Vitamin B (Water Soluble)

1 THE B GROUP OF VITAMINS

1.1 Introduction

Now polished rice is extremely nice
At a high suburban tea,
But Arbuthnot Lane remarks with pain
That it lacks all vitamin B,
And beri-beri is very very
Hard on the nerves, says he.
'Oh take your vitamin B, my dears!'
I heard that surgeon say;
'If I hadn't been fed on standard bread,
I shouldn't be here today.'

This verse, one in a poem of five verses, is actually referring to Vitamin B1 only, but this is often called loosely ‘Vitamin B’. There are actually 11 different substances in the B Complex products that we choose to use, 8 true vitamins and 3 “vitamin-like substances”.

The B Group of Vitamins is characterized by solubility in water, a feature that separates them from Vitamins A, D, E and K. It is not so clear why Vitamin C has always been kept out of this group and given a separate letter of the alphabet. It too is water-soluble and the B Group contains substances with assorted types of chemical structure. However, they all contain nitrogen without exception. With the exception of pantothenic acid they all contain heterocyclic rings (ring structures that include both carbon and nitrogen). Even pantothenic acid combines with a heterocyclic ring to yield its active form, Co-Enzyme A. These rings all contain double bonds. Thiamin, pantothenic acid and biotin also contain sulphur. Vitamin C may be considered somewhat distinct because it contains only a carbon / oxygen ring with no nitrogen or sulphur.

Moreover, for the most part the B Vitamins are known to act as co-factors for enzymes that occupy key places in cell metabolism. Hence they are each individually a sine qua non for an active energy-producing, energy-using cell economy. The majority of them need to be converted into an “active form” before they can be used by the enzymes concerned. Therefore one may have to address the question of the biochemical change needed to produce the “active form” from the basic vitamin. When these vitamins are presented within foods they will typically be partly active and partly in forms that require activation. Therefore manufacturers and marketers of “Food State” or “Food Form” vitamins should not overstate their case by claiming that the vitamins contained in their products are wholly in an “active form”. “Food State” or “Food Form” vitamins, through the way they are produced, are inherently likely to differ significantly from the real state of vitamins in foods and, indeed, the real state of vitamins in foods differs among food types. These manufacturers should publish the precise chemical forms in which the vitamins occur in their products. Most particularly, they should state clearly the levels of the individual co-enzyme forms that are present.
Although the numbers of the true B Vitamins run from 1 to 12 there are significant gaps, especially as there are no numbers in use between 6 and 12. No. 4 is also missing and number 5 has been appended to pantothenic acid as a later thought. This reflects that during the course of the discovery and investigation of the B Vitamins several false vitamins came up for consideration and were later dropped as not fulfilling the criteria for true vitamins. The basis for these criteria is essentiality in the diet. Moreover, withdrawal of the supposed vitamin should lead to a deficiency condition that can be restored to normal by repleting with the vitamin.

The Vitamins B1, B2, B3, B5 and B6 all play roles that are each individually crucial for the cell’s breakdown of food materials to yield ATP energy. Members of the group comprising B1, B2, B3 and B5 each have particularly universal application to this task. It follows quite logically from this that unless a deficiency affects one of these only it will not usually be sufficient to reverse a B vitamin deficiency by giving just one from the group. Diets deficient in one B vitamin will often be deficient also in others. It follows that normal production of cell energy cannot ensue without full restoration of the group as a whole. This has led quite rightly to recognition of the need to administer supplementary B vitamins as a group and not usually as individual vitamins. However, there are cases to be learned in the clinical section of the Course when individual B vitamins may be supplemented specially in addition to providing them in the form of Vitamin B complex.

Another common feature of the B vitamins is that the body has very low storage capacity for most of them, though B12 is a notable exception. This fact makes people vulnerable to short term deficiencies that may possibly be made good some days later when different foods are eaten. It also means that the results of making good a deficiency are experienced virtually at once upon replenishment with either food vitamins or supplementary vitamins. The difficulties experienced in trying to remedy long-standing mineral deficiencies are not experienced. Another feature is that dietary deficiencies of B vitamins, if they are experienced at all, usually affect more than just one of the vitamins, multiple deficiencies are far more common than single deficiencies and the commonest situation of all is likely to be sub-clinical multiple deficiency.

1.2 The Members of the Group

- Thiamin (Also called Thiamine, Aneurin, Vitamin B1)
- Riboflavin (Also called Vitamin B2)
- Nicotinic Acid (also called Niacin, Vitamin B3)
- Nicotinamide (a chemically distinct form of Vitamin B3)
- Pantothenic Acid or Pantothenate (Also called Vitamin B5)
- Pyridoxal (also called Vitamin B6)
- Pyridoxine, Pyridoxamine, Pyridoxal 5’ Phosphate (chemically distinct forms of Vitamin B6)
- Cyanocobalamine (also called Vitamin B12)
- Folic Acid
- Biotin
1.3 Vitamin-like substances

The following are often included in B complex supplements:-

- Choline
- Inositol
- Gamma aminobutyric acid (GABA).

1.4 Pseudovitamins B

The following are often advanced as vitamins by the alternative movement, but fail to fulfil the criteria:-

- Orotic acid (also called Vitamin B13)
- Pangamic Acid (also called Vitamin B15)
- Laetrile (also called Vitamin B17).

Treatment of these pseudovitamins has been left out completely by Garrow et al. However, their story is quite interesting because of the way in which they demonstrate the vulnerability of sick people to deception, to toxic products and even to criminality in the area of nutritional supplements. Anyone interested in following this further can refer to Herbert (1988). Ernst Krebs Jr. was convicted in 1973 for promoting the pseudovitamins B15 and B17 and jailed in 1983 for continuing to promote them in violation of the terms of his probation. No one could ‘pin down’ exactly what pangamic acid was supposed to be and, very suspiciously, it was claimed to be too many different things, some of which would have been toxic. The iniquity of the use of laetrile is that it releases toxic cyanide (a truly notorious poison) upon treatment with saliva and foods. The claim that this cyanide was immediately detoxified proved groundless according to the levels of cyanide found in the blood of users and two deaths have been reported from using 2500mg to 10,500mg of laetrile. This truly is the ‘dark side’ of alternative medicine and has amounted to dangerous exploitation. Even though orotic acid may or may not be harmless, we recommend you to have nothing to do with administering any of these pseudovitamins.

In nutritional medicine practice only some of the B vitamins generally warrant individual prescription as supplements. These are nicotinic acid or nicotinamide, pantothenic acid, B6 and biotin. Thiamin, riboflavin, B12 and folic acid are unlikely to be warranted except as member of a vitamin B complex. Choline and inositol are often prescribed either together or separately, though they are not treated here as a topic. They are also often included in B-vitamin complexes and formulations. They are not recognized in orthodox circles as B vitamins and although they are entirely beneficial, the question of their categorization would seem to be academic. They have been covered from their non-clinical standpoint in the section on lipids and are to be treated from the clinical standpoint in Part 2 of the Course.

Because depletion of either individual or even multiple B vitamins in the body causes a rather generalized form of malfunction within cells, it is not surprising that some of them fail to give rise to any very specific and clearly identifiable “deficiency syndrome”. This fact is quoted repeatedly by orthodox sources as a reason for belittling the nutritional importance of dietary...
supplies these vitamins. Alternative students and practitioners should avoid making the same error. It seems clear to us, and it is inherent in our philosophy and outlook upon the very concept of “chronicity”, that any factor that undermines the general health and metabolic competence of cells will result in much greater exposure to toxic damage and hence, in the long run, to chronic illnesses. Many clinical trial results vouch for the fact that supplementary B vitamins benefit many chronic conditions which are not apparently linked to B vitamin requirements by an obvious biochemical mechanism. These can be assumed to be the outcomes of the “general chronicity” effect.

This idea translates readily into naturopathic terms. Levels of metabolic incompetence of cells and tissues lead to relative inability to detoxify and discharge wastes and xenobiotics. Hence cell damage and tissue damage are bound to increase with increasing levels of metabolic incompetence. Metabolic incompetence results from enzyme damage and damage to DNA and to membranes. Among the most important cell processes to suffer are cell energy generation and protein synthesis. The corollary to this is that giving B vitamins to people who are even marginally deficient in them will tend to have a positive effect upon naturopathic elimination. This is eliminatory treatment, though not so strongly so as magnesium. However, it makes it obvious to use a B vitamin complex and magnesium together, which is what we usually do (among the other ingredients) in most of the broad ranging formulations that we employ. We recommend you never to lose sight of this very significant naturopathic dimension to the B vitamins.

The only course book you will need is Garrow et al (10th Edition). All that Garrow has to say on this subject is potentially relevant, but we have directed your attention to especially important parts and mentioned other parts where he gives more detail than you need.

1.5 References

2 THIAMIN – VITAMIN B1

Please read the account in Garrow & James (10th Edition) of the history of discovery of this vitamin (page 257). Note its strong association with the difference between polished and whole grain rice and with the deficiency disease, beri-beri.

2.1 Chemical Structure

This is given in the diagram below.

This section is for those who would like to know. Chemists call thiamin “a pyrimidyl-substituted thiazole”. That is a way of summarizing the fact that the thiamin molecule contains 2 rings, one a pyrimidine ring and the other a thiazol ring. The pyrimidine ring is the same 6-membered ring as in present in the pyrimidines that are important in nucleic acid structure (see the nucleic acid Side-Book). Two of the 6 positions are occupied by nitrogen atoms. The thiazol ring is a 5-membered ring containing three carbons, one nitrogen and one sulphur atom. The two rings are joined together by a one-carbon bridge called a “methylene bridge”.

2.2 The Formula for Thiamin (Vitamin B1).

Note the bracket to the right-hand side of the CH₂O- group. Everything to the left of the bracket is thiamin. When only H is appended to the formula at the CH₂O- group the thiamin structure is completed. However, although thiamin itself is consumed in foods and is active as a vitamin, the form in which it participates in enzyme reactions (i.e. its “active form”) has two phosphate groups attached and is called thiamin pyrophosphate (TPP) or thiamin diphosphate (TDP). This phosphorylation takes place by attachment to the O of the CH₂O-group. This part formula beyond the bracket on the right represents this pyrophosphate group and its attachment to thiamin gives the active form of the molecule. A much smaller proportion of thiamin in the body occurs as thiamin triphosphate (TTP). This occurs most particularly in brain tissue but the role there is not yet clear.

This means that when thiamin is taken in unphosphorylated the body must convert this to the pyrophosphate form before it can be active in enzyme systems. The body can accomplish this by means of phosphorylating enzymes that use ATP.

There is a fair amount of information available about which features of thiamin structure are needed for biological activity. When the natural structure is modified, some alterations lead to variants that retain thiamin activity whilst other become thiamin antagonists (i.e. they act as analogues that block thiamin action).
2.3 Enzyme Systems that use Thiamin Pyrophosphate

There is a rather brief account of these in Garrow & James (10th Edition) under the heading of “Biological Functions”. They list these as:

<table>
<thead>
<tr>
<th>TPP (TDP) DEPENDENT ENZYMES</th>
<th>PLACE IN METABOLISM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyruvic dehydrogenase</strong></td>
<td>Entry to the Citric Acid Cycle (= Krebs Cycle or Tricarboxylic acid Cycle). Hence essential for breakdown of all carbohydrates and glucogenic amino acids.</td>
</tr>
<tr>
<td><strong>Alpha-ketoglutarate decarboxylase</strong></td>
<td>A component of the Citric Acid Cycle (= Krebs Cycle or Tricarboxylic acid Cycle). Hence essential for breakdown of all bulk nutrients: fats, carbohydrates and proteins.</td>
</tr>
<tr>
<td><strong>Decarboxylases for branched chain keto acids</strong></td>
<td>Breakdown of branched chain amino acids that lead in to lipid pathways at the stage of acetyl CoA (Ketogenic amino acids)</td>
</tr>
<tr>
<td><strong>Transketolases</strong></td>
<td>The Pentose Phosphate Pathway (alternative route from glucose to pyruvate and acetyl CoA)</td>
</tr>
</tbody>
</table>

This information shows us that thiamine pyrophosphate acts as cofactor for enzymes that occupy key positions in metabolism. Without it carbohydrate breakdown could not progress any further than the pyruvate and breakdown of glucogenic amino acids would be blocked at the same stage. Moreover the alternative route of carbohydrate breakdown via the pentose phosphate pathway would also be blocked. The breakdown of the ketogenic amino acids arising from deamination of leucine and isoleucine would also be blocked.

Therefore the syndrome to be expected when thiamin supply is deficient would involve signs of energy (ATP) starvation and possibly also toxic effects from the accumulation of the intermediates that cannot be oxidized away. The deficiency signs associated with thiamin do show that the nervous system is the most likely to be adversely affected. It may be that the nervous system is the most crucially affected by ATP starvation and branched chain keto acid accumulation. Lactic and pyruvic acids are also elevated in deficiency of the vitamin, as you would expect from the blocking of the onward path for pyruvate. Also, due to the blockage of the onward path for alpha-ketoglutarate, alpha-ketoglutaric acid is elevated. Two other “odd-ball” metabolites of carbohydrate also appear, called glyoxalate and methylglyoxal. It is not entirely clear as to which of these metabolite accumulations may prove toxic.

For those who are interested to see more fully how these thiamin-dependent reactions fit into the general scheme of metabolism, Appendix 1 is provided to locate them within a diagram of metabolic pathways.

2.4 The Human Requirements for Thiamin

The dietary requirements can vary considerably because it is coupled with the daily intake of metabolizable carbohydrate. Hence, low carbohydrate intakes or low food intakes reduce the requirement of the individual. Increased carbohydrate to provide for muscular work, pregnancy or lactation will always increase the thiamin requirement. The requirement is therefore not measured in mg/day but in mg/1000 calories. Clinical signs of deficiency can be
averted with from 0.15 to 0.2mg/1000 calories but true adequacy, reflected by normal levels of enzyme activity in the blood cells, requires 0.35-0.4 mg/1000 calories. For a person consuming 2500 calories/day, this amounts to close to 1mg/day, or 1.2mg/day if a safety factor is added. However, since a man doing heavy manual work requires some 5000 calories/day, he would need about 2.0-2.5mg/day. If he satisfies his hunger with a diet high in whole grain cereals he will usually obtain enough. A “bonus” amount of 0.4mg/day is advised for pregnancy and 0.5mg/day for lactation.

2.5 The Prospects of Meeting the Thiamin Requirement from Foods

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN THIAMIN CONTENT IN mg/100g SOLIDS</th>
<th>THIAMIN CONTENT OF THE HIGHEST OF THE GROUP IN mg/100g SOLIDS</th>
<th>THIAMIN CONTENT OF THE LOWEST OF THE GROUP IN mg/100g SOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>0.42</td>
<td>Oats 0.98 Note: Wheat Germ 2.28</td>
<td>Buckwheat, 0.13 Pearl Barley, 0.13</td>
</tr>
<tr>
<td>DAIRY</td>
<td>0.24</td>
<td>Sheep’s Milk 0.47 Skimmed Cows Milk 0.45</td>
<td>Cheddar Cheese 0.05</td>
</tr>
<tr>
<td>FATS</td>
<td>Trace Only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>0.44</td>
<td>Salmon 1.23</td>
<td>Herring – Trace only</td>
</tr>
<tr>
<td>FRUITS</td>
<td>0.30</td>
<td>Orange 0.79</td>
<td>Pears 0.12</td>
</tr>
<tr>
<td>MEATS</td>
<td>0.52</td>
<td>Pork – Leg 1.80</td>
<td>Beef Fore rib 0.09</td>
</tr>
<tr>
<td>NUTS &amp; SEEDS</td>
<td>0.55</td>
<td>Sunflower Seeds 1.67</td>
<td>Coconut 0.03</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>0.25</td>
<td>Crab 0.36</td>
<td>Boiled Winkles 0.06</td>
</tr>
<tr>
<td>PULSES</td>
<td>0.73</td>
<td>Fresh Peas 2.91</td>
<td>Butterbean 0.06</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>1.13</td>
<td>Pumpkin 3.20</td>
<td>Beetroot 0.08</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With reference to the above Table, if we rate food classes that yield an average of less than 0.35mg of thiamine per 100g of dry matter as “poor”, then seafoods, fruits and dairy products fall into that category. If we rate as “medium” food classes yielding from 0.35 to 0.6mg per 100g dry matter, then cereals, fish, meats, nuts and seeds fall into that category. The highest accolade on a dry matter basis goes to pulse and vegetable classes, though we must always appreciate that intake of vegetable solids is limited by their water and fibre content. We should also note that the average for pulses is much affected by the especially favourable content of soya beans, fresh peas, black-eye beans, red kidney beans and peanuts. The rest of the pulses together only average 0.38mg per 100g dry matter.

