ONE

The Vitamins

The vitamins are a disparate group of compounds; they have little in common either chemically or in their metabolic functions. Nutritionally, they form a cohesive group of organic compounds that are required in the diet in small amounts (micrograms or milligrams per day) for the maintenance of normal health and metabolic integrity. They are thus differentiated from the essential minerals and trace elements (which are inorganic) and from essential amino and fatty acids, which are required in larger amounts.

The discovery of the vitamins began with experiments performed by Hopkins at the beginning of the twentieth century; he fed rats on a defined diet providing the then known nutrients: fats, proteins, carbohydrates, and mineral salts. The animals failed to grow, but the addition of a small amount of milk to the diet both permitted the animals to maintain normal growth and restored growth to the animals that had previously been fed the defined diet. He suggested that milk contained one or more "accessory growth factors" – essential nutrients present in small amounts, because the addition of only a small amount of milk to the diet was sufficient to maintain normal growth and development.

The first of the accessory food factors to be isolated and identified was found to be chemically an amine; therefore, in 1912, Funk coined the term *vitamine*, from the Latin vita for "life" and amine, for the prominent chemical reactive group. Although subsequent accessory growth factors were not found to be amines, the name has been retained – with the loss of the final "-e" to avoid chemical confusion. The decision as to whether the word should correctly be pronounced "vitamin" or "veitamin" depends in large part on which system of Latin pronunciation one learned – the *Oxford English Dictionary* permits both.

During the first half of the twentieth century, vitamin deficiency diseases were common in developed and developing countries. At the beginning of the twenty-first century, they are generally rare, although vitamin A deficiency (Section 2.4) is a major public health problem throughout the developing world, and there is evidence of widespread subclinical deficiencies of vitamins B_2 (Section 7.4) and B_6 (Section 9.4). In addition, refugee and displaced populations (some 20 million people according to United Nations estimates in 2001) are at risk of multiple B vitamin deficiencies, because the cereal foods used in emergency rations are not usually fortified with micronutrients [Food and Agriculture Organization/World Health Organization (FAO/WHO, 2001)].

1.1 DEFINITION AND NOMENCLATURE OF THE VITAMINS

In addition to systematic chemical nomenclature, the vitamins have an apparently illogical system of accepted trivial names arising from the history of their discovery (Table 1.1). For several vitamins, a number of chemically related compounds show the same biological activity, because they are either converted to the same final active metabolite or have sufficient structural similarity to have the same activity.

Different chemical compounds that show the same biological activity are collectively known as *vitamers*. Where one or more compounds have biological activity, in addition to individual names there is also an approved generic descriptor to be used for all related compounds that show the same biological activity.

When it was realized that milk contained more than one accessory food factor, they were named A (which was lipid-soluble and found in the cream) and B (which was water-soluble and found in the whey). This division into fat- and water-soluble vitamins is still used, although there is little chemical or nutritional reason for this, apart from some similarities in dietary sources of fat-soluble or water-soluble vitamins. Water-soluble derivatives of vitamins A and K and fat-soluble derivatives of several of the B vitamins and vitamin C have been developed for therapeutic use and as food additives.

As the discovery of the vitamins progressed, it was realized that "Factor B" consisted of a number of chemically and physiologically distinct compounds. Before they were identified chemically, they were given a logical series of alphanumeric names: B_1, B_2, and so forth. As can be seen from Table 1.2, a number of compounds were assigned vitamin status, and were later shown either not to be vitamins, or to be compounds that had already been identified and given other names.

