

Vitamin Basics

The Facts about Vitamins in Nutrition



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Foreword



While plants and micro organism have the capability to produce the vitamins necessary for the metabolism themselves, humans and animals have unfortunately lost this ability during evolution. Because of the lack of specific enzymes for synthesis, vitamins became essential nutrients for them. It was recognized more than 3500 years ago that vitamins are essential food ingredients for maintaining health and well-being. The first records related to the use of specific food items, as we know today contain information on specific vitamins, such as vitamin A in liver, to prevent specific diseases such as night blindness. Only 3000 years later specific conditions of deficiency were recorded that could be attributed to the deficiency of selected nutrients. Well known examples are scurvy (vitamin C deficiency), beriberi (vitamin B₁) and rickets (vitamin D). It took another 400 years until we were able to attribute these disease conditions to specific active substances in our diet named then vitamins. Although we now know that vitamins are not a uniform group of chemical substances like proteins, carbohydrates and lipids, we still use the term to describe the whole group.

Since the beginning of the last century our knowledge on the biological function of vitamins on the molecular and cellular level has increased significantly. This research is reflected by 20 Noble Prize winners between 1928 and 1967. Despite intensive research efforts no additional vitamins have been added to the list of 13 vitamins accumulated between 1897 and 1941.

While in the past, scientist have basically been concerned with the role of vitamins in preventing vitamin related disease and their biochemical functions, today it is recognized that vitamins have an important role in health and well-being beyond the mere prevention of deficiency. This aspect of vitamins is based on the observation that vitamins are not only coenzymes in metabolic processes but also act as potent antioxidants and have hormone-like functions. The later is clearly

visible in the history of vitamin D research. In the late 1970's, research established vitamin D as a hormone essential in bone metabolism. Based on such findings, vitamins are no longer classified into groups defined simply by their physical-chemical properties – such as water-soluble and fat-soluble vitamins. More appropriately, vitamins are now classified according to their biological function in the body; vitamins with coenzyme functions, vitamins with hormone-like properties and vitamins with antioxidants properties. But as expected the borderlines between these groups can not clearly be defined and needs readjustment with the rapid progress in research.

In developing countries chronic, diet-related diseases are still an important public health problem but in the affluent societies, the prevention of degenerative diseases and also acute vitamin deficiencies might be of concern. Regarding the continuing debate of optimal vitamin levels and tolerable upper intake levels (UL) a valid knowledge-base of the daily expanding scientific evidence is necessary. This includes for example the definition of populations at risk, the problem of appropriate biomarkers that not only reflect the dietary intake but also the local status in specific tissues at risk of deficiency as well as environmental factors that influence status and need for certain vitamins.

The following chapters of this book will contribute to the better understanding of the important role of vitamins not only in preventing specific deficiencies but also maintaining and improving human health and well-being by summarizing the actual knowledge-base for the individual vitamins.

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Introduction



Vitamins are essential organic nutrients required in very small amounts for normal metabolism, growth and physical well-being. Most vitamins are not made in the body, or only in insufficient amounts, and are mainly obtained through food. When their intake is inadequate, vitamin deficiency disorders are the consequence. Vitamins are present in food in minute quantities compared to the macronutrients protein, carbohydrates and fat. The average adult in industrialised countries eats about 600g of food per day on a dry-weight basis, of which less than 1 gram consists of vitamins.

No single food contains all of the vitamins and, therefore, a balanced and varied diet is necessary for an adequate intake. Each of the **13 vitamins** known today has specific functions in the body, which makes every one of them unique and irreplaceable. **Vitamins are essential for life!**

Of the 13 vitamins, 4 are fat-soluble, namely vitamins A, D, E and K. The other vitamins are water-soluble: vitamin C and the B-complex, consisting of vitamins B₁, B₂, B₆, B₁₂, folic acid, biotin, pantothenic acid and niacin.

The history of vitamins can be divided into five periods.

Table 1: The History of Vitamins

Vitamin	Discovery	Isolation	Structure	Synthesis
Vitamin A	1909	1931	1931	1947
Provitamin A (Beta-carotene)		1831	1930	1950
Vitamin D	1918	1932	1936	1959
Vitamin E	1922	1936	1938	1938
Vitamin K	1929	1939	1939	1939
Vitamin B ₁	1897	1926	1936	1936
Vitamin B ₂	1920	1933	1935	1935
Niacin	1936	1935	1937	1894
Vitamin B ₆	1934	1938	1938	1939
Vitamin B ₁₂	1926	1948	1956	1972
Folic Acid	1941	1941	1946	1946
Pantothenic Acid	1931	1938	1940	1940
Biotin	1931	1935	1942	1943
Vitamin C	1912	1928	1933	1933

1. The **empirical healing** of diseases, now associated with vitamin deficiency, through consumption of particular foods. An example is the use of liver to treat night blindness (vitamin A deficiency) by the Egyptians (Papyrus Ebers 1550-1570 BC), Assyrians, Chinese, Japanese, Greeks, Romans, Persians and Arabs.
 2. The second phase was characterised by the ability to **induce a deficiency disease** in animals, which started with the classical studies of Lunin and Eijkman around 1890. The ability to produce deficiency diseases, such as beriberi in animals, led to Hopkins' concept that small amounts of "accessory growth factors" are necessary for growth and life, and the coining of the term "vitamine" in 1912 by the Polish-American scientist, Funk.
 3. The third phase consisted in seven decades of exciting research involving the **discovery, isolation, structure elucidation and synthesis** of all the vitamins, and culminating in the synthesis of vitamin B₁₂ in 1972. Most scientists think that the discovery of any new vitamin is quite unlikely, although efforts are still continuing in that quest. Many of the researchers involved in this golden age of the vitamins received a Nobel prize in recognition of their great achievements (Table 2).
 4. During the era of discovery, a fourth period began which was concerned with the **biochemical functions, establishment of dietary requirements and commercial production**. In the early 1930s it was realised that riboflavin (vitamin B₂) was part of the "yellow enzyme", which in time led to the elucidation of the role of the B-vitamins as coenzymes. The subsequent identification of most of the B-vitamins as coenzymes remained a central theme, defining their function for many decades. The first commercial synthesis of vitamin C by Reichstein in 1933 was the start of a successful industrial effort that led to the availability of relatively inexpensive vitamins for research and use in animal feedstuffs, for the fortification of food products, and for supplements.
 5. The accumulation of reports of **health benefits beyond preventing deficiencies** and exciting **new biochemical functions** of vitamins ushered in a fifth period, starting with the report in 1955 of the cholesterol-lowering effect of niacin (1). This is now a well accepted effect of the vitamin, which has nothing at all to do with its classical coenzyme role, and is a clear health effect beyond preventing the deficiency disease pellagra.
- Finally, work on the biochemical function of vitamins in the last three decades has considerably expanded our concept of how vitamins function in the body and has helped provide a chemical basis for the in vivo observation of their health effects (Table 3).

Table 2: Vitamin-Related Nobel Prize Winners

Year	Name	Field	Comments
1928	Adolf Windaus	Chemistry	for his research into the constitution of the steroids and their connection with vitamins
1929	Christiaan Eijkman	Medicine & Physiology	for his discovery of the antineuritic vitamins
	Sir Frederick G. Hopkins	Medicine & Physiology	for his discovery of the growth stimulating vitamin
1934	George R. Minot William P. Murphy George H. Whipple	Medicine & Physiology	for their discoveries concerning liver therapy of anaemias
1937	Sir Walter N. Haworth	Chemistry	for his research into the constitution of carbohydrates and vitamin C
	Paul Karrer	Chemistry	for his research into the constitution of carotenoids, flavins and vitamins A and B ₂
	Albert Szent-Györgyi	Medicine & Physiology	for his discoveries in connection with the biological combustion processes, with particular reference to vitamin C and the catalysis of fumaric acid
1938	Richard Kuhn	Chemistry	for his work on carotenoids and vitamins
1943	Carl Peter Henrik Dam	Medicine & Physiology	for his discovery of vitamin K
	Edward A. Doisy	Medicine & Physiology	for his discovery of the chemical nature of vitamin K
1953	Fritz A. Lipmann	Medicine & Physiology	for his discovery of Coenzyme A and its importance for intermediary metabolism
1955	Axel H.T. Theorell	Medicine & Physiology	for his discoveries concerning the nature and mode of action of oxidation enzymes
1964	Konrad E. Bloch	Medicine & Physiology	for his discoveries concerning the mechanism and regulation of cholesterol and fatty acid metabolism as above
	Feodor Lynen	Medicine & Physiology	
	Dorothy C. Hodgkin	Chemistry	for her structural determination of vitamin B ₁₂
1967	Ragnar A. Granit	Medicine & Physiology	for his research, which illuminated the electrical properties of vision by studying wavelength discrimination in the eye
	Halden K. Hartline	Medicine & Physiology	for his research on the mechanisms of sight
	George Wald	Medicine & Physiology	for his research on the chemical processes that allow pigments in the retina of the eye to convert light into vision

Table 3: Biochemical Function of Vitamins

Vitamin	Classical Role	More Recent Role
Vitamin C	Hydroxylation Reaction	In Vivo Antioxidant
Beta-carotene	Provitamin A	Antioxidant, Immune Function
Vitamin K	Clotting Factors	Calcium Metabolism
Vitamin D	Calcium Absorption, Mineralisation of Bone	Differentiation and Growth, Immune Function
Vitamin B ₆	Coenzyme	Steroid Regulation
Niacin	Coenzyme	Lipid Lowering
Folic Acid	Production and Maintenance of New Cells	Protection Against Neural Tube Birth Defects
Folic Acid, B ₆ and B ₁₂	Energy Metabolism	May Lower Risk of Heart Disease and Stroke*
Antioxidant vitamins		Protection against Cancer and Heart Disease*

*Research ongoing

Dietary Reference Intakes

From 1941 until 1989, RDAs (Recommended Dietary Allowances) were established and used to evaluate and plan menus to meet the nutrient requirements of certain groups. They were also used in other applications such as interpreting food consumption records of populations, establishing standards for food assistance programs, establishing guidelines for nutrition labelling, etc.

The primary goal of RDAs was to prevent diseases caused by nutrient deficiencies.

In the early 1990s, the Food and Nutrition Board (FNB), the Institute of Medicine, the National Academy of Sciences (USA), with the involvement of Health Canada, undertook the task of revising the RDAs, and a new family of nutrient reference values was born – the Dietary Reference Intakes (DRIs).

The primary goal of having these new dietary reference values was not only to prevent nutrient deficiencies, but also to reduce the risk of chronic diseases such as osteoporosis, cancer, and cardiovascular disease.

The first report, Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride, was published in 1997. Since then, three additional vitamin related reports have been released, addressing folate and other B vitamins, dietary antioxidants (vitamins C, E, selenium and the carotenoids), and the micronutrients (vitamins A, K, and trace elements such as iron, iodine, etc). The DRIs are a comprehensive scientific source primarily for nutrition scientists (see References). They are used by health authorities in many countries as a basis for decisions regarding nutritional information on micronutrients.

There are four types of DRI reference values: the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), the Adequate Intake (AI) and the Tolerable Upper Intake Level (UL).

- **Estimated Average Requirement (EAR)** – the amount of a nutrient that is estimated to meet the requirement of half of all healthy individuals in a given age and gender group. This value is based on a thorough review of the scientific literature.

- **Recommended Dietary Allowance (RDA)** – the average daily dietary intake of a nutrient that is sufficient to meet the requirement of nearly all (97-98%) healthy persons. This is the number to be used as a goal for individuals. It is calculated from the EAR.

- **Adequate Intake (AI)** – only established when an EAR (and thus an RDA) cannot be determined because the data are not clear-cut enough; a nutrient has either an RDA or an AI. The AI is based on experimental data or determined by estimating the amount of a nutrient eaten by a group of healthy people and assuming that the amount they consume is adequate to promote health.

- **Tolerable Upper Intake Level (UL)**

- the highest continuing daily intake of a nutrient that is likely to pose no risks of adverse health effects for almost all individuals. As intake increases above the UL, the risk of adverse effects increases. Consistently consuming a nutrient at the upper level should not cause adverse effects. Intake levels at the UL can be interpreted as a ‘warning flag’, not as reason for alarm.

Certain groups at risk of vitamin deficiencies

With the advent of vitamin fortification in the manufacturing of flour, cereals and other foods, specific vitamin deficiency diseases such as scurvy, beriberi, rickets and pellagra have become rare in most industrialised countries. However, in many African, Asian and Latin American countries, chronic, diet-related diseases continue to be a major health problem. In these countries there is a need to eliminate frank vitamin A, C and B-complex deficiencies, as well as other micronutrient deficiencies (iodine, iron, selenium, zinc and calcium).

However, even in highly industrialised countries, numerous large government nutrition surveys of the population indicate that marginal vitamin deficiencies with unspecific symptoms, like fatigue and frequent headaches, are probably not rare. They are difficult for the individual to detect and are largely ignored. Marginal vitamin deficiency is a “state of gradual vitamin depletion in which there is evidence of personal lack of well-being associated with impairment of certain biochemical reactions”. Studies have found that many people have nutritional deficiencies which do not show up in a routine physical examination. It has

also been suggested that marginal deficiencies are linked to behavioural and physiological changes. Extensive surveys have revealed that more than 60% of the elderly have deficient dietary intake of vitamin D, E and folate. Other vitamins – critical not just for the elderly – include thiamin (B₁), panthothenic acid, and biotin.

Many individuals have health problems, habits, or living situations in which chronic or periodic intake of vitamins should exceed the ordinary requirement. High-risk-groups include:

- the elderly
- adolescents
- young or pregnant and lactating women
- alcoholics
- cigarette smokers
- vegetarians
- people fasting or on dietary intervention
- laxative abusers
- users of contraceptives and analgesics and other medications for chronic disease
- people with specific disorders of the gastrointestinal tract.

However, marginal deficiencies are not only limited to those groups listed. The gradual change in the way we live has influenced our diets and has altered our habitual intake of vitamins and minerals. Hectic lifestyles, reduced physical activity and an increase in fast and convenience food have all played a significant role. As a result, a significant proportion of the population fails to reach recommended intake levels.

Antioxidant Vitamins

Vitamin C, vitamin E and carotenoids, such as beta-carotene, are micronutrients with antioxidant properties. Antioxidants are substances that prevent oxidation or chemical reactions involving oxygen.

As the atmosphere changed from being anaerobic to aerobic, oxygen became available in energy production for living organisms, but it also carried a price. When energy is produced, unstable oxygen species known as free radicals are formed. Free radicals are also produced at other sites in the metabolism (e.g., by activated phagocytes as part of the immune defence), and through exogenous sources such as exposure to cigarette smoke, environmental pollutants and ultraviolet light. Free radicals are atoms or molecules that have an unpaired electron which makes them very reactive. They have the potential to damage DNA, proteins, carbohydrates, lipids and cell membranes. In addition to free radicals, there is another highly reactive compound that is a potent generator of free radicals: it is called singlet oxygen. This molecule is unique in that it contains a pair of electrons but exists in an unstable configuration and is very reactive.

The body has an elaborate antioxidant defence system that works to neutralise free radicals and other highly reactive species. The major biological antioxidants are enzymes (superoxide dismutase, catalase and glutathione peroxidase) as well as non-enzymatic scavengers (such as uric acid, CoQ10, glutathione, thiols in proteins) and the antioxidant vitamins (beta-carotene, vitamin C and E).

Each of the antioxidant nutrients has specific characteristics, and they often work synergistically to

strengthen the overall antioxidant capability of the body.

Vitamin E is the principal fat-soluble antioxidant in the body and is responsible for protecting the polyunsaturated fatty acids in cell membranes from oxidation by free radicals. Vitamin E exhibits a sparing effect on beta-carotene by protecting the conjugated double bonds from being oxidised. Exposure to increased oxygen levels, such as reperfusion, results in free radical-mediated tissue damage. However, due to the capability of vitamin E to work at higher oxygen pressures, free radicals are scavenged and tissue injury is minimised.

Beta-carotene also has antioxidant properties and is one of the most powerful quenchers of singlet oxygen. It can dissipate the energy of singlet oxygen, thus preventing this active molecule from generating free radicals.

Vitamin C, a water-soluble antioxidant, interacts with free radicals in the aqueous compartment of cells. Additionally, vitamin C is considered the most important antioxidant in extra-cellular fluids. Vitamin C has the ability to regenerate vitamin E after it has neutralised free radicals and terminated chain reactions.

The balance of free radical production and the level of antioxidant defences have important disease and health implications. If there are too many free radicals produced, and too few antioxidants, to a condition of "oxidative stress" develops which can lead to chronic injury.

It has therefore been suggested that oxidative stress might play a role in the development of a number of diseases:

- cancer
- atherosclerosis
- cardiovascular diseases
- cataracts
- age-related macular degeneration
- Alzheimer's disease
- immune dysfunction
- rheumatoid arthritis

Oxidative stress also plays a role in the aging process.

The scientific literature contains many research articles on the potential roles of the antioxidant nutrients in disease prevention. Many studies are just beginning while others continue to show the positive effects of the antioxidant nutrients. It therefore seems prudent to ensure an adequate intake of beta-carotene, vitamin C and vitamin E in the diet or through supplementation.

Vitamins continue to fascinate, and have become the focus of renewed

attention on the part of researchers, health/nutrition professionals, and government policymakers, as well as the general public.

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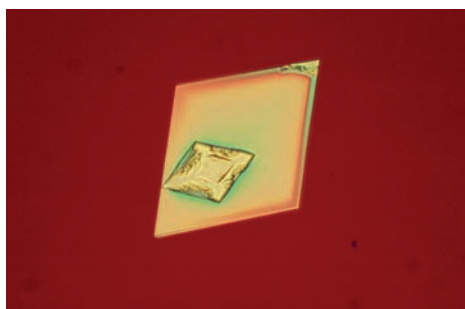
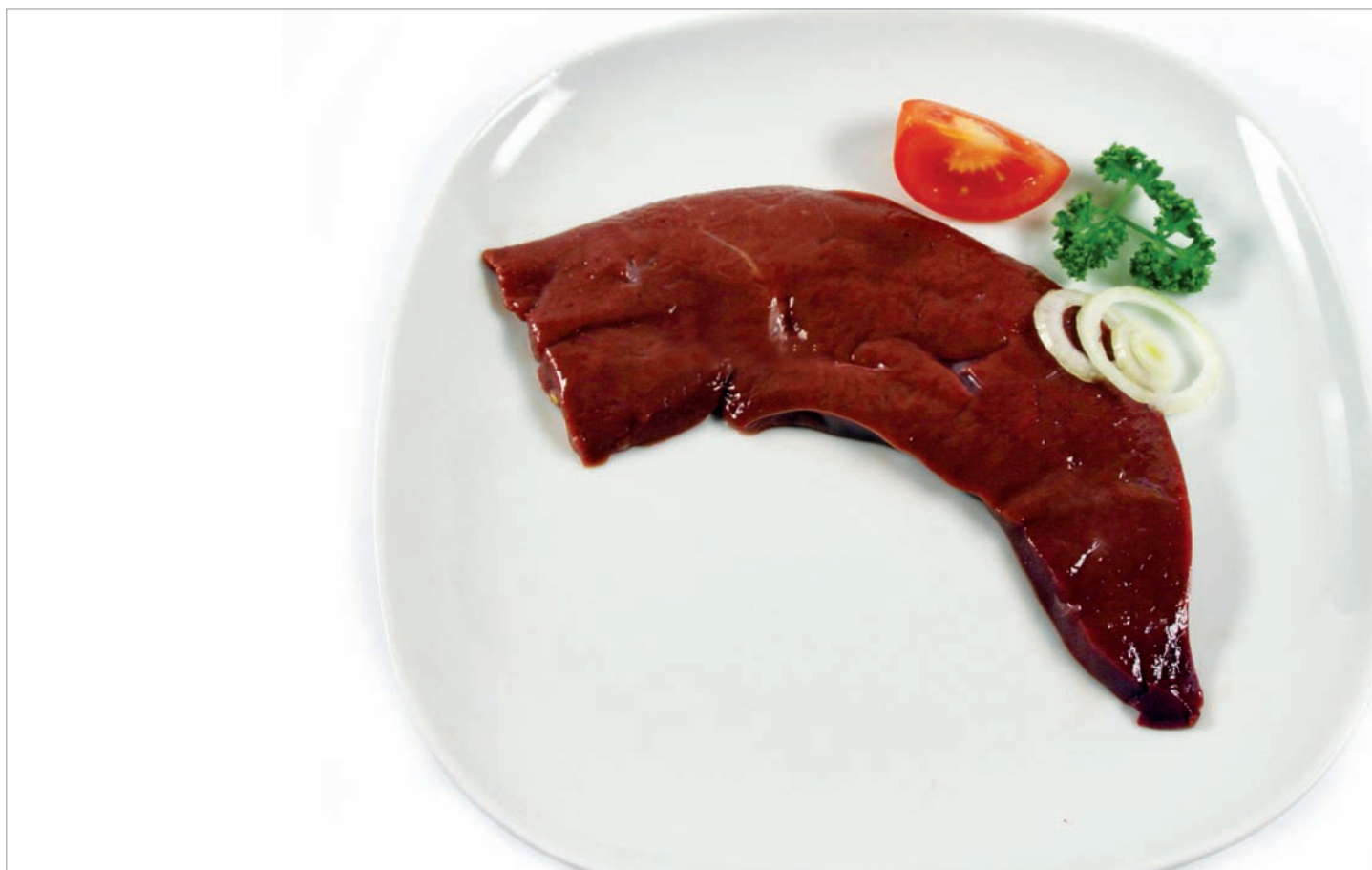
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Vitamin A



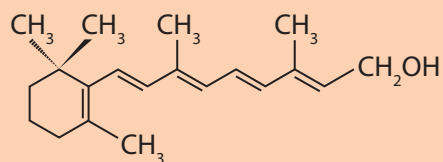
Vitamin A crystals in polarised light

Synonyms

Retinol, axerophthol

Chemistry

Retinol and its related compounds consist of four isoprenoid units joined head to tail and contain five conjugated double bonds. They naturally occur as alcohol (retinol), as aldehyde (retinal) or as acid (retinoic acid).



Molecular formula of vitamin A (retinol)

Introduction

Vitamin A is a generic term for a group of lipid soluble compounds related to retinol. Retinol is often referred to as preformed vitamin A. It is found only in animal sources, mainly as retinyl esters and in food supplements. Many cultures have used ox liver as an excellent source of vitamin A to cure night blindness. The liver was first pressed to the eye and then eaten; the Egyptians described this cure at least 3,500 years ago. Beta-carotene and other carotenoids that can be converted to vitamin A by an enzymatic process in the body are referred to as provitamin A. They are found only in plant sources.

Functions

Retinal, the oxidised metabolite of retinol, is required for the process of vision. Retinoic acid, another vitamin A metabolite, is considered to be responsible for all non-visual functions of vitamin A. Retinoic acid combines with specific nuclear receptor proteins which bind to DNA and regulate the expression of various genes, thereby influencing numerous physiological processes. Retinoic acid is therefore classified as a hormone.

Vision

Receptor cells in the retina of the eye (rod cells) contain a light-sensitive pigment called rhodopsin, which is a complex of the protein opsin and the vitamin A metabolite retinal. The light-induced disintegration of the pigment triggers a cascade of events which generate an electrical signal to the optic nerve. Rhodopsin can only be regenerated from opsin and vitamin A. Rod cells with this pigment can detect very small amounts of light, making them important for night vision.

Cellular differentiation

The many different types of cells in the body perform highly specialised functions. The process whereby cells and tissues become “programmed” to carry out their special functions is called differentiation. Through the regulation of gene expression, retinoic acid plays a major role in cellular differentiation. Vitamin A is necessary for normal differentiation of epithelial cells, the cells of all tissues lining the body, such as skin, mucous membranes, blood vessel walls and the cornea. In vitamin A deficiency, cells lose their ability to differentiate properly.

Growth and development

Retinoic acid plays an important role in reproduction and embryonic development, particularly in the development of the spinal cord and vertebrae, limbs, heart, eyes and ears.

Immune function

Vitamin A is required for the normal functioning of the immune system and therefore helps to protect against infections in a number of ways. It is essential in maintaining

Vitamin A content of foods

Food	Vitamin A (Retinol) µg/100g	RE µg/100g
Veal liver	28000	28000
Carrots	-	1500
Spinach	-	795
Melon (cantaloupe)	-	784
Butter	590	653
Cheese (Cheddar)	390	440
Egg	276	272
Broccoli	-	146
Salmon	41	41
Milk (whole)	35	35

the integrity and function of the skin and mucosal cells, which function as a mechanical barrier and defend the body against infection. Vitamin A also plays a central role in the development and differentiation of white blood cells, such as lymphocytes, killer cells and phagocytes, which play a critical role in the defence of the body against pathogens.

Main functions in a nutshell:

- Vision
- Reproduction
- Growth and development
- Cellular differentiation
- Immune function

Dietary sources

The richest food source of preformed vitamin A is liver, with considerable amounts also found in egg yolk, whole milk, butter and cheese. Provitamin A carotenoids are found in carrots, yellow and dark green leafy vegetables (e.g. spinach, broccoli), pumpkin, apricots and melon.

Until recently, vitamin A activity in foods was expressed as international units (IU). This is still the measurement generally used on food and supplement labels. In order to standardise vitamin A measurement, it has now been internationally agreed to state vitamin A activity in terms of a new unit called the retinol equivalent, or RE, which accounts for the rate of conversion of carotenoids to retinol.

1 RE = 1 µg retinol
= 6 µg beta-carotene
= 12 µg other provitamin A carotenoids
= 3.33 IU vitamin A activity from retinol

Absorption and body stores

Vitamin A is absorbed in the upper part of the small intestine. Provitamin A carotenoids can be cleaved into retinol via an enzymatic process. Preformed vitamin A occurs as retinyl esters of fatty acids. They are hydrolysed and retinol is absorbed into intestinal mucosal cells (i.e. enterocytes). After re-esterification it is incorporated into chylomicrons, excreted into lymphatic channels, delivered to the blood and transported to the liver. Vitamin A is stored in the liver as retinyl esters; stores are enough for one to two years in most adults living in industrialised countries.

Measurement

Vitamin A can be measured in the blood and other body tissues by various modern techniques. For rapid field tests, a method has been developed recently using dried blood spots. Typical serum level is 1.1-2.3 µmol/L. According to the WHO, plasma levels of $\leq 0,35$ µmol/L indicate a vitamin A deficiency.

Stability

Vitamin A is sensitive to oxidation by air. Loss of activity is accelerated by heat and exposure to light. Oxidation of fats and oils (e.g. butter, margarine, cooking oils) can destroy fat soluble vitamins including vitamin A. The presence of antioxidants such as vitamin E therefore contributes to the protection of vitamin A.

Interactions

Positive interactions

- Vitamin E protects vitamin A from being oxidised; hence, adequate vitamin E status protects vitamin A status.

Negative interactions

- Disease and infection, especially measles, compromise vitamin A status and conversely, poor vitamin A status decreases resistance to diseases.
- Chronic heavy alcohol intake can impair liver storage of vitamin A.
- Acute protein deficiency interferes with vitamin A metabolism; similarly, too little fat in the diet interferes with the absorption of both vitamin A and carotenoids.
- Vitamin A deficiency may result in impaired iron absorption and decrease its utilisation for erythropoiesis, thereby potentially exacerbating iron deficiency anaemia.
- Zinc deficiency may adversely affect mobilisation of vitamin A from hepatic stores and absorption of vitamin A from the gut.