In fact the cereals often assume a certain prominence in the provision of thiamine, but this is due more to their large contribution to bulk within the diet than to exceptional content. However, in therapeutic diets employing a lot of brown rice the content of 0.68 places rice (along with oats - see Table) into the “excellent” category as an individual cereal. Manufactured breakfast cereals often has added thiamine – though this is not sufficient reason to justify their use in therapeutic diets. In the Table below we take the hypothetical diet we used in the Vitamin E section, which apart from some separated fats contains nothing but whole foods, to illustrate how well a mainly whole food diet will support thiamine nutrition. The
answer is “very well indeed” since the overall daily intake works out at about 2.68mg / day. The main contributions are clearly coming from cereals and vegetables. This diet is very different from most people’s diets in containing ample whole cereals and an unusual amount of fresh vegetables. Hence it gives a very high safety margin with regard to thiamine status. Whilst this assures us that those who actually hold to a dietary programme of this type do very well for thiamine, if white bread is substituted for whole grain products and vegetables become neglected, then people do become potentially vulnerable to deficiency. Nonetheless, because 1mg/day is sufficient for most people, we are unlikely to see anything worse than marginal thiamine deficiency in populations such as Western ones for whom food is generally plentiful, even though many wrong food choices are being made. There is an exception with regard to alcoholics, in whom it can be more severe.

### 2.6 Severe Thiamin Deficiency

Pigeons and chickens have been the subject of much investigation because they are more susceptible to deficiency than mammals. The characteristic effect is head retraction, which arises from neurological dysfunction. In experimental mammals there is lack of coordination of muscle movements, leading on to paralysis, convulsions and death. The brain is dependent on glucose oxidation for its energy but the decrease in the activity of its enzymes of carbohydrate oxidation does not seem sufficient to directly explain the severity of neurological dysfunction. Probably the severe trouble in the nervous system is caused by quite severe deprivation of the neurotransmitter acetyl choline (because acetyl CoA is not being formed). It is also possible that the triphosphate form of the vitamin (TPP) carries out some as yet unidentified but

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN THIAMIN CONTENT IN mg/100g SOLIDS</th>
<th>ASSUMED WEIGHT OF THE FOOD DRY MATTER EATEN/ DAY (g)</th>
<th>THIAMIN CONTRIBUTION FROM EACH CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>0.42</td>
<td>150</td>
<td>0.63</td>
</tr>
<tr>
<td>DAIRY</td>
<td>0.24</td>
<td>40</td>
<td>0.096</td>
</tr>
<tr>
<td>FATS</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>FISH</td>
<td>0.44</td>
<td>25</td>
<td>0.11</td>
</tr>
<tr>
<td>FRUITS</td>
<td>0.30</td>
<td>40</td>
<td>0.12</td>
</tr>
<tr>
<td>MEATS</td>
<td>0.52</td>
<td>70</td>
<td>0.364</td>
</tr>
<tr>
<td>NUTS &amp; SEEDS</td>
<td>0.55</td>
<td>25</td>
<td>0.137</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>0.25</td>
<td>10</td>
<td>0.025</td>
</tr>
<tr>
<td>PULSES</td>
<td>0.73</td>
<td>40</td>
<td>0.292</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>1.13</td>
<td>80</td>
<td>0.904</td>
</tr>
<tr>
<td>AVERAGE AND TOTAL</td>
<td>0.46</td>
<td>500</td>
<td>2.678</td>
</tr>
</tbody>
</table>
important function in nerve transmission. Loss of appetite, cardiac enlargement, oedema, increased pyruvate and lactate are also seen in these animals.

In deficient humans there are two distinct major deficiency diseases. The best known is beri-beri. Please read Garrow & James (10th Edition) on the subject of the epidemiology of beri-beri on page 260.

This disease is now rare in the countries where it was originally described - Japan, Indonesia and Malaysia. In Western countries occasional cases are seen in alcoholics.

In acute beri-beri there is a form of cardiac failure, with warm extremities bounding pulse, oedema and cardiac enlargement. These features may be the result of intense vasodilatation from accumulation of pyruvate and lactate in blood and tissues (because TPP is not there to catalyze their removal. There are usually no clear changes in the electrocardiogram record. Response to thiamin treatment is prompt, with diuresis and usually a full recovery. A variant is sometimes called “wet” beri-beri where there is right sided cardiac failure leading to respiratory oedema.

In chronic beri-beri, sometimes called “dry” beri-beri, the peripheral nerves are affected, rather than the cardiovascular system. There is muscle weakness and wasting especially in the lower extremities. There is inability to lift the foot up (foot drop), loss of sensation in the feet and ankle jerk reflexes are absent.

Note the reference to the treatment of beri-beri by the injection of substantial doses of thiamin (25mg) under the heading “Therapeutic uses and toxicity” in Garrow & James (10th Edition) p262. Such radical treatment will not be required of the “alternative” nutritional practitioner.

The second deficiency disease is Wernicke’s encephalopathy

It is usually seen in people who have been drinking alcohol heavily for some weeks and eaten very little. Alcohol requires thiamin for its metabolism and alcoholic beverages do not supply much of it. Occasional cases are seen in people on a prolonged fast (such as hunger strikers) or with persistent vomiting. Cases occurred in malnourished soldiers in Japanese prison camps in World War II. Clinically, there is a state of quiet confusion, lowered level of consciousness and lack of coordination. The characteristic feature is paralysis of one or more of the external movements of the eyes. This and the lowered consciousness, respond to injection of thiamin within two days, but if treatment is delayed the memory may never recover. The memory disorder that is a sequel of Wernicke’s encephalopathy is called Korsakoff’s psychosis after the Russian psychologist who first described it. There is an inability to retain new memories and sometimes confabulation. For those who have studied brain anatomy it is interesting to note that in people who die of Wernicke - Korsakoff syndrome, lesions are found in the mamillary bodies, mid brain and cerebellum. Please read Garrow & James (10th Edition) on the subject of Wernicke - Korsakoff syndrome on p261.

Note the reference to the treatment of Wernicke - Korsakoff syndrome by the injection of substantial doses of thiamin (50mg) under the heading “Therapeutic uses and toxicity” in Garrow & James (10th Edition) p262. Such radical treatment will not be required of the “alternative” nutritional practitioner.
It is not clear why one deficient person develops beri-beri and another develops Wernicke-Korsakoff syndrome and why the two diseases seldom coincide. Possibly, the cardiac disease occurs in people who use their muscles for heavy work and so accumulate large amounts of pyruvate, producing vasodilatation and increasing cardiac work, while encephalopathy is the first manifestation in inactive people.

There does not seem to be any significant research on marginal thiamin deficiency. Undoubtedly this must involve relatively slight clinical symptoms that are hard to pick out in individuals. However, in view of the critical vulnerability of the nervous system it is interesting to speculate whether added thiamin contributes to the overall increase in the intelligence of school children when multivitamin / multimineral tablets are given. A drop of Intelligence Quotient by a few points would normally not be noticed in an individual, though it can be measured in a group. It is subtle effects like that that we must look for and expect due to the reduced ability of the nervous system to oxidize glucose, make ATP and synthesize acetylcholine. It is also inherently likely that output of physical work and stamina would suffer from marginal deficiency of this vitamin. There is a biochemical test for thiamin status, based upon the transketolase enzyme activity of the red blood cells. This may show up a relative deficiency of thiamin when no clinical symptoms are presenting. See Garrow & James (10th Edition) pp259-260.

2.7 Absorption, Storage and Excretion

Absorption of thiamin does not generally present much of a problem except in alcoholism, or at any rate high alcohol consumption. Please read what Garrow & James (10th Edition) have to say on these subjects on pp258 (right hand column) and 259 (left hand column).

2.8 Use of Supplementary Thiamin in Nutritional Medicine Practice

As we have mentioned at the outset, we have no need to administer supplementary thiamin as a single nutrient, but rather we give it as a Vitamin B complex or in composite formulae of vitamins and minerals. Our “standard” therapeutic daily intake is 25mg/day, which is very different from giving 25mg, or 50mg, by injection as in orthodox medical practice for severe deficiency. Therefore the maximum that we might give by mouth is 50mg as happens when the “standard” intake is doubled for reasons connected with the intensity of an individual patient’s therapy. These levels are quite high and, it must be admitted, fairly arbitrary. Giving only a tenth of these levels would provide generously for any known and recognised nutritional requirements. However, since these higher intakes have been reported in some work to provide benefit and since thiamin is very thoroughly non-toxic at these levels, and is also inexpensive, we prefer to opt for a clearly generous supply for therapy purposes. The use of this vitamin at these levels, together with the riboflavin that is used with it, turns the patients’ urine yellow, so you must expect to receive many “yellow urine” reports. However, this effect is entirely without significance beyond confirming the fact that the excess of these two vitamins is being excreted. It incidentally also serves to confirm that they have been absorbed in the first place.
2.9 Interactions with Other Nutrients, Foods and Drugs

Anti-thiamin factors in food include the thiaminase enzymes of raw fish, which are abolished by cooking the fish. There are also anti-thiamin factors in tea and coffee. Alcohol is a particularly strong negative influence that, as we have seen, can lead to a severe deficiency condition by virtually abolishing absorption of thiamin. The cytotoxic drug 5-fluorouracil is specifically anti-thiamin (Aksoy et al. 1980). It seems, indeed, that a rather broader spectrum of chemotherapy drugs is also anti-thiamin, such as adriamycin, cisplatin and methotrexate, as is the diuretic drug furosemide (Seligmann et al. 1991).

2.10 The Metabolic Breakdown of Thiamin

Please read what Garrow & James (10th Edition) have to say on this subject on p258 (left hand column, bottom paragraph). Also we provide an appendix on this topic (Appendix 2) for those who are interested.

2.11 References on Thiamin

In general we refer you, should you wish to probe more deeply into the subject of thiamin, to use the very substantial reference list offered by Garrow & James (10th Edition) pp283-287. Here we offer the following references on thiamin/drug interactions.

3 RIBOFLAVIN – VITAMIN B2

3.1 Chemical Structure

Riboflavin contains the heterocyclic ring system that chemists refer to loosely as “flavin” but which is fully named the “isoalloxazine” ring. This is a fused ring system. That is to say that whereas in thiamin the two rings are simply joined flexibly by a one-carbon “bridge” the 3 rings of flavin are fixed firmly and rather rigidly together because each ring shares two common carbon atoms with the adjacent ring. Riboflavin is derived from flavin by the addition of a 5-carbon polyalcohol side-chain, ribitol. Its full structure is given below.

\[
\text{Riboflavin}
\]

3.2 Active Forms

Like thiamin, riboflavin does not act in enzyme systems as the unmodified vitamin. At the very least it has a phosphate group attached to the terminal –OH group of the ribitol moiety. This converts it into a nucleotide. To compare the design of this molecule with the nucleotide building blocks that go to make the nucleic acids, see the Nucleic Acid Side book. The structure “N-base-pentose-sugar-phosphate corresponds to a nucleotide and the only difference here is that we have a ribitol group rather than a ribose group (i.e. sugar alcohol rather than the sugar itself). This active form of riboflavin is called “flavin mononucleotide”, shortened either to FM or to FMN. Its formula is given below:
Flavin Mononucleotide

Nothing has changed since the previous formula except for the addition of the phosphate group.

There is another very important active form in which the FMN above is linked to a second nucleotide, which is adenosine monophosphate (AMP). AMP is the monophosphate version of the all-important ATP, the cells’ energy currency. The two monophosphate nucleotides linked together by their phosphate groups as shown in Garrow & James’ (10th Edition) Fig 14.4 on p263. This is called flavin adenine dinucleotide, or FAD.

FAD and FMN are both co-factors that attach themselves to important oxidizing enzymes.

3.3 Enzyme Systems that Use FMN or FAD

The enzymes that use a flavin cofactor, either FMN or FAD are called flavoproteins. Some of these are:

<table>
<thead>
<tr>
<th>FMN / FAD DEPENDENT ENZYMES</th>
<th>PLACE IN METABOLISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrolipoyl transacylase</td>
<td>A component within the enzyme complex that converts pyruvic acid to acetyl CoA</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>Converts succinic acid to fumaric acid within the citric acid cycle.</td>
</tr>
<tr>
<td>NADH dehydrogenase complex</td>
<td>Major enzyme of the respiratory chain</td>
</tr>
<tr>
<td>Alpha-ketoglutarate dehydrogenase complex</td>
<td>Converts alpha-ketoglutarate to succinyl co-enzyme A within the citric acid cycle</td>
</tr>
</tbody>
</table>

This Table lists the four flavoproteins that are involved in oxidative metabolism of bulk nutrients from pyruvate or acetyl CoA onwards. They are best known for this role. It is therefore common to connect the nutritional need for riboflavin principally with oxidative breakdown of bulk nutrients. Clearly nothing in the body could be much more important that the breakdown of food to supply cell energy. Without riboflavin, since we cannot biosynthesis it in our bodies, we would be completely unable to do this. However, it is interesting to note that flavins are
also needed for an assortment of other essential enzymes including those for synthesis of fatty acids and those for converting Vitamin B6 and folic acid into their active forms. It is noteworthy that a B vitamin can be required in order to make other B vitamins active. This has a bearing upon what was said in (1) above about using the B vitamins as a group. The synthesis of nicotinic acid (Vitamin B3) from the amino acid tryptophan also requires riboflavin. Those wishing to know more about a fuller range of the reactions requiring flavins are referred to Appendix 3.

3.4 The Human Requirements for Riboflavin

The dietary requirements for riboflavin apparently do not rise in line with energy intake as in the case of thiamin (National Academy of Science, Washington, 1980). Nonetheless the requirement is seen to be linked more generally with food intake overall and for convenience the approximation of calculating the requirement on the basis of “per 1000 calories” has often been maintained and is maintained today in USA estimates of requirement.

The USA recommendation is set at 0.6mg/day/1000 calories with an adult minimum of 1.2mg/day. The UK recommendation is set at 1.3mg/day for men and 1.1mg/day for women. A “bonus” amount of 0.3mg/day is advised for pregnancy and 0.5mg/day for lactation.

To assess a person's status in riboflavin it has become usual to measure the activity of the FAD enzyme glutathione reductase of the red blood cell before and after the in vitro addition of external FAD. The activity of this enzyme has been adopted as a biomarker of the adequacy of riboflavin nutrition. The merit of this approach is that it does give a chance for a laboratory method to detect levels of deficiency that may fall short of severe, i.e. marginal deficiency may for once be detectable. The hazard of the approach is that at any given level of nutrurre different enzymes may have different degrees of saturation with their riboflavin dependent cofactors. There may be body enzymes that are more vulnerable to nutritional deficiency of riboflavin than red blood cell glutathione reductase happens to be. That might lead to recommended intakes that nonetheless still permit deficiencies of certain enzyme activities to persist.

The Department of Health (1991) report that in a survey of British adults the median intake of riboflavin was 2.03 mg/day for men and for women 1.56mg/day. These figures leave the great majority of the population in a more than satisfactory position with regard to riboflavin intake. Nonetheless, when we are treating people for clinical conditions that may well decrease absorption and also increase requirements, we do not hesitate to give some excess of this vitamin.
3.5 The Prospects of Meeting Riboflavin Requirements

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN RIBOFLAVIN CONTENT IN mg/100g SOLIDS</th>
<th>RIBOFLAVIN CONTENT OF THE HIGHEST OF THE GROUP IN mg/100g SOLIDS</th>
<th>RIBOFLAVIN CONTENT OF THE LOWEST OF THE GROUP IN mg/100g SOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>0.14</td>
<td>Corn grits 0.28</td>
<td>Pearl Barley 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rye flour 0.26</td>
<td></td>
</tr>
<tr>
<td>DAIRY</td>
<td>1.23</td>
<td>Cows’ milk, skimmed, 1.91</td>
<td>Edam cheese 0.62</td>
</tr>
<tr>
<td>FATS</td>
<td>0.01</td>
<td>Sesame oil 0.07</td>
<td>Most have zero</td>
</tr>
<tr>
<td>FISH</td>
<td>0.55</td>
<td>Trout 1.15</td>
<td>Snapper 0</td>
</tr>
<tr>
<td>FRUITS</td>
<td>0.23</td>
<td>Apricots 0.39</td>
<td>Grapes 0.05</td>
</tr>
<tr>
<td>MEATS (Offals)</td>
<td>0.50</td>
<td>Mince beef 0.87</td>
<td>Pork belly 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Lamb’s liver 10.10)</td>
<td></td>
</tr>
<tr>
<td>NUTS &amp; SEEDS</td>
<td>0.19</td>
<td>Almond 0.78</td>
<td>Pistachio 0.13</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>0.54</td>
<td>Mussels 1.81</td>
<td>Whelks 0.03</td>
</tr>
<tr>
<td>PULSES</td>
<td>0.23</td>
<td>Black gram 0.42</td>
<td>Butterbean 0.03</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>0.48</td>
<td>Mushrooms 4.19</td>
<td>Yam 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turnip tops 2.78</td>
<td></td>
</tr>
<tr>
<td>AVERAGE</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With reference to the above Table, if we rate food classes that yield an average of less than 0.30mg of riboflavin per 100g of dry matter as “poor”, then cereals, fruits, nuts and pulses fall into that category. If we rate as “medium” food classes yielding from 0.30 to 0.5mg per 100g dry matter, then meats and vegetables fall into that category. The highest accolade on a dry matter basis goes to the dairy, sea foods and fish classes, with dairy being way ahead of the others. We should also note that the average for vegetables is much affected by the especially favourable content of mushrooms and turnip tops, which are way above the rest. The value for all the other vegetables only amounts to 0.35mg per 100g dry-matter.