Table 1.1 The Vitamins

Vitamin		Functions	Deficiency Disease
A	Retinol β-Carotene	Visual pigments in the retina; regulation of gene expression and cell differentiation; (β-carotene is an antioxidant)	Night blindness, xerophthalmia; keratinization of skin
D	Calciferol	Maintenance of calcium balance; enhances intestinal absorption of Ca^{2+} and mobilizes bone mineral; regulation of gene expression and cell differentiation	Rickets = poor mineralization of bone; osteomalacia = bone demineralization
E	Tocopherols Tocotrienols	Antioxidant, especially in cell membranes; roles in cell signaling	Extremely rare – serious neurological dysfunction
K	Phylloquinone Menaquinones	Coenzyme in formation of γ-carboxyglutamate in enzymes of blood clotting and bone matrix	Impaired blood clotting, hemorrhagic disease
B_1	Thiamin	Coenzyme in pyruvate and 2-oxo-glutarate dehydrogenases, and transketolase; regulates Cl^- channel in nerve conduction	Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke–Korsakoff syndrome)
B_2	Riboflavin	Coenzyme in oxidation and reduction reactions; prosthetic group of flavoproteins	Lesions of the corner of the mouth, lips, and tongue; sebhorreic dermatitis
Niacin	Nicotinic acid Nicotinamide	Coenzyme in oxidation and reduction reactions, functional part of NAD and NADP; role in intracellular calcium regulation and cell signaling	Pellagra-photosensitive dermatitis; depressive psychosis
B_6	Pyridoxine Pyridoxal Pyridoxamine	Coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase; modulation of steroid hormone action	Disorders of amino acid metabolism, convulsions
	Folic acid	Coenzyme in transfer of one-carbon fragments	Megaloblastic anemia

(*continued*)

Table 1.1 (*continued*)

Vitamin		Functions	Deficiency Disease
B_{12}	Cobalamin	Coenzyme in transfer of one-carbon fragments and metabolism of folic acid	Pernicious anemia = megaloblastic anemia with degeneration of the spinal cord
	Pantothenic acid	Functional part of coenzyme A and acyl carrier protein: fatty acid synthesis and metabolism	Peripheral nerve damage (nutritional melalgia or "burning foot syndrome")
H	Biotin	Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis; role in regulation of cell cycle	Impaired fat and carbohydrate metabolism; dermatitis
C	Ascorbic acid	Coenzyme in hydroxylation of proline and lysine in collagen synthesis; antioxidant; enhances absorption of iron	Scurvy – impaired wound healing, loss of dental cement, subcutaneous hemorrhage

NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.

For a compound to be considered a vitamin, it must be shown to be a dietary essential. Its elimination from the diet must result in a more-or-less clearly defined deficiency disease, and restoration must cure or prevent that deficiency disease.

Demonstrating that a compound has pharmacological actions, and possibly cures a disease, does not classify that compound as a vitamin, even if it is a naturally occurring compound that is found in foods.

Equally, demonstrating that a compound has a physiological function as a coenzyme or hormone does not classify that compound as a vitamin. It is necessary to demonstrate that endogenous synthesis of the compound is inadequate to meet physiological requirements in the absence of a dietary source of the compound. Table 1.3 lists compounds that have clearly defined functions, but are not considered vitamins because they are not dietary essentials; endogenous synthesis normally meets requirements. However, there is some evidence that premature infants and patients maintained on long-term total parenteral nutrition may be unable to meet their requirements for carnitine (Section 14.1.2), choline (Section 14.2.2), and taurine (Section 14.5.3) unless they are provided in the diet, and these are sometimes regarded as

Table 1.2 Compounds that Were at One Time Assigned Vitamin Nomenclature, But Are Not Considered to Be Vitamins

