development of cell membranes.

In the brain and nervous system every neurone requires the myelin sheath (a fatty layer that insulates nerves) for the neurone to function. Vitamin B12 is essential for the integrity of the myelin sheath and also for the formation of neurotransmitters.[16] Some of the most obvious symptoms of B12 deficiency are neurological disorders, whether malfunction of the sensory nerves (pins and

[16] Neurotransmitter chemicals include serotonin, dopamine, acetylcholine and nor-epinephrine.

needles, numbness e.g. “gloves and socks”, phantom pains), or of the motor nerves (paralysis such as Subacute Combined Degeneration (SACD), tremors such as Bell’s Palsy, and eyesight disorders).

Lack of a properly formed myelin sheath, and potentially slow replenishment of neurotransmitter chemicals, may also contribute to poor memory, “the fogs” (feeling cut-off from the activity going on around), dementia, and psychoses and migraines. All of these have been observed in patients with diagnosed B12 deficiency, and all of these symptoms have reversed with appropriate vitamin B12 supplementation.

Another role of vitamin 12 is in the:

- Production of a mood-affecting substance, S-Adenosyl Methionine (SAMe), which is metabolised from methionine.

Vitamin B12 is required in the metabolism of homocysteine to methionine which is then converted to SAMe (elevated levels of homocysteine are a diagnostic marker for B12 deficiency). Homocysteine is a low-mood chemical, associated with depression. It is also associated with heart disease, vascular disease, and death from these diseases. In contrast, SAMe is a mood-raising chemical as well as being a precursor for some essential amino acids. Medical scientists have noted that “deficiencies of folate and vitamin B12, necessary co-factors in the synthesis of SAMe, may account for decreased SAMe levels, especially in patients with depression and dementia” (Sharma et al., 2017).

1.4 Illness groups and conditions linked to B12 deficiency

1.4.1 Neuropsychiatric disorders

Neuropsychiatric symptoms are some of the earliest presenting signs of vitamin B12 deficiency. These include irritability, mood swings, confusion, forgetfulness, fogginess, tension headaches, depression, anxiety/panic attacks, psychosis, hallucinations and delusion. We have found that these conditions are often missed as symptoms of vitamin B12 deficiency or misdiagnosed as other conditions. There is also evidence that vitamin B12 deficiency may contribute to the onset of dementia through its effect on homocysteine (see below). The neuropsychiatric symptoms of vitamin B12 are reviewed in Chapter 8.

1.4.2 Neurological disorders

As we show in Chapter 6, neurological disorders occur frequently in vitamin B12 deficiency and without any haematological signs such as macrocytosis and IF antibody. Some of these disorders are known under other names, such as Bell’s Palsy, Chronic Fatigue Syndrome (CFS), Myalgic Encephalomyelitis (ME) and MS-like presentation, but as they respond well to vitamin B12 therapy – to the extent of complete remission of symptoms in many cases - we conclude that they are predominantly manifestations of B12 deficiency. The most severe of these is Subacute Combined Degeneration of the spinal cord (SACD) which we and others have found to be frequently misdiagnosed.

1.4.3 Illnesses associated with DNA disorders: birth defects and cancer

Vitamin B12 contributes to DNA synthesis through its interaction with folate (see Chapter 4). It is now well known that folate deficiency leads to severe neural tube defects (NTDs) – a fact which led to the fortification of food products with folic acid (the synthetic form of folate) in many countries.

What is less well known is that folate levels in the body appear to be closely related to vitamin B12 levels (see data in Chart 5-1) so that it may be just as important to screen pregnant mothers for B12 deficiency as for folate deficiency (see discussion of vitamin B12 and NTDs in Molloy (2018)). The importance of vitamin B12 in pregnancy is considered in Chapter 5.

Vitamin B12 also contributes to DNA methylation, an epigenetic mechanism which affects gene expression. The roles of vitamin B12 in both DNA synthesis and DNA methylation imply that adequate levels of this vitamin may help to prevent cancer (which is a disorder of DNA). Some researchers have gone farther and suggest that vitamin B12 could be used as an anti-cancer therapy. The role of vitamin B12 in relation to cancer is explored in Chapter 9.

1.4.4 Illnesses associated with high levels of homocysteine

Vitamin B12 deficiency disrupts the methionine cycle, leading to an accumulation of homocysteine. Elevated levels of homocysteine have been linked to cardiovascular disease (Harvard Health Publishing & Harvard Medical School, 2014; Rotter, 2005), the onset of dementia (Smith et al., 2018) and also with early pregnancy loss and neural tube defects in babies (Li et al., 2017).