In the selected diet we have used before the supply of riboflavin would plan out as follows:
As is the case with thiamin, this diet would supply more than enough riboflavin for most people, including the lactating mother. This assures us that those who actually hold to a dietary programme of this type do very well for riboflavin, just as they do for thiamin. However, if white flour products are used, including white bread, with vegetables, fruits and pulses taking a back seat in the diet, then the position becomes much less secure and a great burden is placed upon the supply of riboflavin via dairy products and meat. In the UK diet those two items may provide the main protection against riboflavin deficiency. There is a biochemical technique for assessing the body status in riboflavin. This consists of a measurement of the activity of a riboflavin (FAD)-dependent enzyme in red blood cells, namely “erythrocyte glutathione reductase”. This is covered by Garrow & James (10th Edition) p264, under “methods of assessment”, which we ask you to read.

### 3.6 Severe Riboflavin Deficiency

Deficiency of riboflavin does not usually occur in isolation but is usually accompanied by other nutrient deficits. No clear riboflavin deficiency disease has been characterised. However, the clinical signs of deficiency after almost four months of inadequate intake include lesions of the outside of the lips, corners of the mouth, inflammation of the tongue, red and swollen mouth and/or oedema of the mouth, dermatitis and neuropathy. Deficiency may also give rise to a particular form of anaemia as detailed by Garrow & James (10th Edition), p263-264 under “clinical deficiency”. In boys, scrotal dermatitis may occur. For the reasons cited in 2.2 above there may be effective shortages of B3, B6, and folic acid. There is stunting of growth in children.
Many conditions of serious chronic illness affect riboflavin levels and these include congenital heart disease, some cancers and alcoholism. There is a risk associated with thyroid disease, which alters riboflavin metabolism, while diabetes mellitus, oral contraceptives and stress and trauma all increase riboflavin losses.

3.7 Absorption, Storage and Excretion

Riboflavin and its co-enzyme forms in foods are usually attached to the proteins of the enzyme of which they form a part. The intestinal enzymes break down the protein part and the FMN and FAD co-enzymes are also broken down to riboflavin. There is an active riboflavin absorption mechanism located mainly in the upper part of the small intestine, dependent upon ATP. Absorption is facilitated by bile salts and inhibited by copper, zinc, iron and manganese. The vitamin is transported to the liver mostly as free riboflavin and free riboflavin is also the dominant form in the blood, where it is bound to proteins, especially albumin. Riboflavin is transported across the membrane of tissue cells by an active process. Within the cells riboflavin can be converted into the co-enzyme forms by enzymes that are widely distributed amongst many tissues. The brain is noted to retaining normal levels of riboflavin co-enzymes even in severe riboflavin deficiency. It is presumed tightly bound there by cellular proteins. The hormones ACTH, aldosterone and thyroid hormones all stimulate conversion of riboflavin to the co-enzyme forms. The main excretory form in the urine is free riboflavin.

3.8 Administration of Riboflavin in Nutritional Medicine Practice

The considerations applying to administration of riboflavin are largely identical to those that apply to thiamin. We have no reason to separate out riboflavin to be administered separately from the other B vitamins. The “standard” intake used in our therapy is 25mg.

3.9 Interactions with Other Nutrients, Foods and Drugs

Riboflavin is adversely affected by chemotherapy and has been noted with the anticancer drugs adriamycin, cisplatin and methotrexate.

3.10 References on Riboflavin

In general we refer you, should you wish to probe more deeply into the subject of riboflavin, to use the very substantial reference list on B vitamin offered by Garrow & James (10th Edition) p283-287. Here we have referred specifically to just one:

Checkpoint

One

a) How does thiamin deficiency lead to tissue lack of acetyl CoA?

b) Which food and drinks act as anti-thiamin factors?

c) Name five particularly good food sources of thiamin and five particularly good food sources of riboflavin.

d) What is an adult “standard intake” therapeutic level of supplementation for thiamin and riboflavin in mg/day? Are either of these B vitamins administered as single supplements?

e) Which metabolic process is riboflavin most commonly associated with?

f) Into what type of molecule is riboflavin converted in order to be in active form? What are the active forms of riboflavin.
4 NICOTINIC ACID/NICOTINAMIDE (VITAMIN B3)

4.1 Chemical Structure

The term "niacin" is sometimes applied to nicotinic acid specifically and sometimes applies as a generic term to cover nicotinic acid and nicotinamide together. The activity associated with Vitamin B3 is provided by both of these chemical forms. We give their structures below.

Note that both are based upon a six-membered ring with one nitrogen and five carbon atoms that is unsaturated (i.e. has double bonds). Such a “parent substance” is called “pyridine”. This parent compound differs from the pyrimidines that are present in the nucleotides of the nucleic acids in that the ring contains just one nitrogen, not two. Nicotinic acid is obtained from it (notionally, not in practice) by adding a carboxyl group in a ring position that is two places removed from the nitrogen. Nicotinamide is then derived from that by conversion to the amide form (i.e. replacing the –OH of the carboxyl with an –NH₂ group.

In fact nicotinic acid was first obtained as an oxidation product from the natural alkaloid nicotine, and this fact decided its name. However, its physiological properties are nothing like those of nicotine, which is the habit-forming drug in tobacco. Below we give its structure, only for interest. The pyridine-type ring is present as in nicotinic acid but the side-chain becomes another nitrogen ring that is five-membered. The asterisk represents a positive charge.
4.2 **Active Forms**

The active forms of Vitamin B3 are called:

1. **Nicotinamide adenine dinucleotide (NAD)** The basic structure of this dinucleotide is: Nicotinamide-ribose-phosphate-phosphate-ribose-adenine

2. **Nicotinamide adenine dinucleotide phosphate (NADP)**, which is NAD with an extra phosphate added.

These are co-enzymes that are essential to hundreds of enzymes in the body in a great variety of metabolic processes. Their role is as hydrogen donors and acceptors and hence the reactions in which they are involved are oxidation or reduction (redox) reactions. In that role they play multiple key parts in the glycolysis pathway and the citric acid cycle. The reduced nicotinamide co-enzymes that come from these steps are passed to the respiratory chain, so in this way they are providing the feedstock to the respiratory chain itself and to the entire cascade of reactions that it represents. The respiratory chain is the principal source of oxidative phosphorylation (ATP synthesis). Therefore NAD and NADP could not possibly have more key significance for the body as a whole. The Beta-oxidation pathway of fatty acid breakdown also requires NAD. NADP plays a part in the "pentose phosphate pathway" but it is also often involved in synthetic reactions such as fatty acid synthesis. It has been estimated that some 200 enzymes require these B3-dependent co-factors, primarily dehydrogenases of many kinds. The full structure of NAD and NADP are given in Appendix 5 for those who are interested.

4.3 **The Production of the Active Forms**

Upon entering the body nicotinic acid is transformed to nicotinic acid mononucleotide, which is:

Nicotinic acid-ribose-phosphate

It then reacts with ATP to form nicotinic acid dinucleotide. This is almost identical to NAD except that it lacks the amide group on the nicotinic acid carboxyl. It then reacts again with ATP and glutamine to give NAD. The onward conversion from that point to NADP requires a further reaction with ATP to add the necessary phosphate group. It appears that B3 that enters the body in the form of nicotinamide cannot be converted directly to NAD but must be first deaminated to nicotinic acid and thereafter must follow the pathway just outlined.

4.4 **Enzyme Systems that use NAD and NADP**

We have not attempted to list these out because the sheer number, about 200, is entirely daunting both to compile and to read. Examples are:

- The pyruvate dehydrogenase complex (prior to entry into the citric acid cycle).
- Isocitric dehydrogenase (early reaction within the citric acid cycle).
- Alpha-ketoglutarate dehydrogenase (mid citric acid cycle).
- Malic dehydrogenase (late citric acid cycle).
4.5  Formation of Vitamin B3 from Tryptophan

Part of the usual definition of a vitamin includes the provision that it should be an essential substance that the body cannot synthesise. That provision is usually applied quite rigidly, so it is rather an odd thing that Vitamin B3, which is universally regarded as a vitamin, can be synthesised in the body from the amino acid tryptophan. Consequently the amount of tryptophan you eat affects your Vitamin B3 requirement.

The pathway from tryptophan to nicotinic acid has co-factor requirements of its own (which means that the supply of Vitamin B3 from this source in turn depends upon other nutrients being in good supply. These include iron, riboflavin, Vitamin B6 and, indeed, Vitamin B3 itself in the form of NADP for one of the enzymes involved. These factors may complicate the question of how much Vitamin B3 you can make from tryptophan, even if you have a surplus of tryptophan available to convert. The transformation takes place via an intermediate in tryptophan metabolism called “kynurenine” and the whole process is laid out for those who are interested in Appendix 6.

4.6  The Human Requirements for Vitamin B3

Estimation of Vitamin B3 requirements is complicated, as explained in the last section, by the uncertain factor of the tryptophan-derived NAD. The efficiency with which tryptophan can be converted to the vitamin depends upon a number of factors. These include the relative amounts of Vitamin B3 and tryptophan that are ingested, protein and energy intake, and the nutritional status in Vitamin B6 and riboflavin. There does exist a “rule of thumb” to the effect that 60mg of ingested tryptophan is equivalent to 1mg of Vitamin B3 and that 60mg of tryptophan can therefore be regarded as “one niacin equivalent”, abbreviated “NE”. Based upon this assumption, total intake of “niacin equivalents” amounts to the total of both nicotinic acid and nicotinamide plus one sixtieth of the tryptophan intake. One must remember, though, that as tryptophan intake becomes scarce the relative need for tryptophan for protein synthesis increases and its availability for conversion to Vitamin B3 will tend to diminish.

However, if one employs the “rule of thumb” given above, then a diet that contains 60g/day of protein will typically contain 600mg of tryptophan and be “worth” 10 NE’s. This is 10mg of Vitamin B3 and approaches the daily requirement.

Food Tables can be fairly misleading where Vitamin B3 is concerned because:

- They usually report only preformed niacin, so no weight is given to the benefit of the tryptophan content of the diet.
- Niacin in cereals is usually largely in the form of complex nicotinic acid esters referred to by various names such as “niacinogens” and “niacytin”. These may be largely unavailable and yet, paradoxically, they are reported in the food tables since they represent preformed niacin. Indeed, cereals can appear, from published tables, to be important contributors to Vitamin B3 status, because the values given in the tables are quite good and the cereals may form a major portion of the diet.
Most of the Vitamin B3 in other types of food is usually in the form of the complete co-enzymes, NAD and NADP, and these are completely available. Comparisons between cereal sources and other sources may therefore not be valid.

The available estimates of human requirements come from human depletion and repletion studies carried out in the 1950’s (those of you who sometimes press us to use only the most recent references should note this – much good work was done 50 or more years ago upon which we still rely). They led to adult requirements of 9.2 to 13.3 niacin equivalents/day. However, since the requirement is linked to energy intake, this is often transmuted into 6.6 niacin equivalents per 1000 kcals, subject to a fail-safe minimum intake of no less than 13mg when energy intake is low.

### 4.7 The Prospects of Meeting the Vitamin B3 Requirement from Foods

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN NIACIN CONTENT IN mg/100g SOLIDS</th>
<th>NIACIN CONTENT OF THE HIGHEST OF THE GROUP IN mg/100g SOLIDS</th>
<th>NIACIN CONTENT OF THE LOWEST OF THE GROUP IN mg/100g SOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>3.20</td>
<td>Wholemeal wheat flour 6.63 Brown Rice, 6.15</td>
<td>Oats 0.87</td>
</tr>
<tr>
<td>DAIRY</td>
<td>1.13</td>
<td>Goats milk 2.70 Sheep’s milk 2.35</td>
<td>Cows’ milk, skimmed, 1.91 Edam Cheese 0.18</td>
</tr>
<tr>
<td>FATS</td>
<td>0.01</td>
<td>Sesame Oil 0.10</td>
<td>Most have zero</td>
</tr>
<tr>
<td>FISH</td>
<td>20.9</td>
<td>Anchovy 52.6</td>
<td>Snapper 1.01</td>
</tr>
<tr>
<td>FRUITS</td>
<td>2.96</td>
<td>Plums 6.83</td>
<td>Eating apples 0.65</td>
</tr>
<tr>
<td>MEATS (Offals)</td>
<td>15.0</td>
<td>Chicken 30.5 (Lamb’s liver 43.5)</td>
<td>Breast of Lamb 7.35</td>
</tr>
<tr>
<td>NUTS</td>
<td>1.55</td>
<td>Pine nuts 3.91</td>
<td>Brazil nuts 0.31</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>8.97</td>
<td>Octopus 40.0</td>
<td>Whelks 0.23</td>
</tr>
<tr>
<td>PULSES</td>
<td>4.52</td>
<td>Peanuts 14.7</td>
<td>Butterbean 0.23</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>6.66</td>
<td>Mushrooms 43.2</td>
<td>Beetroot 0.78</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>6.49</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

These figures show us that a truly whole food diet (except for including oil) would give 5 x 6.49 = 32.45mg of Vitamin B3 in a 500g solids / day diet. This is so much above the basic requirement of 13mg/day that there appears to be very little worry about even marginal deficiency in normal circumstances. The problem of the vitamin's availability in cereal foods has not been addressed, but on the other hand the production of the vitamin from tryptophan has not been taken into account either. Given these circumstances the abuse of diet has to go...
a long way in order to produce a deficient condition. It can be noted that, historically, where deficiency has occurred there has usually been a combination of protein deprivation with both low and unavailable niacin. This has involved populations living on maize as a staple crop. It has particularly low tryptophan content, low niacin content, and its niacin suffers from non-availability. This has led to the condition known as “pellagra” in the Southern United States in the 1930’s and elsewhere in developing countries with poor or unreliable food supplies. Please read the introduction to Vitamin B3 in Garrow & James (10th Edition) beginning from “History” on p264 down to the heading “Absorption, Transport and Excretion” on p265.

4.8 Severe Vitamin B3 Deficiency

Classical deficiency of niacin results in the condition known as pellagra. The three D’s – dermatitis, dementia and diarrhoea are often used as a mnemonic device for remembering the signs of the disease, sometimes with the addition of a fourth D – death. The dermatitis is similar to sunburn at first and it appears on areas exposed to the sun such as the face and neck and on the extremities such as the back of the hands, wrists, elbow, knees and feet. It is accentuated by exposure to either heat or trauma. Accounts often omit to mention that these states are typically accompanied by wasting. There are neurological symptoms and these include peripheral neuritis with paralysis of the extremities. More seriously there may be dementia and delirium. There are also gastro-intestinal symptoms apart from the diarrhoea already noted, including nausea and vomiting. There may be achlorhydria (lack of stomach acid). There may be lesions of the lips and at the corners of the mouth as also seen in riboflavin deficiency. These classical signs were noted about 1908.

More recently it has been accepted that manifestations of less severe deficiency include weakness, lassitude, anorexia and indigestion (Campbell et al, 1980). Milder mental signs include mental fatigue, insomnia and apathy. These proceed, however, unless action is taken towards confusion, disorientation, hallucination, loss of memory and eventually psychosis.

In societies with western lifestyle deficiency is only likely to be seen when something else is severely wrong that precipitates the condition. This may be alcoholism or those suffering from chronic diarrhoea before the condition developed or those with inflammatory bowel disease, malabsorption syndromes and some types of cancer. There are certain conditions that involve disturbance of tryptophan metabolism and therefore predispose to Vitamin B3 deficiency. One of these is Hartnup’s disease, an inborn error in which tryptophan absorption is affected. Another is “carcinoid syndrome” in which much tryptophan is directed along an unusual pathway away from niacin biosynthesis.

4.9 Absorption, Storage and Excretion

Please read the small section on this in Garrow & James (10th Edition), p265.
4.10 Use of Supplementary Vitamin B3 in Nutritional Medicine Practice

Our “standard” therapeutic daily intake is 50mg/day in the form of niacinamide, within our various composite formulations. That means that we may give as much as 100mg/day when therapy is intense. However, nicotinic acid as opposed to niacinamide, is noted for its ability to bring about facial flushing. In alternative medicine circles it is not uncommon to make use of its ability to stimulate the circulation in people who have sluggish circulation, especially in those who are shown by the practice of iridology to suffer from poor blood supply to the brain. If this is done the intake of nicotinic acid had best be limited to 100mg/day. One is warned to avoid doing this with people who have asthma or peptic ulcer as they might have their conditions aggravated by the vitamin.

Some work has been done employing doses as high as 3g/day of nicotinic acid for hypercholesterolaemia, and this does have a favourable effect upon the blood lipid picture. In this type of application the vitamin is clearly being used as a drug. However, there is considered to be some risk of hepatic toxicity at such high doses and since we have good hypocholesterolaemic nutrients of other kinds, this application is best avoided. Doses as high as 2g/day of niacinamide have been employed for arthritis, but here there is a risk of causing depression. Therefore on balance mega-dosing is not advisable.