B_3	Assigned to a compound that was probably pantothenic acid, also sometimes used (incorrectly) for niacin
B_4	Later identified as a mixture of arginine, glycine, and cysteine, possibly also riboflavin and vitamin B_6
B_5	Assigned to what was later assumed to be either vitamin B_6 or nicotinic acid; also sometimes used for pantothenic acid
B_7	A factor that prevented digestive disturbance in pigeons (also called vitamin I)
B_8	Later identified as adenylic acid
B_9	Never assigned
B_{10}	A factor for feather growth in chickens, probably folic acid and thiamin
B_{11}	Later identified as a mixture of folic acid and thiamin
B_{13}	A growth factor in rats; orotic acid, intermediate in pyrimidine synthesis
B_{14}	An unidentified compound isolated from urine that increases bone marrow proliferation in culture
B_{15}	Pangamic acid, reported to enhance oxygen uptake
B_{16}	Never assigned
B_{17}	Amygdalin (laetrile), a cyanogenic glycoside with no physiological function
B_c	Obsolete name for folic acid
B_p	Chicken antiperosis factor; can be replaced by choline and manganese salts
B_T	Carnitine, a growth factor for insects
B_W	A growth factor, probably biotin
B_x	Obsolete name for *p*-aminobenzoic acid (intermediate in folate synthesis); also used at one time for pantothenic acid
C_2	A postulated antipneumonia factor (also called vitamin J)
F	Essential fatty acids (linoleic, linolenic, and arachidonic acids)
G	Obsolete name for riboflavin
H_3	"Gerovital," novocaine (procaine hydrochloride) promoted without evidence as alleviating aging, not a vitamin
I	A factor that prevented digestive disturbance in pigeons (also called vitamin B_7)
J	A postulated antipneumonia factor (also called vitamin C_2)
L	Factor isolated from yeast that was claimed to promote lactation
M	Obsolete name for folic acid
N	Extracts from the brain and stomach, purported to have anticancer activity
P	Bioflavonoids
PP	Pellagra-preventing factor, obsolete name for niacin
Q	Ubiquinone (also called Q_{10})
R	Bacterial growth factor, probably folic acid
S	Bacterial growth factor, probably biotin
T	Growth factor in insects, and reported to increase protein uptake in rats, later identified as a mixture of folic acid, vitamin B_{12}, and nucleotides
U	Methylsulfonium salts of methionine
V	Bacterial growth factor, probably NAD
W	Bacterial growth factor, probably biotin
X	Bacterial growth factor, probably biotin
Y	Probably vitamin B_6

NAD, nicotinamide adenine dinucleotide.

Table 1.3 Marginal Compounds that Are Probably Not Dietary Essentials

Carnitine	Required for transport of fatty acids into mitochondria
Choline	Constituent of phospholipids; acetylcholine is a neurotransmitter
Inositol	Constituent of phospholipids; inositol trisphosphate acts as second messenger in transmembrane signaling
Pyrroloquinoline quinone	Coenzyme in redox reactions
Taurine	Osmotic agent in retina and used for conjugation of bile acids; dietary essential for cats
Ubiquinone (coenzyme Q)	Redox coenzyme in mitochondrial electron transport chain

"marginal compounds," for which there is no evidence to estimate requirements.

The rigorous criteria outlined here would exclude niacin (Chapter 8) and vitamin D (Chapter 3) from the list of vitamins, because under normal conditions endogenous synthesis does indeed meet requirements. Nevertheless, they are considered to be vitamins, even if only on the grounds that each was discovered as the result of investigations into once common deficiency diseases, pellagra and rickets.

In addition to the marginal compounds listed in Table 1.3, there are a number of compounds present in foods of plant origin that are considered to be beneficial, in that they have actions that may prevent the development of atherosclerosis and some cancers, although there is no evidence that they are dietary essentials, and they are not generally considered as nutrients.

These compounds are listed in Table 1.4 and discussed in Section 14.7.

1.1.1 Methods of Analysis and Units of Activity

Historically, the vitamins, like hormones, presented chemists with a considerable challenge. They are present in foods, tissues, and body fluids in very small amounts, of the order of μmoles, nmoles, or even pmoles per kilogram, and cannot readily be extracted from the multiplicity of other compounds that might interfere in chemical analyses. Being organic, they are not susceptible to determination by elemental analysis as are the minerals. In addition, for several vitamins, there are multiple vitamers that may have the same biological activity on a molar basis (e.g., the vitamin B_6 vitamers, Section 9.1), or may have very different biological activity (e.g., the vitamin E vitamers, Section 4.1).