1.4.5 Autoimmune disorders

Autoimmune disorders take many forms: they include overactive immune system disorders when the body's immune system attacks and destroys its own tissue and underactive system disorders when the body's defence against disease is reduced. Such disorders are frequent in vitamin B12-deficient patients. The list below from the AARDA (2018) shows some fairly common autoimmune disorders which many with B12 deficiency will immediately recognise:

- Addison's disease
- Amyloidosis
- Ankylosing spondylitis
- Coeliac disease - sprue (gluten-sensitive enteropathy)
- Crohn's disease
- Dermatomyositis
- Graves' disease
- Guillain-Barre Syndrome
- Hashimoto's thyroiditis
- Multiple sclerosis [MS-like presentation/SACD (Subacute Combined Degeneration)]
- Myasthenia gravis
- Pernicious anaemia/B12 deficiency
- Reactive arthritis
- Restless legs syndrome (RLS)
- Rheumatoid arthritis
- Sjögren's syndrome
- Systemic lupus erythematosus (SLE)
- Type I diabetes
- Ulcerative colitis

Note: text in square brackets added by the authors.

Many of these conditions have overlapping symptoms, for example fatigue, general ill-feeling, joint pain and rash. It is our experience that many of these conditions cease to exhibit their symptoms once vitamin B12 balance is restored in the body.

Several important findings have emerged in relation to autoimmune glandular disorders. The first is that over several decades of administering B12 therapy we noticed that autoimmune glandular disorders were particularly prevalent among B12-deficient patients. Some patients also suffered simultaneously from more than one of these disorders, particularly hypoadrenalism and hypothyroidism. It seemed to us that this was not a coincidence and that B12 deficiency was somehow disrupting the immune system pathway in the glandular system. We found that by administering vitamin B12 and the relative hormone-replacement medication, the patients' symptoms subsided without the need for elaborate drugs.

Secondly, the co-occurrence of these conditions in the same patient implied that they were suffering from Autoimmune Polyglandular Syndrome (APS) Type II or III, a condition which is normally considered rare but according to our findings is relatively common. The relationship of vitamin B12 to APS is described in Chapter 7.

Thirdly, our observation of the gastrointestinal symptoms characteristic of early-stage vitamin B12 deficiency suggested that pernicious anaemia (PA) was a progression of these symptoms: in other words, that it is preventable if the B12 deficiency is identified soon enough. PA – the illness through which vitamin B12 deficiency was originally discovered - is itself an autoimmune condition in which the body attacks the parietal cells of the stomach, leading to loss of the glycoprotein IF which is necessary for absorption of vitamin B12. In our view it is an illness which arises *from* vitamin B12 deficiency and then accentuates the deficiency through its effect on IF. We were led to this conclusion partly by the fact that we did not have any cases of PA in our Practice among patients being treated for vitamin B12 deficiency. This issue is considered in Chapter 4.

1.5 Common diagnoses that respond well to B12 supplementation

It is our contention that vitamin B12 deficiency is already observed in the population, but that clusters of symptoms have been given other names than straightforward B12 deficiency, for a variety of reasons including national pride (a developed nation should not admit to a nutritional deficiency), commercial interests (painkillers and anti-psychotics are far more profitable than B12), and academic pride (once you have discovered a new symptom, you may be reluctant to admit that it is not new at all).

Some common diagnoses that respond well to vitamin B12 supplementation include:

1.5.1 ME (Myalgic Encephalomyelitis)/CFS (Chronic Fatigue Syndrome)/ FM (Fibromyalgia)/Anxiety and Depression

The above conditions are often treated with vitamin B12, with excellent results. The likely mechanisms include: poor nerve transmission (poor development of myelin sheath); accumulation of homocysteine; and failure of the Carboxylic Acid (energy-producing) cycle.

1.5.2 MS-like presentation

Multiple Sclerosis (MS) is a characteristic failure of the myelin sheath causing nerves to stop functioning, demonstrated by detecting plaques in areas of the spinal cord and brain where a large

number of myelin sheaths are malformed or missing. These plaques and corresponding symptoms have been induced by restricting intake of vitamin B12, and repaired by B12 supplementation, in laboratory experiments (Scalabrino, 2005, 2009; Scalabrino et al., 2006; Scalabrino et al., 2007).

Not only is the mechanism clear and logically related to B12 deficiency, but the association has been demonstrated and appears to be causal.

1.5.3 Dementia

There is known to be an association between vitamin B12 deficiency and cognitive impairment as in dementia and other signs of brain atrophy because of the role of vitamin B12 in maintaining safe levels of homocysteine (Douaud et al., 2013; Nurk et al., 2010; Smith et al., 2015; Smith et al., 2016). It is known that the body's ability to absorb vitamin B12 reduces with age (Andrès et al., 2004) and that dietary supplementation is recommended. A population study supplementing elderly people with B12 in order to determine if dementia risk could be reduced showed that the group with B12/folate/B6 supplements did indeed have lower homocysteine, suffered less brain atrophy, and also retained their cognitive function better (Smith et al., 2010).

1.5.4 SACD (Subacute Combined Degeneration)/Single Limb Paralysis

Subacute Combined Degeneration (a known consequence of vitamin B12 deficiency) and Single Limb Paralysis are neurological disorders which, in the author's experience, have responded well to B12 supplementation – see Chapter 6 (Chandy, 2006a).