Deficiency

Vitamin A deficiency is rare in the Western world, but in developing countries it is one of the most widespread, yet preventable, causes of blindness. The earliest symptom of vitamin A deficiency is impaired dark adaptation, or night blindness. Severe deficiency causes a condition called xerophthalmia, characterised by changes in the cells of the cornea that ultimately result in corneal ulcers, scarring and blindness. The appearance of skin lesions (follicular hyperkeratosis) is also an early indicator of inadequate vitamin A status. Growth retardation is a common sign in children. Because vitamin A is required for the normal functioning of the immune system, even children who are only mildly deficient in vitamin A have a higher incidence of respiratory disease and diarrhoea, as well as a higher rate of mortality from infectious diseases, than children who consume sufficient vitamin A. Some diseases may themselves induce vitamin A deficiency, most notably liver and gastrointestinal diseases, which interfere with the absorption and utilisation of vitamin A. Vitamin A deficiency during pregnancy leads to malformations during foetal development.



Child suffering from corneal scar

Disease prevention and therapeutic use

Studies have shown that vitamin A supplementation given to children aged over 6 months reduces all-cause mortality by between 23% and 30% in low income countries. The beneficial effect is assumed to be due to the prevention of vitamin A deficiency. The World Health Organisation (WHO) recommends that supplements should be given when children are vaccinated. The currently recommended doses are 100,000 IU at age 6-11 months and 200,000 IU at age ≥ 12 months every 3-6 months. Xerophthalmia is treated with high doses of vitamin A (50,000-200,000 IU according to age). In developing countries, where vitamin A deficiency is one of the most serious health problems, children under the age of 6 years and pregnant and lactating women are the main vulnerable groups. Since vitamin A can be stored in the liver, it is possible to build up a

reserve in children by administration of high-potency doses. In regular periodic distribution programmes for the prevention of vitamin A deficiency, infants < 6 months of age receive a dose of 50,000 IU of vitamin A, and children between six months and one year receive 100,000 IU every 4-6 months, while children > 12 months of age receive 200,000 IU every 4-6 months. A single dose of 200,000 IU given to mothers immediately after delivery of their child has been found to increase the vitamin A content of breast milk. However, caution is necessary when considering vitamin A therapy for lactating women, otherwise a co-existing pregnancy may be endangered: during pregnancy, a daily dose of 10,000 IU vitamin A should not be exceeded. Administration of high doses of vitamin A to children with measles complications, but no overt signs of vitamin A deficiency, decreases mortality by over 50% and significantly lowers morbidity. Natural and synthetic vitamin A analogues have been used to treat psoriasis and severe acne.

Current recommendations in the USA

RDA*

Infants	≤ 6 months	400 μg (Adequate Intake, AI)
Infants	7–12 months	500 μg (AI)
Children	1–3 years	300 μg
Children	4–8 years	400 μg
Children	9–13 years	600 μg
Males	≥ 14 years	900 μg
Females	≥ 14 years	700 μg
Pregnancy	14–18 years	750 μg
Pregnancy	≥ 19 years	770 μg
Lactation	14–18 years	1,200 μg
Lactation	≥ 19 years	1,300 μg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin A varies according to age, sex, risk group and other criteria applied in individual countries (700–1000 µg RE/day for men, 600–800 µg RE/day for women. In the USA the RDA for adults is 900 µg (men) and 700 µg (women) per day of preformed vitamin A (retinol). During lactation, an additional 500–600 µg per day are recommended. Infants and children, due to their smaller body size, have a lower RDA than adults.

Safety

Because vitamin A (as retinyl ester) is stored in the liver, large amounts taken over a period of time can eventually exceed the liver's storage capacity, spill into the blood, and produce adverse effects (liver damage, bone abnormalities and joint pain, alopecia, headaches, vomiting, and skin desquamation). Hypervitaminosis A can occur acutely following very high doses taken over a period of several days, or as a chronic condition from high doses taken over a long period of time. Thus, there is concern about the safety of high intakes of preformed vitamin A (retinol), especially for infants, small children, and women of childbearing age.

Normal foetal development requires sufficient vitamin A intake, but consumption of excess retinol during pregnancy is known to cause malformations in the newborn.

Several recent prospective studies suggest that long-term intakes of preformed vitamin A in excess of 1,500 µg/day are associated with increased risk of osteoporotic fracture and decreased bone mineral density in older men and women. Only excess

intakes of preformed vitamin A, not beta-carotene, were associated with adverse effects on bone health. Current levels of vitamin A in fortified foods are based on RDA levels, ensuring that there is no realistic possibility of vitamin A overdosage in the general population. In the vast majority of cases, signs and symptoms of toxicity are reversible upon cessation of vitamin A intake. Beta-carotene is considered a safe form of vitamin A because it is converted by the body only as needed.

The Food and Nutrition Board of the Institute of Medicine (2001) and the EC Scientific Committee on Food (2002) have set the tolerable upper intake level (UL) of vitamin A intake for adults at 3000 µg RE/day with appropriately lower levels for children.

Supplements and food fortification

Vitamin A is available in soft gelatine capsules, as chewable or effervescent tablets, or in ampoules. It is also included in most multivitamins. Retinyl acetate, retinyl palmitate and retinal are the forms of vitamin A most commonly used in supplements.

Margarine and milk are commonly fortified with vitamin A. Beta-carotene is added to margarine and many other foods (e.g. fruit drinks, salad dressings, cake mixes, ice cream) both for its vitamin A activity and as a natural food colourant.

Industrial production

Nowadays vitamin A is rarely extracted from fish liver oil. The modern method of industrial synthesis of nature-identical vitamin A is a highly complex, multi-step process.



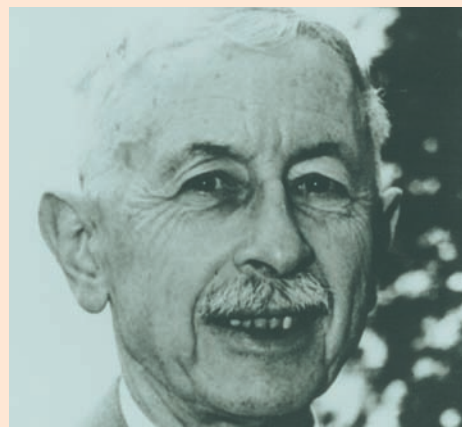
History

Although it has been known since ancient Egyptian times that certain foods, such as liver, would cure night blindness, vitamin A per se was not identified until 1913. Its chemical structure was defined by Paul Karrer in 1931. Professor Karrer received a Nobel Prize for his work because this was the first time that a vitamin's structure had been determined.

- 1831** Wackenroder isolates the orange-yellow colourant from carrots and names it "carotene."
- 1876** Snell successfully demonstrates that night blindness and xerophthalmia can be cured by giving the patient cod liver oil.
- 1880** Lunin discovers that, besides needing carbohydrates, fats and proteins, experimental animals can only survive if given small quantities of milk powder.
- 1887** Arnaud describes the widespread presence of carotenes in plants.
- 1909** Stepp successfully extracts the vital liposoluble substance from milk.
- 1915** McCollum differentiates between "fat-soluble A" and "water-soluble B."
- 1929** The vitamin A activity of beta-carotene is demonstrated in animal experiments.
- 1931** Karrer isolates practically pure retinol from the liver oil of a species of mackerel. Karrer and Kuhn isolate active carotenoids.
- 1946** Isler undertakes the first large-scale industrial synthesis of vitamin A.
- 1984** Sommer demonstrates that vitamin A deficiency is a major cause of infant mortality in Indonesia.
- 1987** Chombon in Strasbourg and Evans in San Diego, and their respective coworkers, simultaneously discover the retinoic acid receptors in cell nuclei.
- 1997** UNICEF, the World Health Organisation (WHO), and the governments of countries including Canada, the United States and the United Kingdom, as well as national governments in countries where vitamin deficiency is widespread, launch a global campaign to distribute high-dose vitamin A capsules to malnourished children.



Elmer V. McCollum

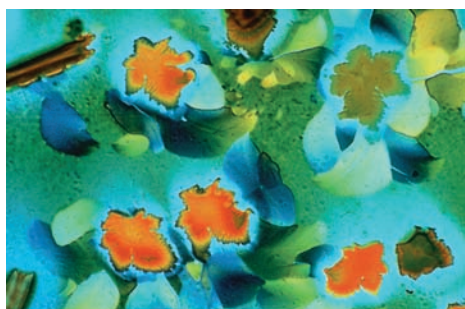


Paul Karrer



Otto Isler

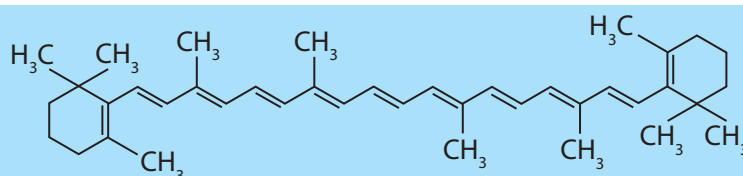
Beta-carotene



Beta-carotene crystals in polarised light

Chemistry

Beta-carotene is a terpene. It is made up of eight isoprene units, which are cyclised at each end. The long chain of conjugated double bonds is responsible for the orange colour of beta-carotene.



Molecular formula of beta-carotene

Introduction

Beta-carotene is one of more than 600 carotenoids known to exist in nature. Carotenoids are yellow, orange and red pigments that are widely distributed in plants. In 1831, beta-carotene was isolated by Wackenroder. Its structure was determined by Karrer in 1931, who received a Nobel prize for his work. About 50 of the naturally occurring carotenoids can potentially yield vitamin A and are thus referred to as provitamin A carotenoids. Beta-carotene is the most abundant and most efficient provitamin A in our foods.

Currently available evidence suggests that in addition to being a source of vitamin A, beta-carotene plays many important biological roles that may be independent of its provitamin status.

Functions

Beta-carotene is the main safe dietary source of vitamin A. Vitamin A is essential for normal growth and development, immune system function, and vision.

Beta-carotene can quench singlet oxygen, a reactive molecule that is generated, for instance, in the skin by exposure to ultraviolet light, and which can induce precancerous changes in cells. Singlet oxygen is capable of triggering free radical chain reactions.

Beta-carotene has antioxidant properties that help neutralise free radicals – reactive and highly energised molecules which are formed through certain normal biochemical reactions (e.g. the immune response, prostaglandin synthesis), or through exogenous sources such as air pollution or cigarette smoke. Free radicals can damage lipids in cell membranes as well as the genetic material in cells, and the resulting damage may lead to the development of cancer.

Main functions in a nutshell:

- Provitamin A
- Antioxidant activity

Dietary sources

The best sources of beta-carotene are yellow/orange vegetables and fruits and dark green leafy vegetables:

- Yellow/orange vegetables – carrots, sweet potatoes, pumpkins, winter squash
- Yellow/orange fruits – apricots, cantaloupes, papayas, mangoes, carambolas, nectarines, peaches
- Dark green leafy vegetables – spinach, broccoli, endive, kale, chicory, escarole, watercress and beet leaves, turnips, mustard, dandelion
- Other good vegetable and fruit sources – summer squash, asparagus, peas, sour cherries, prune plums.

The beta-carotene content of fruits and vegetables can vary according to the season and degree of ripening.

Beta-carotene content of foods

Food	Beta-carotene (mg/100g)
Carrots	7.6
Kale	5.2
Spinach	4.8
Cantaloupes	4.7
Apricots	1.6
Mangoes	1.2
Broccoli	0.9
Pumpkins	0.6
Asparagus	0.5
Peaches	0.1

(Souci, Fachmann, Kraut)

Absorption and body stores

Bile salts and fat are needed for the absorption of beta-carotene in the upper small intestine. Many dietary factors, e.g. fat and protein, affect absorption. Approximately 10-50% of the total beta-carotene consumed is absorbed in the gastrointestinal tract. The proportion of carotenoids absorbed decreases as dietary intake increases. Within the intestinal wall (mucosa), beta-carotene is partially converted into vitamin A (retinol) by the enzyme dioxygenase. This mechanism is regulated by the individual's vitamin A status. If the body has enough vitamin A, the conversion of beta-carotene decreases. Therefore, beta-carotene is a very safe source of vitamin A and high intakes will not lead to hypervitaminosis A.

Excess beta-carotene is stored in the fat tissues of the body and the liver. The adult's fat stores are often yellow from accumulated carotene while the infant's fat stores are white.

Bioavailability of beta-carotene

Bioavailability refers to the proportion of beta-carotene that can be absorbed, transported and utilised by the body once it has been consumed. It is influenced by a number of factors:

- Beta-carotene from dietary supplements is better absorbed than beta-carotene from foods
- Food processing such as chopping, mechanical homogenisation and cooking enhances bioavailability of beta-carotene
- The presence of fat in the intestine affects absorption of beta-carotene. The amount of dietary fat required to ensure carotenoid absorption seems to be low (approximately 3-5g per meal)

Measurement

Plasma carotenoid concentration is determined by HPLC. It reflects the intake of carotenoids. Traditionally, vitamin A activity of beta-carotene has been expressed in International Units (IU; 1 IU = 0.60 µg of all-trans beta-carotene). However, this conversion factor does not take into account the poor bioavailability of carotenoids in humans. Thus, the FAO/WHO Expert Committee proposed that vitamin A activity be expressed as retinol equivalents (RE). 6 µg beta-carotene provide 1 µg retinol.

For labelling, official national directives should be followed.

1 RE = 1 µg retinol
= 6 µg beta-carotene
= 3.33 IU vitamin A activity from retinol
= 10 IU vitamin A activity from beta-carotene

Stability

Carotenoids can lose some of their activity in foods during storage due to the action of enzymes and exposure to light and oxygen. Dehydration of vegetables and fruits may greatly reduce the biological activity of carotenoids. On the other hand, carotenoid stability is retained in frozen foods.

Interactions

Negative interactions

Cholestyramine and colestipol (cholesterol-lowering agents), mineral oil, orlistat (a weight loss medication) and omeprazole (proton-pump inhibitor) can reduce absorption of carotenoids.

Deficiency

Although consumption of provitamin A carotenoids can prevent vitamin A deficiency, there are no known adverse clinical effects of a low carotenoid diet, provided vitamin A intake is adequate.

Disease prevention and therapeutic use

Immune system

In a number of animal and human studies beta-carotene supplementation was found to enhance certain immune responses. Early studies demonstrated the ability of beta-carotene and other carotenoids to prevent infections. Some clinical trials have found that beta-carotene supplementation improves several biomarkers of immune function. It can lead to an increase in the number of white blood cells and the activity of natural killer cells. Both of these are important in combating various diseases. It may be the case that beta-carotene stimulates the immune system once it has undergone conversion to vitamin A. Another explanation could be that the antioxidant actions of beta-carotene protect cells of the immune system from damage by reducing the toxic effects of reactive oxygen species.

Skin

Recent evidence points to a role of beta-carotene in protecting the skin from sun damage. Beta-carotene can be used as an oral sun protectant in combination with sunscreens for the prevention of sunburn. Its effectiveness has been proven both alone and in combination with other carotenoids or antioxidant vitamins.

Cancer and cardiovascular diseases

Epidemiological studies consistently indicate that as consumption of beta-carotene-rich fruits and vegetables increases, the risk of certain cancers (i.e. lung and stomach cancer) and cardiovascular diseases decreases.

Additionally, animal experiments have shown that beta-carotene acts as a cancer risk reduction agent.



This is further supported by studies of biomarkers for the development of certain cancers. There is no evidence that beta-carotene supplementation reduces the risk of cardiovascular diseases.

Erythropoietic protoporphyria

In patients with erythropoietic protoporphyria – a photosensitivity disorder leading to abnormal skin reactions to sunlight – beta-carotene in doses of up to 180 mg has been shown to exert a photoprotective effect.

Recommended Dietary Allowance (RDA)

Until now, dietary intake of beta-carotene has been expressed as part of the RDA for vitamin A. The daily vitamin A requirements for adult men and women are 900 µg and 700 µg of preformed vitamin A (retinol) respectively (FNB, 2001). Apart from its provitamin A function, data continue to accumulate supporting a role for beta-carotene as an important micronutrient in its own right. Consumption of foods rich in beta-carotene is being recommended by scientific and government organisations such as the US National Cancer Institute (NCI) and the US Department of Agriculture (USDA). If these dietary guidelines are followed, dietary intake of beta-carotene (about 6 mg) would be several times the average amount presently consumed in the US (about 1.5 mg daily).

Safety

Beta-carotene is a safe source of vitamin A. Due to the regulated conversion of beta-carotene into vitamin

A, overconsumption does not produce hypervitaminosis A. Excessive intakes of beta-carotene may cause carotenodermia, which manifests itself in a yellowish tint of the skin, mainly in the palms of the hands and soles of the feet. The yellow colour disappears when carotenoid consumption is reduced or stopped.

High doses of beta-carotene (up to 180 mg/day) used for the treatment of erythropoietic protoporphyria have shown no adverse effects.

In two studies investigating the effect of beta-carotene supplementation on the risk of developing lung cancer, an apparent increase of lung cancer in chronic heavy smokers with intakes of more than 20 mg/day over several years has been observed. The reasons for these findings are not yet clear.

The British Expert Committee on Vitamins and Minerals (EVM) recommends a Safe Upper Level for supplementation of 7 mg/day over a lifetime period. Other agencies such as the European DACH Society (German Society of Nutrition, Austrian Society of Nutrition, Swiss Society of Nutrition Research) have concluded that a daily intake of up to 10 mg of beta-carotene is safe.

Supplements and food fortification

Beta-carotene is available in hard and soft gelatine capsules, in multivitamin tablets, and in antioxidant vitamin formulas and as food colour. Margarine and fruit drinks are often fortified with beta-carotene. In 1941, the US Food and Drug Administration (FDA) established a standard of identity for the addition of vitamin A to margarine; since



then, however, vitamin A has been partly replaced by beta-carotene, which additionally imparts an attractive yellowish colour to this product. Due to its high safety margin, beta-carotene has been recognised as more suitable for fortification purposes than vitamin A.

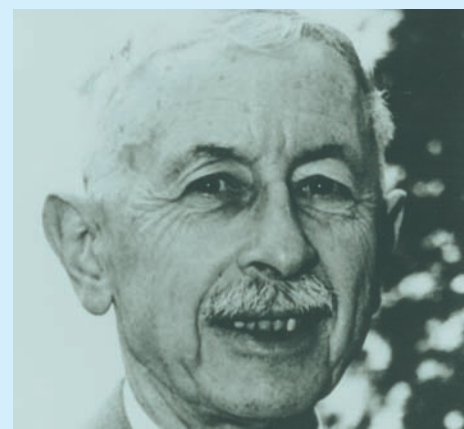
Industrial production

Isler and coworkers developed a method to synthesise beta-carotene, and it has been commercially available in crystalline form since 1954.

History

- 1831** Wackenroder isolates the orange-yellow pigment in carrots and coins the term 'carotene'.
- 1847** Zeise provides a more detailed description of carotene.
- 1866** Carotene is classified as a hydrocarbon by Arnaud and co-workers.
- 1887** Arnaud describes the widespread presence of carotenes in plants.
- 1907** Willstatter and Mieg establish the molecular formula for carotene, a molecule consisting of 40 carbon and 56 hydrogen atoms.
- 1914** Palmer and Eckles discover the presence of carotene and xanthophylls in human blood plasma.
- 1919** Steenbock (University of Wisconsin) suggests a relationship between yellow plant pigments (beta-carotene) and vitamin A.
- 1929** Moore demonstrates that beta-carotene is converted into the colourless form of vitamin A in the liver.
- 1931** Karrer and collaborators (Switzerland) determine the structures of beta-carotene and vitamin A.
- 1939** Wagner and coworkers suggest that the conversion of beta-carotene into vitamin A occurs within the intestinal mucosa.
- 1950** Isler and colleagues develop a method for synthesising beta-carotene.
- 1966** Beta-carotene is found acceptable for use in foods by the Joint FAO/WHO Expert Committee on Food Additives.
- 1972** Specifications for beta-carotene use in foods is established by the U.S. Food Chemicals Codex.
- 1979** Carotene is established as 'GRAS', which means that the ingredient is 'Generally Recognised As Safe' and can be used as a dietary supplement or in food fortification.
- 1981-82** Beta-carotene/carotenoids are recognised as important factors (independent of their provitamin A activity) in potentially reducing the risk of certain cancers. R. Doll and R. Peto: "Can Dietary Beta-carotene Materially Reduce Human Cancer Rates?" (in: Nature, 1981; 290: 201-208) R. Shekelle et al: "Dietary Vitamin A and Risk of Cancer in the Western Electric Study" (in: Lancet, 1981: 1185-1190) "Diet, Nutrition and Cancer" (1982): Review of the U.S. National Academy of Sciences showing that intake of carotenoid-rich foods is associated with reduced risk of certain cancers.

- 1982** Krinsky and Deneke show the interaction between oxygen and oxyradicals using carotenoids.
- 1983-84** The US National Cancer Institute (NCI) launches large-scale clinical intervention trials using beta-carotene supplements alone and in combination with other nutrients.
- 1984** Beta-carotene is demonstrated to be an effective antioxidant in vitro.
- 1988** Due to the large number of epidemiological studies that demonstrate the potential reduction of cancer incidence with increased consumption of dietary beta-carotene, the US National Cancer Institute (NCI) issues dietary guidelines advising Americans to include a variety of vegetables and fruits in their daily diet.
- 1993-94** Availability of results from several large-scale clinical intervention trials using beta-carotene alone or in various other combinations.
- 1997** Evidence indicates that beta-carotene acts synergistically with vitamins C and E.
- 1999** The Women's Health Study shows no increased risk of lung cancer for woman receiving 50 mg beta-carotene on alternate days.
- 2004** Results from the French SU.VI.MAX study indicate that a combination of antioxidant vitamins (C, E and beta-carotene) and minerals lowers total cancer incidence and all-cause mortality in men.

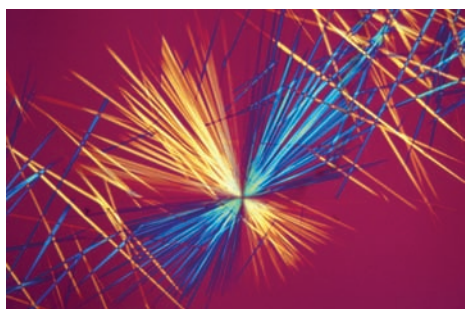


Paul Karrer



Otto Isler

Vitamin D



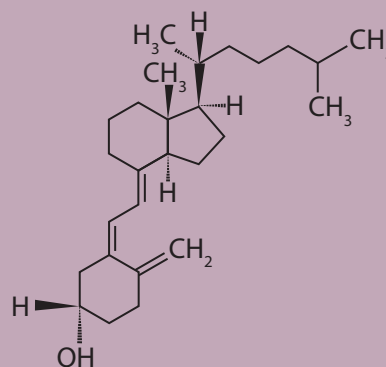
Vitamin D crystals in polarised light

Synonyms

Calciferol; antirachitic factor; "sunshine" vitamin

Chemistry

Vitamin D is a generic term and indicates a molecule of the general structure shown for rings A, B, C, and D with differing side chain structures. The A, B, C, and D ring structure is derived from the cyclopentanoperhydrophenanthrene ring structure for steroids. Technically, vitamin D is classified as a seco-steroid. Seco-steroids are those in which one of the rings has been broken; in vitamin D, the 9,10 carbon-carbon bond of ring B is broken.



Molecular formula of vitamin D₃ (cholecalciferol)

Introduction

Vitamin D is the general name given to a group of fat-soluble compounds that are essential for maintaining the mineral balance in the body. The chemical structure of vitamin D was identified in the 1930s. The main forms are vitamin D₂ (ergocalciferol: found in plants, yeasts and fungi) and vitamin D₃ (cholecalciferol: of animal origin).

As cholecalciferol is synthesised in the skin by the action of ultraviolet light on 7-dehydrocholesterol, a cholesterol derivative, vitamin D does not fit the classical definition of a vitamin. Nevertheless, because of the numerous factors that influence its synthesis, such as latitude, season, air pollution, area of skin exposed, pigmentation, age, etc., vitamin D is recognized as an essential dietary nutrient.

Functions

Following absorption or endogenous synthesis, the vitamin has to be metabolised before it can perform its biological functions. Calciferol is transformed in the liver to 25-hydroxycholecalciferol (25(OH)D, calcidiol). This is the major circulating form, which is metabolised in the kidney to the active forms as required. The most important of these is 1,25-dihydroxy-cholecalciferol (1,25(OH)₂D, calcitriol) because it is responsible for most of the biological functions. The formation of 1,25(OH)₂D, which is considered a hormone, is strictly controlled according to the body's calcium needs. The main controlling factors are the existing levels of 1,25(OH)₂D itself and the blood level of parathyroid hormone, calcium and phosphorus.

To perform its biological functions, 1,25(OH)₂D, like other hormones, binds to a specific nuclear receptor

(vitamin D receptor, VDR). Upon interaction with this receptor, 1,25(OH)₂D regulates more than 50 genes in a wide variety of tissues.

Vitamin D is essential for the control of normal calcium and phosphate blood levels. It is known to be required for the absorption of calcium and phosphate in the small intestine, their mobilisation from the bones, and their reabsorption in the kidneys. Through these three functions it plays an important role for the proper functioning of muscles, nerves and blood clotting and for normal bone formation and mineralisation.

It has been suggested that vitamin D also plays an important role in controlling cell proliferation and differentiation, immune responses and insulin secretion.

Main functions in a nutshell:

- Regulation of calcium and phosphate blood levels
- Bone mineralisation
- Control of cell proliferation and differentiation
- Modulation of immune system

Dietary sources

Vitamin D is found only in a few foods. The richest natural sources of vitamin D are fish liver oils and salt-water fish such as sardines, herring, salmon and mackerel. Eggs, meat, milk and butter also contain small amounts. Plants are poor sources, with fruit and nuts containing no vitamin D at all. The amount of vitamin D in human milk is insufficient to cover infant needs.

Absorption and body stores

Absorption of dietary vitamin D takes place in the upper part of the small intestine with the aid of bile salts. It is incorporated into the chylomicron fraction and absorbed through the lymphatic system. Vitamin D is stored in adipose tissue. It has to be metabolised to become active.

Measurement

Vitamin D status is best determined by the serum 25(OH)D concentration because this reflects dietary sources as well as vitamin D production by UV light in the skin. Usual serum 25(OH)D values are between 25 and 130 nmol/L depending on geographic location.

1 µg vitamin D is equivalent to 40 IU (international unit).

Stability

Vitamin D is relatively stable in foods. Storage, processing and cooking have little effect on its activity, although in fortified milk up to 40% of the vitamin D added may be lost as a result of exposure to light.

Vitamin D content of foods

Food	Vitamin D (µg/100g)
Herring	25
Salmon	16
Sardines	11
Mackerel	4
Egg	2.9
Butter	1.2
Milk (whole)	0.07

(Souci, Fachmann, Kraut)

Interactions

Positive interactions

Women taking oral contraceptives have been found to have slightly elevated blood levels of $1,25(\text{OH})_2\text{D}$.

Negative interactions

Cholestyramine (a resin used to stop reabsorption of bile salts) and laxatives based on mineral oil inhibit the absorption of vitamin D from the intestine. Corticosteroid hormones, anticonvulsant drugs and alcohol can affect the absorption of calcium by reducing the response to vitamin D.

Animal studies also suggest that anticonvulsant drugs stimulate enzymes in the liver, resulting in an increased breakdown and excretion of the vitamin.

Deficiency

Among the first symptoms of marginal vitamin D deficiency are reduced serum levels of calcium and an increase in parathyroid hormone (PTH) production. Serum alkaline phosphatase is elevated in vitamin D deficiency states. This can be accompanied by muscle weakness and tetany, as well as an increased risk of infection. Children may show unspecific symptoms, such as restlessness, irritability, excessive sweating and impaired appetite. Marginal hypovitaminosis D may contribute to bone brittleness in the elderly. Vitamin D deficiency can also cause hearing loss.