The original methods of determining vitamins were biological assays, initially requiring long-term depletion experiments in animals, and later using a

Table 1.4 Compounds that Are Not Dietary Essentials, But May Have Useful Protective Actions

Anthocyanins	Plant (flower) pigments, antioxidants
Bioflavonoids	Polyphenolic compounds with antioxidant action, at one time known as vitamin P
Glucosinolinates	Modify metabolism of foreign compounds and reduce yield of active carcinogens from procarcinogens
Glycosides	Modify metabolism of foreign compounds and reduce yield of active carcinogens from procarcinogens
Polyterpenes	Inhibit cholesterol synthesis
Squalene	Final acyclic intermediate in cholesterol synthesis, acts as feedback inhibitor of cholesterol synthesis
Phytoestrogens	Weak estrogenic and antiestrogenic actions, potentially protective against estrogen- and androgen-dependent tumors and osteoporosis
Polyphenols	Antioxidants
Ubiquinone (coenzyme Q)	Redox coenzyme in mitochondrial electron transport chain, coantioxidant with vitamin E
Vitamin A inactive carotenoids	Antioxidants

variety of microorganisms with more or less defined requirements. Microbiological assays are still commonly used for many of the vitamins; problems of both overestimation and underestimation may occur:

1. Overestimation of the vitamin content of foods will occur if the test organism can use chemical forms and derivatives of the vitamin that are not biologically active in, or available to, human beings.
2. Underestimation will occur if the test organism is unable to use some vitamers, although human beings have appropriate enzymes for interconversion.

Before some of the vitamins had been purified, they were determined in terms of units of biological activity. All should now be expressed in mass or, preferably, molar terms, although occasionally the (now obsolete) international units (iu) are still used for vitamins A (Section 2.1.3), D (Section 3.1), and E (Section 4.1). Where different vitamers differ greatly in biological activity (e.g., the eight tocopherol and tocotrienol vitamers of vitamin E, Section 4.1), it is usual to express total vitamin activity in terms of milligram equivalents of the major vitamer or that with the highest biological activity.

Many of the methods that have been devised for vitamin analysis are now of little more than historical interest, and, in general, unless there is some reason,

no analytical methods are listed in this book. A number of recommended methods for vitamin analysis in foods were published as the outcome of a European Union (EU) COST-91 project (Brubacher et al., 1985); since then, the development of ligand binding assays (radioimmunoassays) and high-performance liquid chromatography techniques has meant that individual chemical forms of most of the vitamins can now be determined with great precision and specificity, often with only a minimal requirement for extraction from complex biological materials. Nevertheless, microbiological assays are still sometimes the method of choice, and biological assay is still essential to determine the relative biological activity of different vitamers.

Although modern analytical techniques have considerable precision and sensitivity, food composition tables cannot be considered to give more than an approximation to vitamin intake. Apart from the problems of biological availability (Section 1.1.2), there is considerable variation in the vitamin content of different samples of the same food, depending on differences between varieties, differences in growing conditions (even of the same variety), losses in storage, and losses in food preparation.

When foods have been enriched with vitamins, because of the requirement for the food to contain the stated amount of vitamin after normal storage, manufacturers commonly add more than the stated amount – so-called overage. One of the problems in the debate concerning folate enrichment of flour (Section 10.12) is the relatively small difference between the amount that is considered desirable and the amount that may pose a hazard to vulnerable population groups, and the precision to which manufacturers can control the amount in the final products. In pharmaceutical preparations, considerable latitude is allowed; the U.S. Pharmacopeia permits preparations to contain from 90% to 150% of the declared amount of water-soluble vitamins and from 90% to 165% of the fat-soluble vitamins.

1.1.2 Biological Availability

The biological availability of a nutrient is the proportion of the nutrient present in a food that can be used by the body. It is determined by the extent to which the nutrient is digested, the extent to which the products of digestion are absorbed, and the metabolism of the products of digestion. A number of factors affect digestion, absorption, and metabolism, and hence biological availability. These factors include the physical properties of the food matrix (e.g., nutrients may be inside intact cells of plant foods, and the plant cell wall is not digested); the chemical nature of the vitamin in the food; and the presence of inhibitors that may be present in the food, taken with food, or taken as drugs or medications (Bates and Heseker, 1994; Ball, 1998).