4.11 Interactions with Other Nutrients, Foods and Drugs

The enzymes for which Vitamin B3 co-enzymes are needed include many that also require zinc, magnesium or manganese. Therefore there is a form of function interaction with these minerals. The negative influence of alcohol upon the vitamin has been noted. The anti-tuberculosis drug isoniazid has a noted anti-vitamin effect upon B3 and can cause deterioration in body status in the vitamin. Like riboflavin it is also adversely affected by the anticancer drugs adriamycin, cisplatin and methotrexate.

4.12 References on Nicotinic Acid and Nicotinamide

In general we refer you, should you wish to probe more deeply into the subject of niacin, to use the very substantial reference list on B vitamin offered by Garrow & James (10th Edition) pp283-287. Here we have referred specifically to just one:

5 PANTOTHENIC ACID (VITAMIN B5)

Pantothenic Acid holds an undisputed position as a member of the B vitamin group but most orthodox sources do not hold with its designation as “Vitamin B5”. We presume this is because such a designation is not historically and chronologically accurate. However, the designation B5 has widespread use throughout alternative medicine circles. As there seems to be no good reason why this vitamin should not have a number we are allowing its use here. The name “pantothenic acid” is a reference to the alleged “universal” distribution of the vitamin in foods. The orthodox position is that all foods contain it and that deficiency is nearly impossible. This is a question in which many in alternative nutrition circles have begged to differ. The question can really only be resolved by agreeing what is a realistic daily intake of the vitamin, but no such resolution has yet occurred.

5.1 Chemical Structure

The structure of pantothenic acid is not very complex and it has no rings. The right hand side of the formula (below) represents a form of the amino acid alanine in which the position of the nitrogen group has been changed from the normal alpha position (next to the carboxyl) to the beta position (one place further away). It is then linked to a 6-carbon branched-chain dihydroxy acid, called D-pantoic acid as a trivial name. The true chemical name is a long one and unnecessary to learn.

\[
\begin{align*}
\text{HO} & \quad \text{CH}_2 \quad \text{C} \quad \text{CH(OH)} \quad \text{CO} \quad \text{NH} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{COOH} \\
\text{CH}_3 & \quad \text{pantothenic acid}
\end{align*}
\]
5.2 Active Forms

Like thiamin, riboflavin and niacin, pantothenic acid does not act in enzyme systems as the unmodified vitamin. The active form is called Co-enzyme A and it has been estimated that some 85% of all the pantothenic acid in foods occurs as Co-enzyme A. The structure of Co-enzyme A can be schematically represented as follows:

Mercaptoethylamine – pantothenic acid – phosphate – phosphate – ribose - adenine

| phosphate

So we see that pantothenic acid represents only a modest proportion of this important molecule. The “phosphate – ribose – adenine” moiety on the right represents adenosine monophosphate derived from ATP and in this respect there is an analogy to the niacin-based structure of NAD and NADP. The mercaptoethylamine portion is a simple 2-carbon amine with a sulphur group attached (sulphydryl, -SH). It is this sulphydryl group that represents the reactive part of this molecule. It is a short-term carrier for acid groups such as “acetyl” as we have seen and of longer groups of the same kind with the formula: CH₃(CH₂)nCO-. These are known as “acyl” groups.

The “acetyl” (CH₃CO-) of “acetyl Co-Enzyme A” is the simplest case in which n = zero. The enzymes using CoA are predominantly carriers, transferors and modifiers of acyl groups.

5.3 Enzyme Systems that use Co-Enzyme A

As in the case of NAD, the enzyme reactions that are CoA-dependent are simply too numerous to list. The matter can be summed up by saying that CoA, and therefore pantothenic acid, are required for the production of energy from carbohydrate, fat and protein. It is the formation of Acetyl-CoA from all three of the bulk nutrients that is a crucial reaction of energy metabolism. This is the form in which 2-carbon units are introduced into the citric acid cycle for oxidation. This is true whether they come from pyruvate, fatty acids or amino acids. As no oxidative metabolism could occur without this step it follows that little ATP could be formed without CoA and the life of complex organisms with oxidative metabolisms would be inconceivable. It is fundamental to cellular metabolism. Since it is common to all known organisms it seems that CoA must have evolved very early in the evolutionary history of the living cell.

Co-Enzyme A is also crucially involved in synthesis of fatty acids, steroids, haem and phospholipids. It is involved in the acetylation of proteins (i.e. adding an acetyl group to) and in this way enables the cell to manage the protection, storage, transportation and relocation of proteins following protein synthesis.
5.4 The Human Requirements for Pantothenic Acid

No recommendations have been made through official channels for daily intakes. This, once again, as in the case of manganese among the minerals, is due to the fact that no clear and specific deficiency syndrome has been identified. Orthodoxy is just not well orientated to take in the fact that in the absence of adequate amounts of such a vitamin the cell simply does not work so efficiently. Relative lack of CoA must necessarily detract from the food oxidizing, ATP synthesizing activity of the cell. The vitality of the cell simply cannot help but be affected by these events. With less energy available for protein synthesizing activity, sodium pump activity and detoxification activity there has to be a tendency for the toxic burden of the cell to build up. The only way that this “wider picture” of the well-being of cells can gain orthodox awareness will be through the development of some test of these indicators of cellular activity.

Different orthodox committees, while avoiding official recommendations, have concluded that a “safe” range of intake lies between either 3 or 4mg/day at the lower end to a possible 7mg/day. Williams (1971) has quoted evidence that individual requirements for pantothenic acid vary across a very wide range. Figures as high as 25mg/day have been both suggested and vigorously defended in alternative nutrition circles as sensible daily intakes for all adults. Meanwhile figures as high as 2g/day or even 10g/day have been employed in mega-vitamin therapy. In fact the best therapeutic diets we can construct tend to offer no more than 10mg/day of pantothenic acid unless they contain unusual amounts of parsley, so the proposed 25mg/day would certainly be impractical for most without the use of supplements. It seems that here is a place where we need to provide you with our own view. It would be that adult intakes between 6 and 10mg/day are feasible from food and should be sufficient and even generous except where there is a reason to use pantothenic acid in a therapeutic role. Since it falls within our range, we would be prepared to accept the orthodox “opinion” that their upper figure of 7mg/day would be enough. We would not accept their lower figures of 3 or 4mg/day. One should note that reasonably modest therapeutic intakes sometimes markedly influence pathological conditions, such as the 12.5mg/day found by Anand (1963a & b) to relieve osteoarthitis. Such an intake level as this can be interpreted as simply effecting the restoration of normal nutritional status within a reasonable time span.

5.5 The Prospects of Meeting the Pantothenic Acid Requirement from Foods

The following Table again uses the same notional diet as before. It illustrates that if we eat a diet that is entirely “whole food” except for the inclusion of fats and take the various food classes in equal proportions, then we shall obtain an average of 2.35mg of pantothenate per 100g of dry solids consumed. Such a diet must contain its share of parsley among the vegetables, but only about 6g of the fresh material will be needed. Trout is the best fish, broad beans are the best pulse and chicken the best meat.

One can conclude that the orthodox nutritionists’ idea of probable minimum daily intake, at 3-4mg is not in danger. As soon as we envisage the possibly more realistic requirement of 7-10mg/day, then a thorough-going whole food approach is essential in order to obtain this much from foods. As soon as we recognise the special position of parsley in connection with pantothenic acid we are well able to counteract shortages. The best and most practical way to consume sizeable amounts may well be in the form of juices, even if parsley represents only a minor part of the feedstock. Parsley is not easy to juice.

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN PANTO-THENATE CONTENT IN mg/100g SOLIDS</th>
<th>PANTOTHENATE CONTENT OF THE HIGHEST OF THE GROUP IN mg/100g SOLIDS</th>
<th>PANTOTHENATE CONTENT OF THE LOWEST OF THE GROUP IN mg/100g SOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>0.71</td>
<td>Oats 1.31, Buckwheat 1.19, Wheat Germ 2.15</td>
<td>Millet 0.5</td>
</tr>
<tr>
<td>DAIRY</td>
<td>2.18</td>
<td>Goats’s Milk 3.69, Skimmed Cows Milk 3.60</td>
<td>Cheddar Cheese 0.56</td>
</tr>
<tr>
<td>FATS</td>
<td>0</td>
<td>Butter Fat 0.05</td>
<td>All others – trace only</td>
</tr>
<tr>
<td>FISH</td>
<td>2.52</td>
<td>Trout 6.79</td>
<td>Tuna 0.66</td>
</tr>
<tr>
<td>FRUITS</td>
<td>1.50</td>
<td>Strawberries 3.24, Gooseberry 2.93</td>
<td>Grapes 0.27</td>
</tr>
<tr>
<td>MEATS</td>
<td>2.22</td>
<td>Chicken 4.69</td>
<td>Breast of Lamb 0.77</td>
</tr>
<tr>
<td>NUTS</td>
<td>1.06</td>
<td>Pecan Nuts 1.77</td>
<td>Brazil Nuts 0.42</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>2.49</td>
<td>Mussels 3.50</td>
<td>Boiled Winkles 0.08</td>
</tr>
<tr>
<td>PULSES</td>
<td>3.11</td>
<td>Broad bean 15.7</td>
<td>Fresh Peas 0.59</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>7.69</td>
<td>Parsley 177.0, Endive 15.3</td>
<td>Runner bean 0.57</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>2.35</td>
<td>21.8</td>
<td>0.44</td>
</tr>
</tbody>
</table>
5.6 Severe Pantothenic Acid Deficiency

As we have noted, severe deficiency is hard to produce experimentally and there is no clear-cut deficiency syndrome. The signs and symptoms are mostly non-specific. However, “burning feet syndrome” has been noted, characterised by abnormal skin sensation of the feet and lower legs, exacerbated by warmth and diminished with cold. There may also be vomiting, fatigue and weakness.

The pantothenate antagonist, omega methylpantothenate has been used to induce experimental low pantothenate status in humans.

5.7 Absorption, Storage and Excretion

Please read the small section on this in Garrow & James (10th Edition), p282.
5.8 Use of Supplementary Pantothenic Acid in Nutritional Medicine Practice

In many patients we have no need to administer supplementary pantothenate as a single nutrient, but rather we give it as a Vitamin B complex or in composite formulae of vitamins and minerals. Our “standard” therapeutic daily intake in those cases is 25mg/day. However, in the case of pantothenic acid we make what is for us a rare concession to those who advocate mega-vitamin therapy. It is permissible to use this vitamin at daily intakes of 500mg, 1g, 2g or even 3g when necessary. These special intakes are provided in the form of calcium pantothenate and are used for cases of adrenal exhaustion. It finds many applications amongst patients who suffer from severe allergic attacks. These people can become quite dependent upon it for relief. We have to make sure therefore that we do not leave them in this dependent condition but apply onward treatment to rid them of the causes of their allergic state.

Garrow & James, although they do not advocate mega-vitamin therapy, concede that pantothenic acid is non-toxic up to 10g/day. 20g/day has been reported to cause mild intestinal stress and diarrhoea. Some other authorities suggest that in gram quantities it may very rarely cause gastric bleeding by irritating the stomach lining. Patients who are known to be vulnerable to this should not be given pantothenic acid above 25mg/day. This would apply to patients who have had bleeds from gastric ulcers in their recent history. For others who take high intakes, these should be accompanied with food and also split between two or three mealtimes per day. Since 3g/day is the maximum we recommend, there will never be any need to administer more than 1g at any one time.

5.9 Interactions with Other Nutrients

Intakes of 100mg/day have been reported to increase the excretion of Vitamin B3. Hence, when high intakes of pantothenic acid are used, modest supplements of Vitamin B3 (as in a “B Complex” or multiple Formula) seem appropriate.
Checkpoint Two

a) What is the name of the specific deficiency syndrome associated with vitamin B3 and what are its 3 notorious symptoms?

b) What side effect may be experienced using nicotinic acid?

c) How does tryptophan intake relate to vitamin B3 requirement?

d) In which cases is the use of pantothenic acid advocated as mega-vitamin therapy? What would be the highest advisable intake?

e) What is the likelihood of meeting the daily pantothenic acid requirement from food alone? Which food is by far the best source of pantothenic acid?

f) Name four foods that are particularly good sources of vitamin B3.

Please turn to the end of this part to check your answers
6  VITAMIN B6

The functions of Vitamin B6 in the body are numerous but are very closely connected with its nitrogen metabolism, especially the interconversions of amino acids. The enzymes involved are the transaminases. These enable amino groups to be shifted from one amino acid carbon skeleton to another. This process permits the efficient use of dietary nitrogen. When the diet offers only an out-of-balance mix of non-essential amino acids, interconversions among them normally permit the deficiencies to be made good and the surpluses to be utilized. Reactions of this type are therefore a support to protein synthesis. They also can initiate the breakdown pathways of the amino acids or the utilization of individual amino acids for the production of key intermediates and products.

6.1 Chemical Structure

The structure of Vitamin B6 is one of the simplest among the B Vitamins. Only nicotinic acid is simpler. Like nicotinic acid it is based upon the simple heterocyclic ring, pyridine. Pyridine is a benzene ring in which one of the six carbons is replaced by nitrogen. There are three non-phosphorylated forms of the vitamin. The differences between them all occur in respect of the carbon atom in the substituent position that is para, that is to say opposite to, the nitrogen. In the following diagram this is the carbon atom at the top of the formula. It can take the form of an alcohol, an aldehyde or an amine, called respectively pyridoxol (or pyridoxine), pyridoxal and pyridoxamine.

Each of these forms of the vitamin occurs also in phosphorylated condition (i.e. combined with phosphate). The phosphate combines with the HOH$_2$C- group that appears on the left hand side of each formula.

The form that acts directly as co-enzyme Vitamin B6 is pyridoxal phosphate. Having the phosphate group positioned as just described, its formula is:
One should note that there have been attempts by the supplements industry to promote pyridoxal phosphate as a ‘better’ form of Vitamin B6 for ingestion because it is the biological active form. The fallacy of this position, however, is that before pyridoxal phosphate can be absorbed through the intestinal wall, it must be dephosphorylated to free pyridoxal. This is true of all the phosphorylated forms.

Pyridoxal phosphate is also the principal form found in the blood, but this must also be hydrolyzed back to simple non-phosphorylated pyridoxal before it can be taken up into cells. Most cells in the body contain pyridoxine kinase – an enzyme that adds a phosphate group to convert it *within the cell* into the co-enzyme form. The conversion of pyridoxine and pyridoxamine to pyridoxal must occur mainly in the liver and the intestine.

### 5.3 Enzymes that Require Vitamin B6

#### 5.3.1 Transaminases (‘aminotransferases’)

As we stated at the outset, the functions of Vitamin B6 are very closely connected with nitrogen metabolism, especially the interconversions of amino acids.

Among the various reactions concerned are the transamination (or ‘aminotransferase’) reactions. These effectively interconvert amino acids by removing the amino group of one, leaving a keto (or ‘oxo’) acid and adding that amino group to another keto acid. The process depends upon the correct ‘recipient’ keto acids being available. Two examples follow.
These reactions have considerable importance in providing the correct balance of non-essential amino acids for protein synthesis and synthesis of other biochemicals, such as neurotransmitters. They are also much involved in the body’s handling of nitrogen that comes from amino acid breakdown, leading to its eventual excretion as urea. These processes are key to understanding why B6 is so important to amino acid and nitrogen metabolism. If you would like to know more about the mechanism of that process, see Appendix B.7.

Much is known about the detailed chemical mechanism of the action of the transaminases and the role of B6. This involves transient combinations between B6 and the amino acid concerned. Although this is very interesting, the information is surplus to your needs.

5.3.2 Other Reactions

Other B6 co-enzyme reactions include the decarboxylation of amino acids (i.e. removal of carboxyl groups). Some of these lead to physiologically active products, such as gamma-amino butyric acid (see below) from glutamic acid, serotonin from tryptophan and histamine from histidine. These are all neurotransmitters. Carnitine synthesis also requires B6 for the same reason. Synthesis of dopamine, another neurotransmitter and taurine, which also modulates nervous system functions.

B6 is involved also in reactions that transfer or remove sulphur from sulphur amino acids, cysteine and methionine (trans sulphydration and desulphydration) and in the cleavage of serine to form glycine and a hydroxymethyl group attached to one of the reduced forms of folic acid (see below).

Racemases also require B6. These are enzymes that interconvert the D- and L- forms of the amino acid. Whilst mainly occurring in bacteria they do also occur in humans.
B6 is needed for the synthesis of haem (as in haemoglobin and the cytochromes). The enzyme is called delta-aminolaevulinic acid synthetase. Delta-aminolaevulinic acid is a key intermediate in haem synthesis. For this reason anaemia appears as one of the symptoms of deficiency. It is hypochromic (i.e. low pigment in the red cells) and microcytic (small red cells). Further, B6 is involved in the breakdown of glycogen through being a co-factor for the glycogen breakdown enzyme, glycogen phosphorylase. It also acts in modulating the actions of steroid hormones. It does this by intervening in the binding of steroid hormones with their receptor proteins in the cell nucleus. It does that by reacting with lysine residues in these proteins, decreasing the intensity of steroid hormone binding.