The most widely recognised manifestations of severe vitamin D deficiency are rickets in children and osteomalacia in adults. Both are characterised by loss of mineral from the bones. This results in skeletal deformities such as bowed legs in children. The ends of the long bones in both the arms and legs are affected, and their growth may be retarded. Rickets also results in

inadequate mineralisation of tooth enamel and dentin.

Osteoporosis, a disorder of older age in which there is loss of bone, not just demineralisation, has also been associated with less obvious states of deficiency.

Groups at risk of deficiency:

- Infants who are exclusively breast fed are at high risk of vitamin D deficiency, because human milk is a poor source of vitamin D. In addition, in premature and low-birth-weight infants, liver and kidney function may be inadequate for optimal vitamin D metabolism.
- The elderly have a reduced capacity to synthesise vitamin D in the skin by exposure to sunlight.
- People with diseases affecting the liver, kidneys, the thyroid gland or fat absorption, as well as vegetarians, alcoholics and epileptics on long-term anticonvulsant therapy have a greater risk of deficiency, as do people who are house-bound.
- Dark-skinned people produce less vitamin D from sunlight and are at risk of deficiency when living far from the equator.
- Populations living at latitudes of around 40 degrees north or south are exposed to insufficient levels of sunlight to cover vitamin D requirements through endogenous production, especially during winter months.

Hereditary vitamin D-dependent rickets (type I and II):

These rare forms of rickets occur in spite of an adequate supply of vitamin D. These are inherited forms in which the formation or utilisation of $1,25(\text{OH})_2\text{D}$ is impaired.

Disease prevention and therapeutic use

In the treatment of rickets, a daily dose of $40\text{ }\mu\text{g}$ (1,600 IU) vitamin D usually results in normal plasma concentrations of calcium and phosphorus within 10 days. The dose can be reduced gradually to $10\text{ }\mu\text{g}$ (400 IU) per day after one month of therapy.

Vitamin D analogues are used in the treatment of psoriasis.

Vitamin D is discussed as a prevention factor for a number of diseases. Results from epidemiological studies and evidence from animal models suggest that the risk of several autoimmune diseases (multiple sclerosis, insulin-dependent diabetes mellitus, rheumatoid arthritis) may be decreased by adequate vitamin D intake.

Vitamin D plays an important role in the prevention of osteoporosis because vitamin D insufficiency can be an important contributing factor in this disease. A prospective study among 72,000 postmenopausal women over 18 years indicated that women consuming at least 600 IU vitamin D/day from food plus supplements had a 37% lower risk of hip fracture. Evidence from most clinical trials suggests that vitamin D supplementation slows bone density losses and decreases the risk of osteoporotic fracture in men and women.

Various surveys and studies suggest that poor vitamin D intake or status is associated with an increased risk of colon, breast and prostate cancer.

Recommended Dietary Allowance (RDA)

Establishing an RDA for vitamin D is difficult because vitamin D can be endogenously produced in the body through exposure to sunlight. Healthy people regularly exposed to the sun have no dietary requirement for vitamin D, under appropriate conditions. As this is rarely the case in temperate zones, however, a dietary supply is needed.

In 1997, the Food and Nutrition Board based adequate intake levels (AI) on the assumption that no vitamin D is produced by UV light in the skin. An AI of 5 µg (200 IU)/day is recommended for infants, children and adults (ages 19-50 years). For the elderly, higher intakes are recommended to maintain normal calcium metabolism and maximise bone health. In other countries, adult recommendations range from 2.5 µg (100 IU) to 10 µg (400 IU).

Safety

Hypervitaminosis D is a potentially serious problem as it can cause permanent kidney damage, growth retardation, calcification of soft tissues and death. Mild symptoms of intoxication are nausea, weakness, constipation and irritability. In general, the toxic dose for adults is around 1.25 mg (50,000 IU) per day. However, certain individuals have an increased sensitivity to vitamin D and present with toxic symptoms after 50 µg (2,000 IU) per day. Hypervitaminosis D is not associated with overexposure to the sun because a regulating mechanism prevents overproduction of vitamin D.

The Food and Nutrition Board (FNB) and the EU Scientific Committee on Food have set the tolerable upper intake level (UL) for vitamin D at 50 µg/day for adolescents and adults.



Supplements and food fortification

Monopreparations of vitamin D and related compounds are available as tablets, capsules, oily solutions and injections. Vitamin D is also incorporated in combinations with vitamin A, calcium, and in multivitamins.

In many countries, milk and milk products, margarine and vegetable oils fortified with vitamin D serve as a major dietary source of the vitamin.

Current recommendations in the USA

RDA*

Infants		5 µg (AI)
Children	1-18 years	5 µg (AI)
Males	19-50 years	5 µg (AI)
Females	19-50 years	5 µg (AI)
Males	51- 70 years	10 µg (AI)
Females	51-70 years	10 µg (AI)
Males	> 70 years	15 µg (AI)
Females	> 70 years	15 µg (AI)
Pregnancy		5 µg (AI)
Lactation		5 µg (AI)

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Industrial production

Cholecalciferol is produced commercially by the action of ultraviolet light on 7-dehydrocholesterol, which is obtained from cholesterol by various methods. Ergocalciferol is produced in a similar manner from ergosterol, which is extracted from yeast. Starting material for the production of calcitriol is the cholesterol derivative pregnenolone.

History

- 1645** Whistler writes the first scientific description of rickets.
- 1865** In his textbook on clinical medicine, Trousseau recommends cod liver oil as treatment for rickets. He also recognises the importance of sunlight and identifies osteomalacia as the adult form of rickets.
- 1919** Mellanby proposes that rickets is due to the absence of a fat-soluble dietary factor.
- 1922** McCollum and coworkers establish the distinction between vitamin A and the antirachitic factor.
- 1925** McCollum and coworkers name the antirachitic factor vitamin D. Hess and Weinstock show that a factor with antirachitic activity is produced in the skin by ultraviolet irradiation.
- 1936** Windaus identifies the structure of vitamin D in cod liver oil.
- 1937** Schenck obtains crystallised vitamin D₃ by activation of 7-dehydro-cholesterol.
- 1968** Haussler and colleagues report the presence of an active metabolite of vitamin D in the intestinal mucosa of chicks.
- 1969** Haussler and Norman discover calcitriol receptors in chick intestine.
- 1970** Fraser and Kodicek discover that calcitriol is produced in the kidney.
- 1971** Norman and coworkers identify the structure of calcitriol.
- 1973** Fraser and associates discover the presence of an inborn error of vitamin D metabolism that produces rickets resistant to vitamin D therapy.
- 1978** De Luca's group discovers a second form of vitamin D-resistant rickets (Type II).
- 1981** Abe and colleagues in Japan demonstrate that calcitriol is involved in the differentiation of bone-marrow cells.
- 1983** Provvedini and colleagues demonstrate the presence of calcitriol receptors in human leukocytes.
- 1984** The same group presents evidence that calcitriol has a regulatory role in immune function.
- 1986** Morimoto and associates suggest that calcitriol may be useful in the treatment of psoriasis.

- 1989** Baker and associates show that the vitamin D receptor belongs to the steroid-receptor gene family.
- 1994** The U.S. Food and Drug Administration approves a vitamin D-based topical treatment for psoriasis, called calcipotriol.
- 2003** A prospective study from Feskanich and coworkers among 72,000 postmenopausal women in the U.S. over 18 years indicated that women consuming at least 600 IU vitamin D/day from food plus supplements had a 37% lower risk of hip fracture.
- 2006** Researchers from the Harvard School of Public Health examined cancer incidence and vitamin D exposure in over 47,000 men in the Health Professionals Follow-Up Study. They found that a high level of vitamin D (~1500 IU daily) was associated with a 17% reduction in all cancer incidences and a 29% reduction in total cancer mortality with even stronger effects for digestive-system cancers.



Sir Edward Mellanby

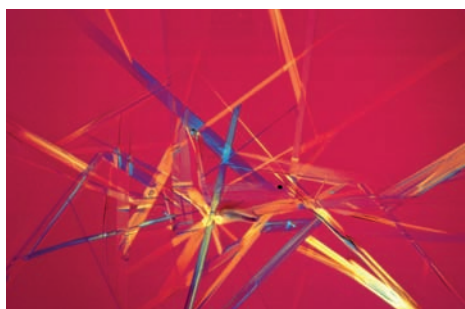


Elmer V. McCollum



Adolf Windaus

Vitamin E



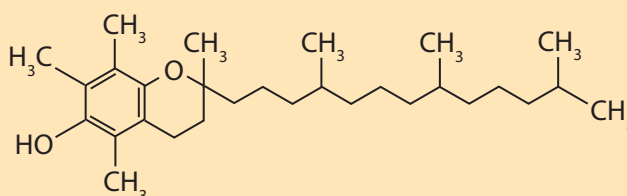
Vitamin E crystals in polarised light

Synonyms

Tocopherol

Chemistry

A group of compounds composed of a substituted chromanol ring with a C₁₆ side chain saturated in tocopherols, with 3 double bonds in tocotrienols.



Molecular formula of α -tocopherol

Introduction

The term vitamin E covers eight fat-soluble compounds found in nature. Four of them are called tocopherols and the other four tocotrienols. They are identified by the prefixes α , β , γ and δ . α -Tocopherol is the most common and biologically the most active of these naturally occurring forms of vitamin E. Natural tocopherols occur in RRR-configuration only (RRR- α -tocopherol was formerly designated as *d*- α -tocopherol). The chemical synthesis of α -tocopherol results in a mixture of eight different stereoisomeric forms which is called *all-rac*- α -tocopherol (or *dl*- α -tocopherol). The biological activity of the synthetic form is lower than that of the natural form.

The name tocopherol derives from the Greek words *tocos*, meaning childbirth, and *pherein*, meaning to bring forth. The name was coined to highlight its essential role in the reproduction of various animal species.

The ending *-ol* identifies the substance as being an alcohol.

The importance of vitamin E in humans was not accepted until fairly recently. Because its deficiency is not manifested by a well-recognised, widespread vitamin deficiency disease such as scurvy (vitamin C deficiency) or rickets (vitamin D deficiency), science only began to recognise the importance of vitamin E at a relatively late stage.

Functions

The major biological function of vitamin E is that of a lipid soluble antioxidant preventing the propagation of free-radical reactions. Free radicals are formed in normal metabolic processes and upon exposure to exogenous toxic agents (e.g. cigarette smoke, pollutants). Vitamin E is located within the cellular mem-



branes. It protects polyunsaturated fatty acids (PUFAs) and other components of cellular membranes from oxidation by free radicals. Apart from maintaining the integrity of the cell membranes in the human body, it also protects low density lipoprotein (LDL) from oxidation.

Recently, non-antioxidant functions of α -tocopherol have been identified.

α -Tocopherol inhibits protein kinase C activity, which is involved in cell proliferation and differentiation. Vitamin E inhibits platelet aggregation and enhances vasodilation. Vitamin E enrichment of endothelial cells downregulates the expression of cell adhesion molecules, thereby decreasing the adhesion of blood cell components to the endothelium.

Main functions in a nutshell:

- Major fat soluble antioxidant of the body
- Non-antioxidant functions in cell signalling, gene expression and regulation of other cell functions

Dietary sources

Vegetable oils (olive, soya beans, palm, corn, safflower, sunflower, etc.), nuts, whole grains and wheat germ are the most important sources of vitamin E. Other sources

are seeds and green leafy vegetables. The vitamin E content of vegetables, fruits, dairy products, fish and meat is relatively low.

The vitamin E content in foods is often reported as α -tocopherol equivalents (α -TE). This term was established to account for the differences in biological activity of the various forms of vitamin E. 1 mg of α -tocopherol is equivalent to 1 TE. Other tocopherols and tocotrienols in the diet are assigned the following values: 1 mg β -tocopherol = 0.5 TE; 1 mg γ -tocopherol = 0.1 TE; 1 mg δ -tocopherol = 0.03 TE; 1 mg α -tocotrienol = 0.3 TE; 1 mg β -tocotrienol = 0.05 TE.

Vitamin E content of foods

Food	Vitamin E (mg α -TE/100g)
Wheat germ oil	174
Sunflower oil	63
Hazelnut	26
Rape seed oil	23
Soya bean oil	17
Olive oil	12
Peanuts	11
Walnuts	6
Butter	2
Spinach	1.4
Tomatoes	0.8
Apples	0.5
Milk (whole)	0.14

(Souci, Fachmann, Kraut)



Absorption and body stores

Vitamin E is absorbed together with lipids in the small intestine, depending on adequate pancreatic function and biliary secretion. Tocopherol esters which are present in food supplements and processed food are hydrolysed before absorption. Vitamin E is incorporated into chylomicrons and transported via the lymphatic system to the liver. α -Tocopherol is the vitamin E form that predominates in blood and tissue. This is due to the action of a liver protein (α -tocopherol transfer protein) preferentially incorporating α -tocopherol into the lipoproteins which deliver it to the different tissues. Vitamin E is found in most human body tissues. The highest vitamin E contents are found in the adipose tissue, liver and muscles. The pool of vitamin E in the plasma, liver, kidneys and spleen turns over rapidly, whereas turnover of the content of adipose tissue is slow.

Measurement

Normal α -tocopherol concentrations in plasma measured by high performance liquid chromatography range from 12–45 μM (0.5–2 mg/100 ml). Plasma α -tocopherol concentrations of <11.6 μM , the level at which erythrocyte haemolysis occurs, indicate poor vitamin E nutritional status. Since plasma levels of α -tocopherol correlate with cholesterol levels, the α -tocopherol concentration is often indicated as α -tocopherol-cholesterol ratio. Vitamin E content is generally expressed by biological activity, using the scale of International Units (IU). According to this system, 1 mg of RRR- α -tocopherol, biologically the most active of the naturally occurring forms of vitamin E, is equivalent to 1.49 IU vitamin E. The

biological activity of 1 mg of *all-rac*- α -tocopheryl acetate, the synthesised form of vitamin E commonly used in food enrichment, is equivalent to 1 IU. Recently, the unit of α -tocopherol equivalent was established (see: Dietary sources).

Stability

Light, oxygen and heat, detrimental factors encountered in long storage of foodstuffs and food processing, lower the vitamin E content of food. In some foods it may decrease by as much as 50% after only two weeks' storage at room temperature. To a large extent, frying destroys the vitamin E in vegetable oils.

Esters of α -tocopherol (α -tocopheryl acetate and α -tocopheryl succinate) are used for supplements because they are more resistant to oxidation during storage.

Interactions

Positive interactions

The presence of other antioxidants, such as vitamin C and beta-carotene, supports the antioxidative, protective action of vitamin E; the same is true of the mineral selenium.

Negative interactions

When taken at the same time, iron reduces the availability of vitamin E to the body; this is especially critical in the case of anaemic newborns.

The requirement for vitamin E is related to the amount of polyunsaturated fatty acids consumed in the diet. The higher the amount of PUFAs, the more vitamin E is required.

Vitamin K deficiency may be exacerbated by vitamin E, thereby affecting blood coagulation.

Various medications decrease absorption of vitamin E (e.g., cholestyramine, colestipol, isoniazid).

Deficiency

Because depletion of vitamin E tissue stores takes a very long time, no overt clinical deficiency symptoms have been noted in otherwise healthy adults. Symptoms of vitamin E deficiency are seen in patients with fat malabsorption syndromes or liver disease, in individuals with genetic defects affecting the α -tocopherol transfer protein and in newborn infants, particularly premature infants.

Vitamin E deficiency results in neurological symptoms (neuropathy), myopathy (muscle weakness) and pigmented retinopathy. Early diagnostic signs are leakage of muscle enzymes, increased plasma levels of lipid peroxidation products and increased haemolysis of erythrocytes (red blood cells). In premature infants, vitamin E deficiency is associated with haemolytic anaemia, intraventricular haemorrhage and retrolental fibroplasia.

Disease prevention and therapeutic use

Research studies suggest that vitamin E has numerous health benefits. Vitamin E is thought to play a role in preventing atherosclerosis and cardiovascular diseases (heart disease and stroke) due to its effects on a number of steps in the development of atherosclerosis (e.g. inhibition of LDL oxidation, inhibition of smooth muscle cell proliferation, inhibition of platelet adhesion, aggregation and platelet release reaction).

Recent studies suggest that vitamin E enhances immunity in the elderly, and that supplementation with vitamin E lowers the risk of contracting an upper respiratory tract infection, particularly the common cold.

Researchers are investigating the

prophylactic role of vitamin E in protecting against exogenous pollutants and lowering the risk of cancer and of cataracts.

Vitamin E in combination with vitamin C may protect the body from oxidative stress caused by extreme sports (e.g. ultra marathon running). A role of vitamin E supplementation in the treatment of neurodegenerative diseases (Alzheimer's disease, amyotrophic lateral sclerosis) is also under investigation.

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin E varies according to age, sex and criteria applied in individual countries. In the USA, the RDA for adults is 15 mg RRR- α -tocopherol/day (FNB, 2000). In Europe, adult recommendations range from 4 to 15 mg α -TE/day for men and from 3 to 12 mg α -TE/day for women.

The RDA for vitamin E of 15 mg can-

not easily be acquired even with the best nutritional intentions, yet most research studies show that optimal intake levels associated with health benefits tend to be high. Vitamin E intake should also be adapted to that of PUFA, which influences the requirement for this vitamin. The EC Scientific Committee on Foods (SCF) has suggested a consumption ratio of 0.4 mg α -TE per gram of PUFA.

Safety

Vitamin E has low toxicity. After reviewing more than 300 scientific studies, the US-based Institute of Medicine (IOM) concluded that vitamin E is safe for chronic use even at doses of up to 1000 mg per day. A recently published meta-analysis suggested that taking more than 400 IU of vitamin E per day brought a weekly increase in the risk of all-cause mortality. However, much of the research was done in patients at high risk of a chronic disease and these findings may not be generalisable to healthy adults. Many human long-term studies with higher doses

Current recommendations in the USA

RDA*

Infants	≤ 6 months	4 mg (6 IU)(AI)
Infants	7-12 months	5 mg (7.5 IU)(AI)
Children	1-3 years	6 mg (9 IU)
Children	4-8 years	7 mg (10.5 IU)
Children	9-13 years	11 mg (16.5 IU)
Males	≥ 14 years	15 mg (22.5 IU)
Females	≥ 14 years	15 mg (22.5 IU)
Pregnancy		15 mg (22.5 IU)
Lactation		19 mg (28.5 IU)

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

of vitamin E have not reported any adverse effects, and it has been concluded that vitamin E intakes of up to 1600 IU (1073 mg RRR- α -tocopherol) are safe for most adults. The Antioxidant Panel of the Food and Nutrition Board (FNB, 2000) has set the UL (tolerable upper intake level) for adults at 1000 mg/day of any form of supplemental α -tocopherol. In 2003 the EC Scientific Committee on Foods (SCF) established the UL of 300 mg α -TE for adults. Also in 2003, the UK Expert group on Vitamins and Minerals (EVM; 2003) set the UL at 540 mg α -TE for supplemental vitamin E. Pharmacologic doses of vitamin E may increase the risk of bleeding in patients treated with anticoagulants. Patients on anticoagulant therapy or those anticipating surgery should avoid high levels of vitamin E.

Supplements, food fortifications and other applications

Vitamin E is available in soft gelatine capsules, or as chewable or effervescent tablets, and is found in most multivitamin supplements. The most common fortified foods are soft drinks and cereals.

The *all-rac*- α -tocopherol form of vitamin E is widely used as an antioxidant in stabilising edible oils, fats and fat-containing food products. Research has shown that vitamin E in combination with vitamin C reduces the formation of nitrosamines (a proven carcinogen in animals) in bacon more effectively than vitamin C alone.

Vitamin E has been used topically as an anti-inflammatory agent, to enhance skin moisturisation and to prevent cell damage by UV light.

In pharmaceutical products tocopherol is used, for example, to stabilise syrups, aromatic components, and vitamin A or provitamin A components.

α -Tocopherol is used as an antioxidant in plastics, technical oils and greases, and in the purified, so-called white oils, employed in cosmetics and pharmaceuticals.

Industrial production

Vitamin E derived from natural sources is obtained by molecular distillation and, in most cases, subsequent methylation and esterification of edible vegetable oil products. Synthetic vitamin E is produced from fossil plant material by condensation of trimethylhydroquinone with isophytol.



History

- 1911** Hart and coworkers publish the first report of a suspected “anti-sterility factor” in animals.
- 1920** Matthill and Conklin observe reproductive anomalies in rats fed on special milk diets.
- 1922** Vitamin E is discovered by Evans and Scott Bishop.
- 1936** Evans and coworkers isolate what turns out to be α -tocopherol in its pure form from wheat germ oil.
- 1938** Fernholz provides the structural formula of vitamin E and Nobel laureate Karrer synthesises dl- α -tocopherol.
- 1945** Dam and coworkers discover peroxides in the fat tissue of animals fed on vitamin E-deficient diets. The first antioxidant theory of vitamin E activity is proposed.
- 1962** Tappel proposes that vitamin E acts as an in vivo antioxidant to protect cell lipids from free radicals.
- 1968** The Food and Nutrition Board of the US National Research Council recognises vitamin E as an essential nutrient for humans.
- 1974** Fahrenholtz proposes singlet oxygen quenching abilities of α -tocopherol.
- 1977** Human vitamin E deficiency syndromes are described.
- 1980** Walton and Packer propose that vitamin E may prevent the generation of potentially carcinogenic oxidative products of unsaturated fatty acids.
- 1980** McKay and King suggest that vitamin E functions as an antioxidant located primarily in the cell membrane.
- 1980s** Vitamin E is demonstrated to be the major lipid-soluble antioxidant protecting cell membranes from peroxidation. Vitamin E is shown to stabilise the superoxide and hydroxyl free radicals.
- 1990** Effectiveness of vitamin E in inhibiting LDL (low density lipoprotein) oxidation is shown.
- 1990** Kaiser and coworkers elucidate the singlet oxygen quenching capability of vitamin E.
- 1991** Azzi and coworkers describe an inhibitory effect of α -tocopherol on the proliferation of vascular smooth muscle cells and protein kinase C activity.
- 2004** Barella and coworkers demonstrate that vitamin E regulates gene expression in the liver and the testes of rats.



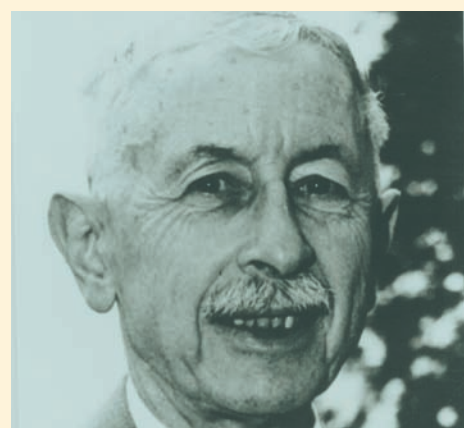
Herbert Evans



Katherine Scott Bishop

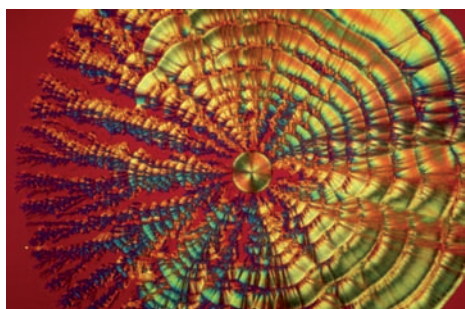


Erhard Fernholz



Paul Karrer

Vitamin K



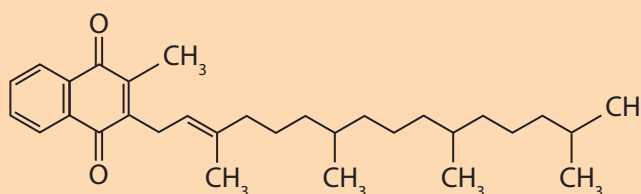
Vitamin K crystals in polarised light

Synonyms

Phylloquinone, menaquinone

Chemistry

Compounds with vitamin K activity are 3-substituted 2-methyl-1,4-naphthoquinones. Phylloquinone contains a phytyl group, whereas menaquinones contain a polyisoprenyl side chain with 6 to 13 isoprenyl units at the 3-position.



Molecular formula of vitamin K₁ (phylloquinone)

Introduction

In 1929 Henrik Dam observed that chicks fed on fat-free diets developed haemorrhages and started bleeding. In 1935 he proposed that the antihaemorrhagic substance was a new fat-soluble vitamin, which he called vitamin K (after the first letter of the German word “Koagulation”). Vitamin K is indeed fat-soluble, and it occurs naturally in two forms: vitamin K₁ (phylloquinone) is found in plants; vitamin K₂ is the term for a group of compounds called menaquinones (MK-n, n being the number of isoprenyl units in the side chain of the molecule) which are synthesised by bacteria in the intestinal tract of humans and various animals. Vitamin K₃ (menadione) is a synthetic compound that can be converted to K₁ in the intestinal tract. It is only used in animal nutrition.

Functions

Vitamin K is essential for the synthesis of the biologically active forms of a range of proteins called vitamin K-dependent proteins. Vitamin K participates in the conversion of glutamate residues of these proteins to γ -carboxylglutamate residues by addition of a carboxyl-group (carboxylation).

In the absence of vitamin K, carboxylation of these proteins is incomplete, and they are secreted in plasma in various so called under-carboxylated forms, which are biologically inactive.

Vitamin K is also essential for the functioning of several proteins involved in blood coagulation (clotting), a mechanism that prevents bleeding to death from cuts and wounds, as well as internal bleeding.

Vitamin K-dependent proteins:

- Prothrombin (factor II), factors VII, IX, and X, and proteins C, S and Z are proteins that are involved in the regulation of blood coagulation. They are synthesised in the liver. Protein S has also been detected in bone.
- The vitamin K-dependent proteins osteocalcin and MGP (matrix Gla-protein) have been found in bone. Osteocalcin is thought to be related to bone mineralisation. Matrix Gla-protein is present in bone, cartilage and vessel walls and has recently been established as an inhibitor of calcification. The role of protein S in bone metabolism is not clear.
- Recently, several other vitamin K-dependent proteins have been identified.

Main functions in a nutshell:

- Coenzyme for a vitamin K-dependent carboxylase
- Blood coagulation
- Bone metabolism

Dietary sources

The best dietary sources of vitamin K₁ are green leafy vegetables such as spinach, broccoli, Brussels sprouts, cabbage and lettuce. Other rich sources are certain vegetable oils. Good sources include oats, potatoes, tomatoes, asparagus and butter. Lower levels are found in beef, pork, ham, milk, carrots, corn, most fruits and many other vegetables.

An important source of vitamin K₂ is the bacterial flora in the anterior part of the gut – the jejunum and ileum.

Vitamin K content of foods

Food	Vitamin K (µg/100g)
Spinach	305
Brussels sprouts	236
Broccoli	155
Rape seed oil	150
Soya bean oil	138
Lettuce	109
Cabbage	66
Asparagus	39
Olive oil	33
Butter	7

(Souci, Fachmann, Kraut)





Absorption and body stores

Vitamin K is absorbed from the jejunum and ileum. As with other fat-soluble vitamins, absorption depends on the presence of bile and pancreatic juices and is enhanced by dietary fat. Although the liver is the main storage site, vitamin K is also found in extrahepatic tissues, e.g. bone and heart. Liver stores consist of about 10% phyloquinones and 90% menaquinones. Compared with that of other fat-soluble vitamins, the total body pool of vitamin K is small and turnover of vitamin K in the liver is rapid. The body recycles vitamin K in a process called the vitamin K cycle, allowing the vitamin to function in the γ -carboxylation of proteins many times over.

Although the liver contains menaquinones synthesised by intestinal bacteria, the absorption of menaquinones and their contribution to the human vitamin K requirement have not yet been fully elucidated.