1. Many vitamins are absorbed by active transport; this is a saturable process, and, therefore, the percentage that is absorbed will decrease as the intake increases.
2. The fat-soluble vitamins (A, D, E, and K) are absorbed dissolved in lipid micelles, and, therefore, absorption will be impaired when the meal is low in fat. Gastrointestinal pathology that results in impaired fat absorption and steattorhea (e.g., untreated celiac disease) will also impair the absorption of fat-soluble vitamins, because they remain dissolved in the unabsorbed lipid in the intestinal lumen. Lipase inhibitors used for the treatment of obesity and fat replacers (e.g., sucrose polyesters such as Olestra™) will similarly impair the absorption of fat-soluble vitamins.
3. Many of the water-soluble vitamins are present in foods bound to proteins, and their release may require either the action of gastric acid (as for vitamin B_{12}, Section 10.7.1) or specific enzymic hydrolysis [e.g., the action of conjugase to hydrolyze folate conjugates (Section 10.2.1) and the hydrolysis of biocytin to release biotin (Section 11.2.3)].
4. The state of body reserves of the vitamin may affect the extent to which it is absorbed (by affecting the synthesis of binding and transport proteins) or the extent to which it is metabolized after uptake into the intestinal mucosa [e.g., the oxidative cleavage of carotene to retinaldehyde is regulated by vitamin A status (Section 2.2.1)].
5. Compounds naturally present in foods may have antivitamin activity. Many foods contain thiaminases and compounds that catalyze nonenzymic cleavage of thiamin to biologically inactive products (Section 6.4.7).
6. Both drugs and compounds naturally present in foods may compete with vitamins for absorption. Chlorpromazine, tricyclic antidepressants, and some antimalarial drugs inhibit the intestinal transport and metabolism of riboflavin (Section 7.4.4); carotenoids lacking vitamin A activity compete with β-carotene for intestinal absorption and metabolism (Section 2.2.2.2); and alcohol inhibits the active transport of thiamin across the intestinal mucosa (Section 6.2).
7. Some vitamins are present in foods in chemical forms that are not susceptible to enzymic hydrolysis during digestion, although they are released during the preparation of foods for analysis. Much of the vitamin B_6 in plant foods is present as pyridoxine glycosides (Section 9.1), which are only partially available, and may also antagonize the metabolism of free pyridoxine (Gregory, 1998); excessive heating can lead to nonenzymic formation of pyridoxyllysine in foods, rendering both the vitamin and the lysine unavailable (Section 9.1); and most of the niacin in cereals

is present as niacytin (nicotinoyl-glucose esters in oligosaccharides and nonstarch polysaccharides), which is only hydrolyzed to a limited extent by gastric acid (Section 8.2.1.1).

Occasionally, protein binding of a vitamin on foods increases its absorption and hence its biological availability. For example, folate from milk is considerably better absorbed than that from either mixed food folates or free folic acid (Section 10.2.1). Folate bound to a specific binding protein in milk is absorbed in the ileum, whereas free folate monoglutamate is absorbed in the (smaller) jejunum.

1.2 VITAMIN REQUIREMENTS AND REFERENCE INTAKES

A priori, it would appear to be a simple matter to determine requirements for vitamins. In practice, a number of problems arise. The first of these is the definition of the word *requirement*. The U.S. usage (Institute of Medicine, 1997) is that the requirement is the lowest intake that will "maintain a defined level of nutriture in an individual" – i.e., the lowest amount that will meet a specified criterion of adequacy. The WHO (1996) defines both a *basal requirement* (the level of intake required to prevent pathologically relevant and clinically detectable signs of deficiency) and a *normative requirement* (the level of intake to maintain a desirable body reserve of the nutrient).

We have to define the purpose for which we are determining the requirement (the criteria of adequacy), then determine the intake required to meet these criteria.

1.2.1 Criteria of Vitamin Adequacy and the Stages of Development of Deficiency

For any nutrient, there is a range of intakes between that which is clearly inadequate, leading to clinical deficiency disease, and that which is so much in excess of the body's metabolic capacity that there may be signs of toxicity. Between these two extremes is a level of intake that is adequate for normal health and the maintenance of metabolic integrity, and a series of more precisely definable levels of intake that are adequate to meet specific criteria and may be used to determine requirements and appropriate levels of intake. These follow.

1. Clinical deficiency disease, with clear anatomical and functional lesions, and severe metabolic disturbances, possibly proving fatal. Prevention of deficiency disease is a minimal goal in determining requirements and is the criterion of the WHO basal requirement (WHO, 1996).