6.4 Human Requirements

Because the keynote of Vitamin B6 action is amino acids it is not surprising that for this vitamin “dietary protein” replaces “calories” as the yardstick for the daily requirement. The UK Committee on “Medical Aspects of Food Policy” has recommended that this value should be 15mcg/g of dietary protein for adult men and women. This will amount to 1.05mg/day for a person eating 70g/day of dietary protein and 1.50mg/day if dietary protein rises to 100g. This estimate (RNI) was based upon studying the changes in tryptophan and methionine metabolism when B6 intake is restricted and upon the levels of blood B6 under these same conditions (Dept. of Health 1991). The infant and child requirement was estimated at 8mcg/g of dietary protein at birth, which is very high in proportion to body weight, with gradual increases towards the adult value.

6.5 Prospects for Fulfilling Daily the Requirement from Foods

We present our usual breakdown of B6 contribution according to food class.

(see over please)
These figures lead to an average value for all whole foods of 0.63mg/100g dry matter. Hence a diet with 500g of dry solids per day will contain 3.15mg. This is more than twice as much as is needed to accompany a high protein diet with 100g of protein per day. It indicates that there should be no problems over B6 intake so long as the diet is mainly composed of whole foods. As with other B vitamins, intakes fall away rapidly when processed foods are eaten, since sugar and separated fats contain none and wholemeal flour is subject to a 70% loss upon milling. The selection of diets low in fish and vegetables will also have a very significant adverse effect. Nonetheless common western diets today that contain breakfast cereals, meat and potatoes can still offer more than sufficient. Many therapeutic diets will contain 100g of dry matter per day from vegetables, i.e. about 1kg/day of fresh vegetables. These will average 3.36mg/day from this source alone. If such a diet also contains 40g/day of solids from fish, then this figure rises to 4.12mg/day from these two sources only. Therapeutic diets starting from a base of vegetables and fish are therefore very safe from this standpoint.

### 6.6 Severe Deficiency

According to orthodox accounts, gross clinical deficiency of B6 is rare and this is in accord with its wide availability in foods. Nonetheless, special circumstances can expose an underlying risk. This risk was highlighted in the 1950s when deficiency occurred in infants due to severe heat treatment of infant milk. The heat caused the B6 to combine with the lysine residues on the proteins, making it unavailable. Those who are most highly vulnerable, apart from babies, are elderly people, alcoholics, renal patients on dialysis and those using certain allopathic drugs exerting an anti-vitamin effect.

The symptoms of deficiency, however caused, include sleepiness, fatigue, anaemia, cheilosis,
glossitis and stomatitis in adults and abnormal electrocardiogram, seizures and convulsions in infants. There is no specific named deficiency illness to compare with beri-beri or pellagra.

6.7 Successful Therapeutic Use

Although B6 deficiency is supposed, in orthodox accounts, to be rare, there have been very numerous accounts of B6 supplementation having a major influence upon disease outcomes. These include several of the pathological conditions that are scourges of our society.

6.7.1 Asthma

Asthma sufferers have been found to show a much-decreased frequency of wheezing and asthmatic attacks when given 50mg of B6 twice daily (Reynolds & Natta, 1985). Double-blind clinical studies were used. The responsiveness of asthmatics may be through the effects of B6 upon partly blocked tryptophan metabolism (see above reference).

6.7.2 Autism

A 1985 study of 60 autistic children showed marked benefit from combined supplementation with B6 and magnesium (Martineau, J. et al, 1985). Autism is associated with a decrease in several brain neurotransmitters that require B6 for their synthesis.

6.7.3 Cardiovascular Disease

This class of condition has long been associated with B6. However, cardiovascular disease is multi-factorial and many other nutrients are also involved, e.g. the essential fatty acids. The connection with B6 is rather dramatic, however, since people with low blood B6 have a five-fold increase in their risk of heart attack compared to those with normal B6 levels, (Kok et al, 1989 and Robinson et al 1995). The mechanism by which B6 is thought to be involved in heart disease are via the reduction in blood homocysteine (see the section on folic acid), the requirement of B6 for cross-linking of collagen and elastin in connective tissues and through inhibition of unwanted blood clotting (thrombosis). B6 as a sole supplement also decreases blood pressure by 10-14 mm of mercury at high intakes of around 150mg/day. This is thought to be due to its effects upon the nervous system.

6.7.4 Carpal Tunnel Syndrome

This is a painful condition of the wrist and hand in which a nerve becomes compressed between bones and ligaments, causing pain and weakness of grip. Doing strenuous work with the hands aggravates it. The sufferers are often B6 deficient and treatment with B6 supplements for 3 months is frequently effective. Hundreds of patients have been reported to benefit (Folkers & Ellis, 1990). Orthodox practitioners appear to have an unjustified predilection to perform surgery for such patients.

6.7.5 Depression
Vitamin B6 levels are often low in depressed patients. Women on the birth control pill are especially vulnerable. This vitamin, due to its crucial involvement in so many reactions of amino acid metabolism, is needed for the formation of many of the brain neurotransmitters. Supplementation with 50-100mg/day of B6 is often effective and the drugs these people are given (e.g. Prozac) may be completely unnecessary.

6.7.6 Diabetic Neuropathy

Neuropathy (nerve malfunction and damage) is one of several “complications” that occur in diabetes apart from the obvious upsets to sugar metabolism. High intakes of B6 (150mg/day) have been reported as effective in protecting against this neuropathy.

6.7.7 Epilepsy

It is really important to recognize that seizures and convulsions in infants are very commonly associated with B6. Surprisingly, such seizures are often increased at first if a zinc supplement is given without B6 alongside. The reason may be B6 deficiency, since zinc and B6 are interdependent. To avoid exacerbating seizures B6 should then be given without zinc for a few weeks before zinc is added. The object is to normalize B6 status. That may require higher than normal intake for a short while, but normal intake will suffice thereafter. Other cases that also relate to B6 require continued high intake to remain free of symptoms, a situation that is referred to as “dependency”, since dietary deficiency is not involved (Crowell & Roach, 1983).

Infant convulsions that are B6-dependent require on-going treatment with high intakes of B6 at 25-50mg/day or more. Nutritional practitioners are unlikely to be asked to help in such cases, which are severe and usually find their way to orthodox medicine for reasons of emergency. For the patients the danger is that the treatment given may be anti-convulsant drugs when it is B6 that is really needed. Although we do not know how this B6 dependency comes about it seems clear that there must be a severe upset to one or more of those neurotransmitters that require B6 in their synthesis, possibly GABA (see below). The fault may be the production of the enzyme that converts glutamic acid to GABA. It is possible that a genetic error results in a version of the enzyme that fails to bind pyridoxal phosphate efficiently. Some workers have applied extremely high doses (e.g. 20-50mg/kg/day) and these can cause liver damage, nausea and vomiting. This is not an area in which nutritional practitioners should ever become involved, due to the inherent dangers. Note that B6 can interfere with the action of anti-convulsant drugs, so B6 therapy and orthodox drug medicine do not mix well together.

6.7.8 Immune Depression

Vitamin B6 is one of many nutrients required by the immune system. In B6 deficiency the system loses much of its competence to fight infections through reduced ability to produce antibodies, reduced numbers and activity of lymphocytes, shrinkage of lymphatic tissues including the thymus gland. Many of these effects are thought to occur because B6 is necessary for normal protein and nucleic acid synthesis (in particular through its effects upon amino acid metabolism). Reduced cell multiplication results. Since antibodies are proteins their output is diminished. The under-function affects cell-mediated immunity as well as the antibodies. This is unlike deficiency of any of the other B vitamins. Furthermore these defects may explain many other conditions of immune under-function or malfunction, not only
diminished response to infections, see Axelrod, A.E. & Traketellis, (1964). AIDS patients tend to have low B6 values. It is unclear whether this precedes a susceptibility to the infection or occurs as a result of it.

6.7.9 Renal Stones

Vitamin B6, magnesium and the amino acid, glutamic acid, are key factors in the prevention of renal stones. At least the first two, but possibly all three, should usually be given to recurrent stone formers. B6 reduces the production and excretion of oxalates. Calcium oxalate is a prime constituent of kidney stones and renal stone formers are usually found to be B6-deficient. In B6 deficiency urinary levels of glutamic acid are reduced, but normal urine levels of glutamic acid are needed to keep calcium oxalate in solution. There are numerous relevant literature references to this work, e.g. Gershoff & Prien, (1967).

6.7.10 Osteoporosis

A Vitamin B6 deficient diet produces osteoporosis in rats (Benke et al, 1972). The mechanism by which dietary B6 is thought to be involved in preventing osteoporosis is similar to the story of B6 in preventing heart disease, i.e. via the reduction in blood homocysteine (see above and also the section on folic acid) and the requirement of B6 for cross-linking of collagen and elastin in connective tissues. That is to say, in bone the B6 is needed for the correct formation of a healthy organic matrix in the bone.

6.7.11 Pregnancy Sickness

There is modest evidence that B6, 30mg/day, can relieve pregnancy sickness in a proportion (only) of expectant mothers with this trouble (Vutyananich et al, 1995). However, ginger seems to produce more benefit and the two things may be used together.

6.7.12 Premenstrual Syndrome (PMS)

Perhaps the most widespread use of B6 is for PMS. At an intake of 50mg/day it is highly prized by many women who are convinced that in their own case it relieves the condition. Clinical trials are also mostly positive (Kliejnen, 1990) but some are not. The reason for this may be that women who do not respond lack other nutrients needed for the phosphorylation of the pyridoxal and therefore require a wider spread of supplementary nutrients, or else need to be given injections of the phosphorylated form. One is tempted to speculate that any efficacy from B6 may be connected with the effect of B6 upon the binding of oestrogens to their nuclear receptors (refer to the section above on enzymes requiring B6).

Note that although there are 12 different medical conditions discussed in this section, you are advised not to get into placing too much importance upon what we may call “symptomatic” nutritional therapy, i.e. giving patients a list of nutrients that have been connected with the particular symptom. Always view B vitamins, individually and conjointly, as agents of naturopathic change. In supporting energy production and the synthesis of proteins, they
generally strengthen the metabolic competence of cells and therefore tend to reverse “general chronicity”.

6.8 Drug and Nutrient Interactions

The drug theophylline depresses PLP levels: birth control pills or premarin have an anti-B6 action and the food colorant tartrazine has a similarly very strong effect. The evidence for these connections can be found in Murray (1996) pp103-105. Anti-vitamin effects are also clearly noted with the drugs isoniazid, penicillamine, corticosteroids and/or anticonvulsants (Groff et al, 1995, p282). The same is true of the vasodilator drug hydralazine, the Parkinson’s disease drug L-dopa, oral contraceptives and the monoamine oxidase inhibitor drug phenelzine.

Among other nutrients B6 is positively supported by magnesium and riboflavin (co-factors for conversion to PLP). In the control of blood homocysteine B6 interacts with both B12 and folic acid, as noted above, with consequences that impinge upon both coronary heart disease and osteoporosis.

Note the interaction between B6 and zinc that was published and discussed in detail by Pfeiffer (1975). Or see back to the zinc section for this.

6.9 Use of Supplementary B6 in Nutritional Medicine Practice

We usually have no need to administer supplementary B6 as a single nutrient, but rather we give it as a Vitamin B complex or in composite formulae of vitamins and minerals. Our “standard” therapeutic daily intake is 25mg/day. Therefore the usual maximum that we might give by mouth is 50mg as happens when the “standard” intake is doubled for reasons connected with the intensity of an individual patient’s therapy. These levels are quite high and, it must be admitted, fairly arbitrary. Giving only a tenth of these levels would provide generously for any known and recognised nutritional requirements. We prefer to opt for a generous supply of B6 for therapy purposes provided 50mg/day has not been exceeded.

As you have seen, some of the 12 medical conditions listed above have been treated on occasion with B6 intakes above 50mg/day. We cannot rule here against such intakes, especially since they often appear to have been effective, but we do not advocate them either. This is because B6 is one of the few vitamins to have been associated with negative effects when given at high intakes. Any action you take in this regard should be taken in a carefully considered ethical manner, taking into account the toxicity issue to be raised below. However, Shrimpton (1995) concluded that in the case of B6 “supplementary daily intakes up to 200mg are safe”.

6.10 Toxicity Issues

Notwithstanding the above favourable conclusion by Shrimpton, B6 is not entirely devoid of toxic effects. Adults ingesting 2 to 6g of pyridoxine/day have been reported to suffer from sensory and peripheral neuropathy (i.e. damage to the nervous system, at least on a temporary basis. Some symptoms include unsteady gait, numbness of hands and feet, impairment of tendon reflexes and areas of sensation loss. Really massive doses for a long time can even cause demyelination of nerves and degeneration of the ‘dorsal root ganglia’ of the spinal cord. These effects are certainly real. On the other hand the use of daily supplements of B6 much closer to the RNI (e.g. 2-10mg/day) are agreed by all to be acceptable. As nutritional practitioners we can easily agree that the daily administration of gram quantities of B6 is hazardous. Somewhere between 10mg/day and 200mg/day the possibility of some level of nerve damage comes into play. Most practitioners and scientists involved appear to believe that 50mg/day is extremely safe and that the possible benefits from such an intake far outweigh any slight risk. The only work that indicates that such modest intakes are hazardous has been widely subjected to criticism for lack of scientific rigour. The consensus seems to be that this work is unacceptable as evidence. Nonetheless regulatory bodies wanting to forbid the use of amounts greater than 10mg/day have generated much pressure. We think that is unreasonable.

Much of the scientific community considers that this pressure is absurd and baseless and this has led to a confrontation. It would entirely thwart the numerous women who wish to use the vitamin for relieving PMS. This pressure has been so at odds with the evidence that regulatory bodies involved have been suspected by some of wanting to suppress the use of nutritional supplements for their own reasons unrelated to the real issue of safety. Their most serious failure has been to omit to attach weight to the immense amount of good that such supplements can do. Intakes of over 50mg/day may not be quite as safe from the possibility of mild neuropathy although there is a great deal of opinion, in accord with Shrimpton, believing that up to 200mg/day is very safe. No one knows at this stage the precise intake threshold at which the risk of neuropathy first appears. It is clear from the literature evidence quoted above that intakes between 50mg and 200mg/day can produce much benefit in certain clinical states. Anyone, physician or otherwise, wishing to employ that range of intakes, should rather carefully balance the risk (however slight) against benefit to be expected and then monitor the patients for any adverse effects.

The mechanism of adverse action at high intakes is not known. However, it is possible that at high intakes the ability of the body to phosphorylate the vitamin is exceeded, resulting in an excess of unphosphorylated forms. It may be that these are responsible for any damage that occurs.
6.11 Absorption, Storage and Excretion

Absorption of B6 from average American diets has been estimated at 71-82% of the intake. In the section on active forms of the vitamin we have stated that the phosphorylated forms must be dephosphorylated before being absorbed. Pyridoxal phosphate is the principal form found in the blood, but this must also be hydrolyzed back to simple non-phosphorylated pyridoxal before it can be taken up into cells. Much of any dietary intake of pyridoxine is excreted in the urine as the oxidized form, pyridoxic acid. The body does not maintain any identifiable store of B6 separate from that which is in use by the tissue enzymes.
6.12 References

7 FOLIC ACID

Folic acid is an unnumbered member of the B Group. It went through a chequered history during which it was referred to as “the Wills factor”, Vitamin M, Vitamin Bc and the “Lactobacillus casei factor”. Its functional roles are strongly associated with those of Vitamin B12. These functions at the molecular level are very complex and they could well fill up a lot of space with explanations of their biochemistry. For nutritionists who are never going to be biochemists the decision has been made to curtail these involved explanations and to focus instead upon the key roles of the vitamin. Nonetheless, your textbook, Garrow et al, nonetheless offers explanations of considerable complexity on pages 272-273 (10th Edition). Frankly the decisions to be taken by practitioners do not gain very much from the detailed biochemistry, so we have limited the learning requirements.