Measurement

Plasma vitamin K concentration is measured by high performance liquid chromatography. The normal range of plasma vitamin K in adults is 0.2-3.2 ng/ml. Levels below 0.5 ng/ml have been associated with impaired blood-clotting functions. However, the value of measuring plasma vitamin K concentration to assess vitamin K status is limited because it responds to changes in

dietary intake within 24 hours.

Overt vitamin K deficiency results in impaired blood clotting, usually demonstrated by laboratory tests that measure clotting time.

Plasma concentration of one of the vitamin K-dependent blood-clotting factors – prothrombin, factor VII, factor IX or factor X – is measured to assess an inadequate intake of vitamin K. The normal range of plasma prothrombin concentration is from 80 to 120 μ g/ml.

Recently, other parameters for assessing vitamin K status have been studied, e.g. measurements of undercarboxylated prothrombin and undercarboxylated osteocalcin in both normal and pathological conditions.

Stability

Vitamin K compounds are moderately stable to heat and reducing agents, but are sensitive to acid, alkali, light and oxidising agents.

Interactions

Negative interactions

- Coumarin anticoagulants (such as warfarin), salicylates and certain antibiotics act as vitamin K antagonists.
- Very high dietary or supplemental intakes of vitamin K may inhibit the anticoagulant effect of vitamin K antagonists (e.g. warfarin).
- High doses of vitamins A and E have been shown to interfere with vitamin K and precipitate deficiency states.
- Absorption of vitamin K may be decreased by mineral oil, bile acid sequestrants (cholestyramine, colestipol) and orlistat (weight loss medication).

Deficiency

Vitamin K deficiency is uncommon in healthy adults but occurs in individuals with gastrointestinal disorders, fat malabsorption or liver disease, or after prolonged antibiotic therapy coupled with compromised dietary intake. Impaired blood clotting is the clinical symptom of vitamin K deficiency, which is demonstrated by measuring clotting time. In severe cases, bleeding occurs. Adults at risk of vitamin K deficiency also include patients taking anticoagulant drugs which are vitamin K antagonists.

Newborn infants have a well established risk of vitamin K deficiency, which may result in fatal intracranial haemorrhage (bleeding within the skull) in the first weeks of life. Breast-fed infants in particular have a low vitamin K status because placental transfer of vitamin K is poor and human milk contains low levels of vitamin K. The concentrations of plasma clotting factors are low in infants due to immaturity of the liver. Haemorrhagic disease in the newborn is a significant worldwide cause of infant morbidity and mortality. Therefore, in many countries vitamin K is routinely administered prophylactically to all newborns.

Disease prevention and therapeutic use

Phylloquinone is the preferred form of the vitamin for clinical use. It is used for intravenous and intramuscular injections as a colloidal suspension, emulsion or aqueous suspension, and as a tablet for oral use. Vitamin K₁ is used in the treatment of hypoprothrombinemia (low amounts of prothrombin), secondary to factors limiting absorption or syn-

thesis of vitamin K. During operations in which bleeding is expected to be a problem, for example, in gall-bladder surgery, vitamin K₁ is administered.

Anticoagulants inhibit vitamin K recycling, which can be corrected rapidly and effectively by the administration of vitamin K₁.

Vitamin K₁ is often given to mothers before delivery and to newborn infants to protect against intracranial haemorrhage.

A putative role of vitamin K in osteoporosis has been investigated since vitamin K-dependent proteins have been discovered in bone. However, further investigations are required to resolve whether vitamin K is a significant etiological component of osteoporosis. A role for vitamin K in the development of atherosclerosis is also under discussion, but studies supporting this hypothesis are limited and future research is recommended.

Recently, studies with cancer cell lines and animal studies have indicated that a combination of vitamin C and vitamin K₃ has antitumor activity and inhibits the development of metastases.

Recommended Daily Allowance (RDA)

The US Food and Nutrition Board of the Institute of Medicine (2001) has established an adequate intake (AI) level for adults based on reported dietary intakes of vitamin K in apparently healthy population groups. Other health authorities have come to similar conclusions.

Safety

Even when large amounts of vitamin K₁ and K₂ are ingested over an extended period, toxic manifestations have not been observed. Therefore, the major health authorities have not established a tolerable upper level of intake (UL) for vitamin K. Allergic reactions have been reported, however. Furthermore, administered menadione (K₃) has been known to cause haemolytic anaemia, jaundice and kernicterus (a grave form of jaundice in the newborn) and is no longer used for treatment of vitamin K deficiency.

Supplements, food fortification and other applications

Supplements of vitamin K are available alone in tablets and capsules, and also in multivitamin preparations.

Infant formula products, beverages and cookies are fortified with vitamin K. Menadione salts are generally preferred for farm animals because of their stability.

Industrial production

The procedure involves the use of monoester, menadiol and an acid catalyst. Purification of the desired product to remove unreacted reagents and side products occurs either at the quinol stage or after oxidation.

Current recommendations in the USA

RDA*

Infants	< 6 months	2 µg (AI)
Infants	7-12 months	2.5 µg (AI)
Children	1-3 years	30 µg (AI)
Children	4-8 years	55 µg (AI)
Children	9-13 years	60 µg (AI)
Children	14-18 years	75 µg (AI)
Males	> 19 years	120 µg (AI)
Females	> 19 years	90 µg (AI)
Pregnancy	14-18 years	75 µg (AI)
Pregnancy	> 19 years	90 µg (AI)
Lactation	14-18 years	75 µg (AI)
Lactation	> 19 years	90 µg (AI)

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

History

- 1929** A series of experiments by Dam results in the discovery of vitamin K.
- 1931** A clotting defect is observed by McFarlane and coworkers.
- 1935** Dam proposes that the antihaemorrhagic vitamin in chicks is a new fat-soluble vitamin, which he calls vitamin K.
- 1936** Dam and associates succeed in preparing a crude plasma prothrombin fraction, and demonstrate that its activity is decreased when it is obtained from vitamin K-deficient chick plasma.
- 1939** Vitamin K₁ is synthesised by Doisy and associates.
- 1940** Brikhous observes haemorrhagic conditions resulting from malabsorption syndromes or starvation, and finds that haemorrhagic disease of the newborn responds to vitamin K.
- 1943** Dam receives half of the Nobel prize for his discovery of vitamin K, the blood coagulation factor.
- 1943** Doisy receives half of the Nobel prize for his discovery of the chemical nature of vitamin K.
- 1974** The vitamin K-dependent step in prothrombin synthesis is demonstrated by Stenflo and associates and Nelsestuen and colleagues.
- 1975** Esmon discovers a vitamin K-dependent protein carboxylation in the liver.

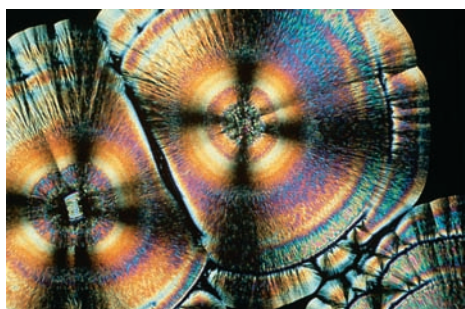


Carl P. H. Dam



Edward A. Doisy

Vitamin C



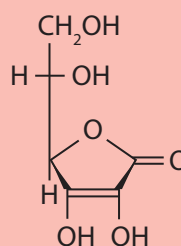
Vitamin C crystals in polarised light

Synonyms

Ascorbic acid, hexuronic acid, anti-scorbutic vitamin

Chemistry

L-ascorbic acid (2,3-endiol-L-gulonic acid- γ -lactone), dehydro-L-ascorbic acid (2-oxo-L-gulonic acid- γ -lactone).



Molecular formula of vitamin C

Introduction

Vitamin C is water-soluble, and probably the most famous of all the vitamins. Even before its discovery in 1932, physicians recognised that there must be a compound in citrus fruits preventing scurvy, a disease that killed as many as 2 million sailors between 1500 and 1800. Later researchers discovered that man, other primates and the guinea pig depend on external sources to cover their vitamin C requirements. Most other animals are able to synthesise vitamin C from glucose and galactose in their body.

Functions

The most prominent role of vitamin C is its immune stimulating effect, which is important for the defence against infections such as common colds. It also acts as an inhibitor of histamine, a compound that is released during allergic reactions. As a powerful antioxidant it can neutralise harmful free radicals and aids in neutralising pollutants and toxins. Thus it is able to prevent the formation of potentially carcinogenic nitrosamines in the stomach (due to consumption of nitrite-containing foods, such as smoked meat). Importantly, vitamin C is also able to regenerate other antioxidants such as vitamin E. Vitamin C is required for the synthesis of collagen, the intercellular “cement” substance which gives structure to muscles, vascular tissues, bones, tendons and ligaments. Due to these functions vitamin C, especially in combination with zinc, is important for the healing of wounds. Vitamin C contributes to the health of teeth and gums, preventing haemorrhaging and bleeding. It also improves the absorption of iron from the diet, and is needed for the metabolism of bile acids, which may have implications

for blood cholesterol levels and gallstones. In addition, vitamin C plays an important role in the synthesis of several important peptide hormones and neurotransmitters and carnitine. Finally, vitamin C is also a crucial factor in the eye's ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration (AMD) and vision-loss in combination with other antioxidant vitamins and zinc.

Main functions in a nutshell:

- Immune stimulation
- Anti-allergic
- Antioxidant
- “Cement” for connective tissues
- Wound healing
- Teeth and gum health
- Aids iron absorption
- Eye health

Dietary sources

Vitamin C is widely distributed in fruits and vegetables. Citrus fruits, blackcurrants, peppers, green vegetables (e.g. broccoli, Brussels sprouts), and fruits like strawberries, guava, mango and kiwi are particularly rich sources. On a quantity basis, the intake of potatoes, cab-

bage, spinach and tomatoes is also of importance. Depending on the season, one medium-sized glass of freshly pressed orange juice (i.e. 100 g) yields from 15 to 35 mg vitamin C.

Absorption and body stores

Intestinal absorption of vitamin C depends on the amount of dietary intake, decreasing with increasing intake levels. At an intake of 30 to 180 milligrams, about 70% to 90% is absorbed; about 50% of a single dose of 1 to 1.5 grams is absorbed; and only 16% of a single dose of 12 grams is absorbed. Up to about 500 milligrams are absorbed via a sodium-dependent active transport process, while at higher doses simple diffusion occurs.

The storage capacity of water-soluble vitamins is generally low compared to that of fat-soluble ones. Humans have an average tissue store of vitamin C of 20 mg/kg body weight. The highest concentration is found in the pituitary gland (400 mg/kg); other tissues of high concentration are the adrenal glands, liver, brain and white blood cells (leukocytes).

Vitamin C content of foods

Food	Vitamin C (mg/100g)
Acerolas	1600
Blackcurrants	200
Peppers	138
Broccoli	115
Fennel	95
Kiwis	71
Strawberries	64
Oranges	49

(Souci, Fachmann, Kraut)

Measurement

Vitamin C can be measured in the blood plasma and other body tissues by various techniques. Also dipstick tests for estimation of vitamin C levels in the urine are available. Less satisfying, however, is the evaluation of the analytical data concerning the true reflection of the body status. Threshold values are difficult to define and the subject of controversial discussion. Typical blood plasma levels are in the range of 20 to 100 µmol/L.

Stability

Vitamin C is sensitive to heat, light and oxygen. In food it can be partly or completely destroyed by long storage or overcooking. Refrigeration can substantially diminish vitamin C loss in food.

Influence of storage and preparation on vitamin C loss in foods

Food	Storage/Preparation	Vitamin C Loss
Potatoes	1 month	50%
Fruit	1 month	20%
Apples	6-9 months	100%
Milk	UHT	25%
Fruit	Sterilisation	50%
Fruit	Air drying	50-70%
Leafy vegetables	Canning	48%

Modified from Oberbeil, Fit durch Vitamine, Die neuen Wunderwaffen, Südwest Verlag GmbH & Co. KG, München 1993

sants, diuretics, birth control pills and aspirin, deplete the tissues of vitamin C. This is also true of certain habits, for example alcohol consumption.

characterised by weakening of collagenous structures, resulting in widespread capillary bleeding. Infantile scurvy causes bone malformations. Bleeding gums and loosening of the teeth are usually the earliest signs of clinical deficiency. Haemorrhages under the skin cause extreme tenderness of extremities and pain during movement. If left untreated, gangrene and death may ensue. Nowadays this is rare in developed countries and can be prevented by a daily intake of about 10-15 mg of vitamin C. However, for optimal physiological functioning much higher amounts are required.

The development of vitamin C deficiency can be caused by:

- Inadequate storage and preparation of food
- Gastrointestinal disturbances
- Stress and exercise
- Infections
- Smoking
- Diabetes
- Pregnancy and lactation

Deficiency

Early symptoms of vitamin C deficiency are very general and could also indicate other diseases. They include fatigue, lassitude, loss of appetite, drowsiness and insomnia, feeling run-down, irritability, low resistance to infections and petechiae (minor capillary bleeding). Severe vitamin C deficiency leads to scurvy,

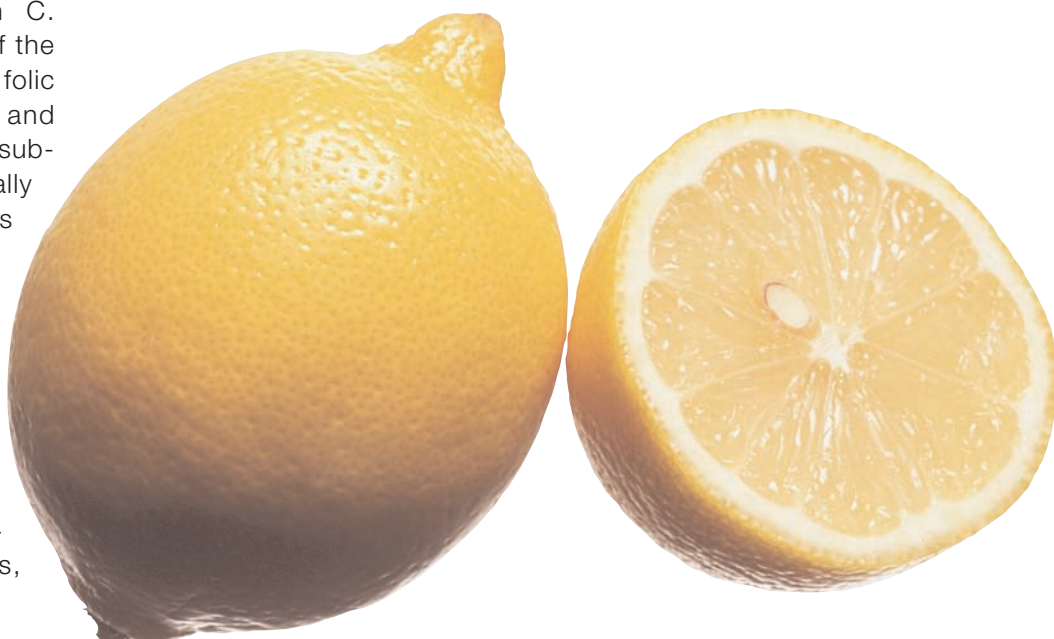
Interactions

Positive interactions

The presence of other antioxidants, such as vitamin E and beta-carotene, supports the protective antioxidant action of vitamin C. Other vitamins, such as those of the B-complex (particularly B₆, B₁₂, folic acid and pantothenic acid) and some pharmacologically active substances, as well as the naturally occurring compounds known as bioflavonoids, may have a sparing effect on vitamin C.

Negative interactions

Due to toxic compounds in smoke, the vitamin C requirement for smokers is about 35 mg/day higher than for non-smokers. Also several pharmacologically active compounds, among them some anti-depres-



Disease prevention and therapeutic use

Dozens of prospective studies suggest that vitamin C plays a role in preventing a variety of diseases. It is also used to treat certain diseases in orthomolecular medicine. As this nutrient is important for a variety of diseases, only a selection of them are presented here in detail.

Cardiovascular diseases (CVD) (heart disease and stroke)

The data for the CVD protective benefits of vitamin C are inconsistent. While some studies have failed to find significant reductions in the risk of coronary heart disease (CHD), numerous prospective cohort studies have found inverse associations between dietary vitamin C intake or vitamin C plasma levels and CVD risk. Vitamin C may protect coronary arteries by reducing the build-up of

plaque, as this helps to prevent the oxidation of LDL cholesterol (the “bad” cholesterol), especially in combination with vitamin E. Some data has shown that vitamin C may also boost blood levels of HDL cholesterol (the “good” cholesterol), which is also considered positive for the prevention of heart diseases. The risk of stroke may be reduced by an adequate intake of vitamin C through fruits, vegetables and supplements. However, due to the inconsistency of the data and its lack of specificity to vitamin C, the interpretation of these results is difficult.

Cancer

The role of vitamin C in cancer prevention has been studied extensively, and until now no beneficial effect has been shown for breast, prostate, or lung cancer. However, a number of studies have associated higher intakes of vitamin C with decreased incidence of cancers of the upper digestive tract, cervix, ovary, bladder, and colon. Studies finding significant cancer risk reduction by dietary intake recommended at least 5 servings of fruits and vegetables per day. Five servings of most fruits and vegetables provide more than 200 mg vitamin C per day. Just significant cancer risk reductions were found in people consuming at least 80 to 110 mg of vitamin C daily.

Common cold

Numerous studies have shown a general lack of effect of prophylactic vitamin C supplementation on the incidence of common cold, but they do show a moderate benefit in terms of the duration and severity of episodes in some groups, especially those who are exposed to substantial physical and/or cold stress. The improve-

ment in severity of colds after vitamin C supplementation may be due to the antihistaminic action of mega doses of vitamin C.

Wound healing

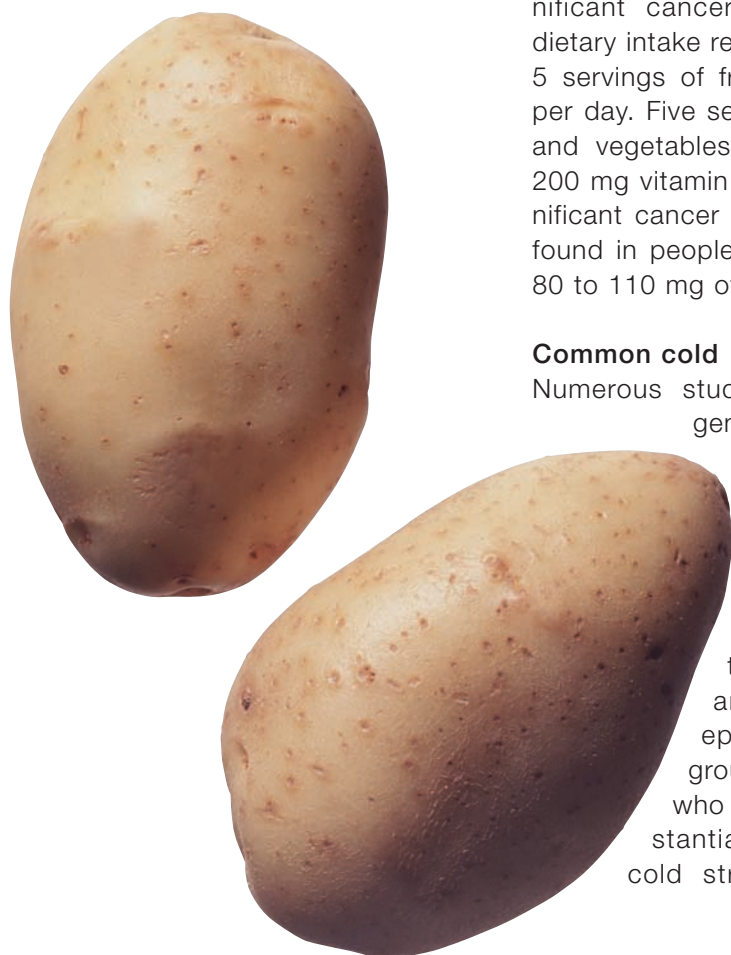
During a postoperative period, or during healing of superficial wounds, supplemental vitamin C contributes to the prevention of infections and promotes skin repair.

Blood pressure

Several studies have shown a blood pressure lowering effect of vitamin C supplementation at about 500 mg per day due to improved dilation of blood vessels.

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin C varies according to age, sex, risk group and criteria applied in individual countries. The RDAs in the USA for vitamin C were recently revised upwards to 90 mg/day for men and 75 mg/day for women, based on pharmacokinetic data. For smokers, these RDAs are increased by an additional 35 mg/day. Higher amounts of vitamin C are also recommended for pregnant (85 mg/day) and lactating women (120 mg/day). The RDAs are in a similar range in other countries. Recent evidence sets the estimate for the maintenance of optimal health in the region of 100 mg daily.



Safety

As much as 6-10 g vitamin C per day (more than 100 times the RDA) has been ingested regularly by many people with no evidence of side effects. Although a number of possible problems with very large doses of vitamin C have been suggested, none of these adverse health effects have been confirmed, and there is no reliable scientific evidence that large amounts of vitamin C (up to 10 g/day in adults) are toxic. In the year 2000 the US Food and Nutrition Board recommended a tolerable upper intake level (UL) for vitamin C of 2 g (2,000 mg) daily in order to prevent most adults from experiencing osmotic diarrhoea and gastrointestinal disturbances.

Supplements and food fortification

Vitamin C is offered in conventional tablets, effervescent and chewable tablets, time-release tablets, syrups, powders, granules, capsules, drops and ampoules, either alone or in multivitamin-mineral preparations. Buffered vitamin C forms are less acidic, which can be an advantage in terms of preventing gastric irritation. Vitamin C can also be used in the form of injections (Rx). A number of fruit juices, fruit flavour drinks and breakfast cereals are enriched with vitamin C. On average in Europe, vitamin C supplements provide between 5.8% and 8.3% of total vitamin C intake.

Uses in food technology

The food industry uses ascorbic acid as a natural antioxidant. This means that ascorbic acid, added to food-stuffs during processing or prior to packing, preserves colour, aroma and nutrient content. This use of ascorbic acid has nothing to do with its vitamin action. In meat processing, ascorbic acid makes it possible to reduce both the amount of added nitrite and the residual nitrite content in the product. The addition of ascorbic acid to fresh flour improves its baking qualities, thus saving the 4-8 weeks of maturation flour would normally have to undergo after milling.

Industrial production

The synthesis of ascorbic acid was achieved by Reichstein in 1933, and this was followed by industrial production five years later by Hoffman La Roche Ltd. (the vitamin division of which is now DSM Nutritional Products Ltd.). Today synthetic vitamin C, identical to that occurring in nature, is produced from glucose on an industrial scale by chemical and biotechnological synthesis.

Current recommendations in the USA

Dietary Reference Intakes*

Infants	< 6 months	40mg (Adequate Intake, AI)
Infants	7-12 months	50mg (AI)
Children	1-3 years	15mg
Children	4-8 years	25mg
Children	9-13 years	45mg
Males	14-18 years	75mg
Females	14-18 years	65mg
Males	> 19 years	90mg
Females	> 19 years	75mg
Pregnancy	< 18 years	80mg
Pregnancy	> 19 years	85mg
Lactation	< 18 years	115mg
Lactation	> 19 years	120mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

History

Ca.

- 400 BC** Hippocrates describes the symptoms of scurvy.
- 1747** British naval physician James Lind prescribes oranges and lemons as a cure for scurvy.
- 1907** Scurvy is experimentally produced in guinea pigs by Hoist and Frohlich.
- 1917** A bioassay is developed by Chick and Hume to determine the anti-scorbutic properties of foods.
- 1930** Szent-Györgyi demonstrates that the hexuronic acid he first isolated from the adrenal glands of pigs in 1928 is identical to vitamin C, which he extracts in large quantities from sweet peppers.
- 1932** In independent efforts, Haworth and King establish the chemical structure of vitamin C.
- 1932** The relationship between vitamin C and anti-scorbutic factor is discovered by Szent-Györgyi and at the same time by King and Waugh.
- 1933** In Basle, Reichstein synthesises ascorbic acid identical to natural vitamin C. This is the first step towards the vitamin's industrial production in 1936.
- 1937** Haworth and Szent-Györgyi receive a Nobel prize for their research on vitamin C.
- 1940** In a self experiment, Crandon proves the mandatory contribution of vitamin C in wound healing
- 1970** Pauling draws worldwide attention with his controversial bestseller "Vitamin C and the Common Cold".
- 1975-79** Experimental studies in vitro illustrate the antioxidant and singlet-oxygen quenching properties of vitamin C.
- 1979** Packer and coworkers observe the free radical interaction of vitamin E and vitamin C.
- 1982** Niki demonstrates the regeneration of vitamin E by vitamin C in model reactions.
- 1985** The worldwide requirement for vitamin C is estimated at 30,000-35,000 tons per year.
- 1988** The National Cancer Institute (USA) recognises the inverse relationship between Vitamin C intake and various forms of cancer, and issues guidelines to increase vitamin C in the diet.

- 1998/99** Three studies show that supplementation with vitamin C can dramatically lower lead levels.
- 2004** A systematic review of thirty studies addressing the effect of supplemented vitamin C on the duration of colds revealed that there was a consistent benefit, with a reduction in duration of 8% to 14%.
- 2005** Levine calls for a re-evaluation of vitamin C as cancer therapy, especially intravenous vitamin C
- 2006** A 5 year Japanese study showed that the risk of contracting three or more colds in the five-year period was decreased by 66% by daily intake of a 500-mg vitamin C supplement.



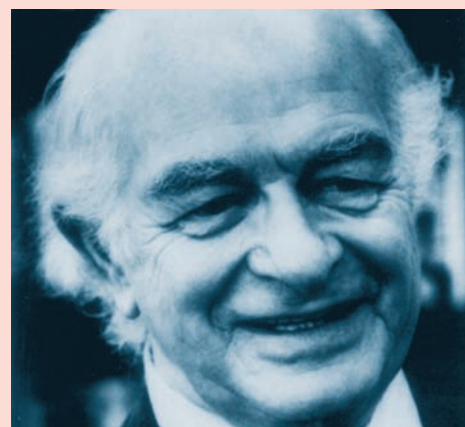
Tadeusz Reichstein



Charles Glen King

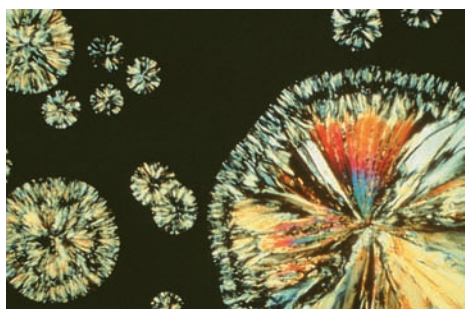


Albert Szent-Györgyi



Linus Carl Pauling

Vitamin B₁



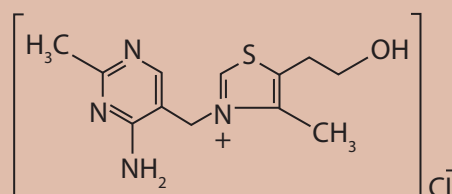
Thiamin crystals in polarised light

Synonyms

Thiamin, thiamine, antiberiberi factor, aneurine, antineuritic factor, nerve vitamin.

Chemistry

Pyrimidine and thiazole moiety linked by a methylene bridge – phosphorylated forms: thiamin monophosphate (TMP), thiamin diphosphate (TDP), thiamin triphosphate (TTP).



Molecular formula of vitamin B₁-chloride

Introduction

Thiamin is a water-soluble B-complex vitamin. It was the first B vitamin to be identified and one of the first organic compounds to be recognised as a vitamin in the 1930s. In fact it was through the discovery and naming of thiamin that the word 'vitamin', from the Latin "vita" = life and "amine" = nitrogen-containing compound, was coined. The notion that the absence of a substance in food could cause a disease (in this case beriberi) was a revolutionary one. Man and other primates rely on their food intake to cover their vitamin B₁ requirements.