7.1 Chemical Structure

The chemical structure is given below.

\[
\begin{align*}
\text{N} & \quad \text{C} \quad \text{OH} \\
\text{H}_2\text{N} & \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{CH}_3 \\
\text{N} & \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{H} \\
\text{NH} & \quad \text{CO} \\
\text{NH} & \quad \text{CH} \quad \text{COOH} \\
& \quad (\text{CH}_2)_2 \\
& \quad \text{COOH}
\end{align*}
\]

pteridine \quad p\text{-aminobenzoic acid} \quad \text{glutamic acid}

\[\begin{align*}
\text{6-Methylpterin} & \quad p\text{-Aminobenzoic acid} & \quad \text{Glutamate} \\
\text{Pteroic acid}
\end{align*}\]

\[\text{Pteroylglutamic acid (folic acid)}\]

This shows that its molecule is composed of three parts. The first, 6-methylpterin, is a heterocyclic compound of two fused rings containing two nitrogen atoms in each ring, as well as the hydroxyl, methyl and amino substituents. The second is p-aminobenzoic acid. This is rather simple, being a benzene ring with an amino group and a carboxyl group in opposite (para) positions. The third component, now well known to you, is the acidic amino acid glutamic acid. The first two components linked together form pteroic acid. This name accounts for the chemical designation of the vitamin itself as pteroylglutamic acid.
7.2 Active Forms

Folic acid is not present in significant quantity in the human body or in foods in the chemical form represented above, though supplements are nearly always presented in the free folic acid form. In the body it occurs in various metabolically active reduced forms (reduced by the addition of hydrogen). Particularly prominent among these is tetrahydrofolic acid (formerly called “folacin”). Garrow et al present the structures of these reduced forms on p 272, though if you want to study them closely you should note that there has been a typographical error in the formula for tetrahydrofolic acid. The addition of the four hydrogen atoms should have the effect of abolishing two double bonds in the left-hand ring of the 6-methylpterin, but that point has been missed and you will find that the formula as represented does not obey the valency rules.

The other reduced coenzyme forms on p272 in Garrow et al represent combinations of tetrahydrofolic acid with various forms of 1-carbon units. This reflects that fact that the role of folic acid in metabolism is very much connected with the transfer of 1-carbon units among other substances. There is no need to memorise these active forms. If you want a simple way to remember the active form, then it can be taken as tetrahydrofolic acid and the conversion into the active form is a 2-step reduction reaction, enzymically catalyzed.

However, there is another way in which folic acid and its co-enzyme forms are modified in tissues and in foods. That is through combination with further molecules of glutamic acid. Note that folic acid or folacin contain one glutamic acid residue as part of the structure, but the further glutamic acid residues add on to that one glutamic acid to form strings of attached glutamic acid moieties. For example a version with two extra glutamic acid residues attached is called pteroyl-gamma-triglutamate. These additional glutamate residues can grow to 8 or even 11 in number. Apparently their addition to the molecule enables the tetrahydrofolic acid to bind more firmly to its dependent enzymes and to resist the tendency for it to be lost from the cell. The polyglutamate forms have to be hydrolysed before they can be absorbed from food.

7.3 Enzyme Systems that use Tetrahydrofolic Acid

The enzymes that use versions of tetrahydrofolic acid coenzymes are those that incorporate single carbon units into the synthesis of purine nucleotides, thymine nucleotides and methionine. The purine nucleotides may be used in the synthesis of RNA and DNA and may comprise both guanidylc acid and adenylic acids. These may also be employed as metabolic co-factors themselves, including high-energy phosphate compounds such as the all-important ATP. Thymidine goes to form DNA. Therefore folic acid deficiency slows DNA synthesis. Inducing a form of folic acid deficiency artificially is one of the strategies in the chemotherapy of cancer. Drugs similar to folic acid and yet different from it are given to block folic acid pathways by competitive inhibition, deliberately blocking the synthesis of DNA. That is obviously toxic to the normal cells of the human body as well as to the cancer cells, but the treatment philosophy is one of “selective toxicity”, relying upon the cancer cells being more in need than normal cells of producing a lot of DNA quickly. The other pathway needing folic acid coenzymes is the synthesis of methionine from homocysteine.
The key folate-dependant enzyme in thymine nucleotide synthesis is “thymidylate synthetase”. The one involved in methionine synthesis is “homocysteine methyltransferase”, also called “methionine synthetase”.

The key folate-dependant enzyme in purine nucleotide synthesis goes by the rather formidable name of “5-aminomidazole-4-carboxamide ribonucleotide transformylase”, or “AICAR transformylase”.

We have named the enzymes involved as users of the single-carbon units carried on folic acid co-enzymes, but there are about 10 more enzymes involved in generating the tetrahydrofolate-1-carbon unit attachments (i.e. in generating the various forms of reduced folate co-enzymes listed by Garrow et al).

As already stated we prefer to take a broad-brush approach to studying these reactions. It really is sufficient to note that folate and its co-enzyme forms are needed for the production of purine and thymine nucleotides and methionine. The main metabolic consequence of folate deficiency is to limit synthesis of nucleotides and nucleic acids and to inhibit the generation of “active” methyl groups via methionine. This latter is important since the active methyl groups of methionine are needed to transfer on to such methyl containing compounds as choline.

S-Adenosylmethionine is the direct donor of the methyl groups in many of these reactions, a role that gives it a very central role in metabolism. These interrelationships are summarized in the diagram below.

For those who wish to follow up the “homocysteine story” in more detail we have prepared an expanded bibliography in Appendix 7.
You may take note here of the involvement of the important intermediate “S-adenosylmethionine”. Although this is in no way a recognised essential nutrient, this substance has found its way into nutritional therapy. As time goes on more and more key metabolic intermediates are found to have beneficial effects when administered to people who suffer from symptoms or labeled illnesses. We shall look more closely at the use of such substances in level II of the Course.

### 7.4 Human Requirements for Folic Acid

The FAO / WHO Expert Group of 1987 recommended a daily intake for adults of 3.1mcg / kg of body weight. This translates into 200mcg / day for a 65kg man and 170mcg / day for a 55kg woman. These amounts will build up stores sufficient to prevent deficiency after 3 to 4 months of zero intake. In the UK the same level (200mcg / day) has been set for men and women, with the woman’s lesser body weight being offset by greater needs for reproduction. The female adult was increased to 300mcg / day during pregnancy. An intake of 50mcg / day has been recommended for formula-fed infants.
7.5 The Prospects of Meeting the Folate Requirement from Foods

Median folate intakes in the UK have been reported as 300mcg by men (range 145-562mcg / day) and 209 mcg / day by women (range 95-385mcg / day). This indicates that adequacy of the diet in folate is the usual experience although a small group of the population suffers from the misdistribution of supply.

With an average of 99mcg / 100g of food dry matter and an estimated 500g / day of solids in the average diet, a value of 495mcg / day intake should be achieved if the diet were a wholefood one, based upon eating all these foods as equal contributors to the diet. We see, however, that vegetables, pulses and liver make disproportionate contributions. As usual though, severely wrong choices could contribute to deficiency, since fat and sugar contain no folate and white four is reduced by 45%. Diets that contain regular intakes of green leafy vegetables are most unlikely to be deficient. On the whole though, only those who make very poor dietary choices should actually become deficient.

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>MEAN FOLATE CONTENT OF IN mcg/100g SOLIDS</th>
<th>FOLATE CONTENT OF THE HIGHEST OF THE GROUP IN mcg/100g SOLIDS</th>
<th>FOLATE CONTENT OF THE LOWEST OF THE GROUP IN mcg/100g SOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHOLE CEREALS</td>
<td>45</td>
<td>Rye flour 92</td>
<td>Pearl barley 22</td>
</tr>
<tr>
<td>DAIRY</td>
<td>60</td>
<td>Cottage cheese 129</td>
<td>Goats’ milk 9</td>
</tr>
<tr>
<td>FATS</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FISH</td>
<td>50</td>
<td>Salmon 81</td>
<td>Herring 14</td>
</tr>
<tr>
<td>FRUITS</td>
<td>91</td>
<td>Raspberries 254</td>
<td>Eating Apples 6.5</td>
</tr>
<tr>
<td>MEATS</td>
<td>21</td>
<td>Chicken 47</td>
<td>Breast of lamb 6</td>
</tr>
<tr>
<td>OFFALS</td>
<td>-</td>
<td>Lamb Liver 673</td>
<td>Lamb Heart 8</td>
</tr>
<tr>
<td>NUTS</td>
<td>46</td>
<td>Hazelnuts 75</td>
<td>Coconut 9</td>
</tr>
<tr>
<td>SEEDS</td>
<td>-</td>
<td>Sesame 102</td>
<td>Pumpkin &amp; Squash 0.1</td>
</tr>
<tr>
<td>SEAFOODS</td>
<td>57</td>
<td>Shrimps 75</td>
<td>Squid 13</td>
</tr>
<tr>
<td>PULSES</td>
<td>203</td>
<td>Black-Eye Beans 706</td>
<td>Butterbean 14</td>
</tr>
<tr>
<td>VEGETABLES</td>
<td>615</td>
<td>Endive 2400</td>
<td>Garlic 14</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>99</td>
<td>386</td>
<td>9.6</td>
</tr>
</tbody>
</table>
7.6 Severe Folate Acid Deficiency

7.6.1 Megaloblastic anaemia

One of the main recognised effects of deficiency of folic acid is a form of anaemia that is accompanied by oversized red blood cells. This is called megaloblastic anaemia, or megaloblastosis. It bears a relationship to the anaemia of pernicious anaemia that comes from Vitamin B12 deficiency, though obviously the cause is different. The precursor cells of the red blood cells in the bone marrow become oversized. They have large abnormal multi-lobed nuclei. In time these emerge into the circulating blood. Even the blood platelets assume giant size. This is almost certainly associated with the inhibition of new DNA formation due to the effect of folate deficiency upon purine nucleotide and thymine nucleotide synthesis. Other tissues with high rates of cell division also suffer adverse effects and one of these is the intestinal mucosal lining.

7.6.2 Growth

In babies and young children growth is affected. Clearly, shortage of folate affects cell division. At one time it was thought that all the effects of folate deficiency could be ascribed to inhibition of nucleic acid synthesis. However, it is now clear that is not the case. A dearth of methylation reactions is more far-reaching in its effects.

7.6.3 Cervical Dysplasia

This is the name for the appearance of abnormal cells in the lining of the cervix. This is a pre-cancerous condition and in recent times has become the subject of regular checks to provide early detection of cancers of the cervix. It is associated with the deficiency of folate and/or inability to use folate in the cells of the cervix. It also has an association with the use of contraceptive pills, which seem to block the access for folate into some cells. Women with cervical dysplasia have low folate in the cervix cells and also in their red blood cells (Butterworth et al, 1992a).

Other risk factors for cervical dysplasia become more hazardous in the presence of a folate deficiency. One example of this is exposure to the human papilloma virus (HPV). On the other hand a high level of folic acid in the cervix cells is very protective against HPV.

Supplementation with folic acid has provided regression or normalization of cervical dysplasia in large numbers of patients, amounting to between 20% and 100% of the patients in different studies (Whitehead et al, 1973, Butterworth et al, 1982a, Butterworth et al, 1992b). Meanwhile women with cervical dysplasia given folate supplementation had a zero progression rate for their condition, even if they were using the contraceptive pill (Butterworth et al, 1992c). For women with cervical hyperplasia not given folate the median time required to progress to carcinoma \textit{in situ} varies between 12 and 86 months depending upon the severity of the hyperplasia when discovered. Regression without the use of folate is very uncommon.
7.6.4 Depression

Folate status is much connected with depression. Between 31 and 35% of depressed patients have been found to be folate deficient (Reynolds et al, 1970, Carney et al, 1990, Godfrey et al, 1990, Crellin et al, 1993). In elderly depressive patients between 35 and 92.6% have been found deficient. It is considered that the role of folic acid in the brain is to enhance methylation (as already described) and thus to improve the formation of the neurotransmitter substances serotonin and dopamine (Reynolds & Strammentsnoli, 1983 and Reynolds et al, 1983). S-adenosylmethionine, which has also been mentioned already, acts in a similar way because it is a powerful methyl donor, and is a very good anti-depressive also. The intakes of folic generally used to influence depression are very high, from 15 to 50mg/day. This borders upon megavitamin therapy, given the low nutritional requirement for folates. However, there is no indication of any lack of safety except in epilepsy and folic acid has been shown to be an effective anti-depressant “drug” (Crellin et al, 1993).

7.6.4 Gout

Some positive results have been reported in the treatment of gout with folic acid (Oster, 1977). However this data is as yet incomplete. The intakes used were 10-40mg/day.

7.6.5 Immune Function

Because of the effect upon blood cell generation in the bone marrow, folic acid deficiency severely affects the immune system, which depends upon high numbers of functional white blood cells. Deficiency leads to thymus gland shrinkage and impaired white blood cell function (Beisel et al, 1981, Dowd & Heatley, 1984, Weiner, 1986).

7.6.6 Osteoporosis

Folic acid is often deficient in the elderly and has been implicated in the pathogenesis of osteoporosis. A deficiency causes defective organic matrix in bone, which then cannot be effectively mineralized. Whilst their greatest influence tends to be in older women, long after the menopause, there is some positive indication for the use of folate for all who are at possible risk of osteoporosis. The role of the folate is thought to be that of reducing the homocysteine levels which, when raised, may interfere with normal bone matrix formation (Brattstrom et al, 1985).

7.6.7 Cardiovascular Disease

Homocysteine (an amino acid that is methionine minus a methyl group) is a metabolite derived in the course of normal metabolism from the breakdown of the amino acid, methionine. Homocysteine, when its blood and tissue levels are elevated, is thought to promote atherosclerosis by directly damaging the artery wall, reducing its integrity. The more homocysteine in the blood the more arterial damage is likely. Elevations of blood homocysteine are found in 20-40% of patients with heart disease (Clarke et al. 1991, Glueck et al. 1995). During metabolism the homocysteine level in the blood is normally controlled.
because this intermediate can be recycled back to form methionine. However, this only happens at a sufficient rate if the conditions in the body are right for active methylation reactions. The individual has an adequate supply of folic acid, Vitamins B6 and B12 and also a supply of "methyl groups, a chemical grouping having the formula –CH₃.

Nutritional deficiencies lead to metabolic imbalances of many kinds, but in this particular case the homocysteine overload can well be life-threatening. The supply of the three vitamins should not be overlooked. Great advantage may be had by the additional use of phospholipids. The choline component of the phospholipids, having three methyl groups per molecule, is a plentiful supplier of methyl groups and hence they can play a key role in reducing the blood homocysteine. In that way, if the subject had a high homocysteine level to start with, then the phospholipids can be expected to afford significant protection against atherosclerosis and therefore coronary heart disease. This conclusion does not come out of any clinical trial but it is a reasonable expectation from our knowledge of homocysteine metabolism, as has been pointed out by Mudd et al (1995).

This then is an important protective role for folate, but it must be seen as a joint effect along with Vitamin B6, B12 and phospholipids. Intakes of folic acid in the region of 400mcg/day are recommended.

7.6.8 Birth Defects

Neural tube defects such as spina bifida can be prevented in large measure by supplementation with folate at the rate of 400mcg/day. Studies showed that supplementation in early pregnancy reduced these defects by between 48% and 80% (Werler et al, 1993, Milunsky et al 1989). This enormous effect was eventually recognised as important by the relevant authorities in the US after the relationship had already been known for 30 years. There is a comment here about the difficulties of making any impact upon orthodox minded national authorities where matters connected with nutritional supplements are concerned.

7.6.9 Other Conditions

Folic acid has been suggested for use in peridontal disease and for restless legs syndrome (35 -60mg/day) and for seborrheic dermatitis (Murray & Pizzorno 1998, pp728, 607 & 795). In these cases the scientific basis is not so clearly established and there is a need for mega-vitamin intakes. In the case of seborrheic dermatitis the best results have been obtained with tetrahydrofolate plus Vitamin B12.

7.7 Absorption, Cell Uptake, Storage and Excretion

Please use the section in Garrow et al (10th Edition), pp272-274. As we see no reason to present this information in any different form here, it is important to read that section.

One can note with interest the key and virtually exclusive role played in intestinal absorption by one from among the reduced coenzyme forms of folic acid listed by Garrow et al. in the Figure on p272. It is one of those forms that have a 1-carbon unit attached: 5-methyltetrahydrofolate.
Under the heading of “Cellular Uptake” in Garrow et al, note that there is an elegant mechanism for controlling the entry of blood folate into cells. You can if you wish study the biochemical mechanism by which that is achieved. It is interesting and impressive but it is not essential to understand it. Note also that there is a role to be played by Vitamin B12 in the process of cellular uptake of folate. This gives us the first clear connection between the actions of these two vitamins.

Please read also the Garrow section on “Catabolism and Excretion” p273. The detailed chemistry of breakdown is not important to us and may be left aside, especially since it is only partly elucidated by research. However, it is more significant to note that non-reduced form of folate usually given as supplements is rapidly excreted, whereas the reduced forms that are characteristic of food folate are well retained. This is a clear indication that food folate is far more nutritionally valuable than the commonest supplementary form. This attaches a special value to supplying folate by diet rather than by supplements. It may also appear to boost the claims of those special “Food Form” or “Food State” folate supplements that pass folate through a biological system, such as yeast, before offering it for use. First, however, one would like to have an analysis of such products to show exactly how much of their folate content is, indeed, in the reduced form.

7.8 Safety in Use

Please read Garrow et al under the heading “Therapeutic uses and toxicity” pp275-276. Of course, the position taken is an orthodox one. Note that intakes of up to 5mg/day (5000mcg) are recommended in specific circumstances even though the general gist of the section is to recognise as safe only intakes of up to 400mcg/day. Shrimpton (1995) expresses this same view in a summary of supplement safety compiled on behalf of the European Federation of Associations of Health Product Manufacturers.