Certain non-coenzyme functions of thiamin are important for nerve tissues and muscles. Its triphosphate form (TTP) in particular plays a role in the conduction of nerve impulses, in the metabolism of the neurotransmitters acetylcholin, adrenalin, and serotonin and in aerobic metabolism.

Main functions in a nutshell:

- Co-enzyme in energy metabolism
- Co-enzyme for pentose metabolism as a basis for nucleic acids
- Nerve impulse conduction and muscle action

Functions

The main functions of thiamin are connected to its role as a coenzyme in the form of thiamin pyrophosphate (TPP). Coenzymes are 'helper molecules' which activate enzymes, the proteins that control the thousands of biochemical processes occurring in the body. TPP acts as a "helper molecule" in about 25 enzymatic reactions and plays an essential role in the production of energy from food in the carbohydrate metabolism as well as in the links between carbohydrate, protein and fat metabolism. It is one of the key compounds for several reactions in the breakdown of glucose to energy. Furthermore, TPP is coenzyme for the metabolism of branched-chain keto acids that are derived from branched-chain amino acids.

Another important function of thiamin is its activation of an enzyme called "transketolase", which in turn catalyses reactions in the pentose phosphate pathway. This pathway is the basis for the production of many prominent compounds, such as ATP, GTP, NADPH and the nucleic acids DNA and RNA.

Dietary sources

Thiamin is found in most foods, but mostly in small amounts. The best source of thiamin is dried brewer's yeast. Other good sources include meat (especially pork and ham products), some species of fish (eel, tuna), whole grain cereals and bread, nuts, pulses, dried legumes and potatoes. Concerning cereal grains, the thiamin-rich bran is removed during the milling of wheat to produce white flour, and during the polishing of brown rice to produce white rice. As a consequence, enriched and fortified grain-products are common today.

Vitamin B₁ content of foods

Food	Vitamin B ₁ (mg/100g)
Brewer's yeast	12
Wheat germ	2
Sunflower seeds	1.5
Brazil nuts	1
Pork	0.9
Beans	0.8
Oatmeal	0.59
Beef	0.23

(Souci, Fachmann, Kraut)

Absorption and body stores

Gastrointestinal absorption of nutritional thiamin occurs in the lumen of the small intestine (mainly the jejunum) by means of a sodium and energy dependent active transport mechanism. For thiamin levels higher than 2 µmol/L, passive diffusion plays an additional role. Thiamin occurs in the human body as free thiamin and its phosphorylated





Stability

Vitamin B₁ is unstable when exposed to heat, alkali, oxygen and radiation. Water solubility is also a factor in the loss of thiamin from foods. About 25% of the thiamin in food is lost during the normal cooking process.

Considerable amounts may be lost in thaw drip from frozen meats or in the water used to cook meats and vegetables. To preserve thiamin, foods should be cooked in a covered pan for the shortest time possible and should not be soaked in water or heated for too long. Juices and cooking water should be re-used in stews and sauces.

Interactions

Positive interactions

The presence of other B-vitamins, such as vitamins B₆, B₁₂, niacin and pantothenic acid, supports the action of thiamin. Antioxidant vitamins, such as vitamins E and C, protect thiamin by preventing its oxidation to an inactive form.

Negative interactions

A number of foods, such as coffee, tea, betel nuts (Southeast Asia) and also some cereals may act as antagonists to thiamin. Chlorogenic acid and other plant polyphenols may be responsible for this anti-thiaminic effect. It is also known that some tropical fish and African silkworms, both traditionally consumed raw in some countries, contain enzymes called "thiaminases" that break down vitamin B₁. Drugs that cause nausea and lack of

appetite, or which increase intestinal function or urinary excretion, decrease the availability of thiamin. Poisoning from arsenic or other heavy metals produces the neurological symptoms of thiamin deficiency. These metals act by blocking a crucial metabolic step involving thiamin in its coenzyme form.

Deficiency

Marginal thiamin deficiency may manifest itself in such vague symptoms as fatigue, insomnia, irritability and lack of concentration, anorexia, abdominal discomfort, constipation and loss of appetite. When there is not enough thiamin, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism has severe consequences. The two principal thiamin deficiency diseases are "beriberi" and "Wernicke-Korsakoff syndrome".

Beriberi, which translated into English means "I can't, I can't", manifests itself primarily in disorders of the nervous and cardiovascular systems. Unfortunately this serious disease is still common in parts of south-east Asia, where polished rice is a staple food and thiamin enrichment programs are not fully in place. Many other countries fortify rice and other cereal grains to replace the nutrients lost in processing.

forms (see Chemistry). Because thiamin has a high turnover rate (10-20 days) and is not appreciably stored in the body (approx. 1 mg/day is used up in tissues), a daily supply is required. The limited stores may be depleted within two weeks or less on a thiamin-free diet, with clinical signs of deficiency beginning shortly after. Regular intake of vitamin B₁ is therefore critical. The heart, kidney, liver and brain have the highest concentrations, followed by the leukocytes and red blood cells.

Measurement

The standard way to assess thiamin status used to be to determine erythrocyte transketolase (α -ETK) activity both with and without stimulation of this enzyme by the addition of TDP cofactor. Technical difficulties led to an increasing use of direct determination of TDP in whole blood, e.g. by HPLC (High Performance Liquid Chromatography), in order to assess thiamin status. The HPLC assay is more robust and easier to perform. Thiamin status determined by this method is considered to be in good correlation with results from transketolase activation assays. Usually, whole blood concentrations are found to be between 66.5 and 200 nmol/L.

Typical serum level <75 nmol/L

"A certain very troublesome affliction, which attacks men, is called by the inhabitants Beriberi (which means sheep). I believe those, whom this same disease attacks, with their knees shaking and legs raised up, walk like sheep. It is a kind of paralysis, or rather Tremor: for it penetrates the motion and sensation of the hands and feet indeed sometimes the whole body..."

Jacobus Bonitus, Java, 1630

The disease exists in three forms:

- dry beriberi, a polyneuropathy with severe muscle wasting
- wet beriberi, which in addition to neurologic symptoms is characterised by cardiovascular manifestations, edema and ultimately heart failure
- infantile beriberi, which occurs in breast-fed infants whose nursing mothers are deficient in thiamin. Symptoms of vomiting, convulsions, abdominal distention and anorexia appear quite suddenly and may be followed by death from heart failure.

The “Wernicke-Korsakoff syndrome” (cerebral beriberi) is the thiamin deficiency disease seen most often in the Western world. It is frequently associated with chronic alcoholism in conjunction with limited food consumption. Symptoms include confusion, paralysis of eye motor nerves, abnormal oscillation of the eyes, psychosis, confabulation, and impaired retentive memory and cognitive function. The syndrome is also seen occasionally in people who fast, have chronic vomiting (hook worm) or have gross malnutrition due to e.g., AIDS or stomach cancer. If treatment of amnesic symptoms is delayed, the memory may be permanently impaired. Recent evidence suggests that oxidative stress plays an important role in the neurologic pathology of thiamin deficiency.

The development of vitamin B₁ deficiency can be caused by:

- Alcoholic disease
- Inadequate storage and preparation of food
- Increased demand due to pregnancy and lactation, heavy physical exertion, fever and stress, or adolescent growth
- Inadequate nutrition
 - high carbohydrate intake (e.g., milled or polished rice, sweetsies)
 - regular heavy consumption of tea and coffee (Tannin = antithiamin)
 - foods such as raw fish or betel nuts (thiaminases)
- Certain diseases (dysentery, diarrhea, cancer, nausea/vomiting, liver diseases, Infections, malaria, AIDS, hyperthyroidism).
- certain drugs (birth-control pills, neuroleptics, some cancer drugs)
- Long-term parenteral nutrition (e.g. highly concentrated dextrose infusions)

Disease prevention and therapeutic use

Thiamin is specific in the prevention and treatment of beriberi and other manifestations of vitamin B₁ deficiency (e.g. Wernicke-Korsakoff, peripheral neuritis). The dosage range is from 100 mg daily in mild deficiency states to 200-300 mg in severe cases. Thiamin administration is often beneficial in neuritis accompanied by excessive alcohol consumption or pregnancy. With alcoholic and diabetic polyneuropathies, the therapeutic dose is most often in the range of 10-100 mg/daily. When alcoholism has led to delirium tremens, large doses of vitamin B₁, together with other vitamins should be given by slow injection. Large doses of thiamin (100-600 mg daily) have been advocated in the treatment of such diverse conditions as lumbago, sciatica, trigeminal neuritis, facial paralysis and optic neuritis. However, the response to such treatment has been variable.

Current recommendations in the USA

RDA*

Infants	< 6 months	0.2mg (Adequate Intake, AI)
Infants	7-12 months	0.3mg (AI)
Children	1-3 years	0.5mg
Children	4-8 years	0.6mg
Children	9-13 years	0.9mg
Males	> 14 years	1.2mg
Females	14-18 years	1.0mg
Females	> 19 years	1.1mg
Pregnancy		1.4mg
Lactation		1.4mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Recommended Dietary Allowance (RDA)

Because thiamin facilitates energy utilisation, requirements are tied to energy intake, which can be very much dependent on activity levels. For adults, the RDA is 0.5 mg per 1000 kcal, which amounts to a range of 1.0-1.1 mg per day for women and 1.2-1.5 mg for men, based on an average caloric intake. An additional 0.4-0.5 mg per day are recommended during pregnancy and lactation. Children's needs are lower: 0.3-0.4 mg/day (infants) and 0.7-1.0 mg/day (children), depending on the age and caloric intake of the child.

Safety

Thiamin has been found to be well tolerated in healthy people, even at very high oral doses (up to 200 mg/day). Due to its very broad safety margin for oral administration and long history of safe use, none of the official regulatory authorities has defined a safe upper limit for this vitamin. The only reaction found in humans is of the hypersensitivity type. In the vast majority of cases these have occurred after injection of thiamin in patients with a history

of allergic reactions. For parenteral administration, the doses that produced these reactions varied from 5 to 100 mg, though most of them occurred at the higher end of this range.

Supplements and food fortification

Thiamin is mostly formulated in combination with other B-vitamins (B-complex) or included in multi-vitamin supplements. Fortification of white flour, cereals, pasta, beverages and rice began in the United States during the second World War (1939-1945), with other countries quickly following suit. Fortification of staple foods has virtually eradicated the B-vitamin deficiency diseases in developed nations.

Industrial production

Chemical synthesis of thiamin is a complicated process, involving some 15-17 different steps. Although commercial production of thiamin was first accomplished in 1937, the production did not develop on a broad scale until the 1950s, when demand rose sharply because of food fortification.



History

- 7th cent.** First classical description of beriberi in a 'General Treatise on the Etiology and Symptoms of Diseases' (author: Ch'ao-Yuan-fang Wu Ching).
- 1882** Takaki, surgeon general, dramatically decreases the incidence of beriberi in the Japanese navy by improving sailors' diets.
- 1897** Dutch medical officers Eijkman and Grijns show that the symptoms of beriberi can be reproduced in chickens fed on polished rice, and that these symptoms can be prevented or cured by feeding them rice bran.
- 1912** Funk isolates the antiberiberi factor from rice bran extracts and calls it a 'vitamine' - an amine essential for life. The name finds ready acceptance and helps to focus attention on the new concept of deficiency diseases.
- 1915** McCollum and Davis propose water-soluble vitamin B₁ as antiberiberi factor
- 1926** Jansen and Donath isolate antiberiberi factor from rice bran.
- 1927** The British Medical Research Council proposes vitamin B₁ as anti- beriberi factor.
- 1936** Williams, who first began experimenting with vitamin B₁ and beriberi in Manila around 1910, identifies and publishes the chemical formula and names it thiamin.
- 1937** The first commercial production of thiamin is accomplished.
- 1943** Williams and coworkers, and Foltz and colleagues carry out dietary studies that document widespread thiamin deficiency in the United States.
- 1943** Standards of identity for enriched flour are created by the US Food and Nutrition Board, requiring that thiamin, niacin, riboflavin and iron be added to white flour.



Christian Eijkman



Casimir Funk

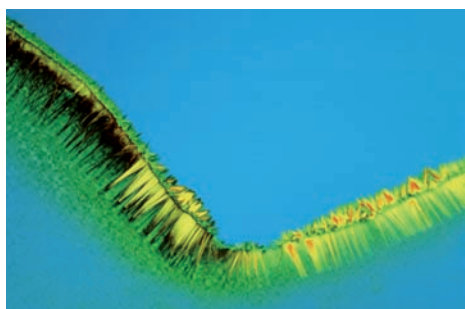


Elmer V. McCollum



Robert R. Williams

Vitamin B₂



Vitamin B₂ crystals in polarised light

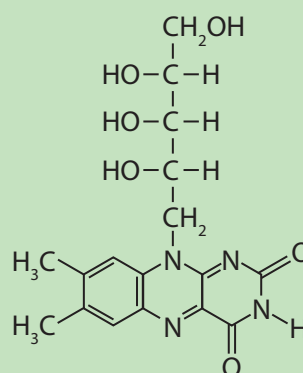
Synonyms

Riboflavin, riboflavine, vitamin B₂, lactoflavin, ovoflavin

Chemistry

7,8-dimethyl-10-(1-D-ribityl)isoalloxazin - different redox states: flavochinon (Fl_{ox}), flavosemichinon (Fl-H), flavohydrochinon (Fl_{red}H₂).

Coenzyme Form(s): FMN (flavin mononucleotide, riboflavin monophosphate), FAD (flavin adenine dinucleotide, riboflavin adenosine diphosphate).



Molecular formula of riboflavin

Introduction

Riboflavin is one of the most widely distributed water-soluble vitamins. The above synonyms, lactoflavin and oboflavin, as well as the terms hepatoflavin, verdoflavin and uroflavin, indicate the source from which the vitamin was originally isolated, i.e. milk, eggs, liver, plants and urine. The term “flavin” originates from the latin word “flavus” referring to the yellow colour of this vitamin. The fluorescent riboflavin is also part of the vitamin B-complex. In the body, riboflavin occurs primarily as an integral component of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Functions

Flavin coenzymes are essential for energy production via the respiratory chain, as they act as catalysts in the transfer of electrons in numerous essential oxidation-reduction reactions (redox reactions). They participate in many metabolic reactions of carbohydrates, fats and proteins. Riboflavin coenzymes are also essential for the conversion of pyridoxine (vitamin B₆) and folic acid into their coenzyme forms and for the transformation of tryptophan to niacin. Vitamin B₂ also promotes normal growth and assists in the synthesis of steroids, red blood cells, and glycogen. Furthermore, it helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system, and is involved in the production of adrenaline by the adrenal glands. Riboflavin is also important for the antioxidant status within cell systems, both by itself and as part of the glutathione reductase and xanthine oxidase system. This defence system may also help defend against bacterial infections and tumour cells.

Main functions in a nutshell:

- Oxidation-reduction reactions
- Energy production
- Antioxidant functions
- Conversion of pyridoxine (vitamin B₆) and folic acid into their active coenzyme forms
- Growth and reproduction
- Growth of skin, hair, and nails

Dietary sources

Riboflavin is present as an essential constituent of all living cells, and is therefore widely distributed. However, there are very few rich sources in food. Yeast and liver have the highest concentrations, but they do not have much relevance in today's human nutrition. The most important and common dietary sources are milk and milk products, lean meat, eggs and green leafy vegetables. Cereal grains, although poor sources of riboflavin, are important for those who rely on cereals as their main dietary component. Fortified cereals and bakery-products supply large amounts. Animal sources of riboflavin are better absorbed than vegetable

sources. In milk from cows, sheep and goats, at least 90% of the riboflavin is in the free form; in most other sources, it occurs bound to proteins.

Absorption and body stores

Most dietary riboflavin is consumed as a food protein with FMN and FAD. These are released in the stomach by acidification and absorbed in the upper part of the small intestine by an active, rapid, saturable transport mechanism. The rate of absorption is proportional to intake and increases when riboflavin is ingested along with other foods. So approximately 15% is absorbed if taken alone versus 60% absorption when taken with food. Passive diffusion plays only a minor role at the physiological doses ingested in the diet.

In the mucosal cells of the intestine, riboflavin is converted to the coenzyme form flavin mononucleotide (FMN). In the portal system it is bound to plasma albumin or to other proteins, mainly immunoglobulins, and transported to the liver, where it is converted to the other coenzyme form, FAD, and bound to specific proteins as flavoproteins.

Riboflavin, mainly as FAD, is distributed in all tissues, but concentrations are low and little is stored. The liver and retinal tissues are the main storage places. Riboflavin is excreted mainly in the urine where it contributes to the yellow colour. Small amounts are also excreted in sweat and bile. During lactation, about 10% of absorbed riboflavin passes into the milk.

Vitamin B₂ content of foods

Food	Vitamin B ₂ (mg/100g)
Brewer's yeast	3.7
Pork liver	3.2
Chicken breast	0.9
Wheat germ	0.7
Camembert/Parmesan	0.6
White mushrooms	0.4
Egg	0.3
Spinach	0.23
Milk/Yoghurt	0.18

(Souci, Fachmann, Kraut)

Measurement

Body status can be determined by direct and indirect methods. Direct methods include the determination of FAD and FMN in whole blood by HPLC (High Performance Liquid Chromatography). Usually, whole blood concentrations (FAD) of 175 - 475 nmol/L are measured. Another possibility for riboflavin status assessment is the monitoring of urinary excretion. Values < 27 µg/g creatinine point to deficiency, 27 - 79 µg/g creatinine are considered marginal, and values > 80 µg/g creatinine are considered normal. Urinary excretion rises sharply after tissue saturation is reached. Indirect methods include determining the activity of the FAD dependent erythrocyte glutathion reductase (EGR). This biochemical method gives a valid indication of riboflavin status. During riboflavin deficiency EGR is no longer saturated with FAD, so enzyme activity increases when FAD is added in vitro. The difference in activity in erythrocytes

with and without added FAD is called the activity coefficient (EGRAC). An EGRAC >1.30 is indicative of biochemical riboflavin deficiency.

Stability

Because riboflavin is degraded by light, loss may be up to 50% if foods are left out in sunlight or any UV light. Because of this light sensitivity, riboflavin will rapidly disappear from milk kept in glass bottles exposed to the sun or bright daylight (85% within 2 hours).

Riboflavin is stable when heated and so is not easily destroyed in the ordinary processes of cooking, but it will leach into cooking water. The pasteurisation process causes milk to lose about 20% of its riboflavin content. Alkalis such as baking soda also destroy riboflavin. Sterilisation of foods by irradiation or treatment with ethylene oxide may also cause destruction of riboflavin.

Interactions

Positive interactions

Thyroxine and triiodothyroxine stimulate the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in mammalian systems. Anticholinergic drugs increase the absorption of riboflavin by allowing it to stay longer at absorption sites.

Negative interactions

Certain drugs have a negative influence on the absorption or metabolism of riboflavin:

- Ouabain (treatment of congestive heart failure), theophylline (muscle relaxant, diuretic, central nervous stimulant), and penicillin displace riboflavin from its binding protein, thus inhibiting transport to the central nervous system.
- Probenecid (anti-gout remedy) inhibits gastrointestinal absorption and renal tubular secretion of riboflavin.
- Chlorpromazine (anti-psychotic drug), barbiturates and possibly tricyclic antidepressants prevent the incorporation of riboflavin into FAD.
- Antibiotics: Riboflavin impairs the antibiotic activity of streptomycin, erythromycin, tyrothricin, carbomycin and tetracyclines, but no inactivation occurs with chloramphenicol, penicillin or neomycin.

Deficiency

Overt clinical symptoms of riboflavin deficiency are rarely seen in developed countries. However, the sub-clinical stage of deficiency, characterised by a change in biochemical indices, is common. Riboflavin deficiency rarely occurs in isolation but usually in combination with deficiencies of other B-complex vitamins, because flavoproteins are also involved in the metabolism of other



B-complex vitamins. Along with other B-vitamins, low vitamin B₂ status has been associated with unfavourably increased plasma homocysteine levels. The absorption of iron, zinc and calcium is impaired in riboflavin deficiency.

Clinically, vitamin B₂-deficiency affects many organs and tissues. Most prominent are the effects on the skin, mucosa and eyes:

- glossitis (magenta tongue, geographical tongue)
- cheilosis, angular stomatitis (fissures at the corners of the mouth)
- sore throat
- burning of the lips, mouth, and tongue
- inflamed mucous membranes
- pruritus (itching)
- seborrheic dermatitis (moist scaly skin inflammation)
- corneal vascularisation associated with sensitivity to bright light, impaired vision, itching and a feeling of grittiness in the eyes

In severe long-term deficiency, damage to nerve tissue can cause depression and hysteria.

Other symptoms are normocytic and normochromic anaemia, and peripheral neuropathy of the extremities (tingling, coldness and pain). Low intracellular levels of flavin coenzymes could effect mitochondrial function, oxidative stress and blood vessel dilation, which have been associated with pre-eclampsia during pregnancy.

Groups at risk of deficiency

Individuals who have inadequate food intake are at risk of deficiency, particularly children from low socioeconomic backgrounds in developing countries, elderly people with poor diets, chronic 'dieters', and people who exclude milk products from their diet (vegans). Riboflavin deficiency may also occur as a result of:

- trauma, including burns and surgery
- chronic disorders (e.g. rheumatic fever, tuberculosis, subacute bacterial endocarditis, diabetes, hypothyroidism, liver cirrhosis)
- intestinal malabsorption, e.g. morbus crohn, sprue, lactose intolerance
- chronic medication (tranquillisers, oral-contraceptives, thyroid hormones, fibre-based laxatives, antibiotics)
- high physical activity
- phototherapy for newborns during icterus

The consequences of a low riboflavin intake may be aggravated

by chronic alcoholism and chronic stress. During pregnancy and lactation riboflavin requirement is increased.

Disease prevention and therapeutic use

Eye-related diseases

Oxidative damage of lens proteins by light may lead to the development of age-related cataracts. Riboflavin deficiency leads to decreased glutathione reductase activity, which can result in cataracts. Therefore, riboflavin is used in combination with other antioxidants, like vitamin C and carotenoids, in disease prevention for age-related cataracts. Riboflavin has been used to treat corneal ulcers, photophobia and noninfective conjunctivitis in patients without any typical signs of deficiency, with beneficial results. Most cases of riboflavin deficiency respond to daily oral doses of 5-10 mg.

Current recommendations in the USA

RDA*

Infants	< 6 months	0.3mg (Adequate Intake, AI)
Infants	7-12 months	0.4mg (AI)
Children	1-3 years	0.5mg
Children	4-8 years	0.6mg
Children	9-13 years	0.9mg
Males	> 14 years	1.3mg
Females	14-18 years	1.0mg
Females	> 19 years	1.1mg
Pregnancy		1.4mg
Lactation		1.6mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Migraines

People suffering from migraine headaches have a modified mitochondrial oxygen metabolism. Because riboflavin plays an important role in energy production, supplemental riboflavin has been investigated as a treatment for migraine. The effect of riboflavin supplementation at 400 mg /day for 3 months was a decrease in gravity and frequency of migraine attacks.

Prevention of deficiencies in high-risk patients

Patients suffering from achlorhydria, vomiting, diarrhoea, hepatic disease, or other disorders preventing absorption or utilisation, should be treated parenterally. Deficiency symptoms begin to improve in 1-3 days, but complete resolution may take weeks.

Recommended Dietary Allowance (RDA)

Dietary recommendations for riboflavin exist in many countries, where mean values for adult males vary between 1.2 and 2.2 mg daily. The recommendations of the Food and Nutrition Board of the US

National Research Council are based on feeding studies conducted in the 1940s, which showed that a riboflavin intake of 0.55 mg or less per day results in clinical signs of deficiency after about 90 days. These data have led to the assumption that an intake of 0.6 mg per 1000 kcal should supply the needs for essentially all healthy people.

Safety

Riboflavin is extremely nontoxic. No cases of toxicity from ingestion of riboflavin have been reported. No toxic or adverse reactions to riboflavin in humans have been identified. A harmless yellow discoloration of urine occurs at high doses. The limited capacity of the gastrointestinal tract to absorb this vitamin makes any significant risk unlikely, and because riboflavin is water-soluble, excess amounts are simply excreted.

Supplements and food fortification

Riboflavin is available as oral preparations, alone or most commonly in multivitamin and vitamin B-complex

preparations, and as an injectable solution. Crystalline riboflavin (E101) is poorly soluble in water, so riboflavin-5'-phosphate (E 106), a more expensive but more soluble form of riboflavin, has been developed for use in liquid formulations. Riboflavin is one of the vitamins often added to flour and bakery products and beverages to compensate for losses due to processing. It is also used to enrich milk, breakfast cereals and dietetic products. Because of its bright yellow colour, riboflavin is sometimes added to other drugs or infusion solutions as a marker.

Industrial production

Riboflavin can be produced by chemical synthesis or by fermentation processes. Chemical processes are usually refinements of the procedures developed by Kuhn and by Karrer in 1934 using o-xylene, D-ribose and alloxan as starting materials. Various bacteria and fungi are commercially employed to synthesise riboflavin, using cheap natural materials and industrial wastes as a growth medium.



History

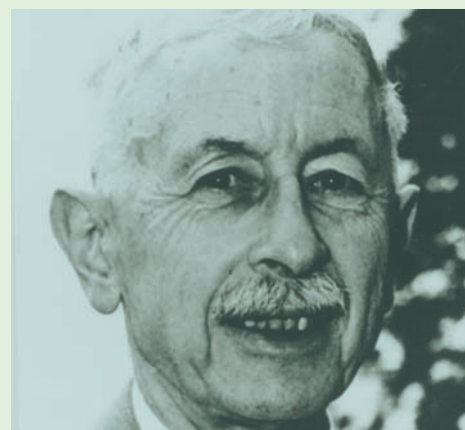
- 1879** Blyth isolates lactochrome – a water-soluble, yellow fluorescent material – from whey.
- 1932** Warburg and Christian extract a yellow enzyme from brewer's yeast and suggest that it plays an important part in cell respiration.
- 1933** Kuhn and coworkers obtain a crystalline yellow pigment with growth-promoting properties from egg white and whey, which they identify as vitamin B₂.
- 1934** Kuhn and associates in Heidelberg, and Karrer and colleagues in Zurich synthesise pure riboflavin.
- 1937** The Council on Pharmacy and Chemistry of the American Medical Association names the vitamin 'riboflavin'.
- 1937** Theorell determines the structure of flavin mononucleotide, FMN.
- 1938** Warburg and Christian isolate and characterise flavin adenine dinucleotide (FAD) and demonstrate its involvement as a coenzyme.
- 1941** Sebrell and coworkers demonstrate clinical signs of riboflavin deficiency in human feeding experiments.
- 1968** Glatzle and associates propose the use of the erythrocyte glutathione reductase test as a measurement of riboflavin status.



Otto Heinrich Warburg

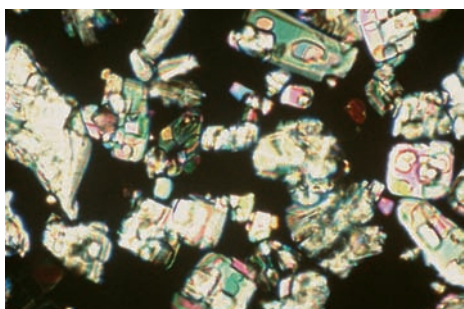


Richard Kuhn



Paul Karrer

Vitamin B₆



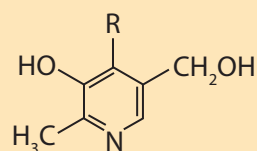
Pyridoxine crystals in polarised light

Synonyms

Vitamin B₆ is composed of three forms (vitamers): **pyridoxine** or pyridoxol (the alcohol), **pyridoxal** (the aldehyde) and **pyridoxamine** (the amine).

Chemistry

Pyridoxine (3-hydroxy-2-methylpyridine) is a basal compound of the group. Substitution (R) is carried out on 5'-C. Pyridoxic acid is an inactive catabolite of the compounds.



R = CH₂OH = Pyridoxine

R = CHO = Pyridoxal

R = CH₂NH₂ = Pyridoxamine

Molecular formulae of vitamin B₆

Introduction

Pyridoxine is a water-soluble vitamin. Man and other primates depend on external sources to cover their vitamin B₆ requirements. Vitamin B₆ was discovered in the 1930s almost as a by-product of the studies on pellagra, a deficiency disease caused by the absence in the body of the vitamin niacin. Negligible amounts of vitamin B₆ can be synthesised by intestinal bacteria. There are three different natural forms (vitamers) of vitamin B₆, namely pyridoxine, pyridoxamine, and pyridoxal, all of which are normally present in foods. For human metabolism the active derivative of the vitamin, pyridoxal 5'-phosphate (PLP), is of major importance as the metabolically active coenzyme form.

Functions

Vitamin B₆ serves as a coenzyme of approximately 100 enzymes that catalyse essential chemical reactions in the human body. It plays an important role in protein, carbohydrate and lipid metabolism. Its major function is the production of serotonin from the amino acid tryptophan in the brain and other neurotransmitters, and so it has a role in the regulation of mental processes and mood. Furthermore, it is involved in the conversion of tryptophan to the vitamin niacin, the formation of haemoglobin and the growth of red blood cells, the absorption of vitamin B₁₂, the production of prostaglandines and hydrochloric acid in the gastrointestinal tract, the sodium-potassium balance, and in histamine metabolism. As part of the vitamin B-complex it may also be involved in the downregulation of the homocysteine blood level. Vitamin B₆ also plays a role in the improvement of the immune system.

Main functions in a nutshell:

- Nervous system (neurotransmitter synthesis)
- Red blood cell formation
- Niacin formation
- Homocysteine downregulation (preventing atherosclerosis)
- Immune system (antibody production)
- Steroid hormones (inhibition of the binding of steroid hormones)

Dietary sources

Vitamin B₆ is widely distributed in foods, mainly in bound forms. Pyridoxine is found especially in plants, whereas pyridoxal and pyridoxamine are principally found in animal tissue, mainly in the form of PLP. Excellent sources of pyridoxine are chicken and the liver of beef, pork and veal. Good sources include fish (salmon, tuna, sardines, halibut, herring), nuts (walnuts, peanuts), bread, corn and whole grain cereals. Generally, vegetables and fruits are rather poor sources of vitamin B₆, although there are products in these food classes which contain considerable amounts of pyridoxine, such as lentils, courgettes and bananas.

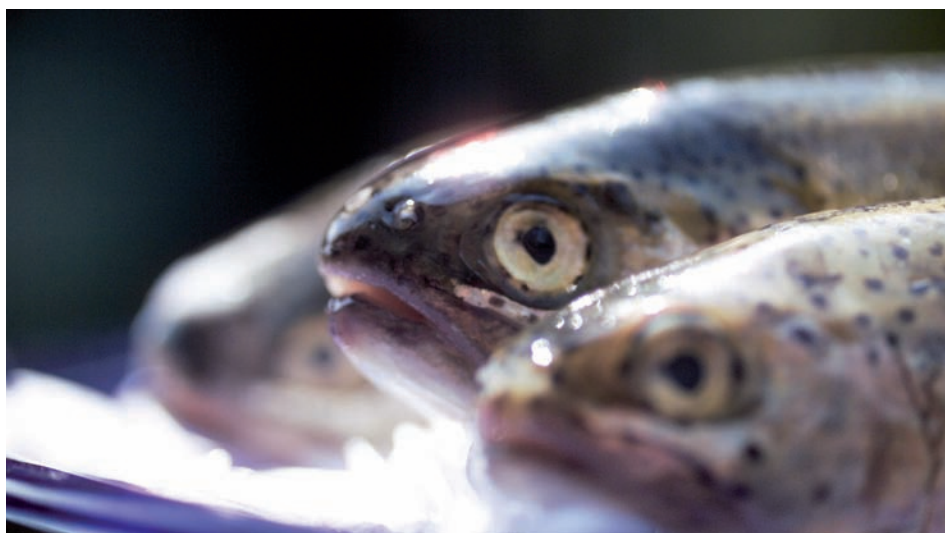
Vitamin B₆ content of foods

Food	Vitamin B ₆ (mg/100g)
Brewer's yeast	4.4
Salmon	0.98
Walnuts	0.87
Wheat germ	0.72
Pork liver	0.59
Lentils	0.57
Avocado	0.53
Chicken	0.5
Courgettes	0.46
Bananas	0.36

(Souci, Fachmann, Kraut)

Absorption and body stores

All three forms of vitamin B₆ (pyridoxine, pyridoxal and pyridoxamine) are readily absorbed in the small intestine by an energy dependent process. All three are converted to pyridoxal phosphate in the liver, a process which requires zinc and riboflavin. The bioavailability of plant-based foods varies considerably, ranging from 0% to 80%. Some plants contain pyridoxine glycosides that cannot be hydrolysed by intes-



tinal enzymes. Although these glycosides may be absorbed, they do not contribute to vitamin activity.

The storage capacity of water-soluble vitamins is generally low compared to that of fat-soluble ones. Small quantities of pyridoxine are widely distributed in body tissue, mainly as PLP in the liver and muscle. PLP is tightly bound to the proteins albumin and haemoglobin in plasma and red blood cells. Because the half-life of pyridoxine is 15-20 days and it is not significantly bound to plasma proteins, and the limited stores may be depleted within two to six weeks on a pyridoxine-free diet, a daily supply is required. Excess pyridoxine is excreted in the urine.



determination of urinary excretion of 4-pyridoxic acid (4-PA). The method of choice for quantification of both compounds is high performance liquid chromatography. Whole blood concentrations usually 35-110 nmol/L PLP. Concentrations of PLP have been found to correlate well with the pyridoxine status determined by indirect methods. Indirect methods measure the stimulated activity of pyridoxine dependent enzymes in erythrocytes by addition of PLP. This mainly determines the erythrocyte alanine aminotransferase activation coefficient (EAST-AC) or the erythrocyte aspartate aminotransferase activation coefficient. The coefficient of activity with stimulation to activity without stimulation indicates the pyridoxine status. For EAST-AC, values > 1.8 are considered to show deficiency,

1.7-1.8 to be marginal, and < 1.7 to be adequate. For large-scale population surveys there is another method of assessing a pyridoxine deficiency state: the tryptophan load test.

Vitamin B₆ participates in the conversion of tryptophan to the vitamin niacin. A pyridoxine deficiency

blocks this process, producing more xanthurenic acid. If the administration of tryptophan leads to an increased excretion of xanthurenic acid, a pyridoxine deficiency can be diagnosed.

Typical serum level of pyridoxine = 15-37 nmol/L.

Stability

Pyridoxine is relatively stable to heat, but pyridoxal and pyridoxamine are not. Pasteurisation therefore causes milk to lose up to 20% of its vitamin B₆ content. Vitamin B₆ is decomposed by oxidation and ultraviolet light, and by an alkaline environment. Because of this light sensitivity, vitamin B₆ will disappear (50% within a few hours) from milk kept in glass bottles exposed to the sun or bright daylight. Alkalis, such as baking soda, also destroy pyridoxine. Freezing of vegetables causes a reduction of up to 25%, while milling of cereals leads to wastes as high as 90%. Cooking losses of processed foods may range from a few percent to nearly half the vitamin B₆ originally present. Cooking and storage losses are greater with animal products.

Interactions

Positive interactions

Certain vitamins of the B-complex (niacin, riboflavin, biotin) may act synergistically with pyridoxine.

Negative interactions

Pyridoxine requires riboflavin, zinc and magnesium to fulfil its physiological function in humans. It has been claimed that women taking oral contraceptives may have an increased requirement for pyridoxine. There are more than 40 drugs that interfere with vitamin B₆, potentially causing decreased availability and poor vitamin B₆ status. Supplementation with the affected nutrient may be necessary. Principal antagonists include:

- Phenytoin (an antiepileptic drug)
- Theophylline (a drug for respiratory diseases)
- Phenobarbitone (a barbiturate mainly used for its antiepileptic properties)

Measurement

There are several direct and indirect methods that can be used for assessing a person's vitamin B₆ status. Direct methods include determination of PLP in whole blood, and

- Desoxypyridoxine, an effective antimetabolite
- Isoniazid (a tuberculostatic drug)
- Hydralazine (an antihypertensive)
- Cycloserine (an antibiotic)
- Penicillamine (used in treatment of Wilson's disease)

Vitamin B₆, for its part, reduces the therapeutic effect of levodopa – a naturally occurring amino acid used to treat Parkinson's disease – by accelerating its metabolism.

Deficiency

A deficiency of vitamin B₆ alone is uncommon, because it usually occurs in combination with a deficit in other B-complex vitamins, especially with riboflavin deficiency, because riboflavin is needed for the formation of the coenzyme PLP. A dietary deficiency state showing definable clinical deficiency symptoms is rare, although recent diet surveys revealed that a significant part of the following population groups have B₆ intakes below the RDA:

- pregnant and lactating women (additional demands)
- most women in general, especially those taking oral contraceptives
- the elderly (due to lower food intake)
- underweight people
- chronic alcoholics (heavy drinking may severely impair the ability of the liver to synthesise PLP, low intake)
- people with a high protein intake

A pyridoxine depleted diet, an antagonist-induced deficiency or certain genetic errors of amino acid metabolism may result in various symptoms, such as:

- hypochromic anaemia (abnormal decrease in the haemoglobin content of erythrocytes)
- nervous system dysfunction (decrease in the metabolism of

- glutamate in the brain)
- impairment of the immune system (decrease in circulating lymphocytes)
- epileptiform convulsions in infants
- skin lesions, e.g. seborrheic dermatitis (similar to pellagra)
- abdominal distress, nausea, vomiting
- kidney stones
- electroencephalographic abnormalities
- peripheral neuritis, nerve degeneration
- poor growth
- depression, insomnia, lethargy, decreased alertness
- elevated homocysteine



Disease prevention and therapeutic use

Sideroblastic anaemias and pyridoxine-dependent abnormalities of metabolism

Pyridoxine is an approved treatment for sideroblastic anaemias and pyridoxine-dependent abnormalities of metabolism. In such cases, therapeutic doses of approximately 40-200 mg vitamin B₆ per day are indicated.

PMS (premenstrual syndrome)

Some studies suggest that vitamin B₆ doses of up to 100 mg/day may be of value for relieving the symptom complex of premenstrual syndrome. However, final conclusions are still limited and more research is needed.

Current recommendations in the USA

RDA*

Infants	< 6 months	0.1 mg (Adequate Intake, AI)
Infants	7-12 months	0.3mg (AI)
Children	1-3 years	0.5mg
Children	4-8 years	0.6mg
Children	9-13 years	1mg
Males	14-50 years	1.3mg
Females	14-18 years	1.2mg
Females	19-50 years	1.3mg
Males	> 51 years	1.7mg
Females	> 51 years	1.7mg
Pregnancy		1.9mg
Lactation		2mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Hyperemesis gravidarum

Pyridoxine is often administered in doses of up to 40 mg/day in the treatment of nausea and vomiting during pregnancy (hyperemesis gravidarum). However, as “morning sickness” improves even without treatment it is difficult to prove the therapeutic benefit.

Depression

Pyridoxine is also used to assist in the relief of depression (especially in women taking oral contraceptives). However, clinical trials have not yet provided evidence for its efficacy.

Carpal tunnel syndrome

Pyridoxine has also been claimed to alleviate the symptoms of carpal tunnel syndrome. Some studies report benefits while others do not.

Hyperhomocystinaemia / cardiovascular disease

Elevated homocysteine levels in the blood are considered a risk factor for atherosclerotic disease. Several studies have shown that vitamin B₆, vitamin B₁₂ and folic acid can lower critical homocysteine levels.

Immune function

The elderly are a group that often suffers from impaired immune function. Adequate B₆ intake is thus important, and it has been shown that the amount of vitamin B₆ required to improve the immune system is higher (2.4 mg/day for men; 1.9 mg/day for women) than the current RDA.

Asthma

Asthma patients taking vitamin B₆ supplements may have fewer and less severe attacks of wheezing, coughing and breathing difficulties.

Diabetes

Research has also suggested that certain patients with diabetes mellitus or gestational diabetes experience an improvement in glucose tolerance when given vitamin B₆ supplements.

Kidney stones

Glyoxylate can be oxidised to oxalic acid that may lead to calcium oxalate kidney stones. Pyridoxal phosphate is a cofactor for the degradation of glyoxylate to glycine. There is some evidence that high doses of vitamin B₆ (> 150 mg/day) may be useful for normalising the oxalic acid metabolism to reduce the formation of kidney stones. However, the relationship between B₆ and kidney stones must be studied further before any definite conclusions can be drawn.

Chinese restaurant syndrome

People who are sensitive to glutamate, which is often used for the preparation of Asiatic dishes, can react with headache, tachycardia (accelerated heart rate), and nausea. 50 to 100 mg of pyridoxine can be of therapeutic value.

Autism

High dose therapy with pyridoxine improves the status of autistics in about 30% of cases.

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin B₆ varies according to age, sex, risk group (see ‘Groups at risk’) and criteria applied. The vitamin B₆ requirement is increased when high-protein diets are consumed, since protein metabolism can only function properly with the assistance of pyridoxine. Pregnant and lactating women need an additional 0.7 mg to compensate for increased demands made by the foetus or baby.

Safety

Vitamin B₆ in all its forms is well tolerated, but large excesses are toxic. Daily oral doses of pyridoxine of up to 50 times the RDA (ca. 100 mg) for periods of 3-4 years have been administered without adverse effects. Daily doses of 500 mg and more may cause sensory neuropathy after several years of ingestion, whereas the intake of amounts in excess of 1 gram daily may lead to reversible sensory neuropathy within a few months. Sensory neuropathy has been selected as a critical end-point on which to base a tolerable upper intake level (UL) of 100 mg/day for adults, although supplements somewhat higher than this may be safe for most individuals. Fortunately these side-effects are largely reversible upon cessation of vitamin B₆ intake. Today, prolonged intake of doses exceeding 500 mg a day is considered to carry the risk of adverse side-effects.

Supplements and food fortification

The most commonly available form of vitamin B₆ is pyridoxine hydrochloride, which is used in food fortification, nutritional supplements and therapeutic products such as capsules, tablets and ampoules. Vitamins, mostly of the B-complex, are widely used in the enrichment of cereals. Dietetic foods such as infant formulas and slimming diets are often fortified with vitamins, including pyridoxine.

History

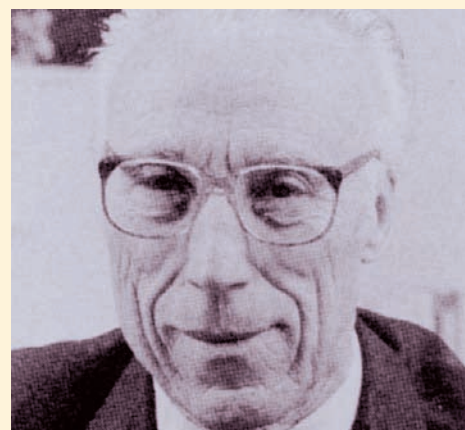
- 1926** Goldberger and coworkers feed rats a diet deficient in what is considered to be the pellagra-preventive factor; these animals develop skin lesions.
- 1934** György first identifies the factor as vitamin B₆ or adermin, a substance capable of curing a characteristic skin disease in rats (dermatitis acrodynia). The factor is then called the rat anti-acrodynia factor, deficiency of which causes so-called “rat-pellagra”
- 1935** Birch and György succeed in differentiating riboflavin and vitamin B₆ from the specific pellagra preventive factor (P-P) of Goldberger and his associates.
- 1938** Lepkovsky is the first to report the isolation of pure crystalline vitamin B₆. Independently, but slightly later, several other groups of researchers also report the isolation of crystallised vitamin B₆ from rice polishings (Keresztesy and Stevens; György; Kuhn and Wendt; Ichiba and Michi).
- 1939** Harris and Folkers determine the structure of pyridoxine and succeed in synthesising the vitamin. György proposes the name pyridoxine.
- 1945** Snell demonstrates that two other natural forms of the vitamin exist, namely pyridoxal and pyridoxamine.
- 1957** Snyderman determines the levels of vitamin B₆ required by humans.



Joseph Goldberger

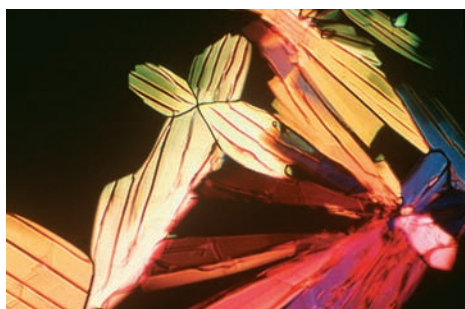


Paul György



Esmond Emerson Snell

Vitamin B₁₂



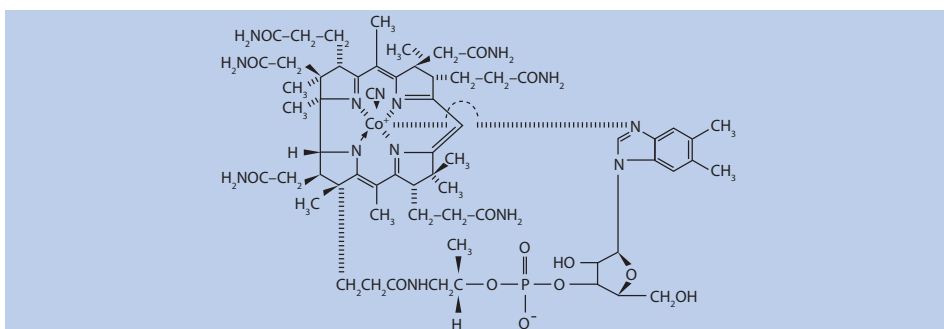
Cyanocobalamin crystals in polarised light

Synonyms

Cobalamin, antipernicious-anaemia factor, Castle's extrinsic factor, or animal protein factor.

Chemistry

The structure of vitamin B₁₂ is based on a corrin ring, which has two of the pyrrole rings directly bonded. The central metal ion is Co (cobalt). Four of the six coordinations are provided by the corrin ring nitrogens, and a fifth by a dimethylbenzimidazole group. The sixth coordination partner varies, being a cyano group (-CN) (cyanocobalamin), a hydroxyl group (-OH) (hydroxocobalamin), a methyl group (-CH₃) (methylcobalamin) or a 5'-deoxyadenosyl group (5-deoxyadenosylcobalamin).



Molecular formula of cyanocobalamin

Introduction

Vitamin B₁₂ is the largest and most complex of all the vitamins. The name vitamin B₁₂ is generic for a specific group of cobalt-containing corrinoids with biological activity in humans. Interestingly it is the only known metabolite to contain cobalt, which gives this water-soluble vitamin its red colour. This group of corrinoids is also known as cobalamins. The main cobalamins in humans and animals are hydroxycobalamin, adenosylcobalamin and methylcobalamin, the last two being the active coenzyme forms. Cyanocobalamin is a form of vitamin B₁₂ that is widely used clinically due to its availability and stability. It is transformed into active factors in the body.

In 1934, three researchers won the Nobel prize in medicine for discovering the lifesaving properties of vitamin B₁₂. They found that eating large amounts of raw liver, which contains high amounts of vitamin B₁₂, could save the life of previously incurable patients with pernicious anaemia. This finding saves 10,000 lives a year in the US alone. Vitamin B₁₂ was isolated from liver extract in 1948 and its structure was elucidated 7 years later.

Functions

Vitamin B₁₂ is necessary for the formation of blood cells, nerve sheaths and various proteins. It is therefore, essential for the prevention of certain forms of anaemia and neurological disturbances. It is also involved in fat and carbohydrate metabolism and is essential for growth. In humans, vitamin B₁₂ functions primarily as a coenzyme in intermediary metabolism. Two metabolic reactions are dependent on vitamin B₁₂:

- 1) The methionine synthase reaction with methylcobalamin
- 2) The methylmalonyl CoA mutase reaction with adenosylcobalamin

In its methylcobalamin form vitamin B₁₂ is the direct cofactor for methionine synthase, the enzyme that recycles homocysteine back to methionine. There is evidence that vitamin B₁₂ is required in the synthesis of folate polyglutamates (active coenzymes required in the formation of nerve tissue) and in the regeneration of folic acid during red blood cell formation.

Methylmalonyl CoA mutase converts 1-methylmalonyl CoA to succinyl CoA (an important reaction in lipid and carbohydrate metabolism). Adenosylcobalamin is also the coenzyme in ribonucleotide reduction (which provides building blocks for DNA synthesis).

Main functions in a nutshell:

- Essential growth factor
- Formation of blood cells and nerve sheaths
- Regeneration of folic acid
- Coenzyme-function in the intermediary metabolism, especially in cells of the nervous tissue, bone marrow and gastrointestinal tract

Dietary sources

Vitamin B₁₂ is produced exclusively by microbial synthesis in the digestive tract of animals. Therefore, animal protein products are the source of vitamin B₁₂ in the human diet, in particular organ meats (liver, kidney). Other good sources are fish, eggs and dairy products. In foods, hydroxo-, methyl- and 5'-deoxyadenosyl-cobalamins are the main cobalamins present. Foods of plant origin contain no vitamin B₁₂ beyond that derived from microbial contamination. Bacteria in the intestine synthesise vitamin B₁₂, but under normal circumstances not in areas where absorption occurs.



Vitamin B₁₂ content of foods

Food	Vitamin B ₁₂ (µg/100g)
Beef liver	65
Crab	27
Blue mussel	8
Steak	5
Coalfish	3.5
Cheese (Camembert)	3
Egg	1-3

(Souci, Fachmann, Kraut)

Absorption and body stores

Vitamin B₁₂ from food sources is bound to proteins and is only released by an adequate concentration of hydrochloric acid in the stomach. Free vitamin B₁₂ is then immediately bound to glycoproteins originating from the stomach and salivary glands. This glycoprotein complex protects vitamin B₁₂ from chemical denaturation. Gastrointestinal absorption of vitamin B₁₂ occurs in the small intestine by an active process requiring the presence of intrinsic factor, another glycoprotein, which the gastric parietal cells secrete after being stimulated by food. The absorption of physiological doses of vitamin B₁₂ is limited to approximately 10 µg/dose. The vitamin B₁₂ intrinsic factor complex is then absorbed through phagocytosis by specific ileal receptors. Once absorbed, the vitamin is transferred to a plasma-transport protein which delivers the vitamin to target cells. A lack of intrinsic factor results in malabsorption of cobalamin. If this is untreated, potentially irreversible neurological damage and life-threatening anaemia develops (see deficiency).

Regardless of dose, approximately 1% of vitamin B₁₂ is absorbed by passive diffusion, so this process becomes quantitatively important at pharmacological levels of exposure. Once absorbed, vitamin B₁₂ is stored principally (60%) in the liver. The average B₁₂ content is approximately 1.0 mg in healthy adults, with 20-30 µg found in the kidneys, heart, spleen and brain. Estimates of total vitamin B₁₂ body content for adults range from 0.6 to 3.9 mg with mean values of 2-3 mg. The normal range of vitamin B₁₂ plasma concentrations is 150-750 pg/ml, with peak levels achieved 8-12 hours after ingestion.

Excretion of vitamin B₁₂ is proportional to stores and occurs mainly by urinary and faecal routes. Vitamin B₁₂ is very efficiently conserved by the body, with 65-75% re-absorption in the ileum of the 0.5-5 µg excreted into the alimentary tract per day (mainly into the bile). This helps to explain the slow development (over several years) of deficiency states in subjects with negligible vitamin B₁₂ intake, such as vegans. Subjects with a reduced ability to absorb cobalamin via the intestine (lack of intrinsic factor) develop a deficiency state more rapidly.

Measurement

Measurement of vitamin B₁₂ in plasma is routinely used to determine deficiency, but may not be a reliable indication in all cases. In pregnancy, for example, tissue levels are normal but serum levels are low. Vitamin B₁₂ can be measured by chemical, microbiological or immunoassay isotope dilution methods. Microbiological assays, which are widely used for blood and tissue samples, are sensitive but non-specific. Serum cobalamin concentration is often determined by automated immunoassays using intrinsic factor as a binding agent. These assays have mainly replaced microbiological methods. Data in the literature about vitamin B₁₂ concentration in serum varies. However, values < 110 – 150 pmol/L are considered to reflect deficiency, whereas values > 150 – 200 pmol/L represent an adequate status.

Major vitamin B₁₂-dependent metabolic processes include the formation of methionine from homocysteine, and the formation of succinyl coenzyme A from methylmalonyl coenzyme A. Thus, apart from directly determining vitamin B₁₂ concentration in the blood, elevated levels of both methylmalonic acid (MMA) and homocysteine may

indicate a vitamin B₁₂ deficiency.

The Schilling test (which quantifies ileal absorption by measuring radioactivity in the urine after oral administration of isotopically labelled vitamin) enables detection of impaired vitamin B₁₂ absorption. The measurement of urinary methylmalonate (0-3.5 mg/day is normal; a vitamin B₁₂-deficient patient will excrete up to 300 mg/day) can be used as a diagnostic means to assess vitamin B₁₂ status. (Other tests include the cobalamin absorbance test and the serum gastrin deoxyuridine suppression test).

Stability

Vitamin B₁₂ is stable to heat, but slowly loses its activity when exposed to light, oxygen and acid or alkali-containing environments. Loss of activity during cooking is due to the water solubility of vitamin B₁₂ (loss through meat juices or leaching into water) rather than to its destruction.

Interactions

Negative interactions

Absorption of cobalamins is impaired by alcohol and vitamin B₆ (pyridoxine) deficiency. Furthermore, a number of drugs reduce the absorption of vitamin B₁₂, and supplementation with the affected nutrient may be necessary:

- Stomach medication: proton pump inhibitors, H₂ receptor antagonists
- Liver medication: cholestyramine
- Tuberculostatics: para-aminosalicylic acid
- Anti-gout medication: colchicine
- Antibiotics: neomycin, chloramphenicol
- Anti-diabetics: oral biguanides metformin and phenformin
- Potassium chloride medications
- Oral contraceptives

A number of anticonvulsants – phenobarbitone, primidone, phenytoin and ethylphenacemide – can alter the metabolism of cobalamins in the cerebrospinal fluid and lead to neuropsychic disturbances. Several substituted amide, lactone and lactam analogues of cyanocobalamin compete with binding sites on intrinsic factor and lead to depressed absorption of the vitamin. Nitrous oxide (anaesthetic) also interferes with cobalamin metabolism.

Deficiency

Clinical cobalamin deficiency due to dietary insufficiency is rare in younger people, but occurs more frequently in older people. Vitamin B₁₂ deficiency affects 10-15% of individuals over the age of 60.

Deficiency of vitamin B₁₂ leads to defective DNA synthesis in cells, which affects the growth and repair of all cells. Tissues most affected are those with the greatest rate of cell turnover, e.g. those of the haematopoietic system. This can lead to megaloblastic anaemia (characterised by large and immature red blood cells) and neuropathy, with numerous symptoms including: glossitis, weakness, loss of appetite, loss of taste and smell, impotence, irritability, memory impairment, mild depression, hallucination, breathlessness (dyspnea) on exertion, tingling and numbness (paraesthesia). Vitamin B₁₂ deficiency can also lead to hyperhomocysteinaemia, a possible risk factor for occlusive vascular disease.