Other sources generally acknowledge the safety of intakes of up to 400mcg/day, or in some cases, as much as 800 or 1000mcg/day. Higher intakes (5-10mg/day) are widely thought of as being justified where there is a specific therapeutic objective, especially in cervical hyperplasia or depression. This implies that any toxic effects are thought of as very minor up to these levels. Any such risk is therefore considered justified by the therapeutic need. High intakes require medical prescription.

Known effects of milligram/day intakes of folate include flatulence, nausea and loss of appetite. They are not particularly dangerous symptoms and they only occur in some cases, not all.

Folate supplements do carry risk in two particular circumstances. One is the presence of epilepsy, which can be aggravated by it. The other is the simultaneous existence of Vitamin B12 deficiency at a level that leads to neurological damage. High folate intake in such a case may mask the Vitamin B12 deficiency while allowing the neurological damage to continue. Safety can be much better ensured by giving folate and B12 together.

Of particular interest is the question as to whether of not food folate could conceivably be hazardous by reason of the level of intake. A kilogram of spinach contains 1500mcg of folate.
It would only represent about 100g of spinach solids. It could certainly be eaten in a day but one is unlikely to choose to do so. According to Garrow it could be a hazard. But even 300g of fresh spinach would deliver 450mcg folate/day, which is still above Garrow's safety limit. However, Garrow et al add on p276 that any toxicity may be associated with the non-reduced folate form and not with the reduced folates of food. That could be a complete 'let out' for diets containing more that 400mcg/day of food folate. Indeed, most therapeutic diets would contain more than that amount.

### 7.9 Use of Supplementary Folic Acid in Nutritional Medicine Practice

Given all the considerations above the best therapeutic strategy is to ensure that diets contain more than 400mcg/day of food folate. In addition the principal mineral / vitamin formulations that we use contain folic acid (even though it is non-reduced). Typically we give about 40mcg/day as an extra insurance policy. Food State folic acid is also available in 400mcg tablets but one should note that we do not know the proportions of reduced and non-reduced forms in this product. Obviously a supplement of reduced folic acid (eg folinic acid) would be a surer and safer way to supplement (Bailey, 1995).

### 7.10 Interactions with Other Nutrients and with Drugs

The principal interactions of folic acid with other nutrients are those with Vitamin B12 as already described, and the interactions with Vitamin B6 and with methyl group sources such as methionine and choline relating especially to the protective effects in cardiovascular disease.

The increase in folic acid excretion in rheumatoid arthritis patients has been linked to intakes of aspirin and other non-steroidal anti-inflammatory drugs. The cancer chemotherapy drugs adriamycin, cisplatin and methotrexate, and possibly others, have an anti-folate effect as does also the bile acid sequestrant drugs cholestyramine and colestipol and the anaesthetic nitrous oxide. The adverse effect on folate from the oral contraceptive have been stressed already. The anti-convulsants phenobarbital and phenytoin. The drug sulfasalazine, used for ulcerative colitis, antagonizes folate (Murray & Pizzorno p597).
7.11 References on Folic Acid

Checkpoint Three

a) Why is vitamin B6 so important to amino acid and nitrogen metabolism?

b) Against what gauge is the RNI for vitamin B6 measured?

c) Which groups of the population are most at risk from vitamin B6 deficiency? What are the deficiency symptoms?

d) Which birth defect can be substantially reduced with folate supplements?

e) Name 7 effects of folic acid deficiency.

f) Why is it advisable for supplements of folate and vitamin B12 to be used together?

Please turn to the end of this part to check your answers
8  VITAMIN B12

Vitamin B₁₂ was discovered through studies of a formerly incurable disease, pernicious anemia. This condition begins with a megaloblastic anemia, which is virtually identical to that seen in folate deficiency but which leads to an irreversible degeneration of the nervous system if it is untreated. In 1926 two Harvard physicians, George Minor and William Murphy, found that symptoms of the disease could be alleviated by feeding patients large amounts of raw liver. The active material in the liver, which was named vitamin B₁₂, was present in exceedingly small amounts, so many years passed until sufficient material had been isolated for characterization. In 1964 Dorothy Hodgkin and her colleagues, in England, used X-ray crystallography to complete the structure determination for this active substance. Hodgkin was awarded the Nobel Prize for this work.

The action of Vitamin B₁₂ is closely related to that of folic acid and both vitamins are involved together in the metabolism of 1-carbon units. This vitamin is not synthesized by plants and hence its supply represents potentially a crucial problem to vegans. It is not generally a problem to those who simply do not eat meat, since dairy products and fish will provide for one’s needs. It is also produced by microorganisms. This makes yeast, which is a modest source, available to vegans and the vitamin is also found in fermentation products, especially those that still contain the organisms that carried out the fermentation. Therefore tofu can be a vegan B₁₂ source, as can the rootlets of sprouting seeds of the Leguminosae – the pea and bean family – on account of the Rhizobia bacteria that live symbiotically in the roots of these plants.

Nutritional Medicine practitioners do not have to take too many treatment decisions about Vitamin B₁₂. However, you need to take note that many therapeutic diets are either vegan or nearly so. We overcome the problem in most cases by providing a combined formula that contains a “B-Complex” component that includes B₁₂, typically about 25 mcg/day. One needs to be alert to the possible hazard of long-term vegan diets in combination with supplement programmes that exclude such a formula. In those cases microbial products can make a real difference, but you do have to remember to provide them explicitly in the dietary guidelines.

The daily requirement for this vitamin is very small. The lack of B₁₂ deficiency in Indian vegans had long been noted. Then it was found that Indian vegans are “let off the hook” of B₁₂ deficiency by the imperfect hygiene conditions affecting the storage of their vegetable-derived foods. These foods, many of them sun-dried in the open, contained sufficient insects to provide the B₁₂ requirement. Hence these people were found to be to a very minor extent, accidentally non-vegan.

There has long been a theory promulgated by vegan enthusiasts, to the effect that, after all, plants do synthesize B₁₂ and that plant foods are, indeed, a source of B₁₂. No satisfactory scientific support has been found for such a theory, which appears to be a piece of quite dangerous “wishful thinking”. Any tiny amounts of B₁₂ that have been found associated with plant material are so small as to have been derived from microbes on the plant surface or with residues of soil splashed onto crops by rain. Soil contains both microbes and microbial products. These amounts are insufficient to be useful and are often close to the limits of detection. Another theory that may also be associated with “wishful thinking” is the one that proposes that intestinal bacteria synthesize enough B₁₂ to meet our requirements. There is
no doubt that these bacteria do synthesize B12 and they may well synthesize enough for our purposes. What is in doubt is whether it, or sufficient of it, is available for absorption. What evidence there is on this point suggests that the B12 may be “locked” into bacterial cells and hence be unavailable. Intestinal bacteria can be significant in the supply of certain other B vitamins.

8.1 Chemical Structure

The detail of the fairly complex chemical structure is given in Appendix 8. At the core of the structure is a porphyrin type ring similar to (but not identical with) the rings of haem and of chlorophyll. This is known as the “corrin” ring. At the centre of the ring is a single cobalt atom. This represents the only known function for cobalt as a trace element in human nutrition. Another part of the structure is a heterocyclic portion with a five-membered and a six-membered ring fused together. There is a ribose sugar unit and the side-chains attached to the corrin ring bear several nitrogen atoms of the “amide” type in which the –NH₂ group is substituted into a carboxyl group. Because of the presence of cobalt and also the multiple amide nitrogens, B12-derived compounds have been called “cobalamines”.

8.2 Active Forms

The Vitamin as isolated from natural sources is a form in which a “spare” attachment position on the cobalt atom is occupied by a cyanide group, -CN. This form is “cyanocobalamine”. The actual intracellular forms that participate in enzymic reactions are “methylcobalamine” and “deoxyadenosylcobalamine”.

8.3 Enzyme Systems that use Vitamin B12-derived Co-Enzymes

In mammalian systems, and therefore in humans, there are only three enzymic reactions known to require these co-enzymes. These are:

1. Synthesis of methionine from homocysteine, as detailed in the section on folic acid (methionine synthetase).
2. Conversion of methylmalonyl co-enzymeA into succinyl CoA: this is a reaction needed for the breakdown of those more rare fatty acids that have odd-numbered chain-lengths and the breakdown of the amino acids methionine, isoleucine and threonine (methylmalonyl-CoA transmutase).
3. Breakdown of the amino acid leucine and a product of the intestinal bacteria, beta-leucine (leucine aminotransmutase).

The first of these uses methylcobalamine as a co-factor and the second and third use deoxyadenosylcobalamine.

Understanding the way in which folic acid and Vitamin B12 co-enzymes work together is the subject of some quite complex biochemistry. There is no real need to probe into all of that. A more broad and general understanding can be stated as follows. Folic acid and Vitamin B12
Co-enzymes are both directly involved in methionine synthesis. Consequently in Vitamin B12 deficiency methionine synthesis is restricted but there is usually sufficient dietary methionine to avoid serious upset to protein metabolism. However, this Vitamin B12 deficiency nonetheless upsets the balance between the various different reduced co-enzyme forms of folic acid. This disturbance to the balance among the reduced folic acid co-enzymes has knock-on effects that in turn restrict the synthesis of nucleic acid precursors.

The combined involvement of folic acid and Vitamin B12 co-enzymes in methionine synthesis can be summarized in the following diagram:

These details do not explain, of course, why it is that Vitamin B12 deficiency has such serious neurological effects. One suspected mechanism is via the enzyme methylmalonyl-CoA transmutase (above). The brain tissue has a high content of lipid, especially phospholipids. These have a much wider range of structural types than phospholipids in the rest of the body and they contain many unusual fatty acids. Since these can include fatty acids of odd-numbered chain-lengths, Vitamin B12 is needed for their breakdown. Based upon this hypothesis, it is supposed that the neurological defects of B12 deficiency are due to adverse effects upon the composition of the brain’s unusual lipoidal constituents.

However, Garrow et al (10th Edition) on p278 favours an explanation for the neuropathy that depends upon a deficiency of methylation capacity, supposing that this capacity is required to synthesize important brain lipids. In either event, the brain lipids are seen as the most likely focus of the malfunction.
8.4 The Human Requirements for Vitamin B12

In the UK the reference Nutrient Intake (RNI) for Vitamin B12 was set in 1991 at 1.5mcg/day for adults, either male or female. This was based upon published observations upon vegan groups in Australia, Sweden and India, showing that they both survived and avoided megaloblastic anaemia on daily intakes of only 0.25-0.65mcg/day. The figure of 1.5mcg was chosen to allow for individual variation of requirement and to permit people to build up body stores of the Vitamin to enable them to survive any period of zero intake. Children are considered to require 0.3mcg/day in infancy, advancing with the years towards the adult intake.

8.5 Meeting the Vitamin B12 Requirement from Foods

As already stated, the only foods that contain Vitamin B12 are animal-derived foods and microbial foods. The following Table shows the levels of the vitamin in animal foods and the amounts of these foods needed to supply the current UK RNI.

<table>
<thead>
<tr>
<th>FOOD CLASS</th>
<th>B$_{12}$ in mcg/100g dry matter</th>
<th>Amount of the food dry matter needed to provide the RNI (g)</th>
<th>Amount of the fresh food needed to provide the RNI (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meats (average)</td>
<td>3.09</td>
<td>48.5</td>
<td>135</td>
</tr>
<tr>
<td>‘Best’ meat – stewing steak</td>
<td>6.39</td>
<td>23.5</td>
<td>75</td>
</tr>
<tr>
<td>Offal – Lamb heart</td>
<td>32.8</td>
<td>4.5</td>
<td>19</td>
</tr>
<tr>
<td>Offal – Lamb kidney</td>
<td>260</td>
<td>0.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Offal – Lamb liver</td>
<td>257</td>
<td>0.58</td>
<td>1.8</td>
</tr>
<tr>
<td>Fish (average)</td>
<td>12.42</td>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>‘Best’ fish - mackerel</td>
<td>27.8</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Seafood (average)</td>
<td>28.3</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>‘Best’ seafood (mussels)</td>
<td>105</td>
<td>1.4</td>
<td>7</td>
</tr>
<tr>
<td>Dairy (average)</td>
<td>2.63</td>
<td>57</td>
<td>322</td>
</tr>
<tr>
<td>‘Best’ dairy – milk (skimmed cow’s)</td>
<td>4.5</td>
<td>33</td>
<td>374</td>
</tr>
<tr>
<td>Eggs</td>
<td>10</td>
<td>15</td>
<td>60</td>
</tr>
</tbody>
</table>
These figures show that in diets with significant intakes of these foods, there will be little or no chance of developing dietary deficiency of the vitamin. For example, one egg per day will provide more than the RNI. Lactovegetarians usually obtain ample supplies from dairy products and perhaps also eggs.

As we have said, strict vegans will be dependent upon microbial products. Unfortunately even yeasts and fermented products such as wine contain only a trace of B12. Strong ale will yield 0.4mcg/100ml, so “half a pint” daily (280ml) could bring a vegan to near adequacy at an intake of 1.12mcg/day. However, most ordinary beers provide less. However, the position of vegans, especially vegans who take no beer, is not secure. Many of them may well be living within the rather generous margin that exists between the very low intakes that give rise to megaloblastic anaemia and the RNI. Tofu and sprouted leguminous seeds may also help when these are used.

8.6 Severe Vitamin B12 Deficiency

Inadequate absorption of the vitamin rather than inadequate dietary intake is responsible for some 95% of the B12 deficiency states seen in N. America and Europe. Although, as we have seen, a strict vegan diet may produce deficiency, clinical symptoms may not appear for 20 or 30 years on such a diet (Davis 1984). An exception would be an infant or very young child maintained on exclusively vegetable-derived foods without supplements.

Vitamin B12 deficiency occurs in stages:

- Serum concentrations diminish
- Cell concentrations also diminish
- Biochemical deficiency occurs, with less DNA synthesis, raised homocysteine and Methylmalonic acid in the serum
- Finally anaemia occurs.

This means, of course that subclinical deficiencies cannot be diagnosed without biochemical tests. The best policy for nutritional therapists is to ensure adequate intakes of the vitamin for patients at all times. Those that do not have animal-derived foods are best given supplements of B12, which is available from the combined formulations. Then, any sign of actual anaemia should be investigated. In particular, anaemias not responding to iron and other nutritional supplements require a laboratory investigation.

The megaloblastic anaemia of Vitamin B12 deficiency is not different from that of folic acid deficiency. The other major disease state that arises from deficiency is the B12 neuropathy, which is also called subacute combined degeneration (SCD).

The signs and symptoms of Vitamin B12 come from inadequate formation of the myelin sheath around the nerves. The myelin is composed of lipoprotein and this gives us the connection between the disease and the brain lipids. They are:
- Numbness and tingling in the hands and feet
- Diminished vibration sense and position sense (commonly occurring first in the ankles and feet)
- Unsteadiness, poor muscular co-ordination with ataxia (walking difficulties)
- Moodiness, mental slowness and poor memory
- Confusion, agitation and depression
- Dim vision (sometimes)
- Delusions, hallucination and even overt psychosis

Many of these mental symptoms are distinguishable from forms of senile dementia only by their ready response to treatment with Vitamin B12.

Raised levels of homocysteine are connected, as we have already seen, with increased risk of coronary heart disease (CHD). Hence B12 deficiency poses a threat of CHD.

Another biochemical manifestation of B12 deficiency is reduction of the tissue levels of reduced glutathione. Since that is a parameter of the ability of the cells to detoxify, this could be expected to have consequences for the body’s ability to process and excrete its toxic burden.

There is a very important warning that all nutritionists should note. Folic acid administration can dispel the symptoms of a megaloblastic anaemia caused by B12 deficiency. However, folic acid does nothing to correct the myelination problems that arise from B12 deficiency. Danger for the patient arises if folic acid is given and the megaloblastic anaemia is corrected by folic acid alone, because this leaves the neurological symptoms and damage associated with Vitamin B12 to continue to develop unabated. Hence practitioners must avoid this pitfall.

8.7 Absorption, Cell Uptake, Storage and Excretion

Absorption is a critical factor in the body's handling of Vitamin B12. Recall that the vitamin was first isolated as a factor that could cure pernicious anaemia. However, pernicious anaemia is a disease of the stomach. Gastric tissue (parietal cells) secretes a glycoprotein called “intrinsic factor” (IF), which complexes with ingested B12 and promotes its absorption through the small intestine. Pernicious anaemia is the outcome of insufficient production of IF from the stomach. The commonest reason for the trouble is an auto-immune attack upon the cells of the stomach responsible for producing intrinsic factor, but the same disease can arise from surgical removal of the stomach. The uncomplexed vitamin can be absorbed but so poorly that massive doses must be given to cure or prevent the disease. Alcohol acts against B12 absorption.

Cobalamin in the diet are in the form of combinations with proteins that must be hydrolysed to yield cobalamin in the free form. The free forms combine with other proteins produced from the stomach (called ‘R proteins’) to be conveyed through to the intestine. There the R proteins are digested by pancreatic enzymes. The free cobalamin then bind to IF to be transported across the intestinal wall. The receptors for B12 are present only in the ileum, especially the distal third of it. Therefore the IF-cobalamin complexes must travel almost the
length of the small intestine. Absorption is slow and peak levels in blood are not reached until 8-12 hours after ingestion.