The symptoms of vitamin B₁₂ deficiency are similar to those of folic acid deficiency, the major difference being only that vitamin B₁₂ deficiency is associated with spinal cord degeneration. If folic acid is used to treat vitamin B₁₂ deficiency, anaemia may be alleviated but the risk of

damage to the nervous system remains. It is therefore essential to diagnose the deficiency accurately before starting therapy. The Food and Nutrition Board advises adults to limit their folic acid intake (through supplements and fortification) to 1 mg per day.

Cause of deficiency is not usually insufficient dietary intake but lack of intrinsic factor secretion. Without intrinsic factor, absorption is not possible and a severe and persistent deficiency develops that cannot be prevented by the usual dietary intakes of vitamin B₁₂. This occurs in people with:

- pernicious anaemia (a hereditary autoimmune disease that chiefly affects persons post middle age),
- food-bound vitamin B₁₂ malabsorption, reported in patients on long-term treatment with certain drugs, and in elderly patients with gastric atrophy.
- after gastrectomy
- after ingestion of corrosive agents with destruction of gastric mucosa.
- lesions of the small bowel (blind loops, stenoses, strictures, diverticula). Bacterial overgrowth may lead to competitive utilisation of available vitamin. Impaired absorption also occurs in patients with small intestinal defects (e.g. sprue, celiac disease, ileitis, ileal resection) and those with inborn errors of cobalamin metabolism, secretion of biologically abnormal intrinsic factor, or Zollinger-Ellison syndrome.
- pancreatic insufficiency
- in alcoholics vitamin B₁₂ intake and absorption are reduced, while elimination is increased
- AIDS also brings an increased risk of deficiency

The risk of nutritional deficiency is increased in vegans; a high intake of fibre has been shown to aggravate a precarious vitamin balance. There have also been reports of vitamin B₁₂ deficiency in infants breast-fed

by vegetarian mothers. Strict vegetarians are urged to use a vitamin B₁₂ supplement.

Pernicious anaemia:

Pernicious anaemia is the classical symptom of B₁₂ deficiency, but it is actually the end-stage of an autoimmune inflammation of the stomach, resulting in destruction of stomach cells by the body's own antibodies. Anaemia is a condition in which red blood cells do not provide adequate oxygen to body tissues. Pernicious anaemia is a type of megaloblastic anaemia.

Gastric atrophy:

Gastric atrophy is a chronic inflammation of the stomach resulting in decreased stomach acid production. Because this is necessary for the release of vitamin B₁₂ from the proteins in food, vitamin B₁₂ absorption is reduced.

Disease prevention and therapeutic use

Pernicious anaemia

Patients with lack of intrinsic factor secretion can be effectively treated using oral vitamin B₁₂ but require lifetime vitamin B₁₂ therapy. When used alone, oral doses of at least 150 µg/day are necessary, although single weekly oral doses of 1000 µg have proved satisfactory in some cases. Combinations of vitamin B₁₂ and intrinsic factor may be given, but as a variable number of patients become refractory to intrinsic factor after prolonged treatment, parenteral therapy with cyanocobalamin or hydroxocobalamin is preferred.

Hyperhomocysteinaemia

Homocysteine appears to be a nerve and vessel toxin, promoting mortality and cardiovascular disease (CVD) as well as stroke, Alzheimer's disease, birth defects, recurrent pregnancy loss, and eye disorders. Keeping homocysteine at levels associated with lower rates of disease requires adequate B₁₂, folic acid and B₆ intake.

Cancer

Vitamin B₁₂ deficiency may lead to an elevated rate of DNA damage and altered methylation of DNA. These are obvious risk factors for cancer. In a recent study, chromosome breakage was minimised in young adults by supplementation with 700 µg of folic acid and 7 µg of vitamin B₁₂ daily in cereal for two months.

Recommended Dietary Allowance (RDA)

RDA intakes for vitamin B₁₂ range from 0.3 to 5.0 µg/day in 25 countries. An increase to 2.2 µg/day is recommended during pregnancy and to 2.6 µg/day for lactation to cover the additional requirements of the foetus/infant. The Committee on Nutrition of the American Academy of Paediatrics recommends a daily vitamin B₁₂ intake of 0.15 µg/100 kcal energy intake for infants and preadolescent children. Other authorities have suggested intakes of 0.3-0.5 µg (0-1 year of age), 0.7-1.5 µg (1-10 years of age) and 2 µg (> 10 years). The "average" western diet probably supplies 3-15 µg/day, but can range from 1-100 µg/day.

Safety

Large intakes of vitamin B₁₂ from food or supplements have caused no toxicity in healthy people. No adverse effects have been reported from single oral doses as high as 100 mg and chronic administration of 1 mg (500 times the RDA) weekly for up to 5 years. Moreover, there have been no reports of carcinogenic or mutagenic properties, and studies to date indicate no teratogenic potential. The main food safety authorities have not set a tolerable upper intake level (UL) for vitamin B₁₂ because of its low toxicity.

Supplements and food fortification

Current recommendations in the USA

RDA*

Infants	< 6 months	0.4 µg (Adequate Intake, AI)
Infants	7-12 months	0.5 µg (AI)
Children	1-3 years	0.9 µg
Children	4-8 years	1.2 µg
Children	9-13 years	1.8 µg
Adults	> 14 years	2.4 µg
Pregnancy		2.6 µg
Lactation		2.8 µg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

The principal form of vitamin B₁₂ used in supplements is cyanocobalamin. It is available in the form of injections and as a nasal gel for the treatment of pernicious anaemia. Cyanocobalamin is also available in tablet and oral liquid form for vitamin B-complex, multivitamin and vitamin B₁₂ supplements.

Vitamin B₁₂ is widely used to enrich cereal products and certain beverages. Dietetic foods such as slimming foods and infant formulas are often fortified with vitamins, including vitamin B₁₂. Fortification with vitamin B₁₂ is especially important for products aimed at people with a low dietary intake, such as vegans.

Industrial production

Vitamin B₁₂ is produced commercially from bacterial fermentation, usually as cyanocobalamin.

History

- 1824** The first case of pernicious anaemia and its possible relation to disorders of the digestive system is described by Combe.
- 1855** Combe and Addison identify clinical symptoms of pernicious anaemia.
- 1925** Whipple and Robscheit-Robbins discover the benefit of liver in the regeneration of blood in anaemic dogs.
- 1926** Minot and Murphy report that a diet of large quantities of raw liver to patients with pernicious anaemia restores the normal level of red blood cells. Liver concentrates are developed and studies on the presumed active principle(s) ("antipernicious anaemia factor") are initiated.
- 1929** Castle postulates that two factors are involved in the control of pernicious anaemia: an "extrinsic factor" in food and an "intrinsic factor" in normal gastric secretion. Simultaneous administration of these factors causes red blood cell formation which alleviates pernicious anaemia.
- 1934** Whipple, Minot and Murphy are awarded the Nobel prize for medicine for their work in the treatment of pernicious anaemia.
- 1948** Rickes and associates (USA) and Smith and Parker (England), working separately, isolate a crystalline red pigment which they name vitamin B₁₂.
- 1948** West shows that injections of vitamin B₁₂ dramatically benefit patients with pernicious anaemia.
- 1949** Pierce and coworkers isolate two crystalline forms of vitamin B₁₂ equally effective in combating pernicious anaemia. One form is found to contain cyanide (cyanocobalamin) while the other is not (hydroxocobalamin).
- 1955** Hodgkin and coworkers establish the molecular structure of cyanocobalamin and its coenzyme forms using X-ray crystallography.
- 1955** Eschenmoser and colleagues in Switzerland and Woodward and coworkers in the USA synthesise vitamin B₁₂ from cultures of certain bacteria/fungi.
- 1973** Total chemical synthesis of vitamin B₁₂ by Woodward and coworkers.



Georg Richard Minot



William Parry Murphy



Dorothy Crowfoot Hodgkin



Robert Burns Woodward

Niacin:

Nicotinic Acid and Nicotinamide



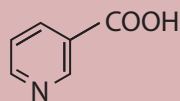
Nicotinic acid crystals in polarised light

Synonyms

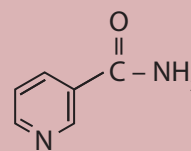
Vitamin B₃, vitamin B₄, PP factor (pellagra-preventative factor)

Chemistry

Nicotinic acid (pyridine-3-carboxylic acid), nicotinamide (pyridine-3 carboxamide)



Nicotinic Acid



Nicotinic Amide

Molecular formula of nicotinic acid

Introduction

The term niacin refers to both nicotinic acid and its amide derivative, nicotinamide (niacinamide). Both are used to form the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Niacin is a member of the water soluble B- vitamin complex. The amino acid tryptophan can be converted to nicotinic acid in humans, therefore niacin is not really a vitamin provided that an adequate dietary supply of tryptophan is available.

Nicotinic acid was isolated as early as 1867. In 1937 it was demonstrated that this substance cures the disease pellagra. The name niacin is derived from nicotinic acid + vitamin.

Functions

The coenzymes NAD and NADP are required for many biological oxidation-reduction (redox) reactions. About 200 enzymes require NAD or NADP. NAD is mainly involved in reactions that generate energy in tissues by the biochemical degradation of carbohydrates, fats and proteins. NADP functions in reductive biosyntheses such as the synthesis of fatty acids and cholesterol.

NAD is also required as a substrate for non-redox reactions. It is the source of adenosine diphosphate (ADP)-ribose, which is transferred to proteins by different enzymes. These enzymes and their products seem to be involved in DNA replication, DNA repair, cell differentiation and cellular signal transduction.

Main functions in a nutshell:

- Coenzymes (NAD and NADP) in redox reactions
- NAD is a substrate for non-redox reactions

Dietary sources

Nicotinamide and nicotinic acid occur widely in nature. Nicotinic acid is more prevalent in plants, whereas in animals nicotinamide predominates. Yeast, liver, poultry, lean meats, nuts and legumes contribute most of the niacin obtained from food. Milk and green leafy vegetables contribute lesser amounts.

In cereal products (corn, wheat), nicotinic acid is bound to certain components of the cereal and is thus not bioavailable. Specific food processing, such as the treatment of corn with lime water involved in the traditional preparation of tortillas in Mexico and Central America, increases the bioavailability of nicotinic acid in these products.

Tryptophan contributes as much as two thirds of the niacin activity required by adults in typical diets. Important food sources of tryptophan are meat, milk and eggs.

Niacin content of foods

Food	Niacin (mg/100g)
Veal liver	15
Chicken	11
Beef	7.5
Salmon	7.5
Almonds	4.2
Peas	2.4
Potatoes	1.2
Peach	0.9
Tomatoes	0.5
Milk (whole)	0.1

(Souci, Fachmann, Kraut)

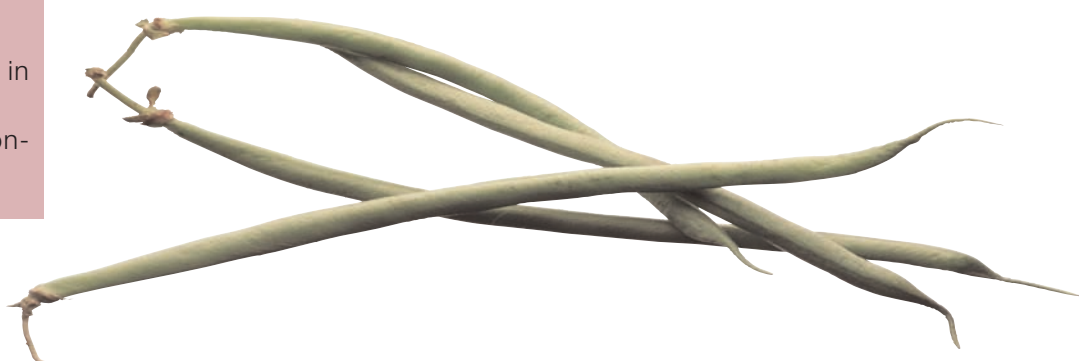
Absorption and body stores

Both acid and amide forms of the vitamin are readily absorbed from the stomach and the small intestine. At low concentrations the two forms are absorbed by a sodium-dependent facilitated diffusion, and at higher concentrations by passive diffusion. Niacin is present in the diet mainly as NAD and NADP, and nicotinamide is released from the coenzyme forms by enzymes in the intestine. The main storage organ, the liver, may contain a significant amount of the vitamin, which is stored as NAD. The niacin coenzymes NAD and NADP are synthesised in all tissues from nicotinic acid or nicotinamide.

Measurement

Determination of the urinary excretion of two niacin metabolites, N-methyl-nicotinamide and N-methyl-2-pyridone-5-carboxamide has been used to assess niacin status. Excretion of 5.8 ± 3.6 mg N-methyl-nicotinamide/24hrs and 20.0 ± 12.9 mg N-methyl-2-pyridone-5-carboxamide/24hrs are considered normal.

Recent studies suggest that the measurement of NAD and NADP concentrations and their ratio in red blood cells may be sensitive and reliable indicators for the determination of niacin status. A ratio of erythrocyte NAD to NADP < 1.0 may identify subjects at risk of developing niacin deficiency.



Stability

Both nicotinamide and nicotinic acid are stable when exposed to heat, light, air and alkali. Little loss occurs in the cooking and storage of foods.

Interactions

Negative interactions

Copper deficiency can inhibit the conversion of tryptophan to niacin. The drug penicillamine has been demonstrated to inhibit the tryptophan-to-niacin pathway in humans; this may be due in part to the copper-chelating effect of penicillamine. The pathway from tryptophan to niacin is sensitive to a variety of nutritional alterations. Inadequate iron, riboflavin, or vitamin B₆ status reduces the synthesis of niacin from tryptophan.

Long-term treatment of tuberculosis with isoniazid may cause niacin deficiency because isoniazid is a niacin antagonist. Other drugs which interact with niacin metabolism may also lead to niacin deficiency, e.g. tranquillisers (diazepam) and anticonvulsants (phenytoin, phenobarbital).

Deficiency

Symptoms of a marginal niacin deficiency include: insomnia, loss of appetite, weight and strength loss, soreness of the tongue and mouth, indigestion, abdominal pain, burning sensations in various parts of the body, vertigo, headaches, numbness, nervousness, poor concentration, apprehension, confusion and forgetfulness.

Severe niacin deficiency leads to pellagra, a disease characterised by dermatitis, diarrhoea and dementia. In the skin, a pigmented rash develops symmetrically in areas exposed

to sunlight (the term pellagra comes from the Italian phrase for raw skin). Symptoms affecting the digestive system include a bright red tongue, stomatitis, vomiting, and diarrhoea. Headaches, fatigue, depression, apathy, and loss of memory are neurological symptoms of pellagra. If untreated, pellagra is fatal.

Since the synthesis of NAD from tryptophan requires an adequate supply of riboflavin and vitamin B₆, insufficiencies of these vitamins may also contribute to niacin deficiency, resulting in pellagra.

Pellagra is rarely seen in industrialised countries, except for its occurrence in people with chronic alcoholism. In other parts of the world where maize and jowar (barley) are the major staples, pellagra persists. It also occurs in India and parts of China and Africa.

Patients with Hartnup's disease, a genetic disorder, develop pellagra because their absorption of tryptophan is defective. Carcinoid syndrome may also result in pellagra because dietary tryptophan is preferentially used for serotonin synthesis and NAD synthesis is therefore restricted.

Disease prevention and therapeutic use

Niacin is specific in the treatment of glossitis, dermatitis and the mental symptoms seen in pellagra.

High doses of nicotinic acid (1.5-4 g/day) can reduce total and low-density lipoprotein cholesterol and triacylglycerols and increase high-density lipoprotein cholesterol in patients at risk of cardiovascular disease. There is a flush reaction to high doses of nicotinic acid, which is

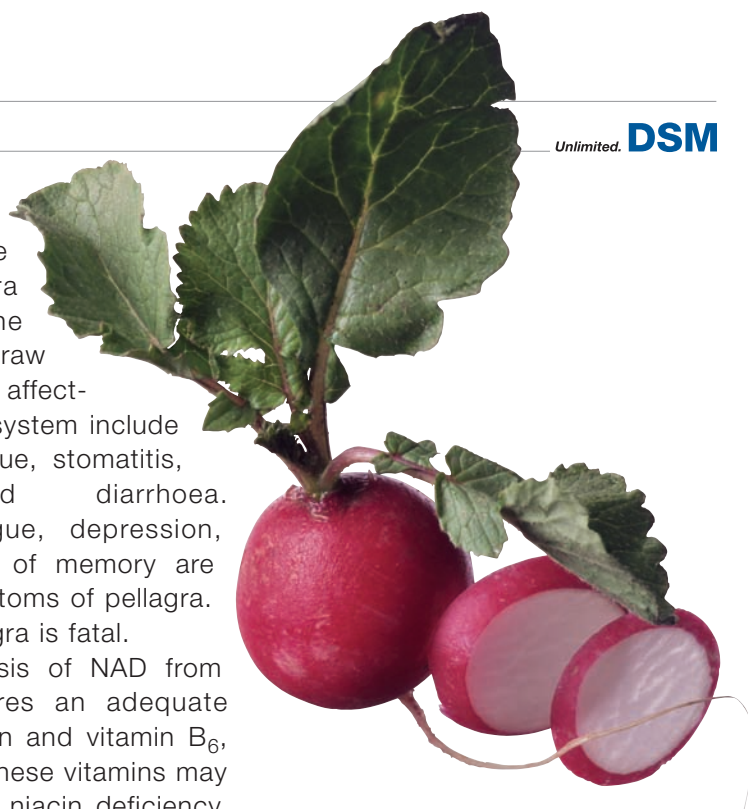
seen primarily with a rising blood level and may wear off once a plateau level has been reached.

Nicotinic acid has also been used in doses of 100 mg as a vasodilator in patients suffering from diseases causing vasoconstriction.

Type 1 diabetes mellitus results from the autoimmune destruction of insulin-secreting β -cells in the pancreas. There is evidence that nicotinamide may delay or prevent the development of diabetes. Clinical trials are in progress to investigate this effect of nicotinamide.

Recent studies suggest that infection with human immunodeficiency virus (HIV) increases the risk of niacin deficiency. Higher intakes of niacin were associated with decreased progression rate to AIDS in an observational study of HIV-positive men.

DNA damage is an important risk factor for cancer. NAD is consumed as a substrate in ADP-ribose transfer reactions to proteins which play a role in DNA repair. This has aroused interest in the relationship between niacin and cancer. A large case-control study found increased consumption of niacin, along with antioxidant nutrients, to be associated with decreased incidence of cancers of the mouth, throat and oesophagus.



Recommended Dietary Allowance (RDA)

The actual daily requirement of niacin depends on the quantity of tryptophan in the diet and the efficiency of the tryptophan to niacin conversion. The conversion factor is 60 mg of tryptophan to 1 mg of niacin, which is referred to as 1 niacin equivalent (NE). This conversion factor is used for calculating both dietary contributions from tryptophan and recommended allowances of niacin.

In the USA, the RDA for adults is 16 mg NEs for men and 14 mg NEs for women. Other regulatory authorities have established similar RDAs.

Safety

There is no evidence that niacin from foods causes adverse effects. Pharmacological doses of nicotinic acid, but not nicotinamide, exceeding 300 mg per day have been associated with a variety of side effects

including nausea, diarrhoea and transient flushing of the skin. Doses exceeding 2.5 g per day have been associated with hepatotoxicity, glucose intolerance, hyperglycaemia, elevated blood uric acid levels, heartburn, nausea, headaches. Severe jaundice may occur, even with doses as low as 750 mg per day, and may eventually lead to irreversible liver damage. Doses of 1.5 to 5 g/day of nicotinic acid have been associated with blurred vision and other eye problems.

Tablets with a buffer and time release capsules are available to reduce flushing and gastrointestinal irritation for persons with a sensitivity to nicotinic acid. These should be used with caution, however, because time-release niacin tablets used at high levels are linked to liver damage.

The Food and Nutrition Board (1998) set the tolerable upper intake level (UL) for niacin (nicotinic acid plus nicotinamide) at 35 mg/day. The EU Scientific Committee on Food (2002) developed different upper levels for nicotinic acid and nicotinamide: the UL for nicotinic acid has been set at 10 mg/day, for nicotinamide at 900 mg/day.

Supplements and food fortification

Single supplements of nicotinic acid are available in tablets, capsules and syrups. Multivitamin and B-complex vitamin infusions, tablets and capsules also contain nicotinamide.

Niacin is used to fortify grain including corn and bran breakfast cereals and wheat flour (whole meal, white and brown). US standards of identity and state standards require enrichment of bread, flour, farina, macaroni, spaghetti and noodle products, corn meal, corn grits and rice.

Industrial production

Although other routes are known, most nicotinic acid is produced by oxidation of 5-ethyl-2-methylpyridine. Nicotinamide is produced via 3-methylpyridine. This compound is derived from two carbon sources, acetaldehyde and formaldehyde, or from acrolein plus ammonia. 3-Methylpyridine is first oxidised to 3-cyanopyridine, which in a second stage converts to nicotinamide by hydrolysis.

Current recommendations in the USA

RDA*

Infants	< 6 months	2mg (AI)
Infants	7-12 months	4mg (AI)
Children	1-3 years	6mg
Children	4-8 years	8mg
Children	9-13 years	12mg
Males	> 14 years	16mg
Females	> 14 years	14mg
Pregnancy		18mg
Lactation		17mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

History

- 1755** The disease pellagra is first described by Thiery who calls the disease “mal de la rosa”.
- 1867** Huber provides the first description of nicotinic acid.
- 1873** Weidel describes the elemental analysis and crystalline structure of the salts and other derivatives of nicotinic acid in some detail.
- 1894** First preparation of nicotinamide by Engler.
- 1913** Funk isolates nicotinic acid from yeast.
- 1915** Goldberger demonstrates that pellagra is a dietary deficiency disease.
- 1928** Goldberger and Wheeler use the experimental model of black tongue disease in dogs as an experimental model for the human disease pellagra.
- 1937** Elvehjem and coworkers show the effectiveness of nicotinic acid and nicotinamide in curing canine black tongue.
- 1937** Spies cures human pellagra using nicotinamide.
- 1945** Krehl discovers that the essential amino acid tryptophan is transformed into niacin by mammalian tissues.
- 1955** The concept of niacin equivalents is proposed by Horwitt.
- 1955** Altschul and associates report that high doses of nicotinic acid reduce serum cholesterol in man.
- 1961** Turner and Hughes demonstrate that the main absorbed form of niacin is the amide.
- 1979** Shepperd and colleagues report that high doses of nicotinic acid lower both serum cholesterol and triglycerides.
- 1980** Bredehorst and colleagues show that niacin status affects the extent of ADP-ribosylation of proteins.



Casimir Funk

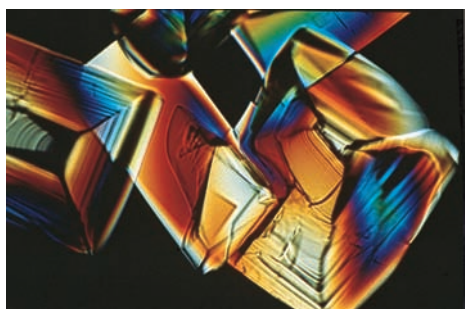


Conrad Elvehjem



Tom Spies

Pantothenic Acid



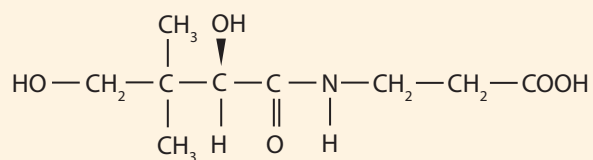
Calcium pantothenate crystals in polarised light

Synonyms

Vitamin B₅, antidermatosis vitamin, chick antidermatitis factor, chick antipellagra factor

Chemistry

Pantothenic acid is composed of beta-alanine and 2,4-dihydroxy-3,3-dimethylbutyric acid (pantoic acid), acid amide-linked. Pantetheine consists of pantothenic acid linked to a β -mercaptoethylamine group.



Molecular formula of pantothenic acid

Introduction

Pantothenic acid was discovered in 1933 and belongs to the group of water-soluble B vitamins. Its name originates from the Greek word "pantos", meaning "everywhere", as it can be found throughout all living cells.

Functions

Pantothenic acid, as a constituent of coenzyme A (a coenzyme of acetylation), plays a key role in the metabolism of carbohydrates, proteins and fats, and is therefore important for the maintenance and repair of all cells and tissues. Coenzyme A is involved in reactions that supply energy, in the synthesis of essential lipids (e.g. sphingolipids, phospholipids), sterols (e.g. cholesterol), hormones (e.g. growth, stress and sex hormones), neurotransmitters (e.g. acetylcholine), porphyrin (a component of haemoglobin, the oxygen-carrying red blood cell pigment) and antibodies, and in the metabolism of drugs (e.g. sulphonamides) and in alcohol detoxification. Another essential role of pantothenic acid concerns acyl carrier protein, an enzyme involved in the synthesis of fatty acids. In the process of fat burning, pantothenic acid works in concert with coenzyme Q10 and L-carnitine.

Main functions in a nutshell:

- Metabolism of carbohydrates, proteins and fats
- Supply of energy from foods
- Synthesis of essential lipids, sterols, hormones, neurotransmitters, and porphyrin
- Metabolism of drugs and alcohol detoxification

Dietary sources

The active vitamin is present in virtually all plant, animal and microbial cells. Thus pantothenic acid is widely distributed in foods, mostly incorporated into coenzyme A. Its richest sources are yeast and organ meats (liver, kidney, heart, brain), but eggs, milk, vegetables, legumes and whole-grain cereals are more common sources.

Pantothenic acid is synthesised by intestinal micro-organisms, but the extent and significance of this enteral synthesis is unknown.

Pantothenic acid content of foods

Food	Pantothenic acid (mg/100g)
Veal liver	7.9
Brewer's yeast	7.2
Peanuts	2.1
White mushrooms	2.1
Egg	1.6
Wheat germ	1
Herring	0.94
Milk	0.35
Vegetables	0.2-0.6

(Souci, Fachmann, Kraut)

Absorption and body stores

Most of the pantothenic acid in food exists in the form of coenzyme A, and pantothenic acid is released by a series of enzyme reactions in the small intestine. It is then absorbed by passive diffusion into the portal circulation and transported to the tissues, where re-synthesis of the coenzyme occurs. About half of the pantothenic acid in the diet is actually absorbed. If calcium pantothenate or pan-

tothenic acid are ingested as nutritional supplements, they must first be converted to pantetheine by intestinal enzymes before being absorbed. Topical and orally applied D-pantethenol (the alcoholic form of pantothenic acid that can, e.g., be found in many cosmetic products) is also absorbed by passive diffusion and transformed to pantothenic acid by enzymatic oxidation. The highest concentrations in the body are in the liver, adrenal glands, kidneys, brain, heart and testes. Total pantothenic acid levels in whole blood are at least 1 mg/L in healthy adults; most of it exists as coenzyme in the red blood cells. Urinary excretion in the form of pantothenic acid generally correlates with dietary intake, but variation is large (2-7 mg daily). During lactation, a large proportion of the intake reaches the milk (1-5 mg daily).

Measurement

Due to the fact that dietary deficiency is practically unknown, little research has been conducted through assays to assess pantothenate status in man. Nutritional status can be deduced from amounts of pantothenate excreted in urine. Less than 1 mg daily is considered abnormally low. A more convenient approach is determination of pantothenate in serum, or preferably whole blood, by microbiological methods. Although these assays are highly sensitive and specific, they are slow and tedious to perform. New methods, such as HPLC/MS (High Performance Liquid Chromatography / mass spectrometry) and immunologic methods, have also been applied. Another method suggested for assessing nutritional status is the sulphanilamide acetylation test, which measures the activity of coenzyme A in the blood. Whole blood levels typically range from 0.9 – 1.5 µmol/L.