Vitamin B12 is transported in the blood attached to transporter proteins called “transcobalamines” I, II and III. These are related to the R proteins of the gut. They perform different functions of B12 transport. Transcobalamine II is the one that attaches, in the main, to the newly absorbed vitamin. About 60-80% of the B12 in the blood is methylcobalamine and about 20% is adenosylcobalamine.

Excretion is via the faecal route due to B12 being passed out in the bile. Indeed, there is apparently a quite strong enterohepatic circulation of B12. Any interruption of the re-absorption of biliary B12 from the intestine results in a major increase in B12 requirements. There appears to be little or no metabolic breakdown of B12.

This vitamin, unlike other water-soluble vitamins, can be stored and retained in the body for long periods. These periods may be measured in years. Hence, upon transferring from a B12 adequate diet to a virtually B12-free diet, many years ensue before any trouble develops. Storage is mainly in the liver, with lesser amounts in muscle, bone, kidneys, heart, brain and spleen. Adenosylcobalamine is the primary storage form in the liver. The transport forms in the blood are in equilibrium with these stores.

8.9 Supplementary Vitamin B12 in Nutritional Practice

There are no records of hazard associated even with quite large amounts of B12 ingestion, so our use of the vitamin is not limited by any toxic considerations. With many good therapeutic diets that contain fish there will be little to worry about concerning B12 because, as we have seen, only 51g fresh weight of fish daily (or 153g every 3 days) will provide the full RNI. Vegan diets will always require to be supplemented, at least in the long term, though one may rely upon the liver stores of those patients who were previously non-vegan for a considerable period. The main composite formulae we use contain 25mcg/day in a standard intake – a very generous supplement. Plenty of other single-vitamin generic B12 supplements exist but we rarely have need to call upon them.

8.10 Interactions with Other Nutrients and with Drugs

Please see the connections given by Garrow et al (10th Edition) p278 to the drugs colchicine, neomycin, p-aminosalicylate, cimitidine, ranitidine and omeprazole, that all cause B12 malabsorption. Note also the connection with the anaesthetic nitrous oxide, which inhibits B12 action. The principal nutrient interaction of B12 is that with folic acid.

8.11 Reference

Otherwise please make use of the references provided by Garrow et al (10th Edition) throughout the section on Vitamin B12 pp276-280.
9 Biotin

Biotin is a true B group vitamin, yet it receives scant attention. It was discovered originally as part of a complex that promoted the growth of yeast. In orthodox nutrition circles it ‘suffers’ from the fact that it is hard to produce a clear-cut human deficiency syndrome associated with biotin. This is a certain factor to direct orthodox attention away from a vitamin as having any nutritional significance. Yet we re-quote here what we said above under the heading of pantothenic acid “Orthodoxy is just not well orientated to take in the fact that in the absence of adequate amounts of such a vitamin the cell simply does not work so efficiently”. Biotin plays key metabolic roles within the cell just as pantothenic acid does. Regardless of the existence or not of any human deficiency syndrome, it is inherently reasonable to suppose that in the absence of optimal amounts of it human cells will function less well in a general sense and supplies of metabolic energy may well be restricted, resulting in a generalized “chronicity”. This is a hypothesis rather than a proven fact, but it is one that fits in well with other biochemical findings relating to B vitamins and metabolism.

The ability to produce in humans experimentally any form of syndrome deficiency associated with biotin depends upon the rather artificial approach of feeding diets high in raw eggs. These contain a ‘avidin’ that binds biotin making it unavailable. This produces a deficiency condition that is not necessarily linked in any way to low dietary intake, although simultaneous dietary depletion obviously speeds up the appearance of the deficiency.

The ‘status’ of biotin as a nutritionally important vitamin is also undermined by the knowledge that it is produced by the intestinal bacteria and may be absorbed from the low gut (Bowman & Rosenberg, 1987, Noda et al 1994). The heavy production of the vitamin by the bowel flora is emphasized by the fact that its rate of faecal excretion is 3-6 times the dietary intake. Yet no one knows the extent of effective absorption of the bacterially generated vitamin through the intestinal wall. This unknown contribution obviously throws some doubt onto the importance of the orally ingested vitamin.

When deficiency is induced with avidin the symptoms are a fine scaly dermatitis with hair loss. The hairs are particularly affected, with loss of the sebaceous glands at the base of the hairs and atrophy of the hair follicles. The condition can amount to a seborrheic dermatitis in which there is excessive oiliness and dandruff. In infants this is known as “cradle cap” (Nisenson, 1957). Also there may be depression, hallucinations, muscle pain, local paraesthesia (numbness), anorexia and nausea.
9.1 Chemical Structure

Biotin is a 2-ring heterocyclic compound containing both nitrogen and sulphur within the rings. As it is pictured below the left-hand side of the formula resembles urea and the right-hand side of the ring structure resembles dimethyl sulphide. The formula as a whole is based upon a 9-carbon acid of which the first 5 carbons constitute the biotin side-chain (valeric acid side-chain).

\[
\begin{align*}
\text{O} & \quad \text{NH} \quad \text{CH} \quad \text{CH}_2 \\
\text{S} & \quad \text{NH} \quad \text{CH} \quad \text{CH}-(\text{CH}_2)_4-\text{COOH}
\end{align*}
\]

Biotin is often found in foods in the combined form “biocytin”, which is a combination between biotin and the amino acid lysine. However, biocytin is not exactly an “active form”. The biotin produces its co-enzyme effects in a form in which it is directly combined with an enzyme protein. However, when it combines with the protein it does so via a lysine residue. It clearly has a strong affinity for lysine.
9.2 Enzymes Requiring Biotin

Biotin-requiring enzymes are enzymes that catalyze reactions that involve the addition of or removal of carbon dioxide (CO₂) to or from substrate molecules. These include pyruvate carboxylase, which adds CO₂ to pyruvate to form oxaloacetate. This enzyme, by producing oxaloacetate, replenishes the intermediates of the citric acid cycle (Krebs cycle) and, through that mechanism it has a controlling influence upon the rate of oxidative metabolism. Also, working in reverse, it promotes gluconeogenesis. Another such enzyme is acetyl CoA carboxylase, which has the effect of committing acetate units towards the de novo synthesis of fatty acids. Still further enzymes of the group allow for the breakdown of odd-numbered fatty acid chains and of the amino acids isoleucine, threonine and methionine. These are the same reactions that also involve B12, though a different enzymic stage is affected. A fourth enzyme of this group provides for the breakdown of leucine. These 4 processes make biotin important in metabolism.

9.3 Human Requirements

Due to the uncertainties given above, no one is sure about the dietary requirement. The average intake by British men is given by “The Dietary and Nutritional Survey of British Adults” (Gregory et al 1990) as 39mcg/day and for women 26mcg/day. These values are considered to protect against deficiency and although no RNI has been set, a value as low as 10mcg/day is considered safe and adequate.
9.4 Biotin in Foods

With so many unknowns about biotin requirement, assessment of the exact quantities obtainable from foods assumes lesser importance because exact information cannot be used to good effect. We give only the mean biotin content of the main food classes. These are certainly generous amounts in relation to the estimated safe intakes. For example, if you were to eat a diet containing 500g of food solids/day, equally divided between these classes, all in "whole food" form, you would take in 85mcg/day.

<table>
<thead>
<tr>
<th>FOOD</th>
<th>BIOTIN CONTENT (mcg/100g dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>9.5</td>
</tr>
<tr>
<td>Dairy Products</td>
<td>13.3</td>
</tr>
<tr>
<td>Fish</td>
<td>21.6</td>
</tr>
<tr>
<td>Fruit</td>
<td>5.3</td>
</tr>
<tr>
<td>Meats</td>
<td>3.6</td>
</tr>
<tr>
<td>Nuts</td>
<td>32.3</td>
</tr>
<tr>
<td>Pulses</td>
<td>35.8</td>
</tr>
<tr>
<td>Sea Foods</td>
<td>18.9</td>
</tr>
<tr>
<td>Vegetables</td>
<td>12.8</td>
</tr>
<tr>
<td>Average of all</td>
<td>17.0</td>
</tr>
</tbody>
</table>

9.5 The Use of Supplementary Biotin

The main composite formulae we use contain 25mcg/day in a standard intake. By relating this to the figures for intake given above you can see that this is a significant supplement, being of the same order of magnitude as the likely intake from foods. In using such a formula the practitioner may halve the intake or double it, in accord with the perceived need for other components in the formula, and the resulting supplementary intake of biotin will still be both useful and safe.

However, fully adequate levels of biotin are clearly needed in diabetes because the vitamin increases insulin sensitivity and enhances the activity of the enzyme hexokinase, which catalyzes the first step in glucose metabolism (Coggeshall et al 1985, Reddi et al 1988).

In certain other situations high intakes have been advocated that are well above those that could be obtained from food and which could be dubbed “megavitamin therapy”. These include the strengthening of nails and hair with biotin intakes of 2500mcg/day. In the case of ‘cradle cap’, 3000mcg twice per day has been advocated for the nursing mother.

Finally high intakes of biotin are said to be necessary for correcting dysbiosis, especially that which involves Candida overgrowth. This dates back to an in vitro experimental study by Yamaguchi (1974) who showed that under the conditions of the experiments biotin deficiency
promoted the conversion of Candida into its more pathogenic fungal form. This does not appear to be well supported by other scientific studies but it is nonetheless quite common practice to give supplements of biotin, up to 1000mcg/day to help rectify dysbiosis and Candidiasis. Indeed, intakes of 5000mcg/day have been recommended, apparently in an effort to raise the intestinal concentrations of biotin to those indicated to be needed in the Yamaguchi experiments (DeSchepper, 1986, pp82 & 119, Chaitow & Trenev, 1990, pp 87, 91, 97). No ill-effects have been observed from these large intakes of biotin.

9.6 Absorption, Cell Uptake, Storage and Excretion

With the state of knowledge about biotin requirements being rather rudimentary, there is less reason than in the case of other B vitamins, to focus upon the details of absorption, cell uptake, storage and excretion. Nonetheless a good many facts are known. Several degradation products of biotin are known. Protein-bound dietary biotin is first hydrolyzed by protein-digesting enzymes in the gut to biocytin or peptides that contain it. Biocytin itself is hydrolyzed in the intestinal cells to free biotin. Absorption occurs in the upper part of the small intestine. Biotin in blood plasma is partly free and partly protein-bound. There does not appear to be significant body stores of the vitamin.

9.7 References

- DeSchepper, L., “Candida: the symptoms, the causes, the cure”, Publ. by the author, Santa Monica California, USA (1986).
10  GAMMA AMINO BUTYRIC ACID (GABA)

Gamma amino butyric acid has no proper place among the B vitamins and is mentioned here only because it is included among the B vitamin component of many of the supplement formulations we commonly use. GABA is readily produced from the amino acid, glutamic acid. The conversion involves removal of the alpha carboxyl group of glutamate by the B6-dependent enzyme glutamate decarboxylase. GABA is believed to be the neurotransmitter for brain cells that exert inhibitory effects on other brain cells. GABA and glutamate itself are thought to have opposite effects in the brain, glutamate being excitatory and GABA being inhibitory. The use of GABA as a nutritional supplement is done on the basis of influencing brain cell levels of this neurotransmitter.

Checkpoint Four

- a) Why are vegans particularly prone to vitamin B12 deficiency? What non-meat sources of vitamin B12 are available to vegans?
- b) Name five signs and symptoms of vitamin B12 deficiency. What are these symptoms the result of?
- c) What is the difference between vitamin B12 and the other water-soluble vitamins? What consequences does this have on symptom development?
- d) Why is there doubt as to the importance of orally digested biotin?
- e) Why do diabetic patients benefit from biotin supplementation?
- f) In what circumstances might high doses of biotin be used as treatment?

*Please turn to the end of this part to check your answers*
11 ANSWERS TO CHECKPOINTS

11.1 Checkpoint One

a) Thiamine deficiency leads to tissue lack of acetyl CoA because thiamine is a cofactor for decarboxylase and transketolase enzymes in pathways that form Acetyl CoA during carbohydrate and amino acid breakdown. Without thiamine these pathways become blocked and Acetyl CoA is not formed.

b) The food and drinks that act as anti-thiamine factors and therefore reducing absorption of the vitamin are tea, coffee, raw fish and alcohol.

c) Any of the list below are good food sources of thiamine:
   - Fresh peas
   - Soya beans
   - Black-eye beans
   - Red kidney beans
   - Peanuts
   - Pumpkin
   - Wheat germ
   - Pork
   - Sunflower seeds
   - Salmon

Any of the list below are good sources of riboflavin:
   - Lamb’s liver
   - Mushrooms
   - Turnip tops
   - Skimmed milk
   - Mussels
   - Trout

d) The standard therapeutic dose of both thiamine and riboflavin is 25mg/day. There is usually no need to administer either vitamin separately from the other B vitamins.

e) Riboflavin is most commonly associated with oxidative metabolism of the bulk nutrients. It is a cofactor for enzymes essential for the breakdown of food into cell energy.

f) Riboflavin is converted into a nucleotide in order to be in an active form. The active forms of riboflavin are flavin mononucleotide (FM or FMN) and flavin adenosine dinucleotide (FAD).

11.2 Checkpoint Two

a) The specific deficiency syndrome associated with vitamin B3 is pellagra. Its notorious symptoms are dermatitis, dementia and diarrhoea.

b) Nicotinic acid may produce facial flushing especially at high doses.

c) Tryptophan intake relates to vitamin B3 requirement as vitamin B3 can be synthesised in the body from tryptophan. Therefore the amount of tryptophan you eat affects your vitamin B3 requirement.

d) Pantothenic acid has been shown to be useful in mega-doses in patients with adrenal exhaustion or with severe allergies. The highest advisable intake would be 3g/day split between mealtimes.
e) If a varied whole food diet were consumed then it would contain around about the orthodox nutritionists idea of the daily minimum requirement of pantothenic acid (3-4mg/day). However it would not reach the alternative target of 7-10mg. This could only be achieved by including specific foods in the diet, which are high in the vitamin. Parsley is by far and away the best food source of pantothenic acid.

f) Any of the list below are foods that are particularly good sources of vitamin B3:
- Anchovy
- Lamb’s liver
- Mushrooms
- Octopus
- Chicken

11.3 Checkpoint Three

a) Vitamin B6 is the cofactor for the transaminases. These are enzymes responsible for providing the correct balance of non-essential amino acids for protein synthesis and synthesis of other biochemicals such as neurotransmitters. They are also involved in the bodies handling of nitrogen that comes from amino acid breakdown, leading to its excretion as urea.

b) The RNI for vitamin B6 is calculated according to protein intake. The recommended intake is 15mcg/g of dietary protein consumed.

c) The members of the population most at risk from vitamin B6 deficiency are babies, the elderly, alcoholics, renal patients on dialysis and those using allopathic drugs with antivitamin effects. Deficiency symptoms include fatigue, anaemia, cheilosis, glossitis, stomatitis. Children may display seizures, convulsions and abnormal electrocardiogram.

d) Neutral tube defects such as spina bifida can be prevented in large measure by supplementation with folate at a rate of 400mcg/day.

e) Any of the list below are effects of folic acid deficiency:
- Megaloblastic anaemia
- Impaired growth in children
- Cervical dysplasia
- Depression
- Gout
- Impaired immune function
- Osteoporosis
- Cardiovascular disease
- Birth defects

f) Supplements of folate and vitamin B12 should be used together due to the risk of vitamin B12 deficiency simultaneous to folate deficiency. B12 deficiency can lead to neurological damage and can be masked by folate supplementation, allowing the damage to continue unnoticed.

11.4 Checkpoint Four

a) Vegans are prone to vitamin 12 deficiency because it cannot be synthesised by plants. Vegan sources of vitamin B12 are yeast, fermentation products such as tofu and the rootlets of sprouting seeds due to the microorganisms present in these foods.
b) Any of the list below are signs and symptoms of vitamin B12 deficiency:

- Numbness and tingling in the hands and feet
- Diminished vibration sense and position sense
- Unsteadiness, poor muscular co-ordination with ataxia
- Moodiness, mental slowness and poor memory
- Confusion, agitation and depression
- Dim vision
- Delusions, hallucination and overt psychosis

These symptoms are the result of inadequate formation of the myelin sheath around the nerves.

c) Unlike all other water-soluble vitamins, B12 can be stored in the body for long periods, even years. This means that a person's diet could have been deficient in B12 for years before any symptoms occur.

d) Biotin is produced by the intestinal bacteria and may be absorbed into the lower gut. The rate of faecal excretion is actually 3-6 times the dietary intake.

e) Diabetic patients benefit from biotin supplementation as it increases insulin sensitivity and enhances the activity of the enzyme hexokinase, which catalyses the first step in glucose metabolism.

f) High doses of biotin have been shown to strengthen hair and nails and reduce cradle cap in babies if given to the nursing mother. High levels are also required for correcting dysbiosis, especially candida overgrowth.