Stability

Pantothenic acid is stable under neutral conditions, but is readily destroyed by heat in alkaline or acid solutions. Up to 50% may be lost during cooking (due to leaching) and up to 80% as a result of food processing and refining (canning, freezing, milling etc.). Pasteurisation of milk only causes minor losses.

Interactions

Positive interactions

Various studies have indicated that vitamin B₁₂ may aid in the conversion of free pantothenic acid into coenzyme A. In the absence of B₁₂, coenzyme A production is decreased and fat metabolism impaired. In animal experiments, ascorbic acid (vitamin C) was shown to lessen the severity of symptoms due to pantothenic acid deficiency; vitamin A, vitamin B₆, folic acid and biotin are also necessary for proper utilisation of pantothenic acid.

Negative interactions

Ethanol causes a decrease in the amount of pantothenic acid in tissues, with a resulting increase in serum levels. It has therefore been suggested that pantothenic acid utilisation is impaired in alcoholics. Birth control pills containing estrogen and progestin may increase the requirement for pantothenic acid. The most common antagonist of pantothenic acid used experimentally to accelerate the appearance of deficiency symptoms is omega-methyl pantothenic acid. L-pantothenic acid has also been shown to have an antagonistic effect in animal studies. Methyl bromide, a fumigant used to control vermin in places where food is stored, destroys the pantothenic acid in foods exposed to it.

Deficiency

Since pantothenic acid occurs to some extent in all foods, it is generally assumed that dietary deficiency of this vitamin is extremely rare. However, pantothenic acid deficiency in humans is not well documented and probably does not occur in isolation but in conjunction with deficiencies of other B vitamins. Clinical manifestations that can be clearly ascribed to dietary deficiency of pantothenic acid have not been identified, although it has been implicated in "burning feet" syndrome, a condition observed among malnourished prisoners of war in the 1940s. Deficiency symptoms have been produced experimentally by administering the antagonist omega-methyl pantothenic acid. They include fatigue, headaches, insomnia, nausea, abdominal cramps, vomiting and flatulence. The subjects complained of tingling sensations in the arms and legs, muscle cramps and impaired coordination. There was cardiovascular instability and impaired responses to insulin, histamine and ACTH (a stress hormone).

Homopantothenate is a pantothenic acid antagonist that has been used in Japan to enhance mental function, especially in Alzheimer's disease. A rare side effect was an abnormal brain function resulting from the failure of the liver to eliminate toxins (hepatic encephalopathy). This condition was reversed by pantothenic acid supplementation, suggesting it was due to pantothenic acid deficiency caused by the antagonist. In experiments with mice it has been shown that a deficiency of pantothenic acid leads to skin irritation and greying of the fur, which were reversed by giving pantothenic acid. Pantothenic acid has since been added to shampoo, although it has never been successful in restoring hair colour in humans.

Groups at risk of deficiency

- Alcoholics
- Women on oral contraceptives
- People with insufficient food intake (e.g. elderly, post-operative)
- People with impaired absorption (due to certain intestinal diseases)

Current recommendations in the USA

RDA*

Infants	< 6 months	1.7mg (AI)
Infants	7-12 months	1.8mg (AI)
Children	1-3 years	2mg (AI)
Children	4-8 years	3mg (AI)
Children	9-13 years	4mg (AI)
Adults	>14 years	5mg (AI)
Pregnancy		6mg (AI)
Lactation		7mg (AI)

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Disease prevention and therapeutic use

Although isolated deficiency states are rarely observed, various investigators have noted changes in pantothenic acid levels in various diseases, and pharmacological amounts of the vitamin are used in the treatment of numerous conditions. In most cases, however, the claimed therapeutic responses have not been confirmed by controlled studies in humans.

For the treatment of deficiency due to impaired absorption, intravenous or intramuscular injections of 500 mg several times a week are recommended. Postoperative ileus (paralysis of the intestine) requires doses of up to 1000 mg every six hours.

Panthenol is applied topically to skin and mucosa to speed healing of wounds, ulcers and inflammation, such as cuts and grazes, burns, sunburn, nappy rash, bed sores, laryngitis and bronchitis. In combination, pantothenic acid and ascorbic acid significantly enhance post surgical therapy and wound healing. The healing process of conjunctiva and the cornea after reconstructive surgery of the epithelium has also been accelerated. Pantothenic acid has been tried, with varying results, to treat various liver conditions, arthritis, and constipation in the elderly; to prevent urinary retention after surgery or childbirth; and (together with biotin) to prevent baldness. It has also been reported to have a protective effect against radiation sickness.

Pantethine is used to normalise lipid profiles, as it lowers elevated triglycerides and LDL cholesterol while raising levels of the beneficial HDL cholesterol. Pantethine actually consists of two molecules of pantetheine joined by two molecules of sulphur (a disulphide bridge). It is especially effective at lowering elevated blood lipids in patients with diabetes without hindering blood sugar control.

Recommended Dietary Allowance (RDA)

It is widely agreed that there is insufficient information available on which to base an RDA for pantothenic acid. Most countries that make recommendations therefore give an estimate of safe and adequate levels for daily intake. These adequate intake levels (AI) are based on estimated dietary intakes in healthy population groups and range, depending on the health authority concerned, from 2 to 14 mg for adults.

Safety

Pantothenic acid is essentially considered to be nontoxic, and no cases of hypervitaminosis have ever been reported. As much as 10 g daily in humans produces only minor gastrointestinal disturbance (diarrhoea). Due to the lack of reports of adverse effects the main regulatory authorities have not defined a tolerable upper level of intake (UL) for pantothenic acid.

Supplements, food fortification and cosmetics

Pure pantothenic acid is a viscous hygroscopic oil that is chemically not very stable. Supplements therefore usually contain the calcium salt, or alcohol, panthenol. Both are highly water soluble and are rapidly converted to free acid in the body. Calcium pantothenate is often included in multivitamin preparations; panthenol is the more common form used in mono-preparations, which are available in a wide variety of pharmaceutical forms (e.g. solutions for injection and local

application, aerosols, tablets, ointments and creams). Pantethine, a derivative of pantothenic acid, is used as a cholesterol and triglyceride-lowering drug in Europe and Japan and is available in the U.S. as a dietary supplement.

Pantothenate is added to a variety of foods, the most important of which are breakfast cereals and beverages, dietetic and baby foods.

D-Panthenol is often used in cosmetic products. In skin care products, it helps to keep the skin moist and supple, stimulates cell growth and tissue repair, and inhibits inflammation and reddening. As a moisturiser and conditioner in hair care products, it protects against and repairs damage due to chemical and mechanical procedures (brushing, combing, shampooing, perming, colouring etc.), and imparts sheen and luster.

Industrial production

Pantothenic acid is chemically synthesised by condensation of D-pantolactone with P-alanine. Addition of a calcium salt produces colourless crystals of calcium pantothenate. Panthenol is produced as a clear, almost colourless, viscous hygroscopic liquid.



History

- 1931** Williams and Truesdail separate an acid fraction from “bios”, the growth factor for yeast discovered in 1901 by Wildiers.
- 1933** Williams and coworkers show this fraction to be a single acid substance essential for the growth of yeast. Because they find it in a wide range of biological materials, they suggested calling it “pantothenic acid”.
- 1938** Williams and associates establish the structure of pantothenic acid
- 1939** Jukes and colleagues show the similarity between pantothenic acid and the chick antidermatitis factor.
- 1940** Total synthesis of the vitamin is achieved independently by Williams and Major, Stiller and associates, Reichstein and Grüssner, and Kuhn and Wieland.
- 1947** Lipmann and his associates identify pantothenic acid as one of the components of the coenzyme they had discovered in liver two years earlier.
- 1953** The full structure of coenzyme A is elucidated by Baddiley and colleagues. Lipmann receives the Nobel Prize, together with Krebs, for his work on coenzyme A and its role in metabolism.
- 1954** Bean and Hodges report that pantothenic acid is essential in human nutrition. Subsequently, they and their colleagues conduct several further studies to produce deficiency symptoms in healthy humans using the antagonist omega-methyl pantothenic acid.
- 1965** Pugh and Wakil identify the acyl carrier protein as an additional active form of pantothenic acid.
- 1976** Fry and her associates measure the metabolic response of humans to deprivation of pantothenic acid without involvement of an antagonist.



Roger John Williams

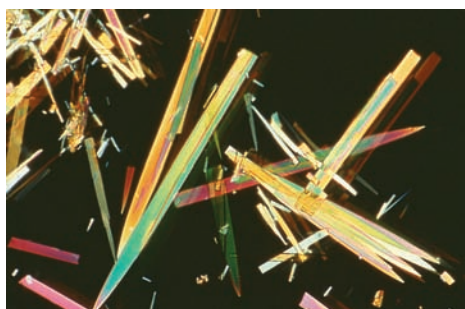


Fritz Albert Lipmann



Tadeusz Reichstein

Folic Acid



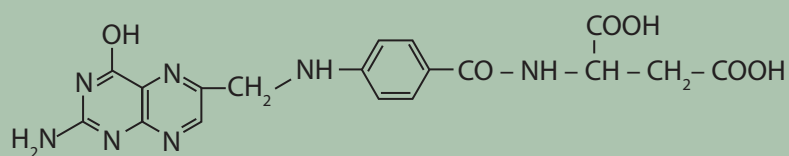
Folic acid crystals in polarised light

Synonyms

Folacin, vitamin B₉, vitamin B₉, *Lactobacillus casei* factor

Chemistry

Folic acid consists of a pteridine ring system, p-aminobenzoic acid and one molecule of glutamic acid (chemical name: pteroylglutamic acid). Naturally occurring folates are pteroylpolyglutamic acids with two to eight glutamic acid groups.



Molecular formula of folic acid

Introduction

Folate is a generic term for a water-soluble group of B vitamins including folic acid and naturally occurring folates. Folic acid is a synthetic folate compound used in vitamin supplements and fortified food because of its increased stability. The name comes from folium, which is the Latin word for leaves, because folates were first isolated from spinach in 1941. In 1962 Herbert consumed a folate-deficient diet for several months and records his development of deficiency symptoms. His findings set the criteria for the diagnosis of folate deficiency.

Functions

Tetrahydrofolic acid, which is the active form of folate in the body, acts as a coenzyme in numerous essential metabolic reactions. Folate coenzymes act as acceptors and donors of one-carbon units in these reactions. Folate coenzymes play an important role in the metabolism of several amino acids, the constituents of proteins. The synthesis of the amino acid methionine from homocysteine requires a folate coenzyme and, in addition, vitamin B₁₂. Tetrahydrofolic acid is involved in the synthesis of nucleic acids (DNA and RNA) – the molecules that carry genetic information in cells – and also in the formation of blood cells. Folates are therefore essential for normal cell division, proper growth and for optimal functioning of the bone marrow.

Main functions in a nutshell:

- Coenzyme in amino acid metabolism
- Coenzyme in the synthesis of nucleic acids
- Blood cell formation in the bone marrow

Dietary sources

Folates are found in a wide variety of foods. Its richest sources are liver, dark green leafy vegetables, beans, wheat germ and yeast. Other sources are egg yolk, milk and dairy products, beets, orange juice and whole wheat bread. Folates synthesised by intestinal bacteria do not contribute significantly to folate nutrition in humans because bacterial folate synthesis is usually restricted to the large intestine (colon), whereas absorption occurs mainly in the upper part of the small intestine (jejunum).

Folate content of foods

Food	Folate (µg/100g)
Beef liver	592
Peanuts	169
Spinach	145
Broccoli	114
Asparagus	108
Egg	67
Strawberries	43
Orange juice (freshly squeezed)	41
Tomatoes	22
Milk (whole)	6.7

(Souci, Fachmann, Kraut)

Absorption and body stores

Most dietary folates exist as polyglutamates, which have to be converted to the monoglutamate form in the gut before absorption. The monoglutamate form is absorbed in the proximal small intestine by an active carrier-mediated transport mechanism, and also by passive diffusion. Ingested folic acid is enzymatically reduced and methylated in the mucosa cells. The predominant form

of folate in the plasma is 5-methyltetrahydrofolate. Folates are widely distributed in tissues, most of them as polyglutamate derivatives. The main storage organ is the liver, which contains about half of the body's stores.

Bioavailability

Absorption of folic acid is almost 100% when consumed under fasting conditions. When folic acid is consumed with a portion of food, bioavailability is estimated from experimental data to be 85%. The bioavailability of food folates is variable and incomplete, and has been estimated to be no more than 50% that of folic acid.

Measurement

Different methods are used for the measurement of folates. They can be measured by microbiological assays using *Lactobacillus casei* as test organism. Radioassays based on competitive protein binding are simpler to perform and are not affected by antibiotics, which give false low values in microbiological assays. High-performance liquid chromatography (HPLC) methods have also been established for the analysis of folates. Folate status is assessed by measuring serum and red blood cell folate levels of methyltetrahydrofolate, which is the predominant folate. Serum folate level is not a reliable indicator of folate deficiency, but is considered a sensitive indicator of recent folate intake. Serum concentrations < 7 nmol/L (3 ng/ml) are suggested to indicate negative folate balance. Levels in the red blood cells are considered to be an indicator of long-term status, and to be representative of tissue folate stores. Levels < 305 nmol/L (140 ng/ml) indicate inadequate folate

status. A recent development has been a method for the measurement of whole blood cell folate in dried blood spots on filter paper.

Increased homocysteine levels may also indicate folate deficiency. Methyltetrahydrofolate is necessary for the conversion of homocysteine to methionine. Therefore plasma homocysteine concentration increases when folate is not available in sufficient amounts. Although plasma homocysteine concentration is a sensitive indicator, it is not highly specific because it may be influenced by other nutrient deficiencies (vitamin B₁₂, B₆), genetic abnormalities and renal insufficiency.

Stability

Most forms of folate in food are unstable. Fresh leafy vegetables stored at room temperature may lose up to 70% of their folate activity within three days. Considerable losses also occur through leaching into cooking water (up to 95%) and through heating.

Interactions

Positive interactions

Proper folate utilisation depends on an adequate supply of other vitamins of the B group such as vitamin B₁₂ and B₆ and vitamin C, which are involved in the chemical reactions needed for folate metabolism. Vitamin C may also provide the reducing conditions needed to preserve folates in the diet, and a diet deficient in folates is also likely to be deficient in vitamin C.

Negative interactions

Several chemotherapeutic agents (e.g. methotrexate, trimethoprim, pyrimethamine) inhibit the enzyme dihydrofolate reductase, which is necessary for the metabolism of folates.

When nonsteroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen) are taken in very large therapeutic doses, for example in the treatment of severe arthritis, they may interfere with folate metabolism.

Many drugs may interfere with the absorption, utilisation and storage of folates. These include alcohol, cholestyramine and colestipol (drugs used to lower blood cholesterol), antiepileptic agents such as barbiturates and diphenylhydantoin, and sulfasalazine, which is used in the treatment of ulcerative colitis. Drugs that reduce acidity in the intestine, such as antacids and modern anti-ulcer drugs, have also been reported to interfere with the absorption of folic acid.

Early studies of oral contraceptives containing high levels of oestrogen suggested an adverse effect on folate status, but this has not been supported by more recent studies on low dose oral contraceptives.

Deficiency

Folate deficiency is one of the commonest vitamin deficiencies. It can result from inadequate intake, defective absorption, abnormal metabolism or increased requirements.

Diagnosis of a subclinical deficiency relies on demonstrating reduced red cell folate concentration or on other biochemical evidence such as increased homocysteine concentration, since haematological manifestations are usually absent. Early symptoms of folate deficiency are non-specific and may include tiredness, irritability and loss of appetite. Severe folate deficiency leads to megaloblastic anaemia, a condition in which the bone marrow produces giant, immature red blood cells. At an advanced stage of anaemia symptoms of weakness, fatigue,

shortness of breath, irritability, headache, and palpitations appear. If left untreated, megaloblastic anaemia may be fatal. Gastrointestinal symptoms also result from severe folate deficiency. Deficiency during pregnancy may result in premature birth, infant low birth weight and foetal growth retardation. In children, growth may be retarded and puberty delayed.

Folate deficiency is very common in many parts of the world and is part of the general problem of undernutrition. In developed countries, nutritional folate deficiency may be encountered above all in economically underprivileged groups (e.g., the elderly). Reduced folate intake is also often seen in people on special diets (e.g. weight-reducing diets). Disorders of the stomach (e.g. atrophic gastritis) and small intestine (e.g. celiac disease, sprue, Crohn's disease) may lead to folate deficiency as a result of malabsorption. In conditions with a high rate of cell turnover (e.g. cancer, certain anaemias and skin disorders), folate requirements are increased. This is also the case during pregnancy and lactation, due to rapid tissue growth during pregnancy and to losses through the milk during lactation. People undergoing drug treatment, e.g. for epilepsy, cancer or an infection, are at high risk of developing a



folate deficiency, as are patients with renal failure who require regular haemodialysis. Acute folate deficiencies have been reported to occur within a relatively short time in patients undergoing intensive care, especially those on total parenteral nutrition.

Disease prevention and therapeutic use

In situations where there is a high risk of folate deficiency, oral folic acid supplementation is recommended, usually in a multivitamin preparation containing 400-500 µg of folic acid.

In acute cases of megaloblastic anaemia, treatment often has to be started before a diagnosis of the cause (vitamin B₁₂ or folate deficiency) has been made. To avoid complications that may arise by treating a B₁₂ deficiency with folic acid in such circumstances (see below), both folic acid and vitamin B₁₂ need to be administered until a specific diagnosis is available.

It has been demonstrated that periconceptional (before and during the first 28 days after conception) supplementation of women with folic acid can decrease the risk of neural tube defects (malformations of the brain and spinal cord, causing anencephaly or spina bifida). Therefore, a daily intake of 400 µg folic acid in addition to a healthy diet 8 weeks prior to and during the first 12 weeks after conception is recommended. There is evidence that adequate folate status may also prevent the incidence of other birth defects, including cleft lip and palate, certain heart defects and limb malformations.

Results from intervention studies have shown that a multivitamin supplement containing folic acid is more

effective in decreasing the risk of neural tube defects and other birth defects than folic acid alone.

Numerous studies have shown that even moderately elevated levels of homocysteine in the blood increase the risk of atherosclerosis. Folic acid has been shown to decrease homocysteine levels. Several randomised placebo-controlled trials are presently being conducted to establish whether folic acid supplementation reduces the risk of cardiovascular diseases by lowering homocysteine blood levels.

A number of different observational studies have found poor folate status to be associated with increased cancer risk. There is evidence that folate plays a role in preventing colorectal cancer. The results of two large epidemiological investigations suggest that increased folate intake may reduce breast cancer risk associated with regular alcohol consumption.

Low folate levels have also been associated with Alzheimer's disease, dementia and depression.

Recommended Dietary Allowance (RDA)

In the USA the recommendations of the Food and Nutrition Board (1998) are expressed as DFEs. This organisation recommends a daily intake of 400 µg of DFE for adult females and males. To cover increased needs during pregnancy and lactation, it recommends 600 µg/day and 500 µg/day respectively. In Europe, the RDA varies between 200-400 µg/day for adults in different countries.

Dietary Folate Equivalents (DFE) have been introduced because of the different bioavailability of folates and folic acid.

1 µg DFE = 1 µg of food folate
= 0.5 µg of folic acid taken on an empty stomach
= 0.6 µg of folic acid from fortified food or as a supplement taken with meals

Current recommendations in the USA

RDA*

RDA listed as dietary folate

Infants	< 6 months	65 µg (AI)
Infants	7-12 months	80 µg (AI)
Children	1-3 years	150 µg
Children	4-8 years	200 µg
Children	9-13 years	300 µg
Adults	> 14 years	400 µg
Pregnancy		600 µg
Lactation		500 µg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Safety

Oral folic acid is not toxic to humans. Even with daily doses as high as 15 mg there have been no substantiated reports of toxicity, and a daily supplement of 10 mg has been taken for five years without adverse effect.

It has been claimed that high doses of folic acid may counteract the effect of antiepileptic medication and so increase the frequency of seizures in susceptible patients.

A high intake of folic acid can mask vitamin B₁₂ deficiency. It should therefore not be used indiscriminately in patients with anaemia because of the risk of damage to the nervous system due to B₁₂ deficiency.

The US Food and Nutrition Board (1998) set the tolerable upper intake level (UL) of folic acid from fortified foods or supplements at 1,000 µg/day for adults. The EU Scientific Committee on Food (2000) also established a UL of 1,000 µg for folic acid.

Supplements and food fortification

Folic acid is available as oral preparations, alone or in combination with other vitamins or minerals (e.g. iron), and as an aqueous solution for injection. As the acid is only poorly soluble in water, folate salts are used to prepare liquid dosage forms. Folinic acid (also known as leucovorin or citrovorum factor) is a derivative of folic acid administered by intramuscular injection to circumvent the action of dihydrofolate reductase inhibitors, such as methotrexate. It is not otherwise indicated for the prevention or treatment of folic acid deficiency.

Folic acid is added to a variety of foods, the most important of which are flour, salt, breakfast cereals and beverages, soft drinks and baby foods.

To reduce the risk of neural tube defects, cereal grains are fortified with folate in some countries. In the USA and Canada all enriched cereal grains (e.g., enriched bread, pasta, flour, breakfast cereals, and rice) are required to be fortified with folic acid. In Hungary and Chile, wheat flour is fortified with folic acid.

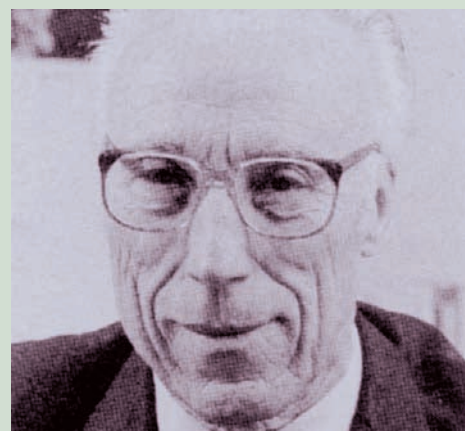
Industrial production

Folic acid is manufactured on a large scale by chemical synthesis. Various processes are known. Most synthesised folic acid is used in animal feed.



History

- 1931** Wills in India observes the effect of liver and yeast extracts on tropical macrocytic anaemia and concludes that this disorder must be due to a dietary deficiency. She recognises that yeast contains a curative agent equal in potency to that of liver.
- 1938** Day and coworkers find an antianaemia factor for monkeys in yeast and designate it "vitamin M." Around the same time, Stokstad and Manning discover a growth factor for chicks, which they call "Factor U".
- 1939** Hogan and Parrott identify an antianaemia factor for chicks in liver extracts, which they name "Vitamin BC".
- 1940** Discovery of growth factors for *Lactobacillus casei* and *Streptococcus lactis*. Snell and Peterson coin the term "norite-eluate factor".
- 1941** Mitchell and colleagues suggest the name "folic acid" (folium, Latin for leaf) for the factor responsible for growth stimulation of *Streptococcus lactis*, which they isolate from spinach and suspect of having vitamin-like properties for animals.
- 1945** Angier and coworkers report the synthesis of a compound identical to the *L. casei* factor isolated from liver. They later describe the chemical structures of the basic and related compounds.
- 1945** Spies demonstrates that folic acid cures megaloblastic anaemia during pregnancy.
- 1962** Herbert consumes a folate-deficient diet for several months and records his development of deficiency symptoms. His findings set the criteria for the diagnosis of folate deficiency. In the same year, Herbert estimates the folic acid requirements for adults, which still serve as a basis for many RDAs.
- 1991** Wald establishes that folic acid supplementation reduces risk of neural tube defects by 70% among women who have already given birth to a child with such birth defects.
- 1992** Butterworth finds that higher than normal serum levels of folic acid are associated with decreased risk of cervical cancer in women infected with human papillomavirus. Also, Czeizel demonstrates that first-time occurrence of neural tube defects may be largely eliminated with a multivitamin containing folic acid taken in the periconceptional period.
- 1993** The US Public Health Service recommends that all women of childbearing potential consume 0.4 mg (400 µg) of folate daily in order to reduce the risk of foetal malformations such as spina bifida and other neural tube defects.
- 1998** Fortification of all enriched cereal grains (e.g., enriched bread, pasta, flour, rice and breakfast cereals) with folic acid becomes mandatory in the USA and in Canada. In Hungary, wheat flour is fortified with folic acid.
- 2000** Fortification of wheat flour with folic acid is established in Chile.



Esmond Emerson Snell

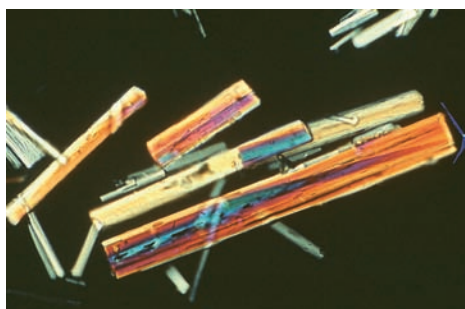


Robert B. Angier



Tom Spies

Biotin



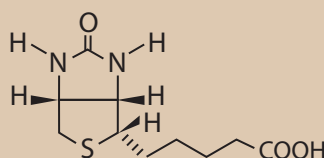
Biotin crystals in polarised light

Synonyms

Vitamin H ("**H**aar und **H**aut", German words for "hair and skin"), vitamin B₈ and co-enzyme R.

Chemistry

Biotin has a bicyclic ring structure. One ring contains a ureido group and the other contains a heterocyclic sulphur atom and a valeric acid side-group. (Hexahydro-2-oxo-1H-thieno [3,4-d]imidazole-4-pentanoic acid). Biologically active analogues: biocytin (ε-N-biotinyl-L-lysine), oxybiotin (S substituted with O).



Molecular formula of biotin

Introduction

Biotin is a colorless, water-soluble member of the B-complex group of vitamins. Although biotin was discovered already in 1901 as a special growth factor for yeast, it took nearly forty years of research to establish biotin as a vitamin. Due to its beneficial effects for hair, skin and nails, biotin is also known as the “beauty vitamin”. There are eight different forms of biotin, but only one of them – D-biotin – occurs naturally and has full vitamin activity. Biotin can only be synthesised by bacteria, moulds, yeasts, algae, and by certain plant species.

Functions

Biotin plays a key role in the metabolism of lipids, proteins and carbohydrates. It acts as a critical coenzyme of four carboxylases (enzymes):

- acetyl-CoA carboxylase (involved in the synthesis of fatty acids from acetate)
- propionyl-CoA carboxylase (involved in gluconeogenesis, i.e. the generation of glucose from lactate, glycerol, and amino acids)
- β -methylcrotonyl-CoA carboxylase (necessary for the metabolism of leucin, an essential amino acid)
- pyruvate carboxylase (involved in energy metabolism, necessary for the metabolism of amino acids, cholesterol, and odd chain fatty acids)

Biotin also plays a special role in enabling the body to use blood glucose as a major source of energy for body fluids. Furthermore, biotin may have a role in DNA replication and transcription arising from its interaction with nuclear histone proteins. It owes its reputation as the “beauty vitamin” to the fact that it activates protein/amino acid metabolism in the hair roots and fingernail cells.

Main functions in a nutshell:

- Synthesis of fatty acids, amino acids and glucose
- Energy metabolism
- Excretion of by-products from protein metabolism
- Maintenance of healthy hair, toenails and fingernails

Dietary sources

Biotin is widely distributed in most foods but at very low levels compared to other water-soluble vitamins. It is found in free and protein-bound forms in foods. Its richest sources are yeast, liver and kidney. Egg yolk, soybeans, nuts and cereals are also good sources. 100 g of liver contains approximately 100 μ g biotin, whereas most other meats, vegetables and fruits only contain approximately 1 μ g biotin /100 g. In animal experiments, biotin bioavailability has been shown to vary considerably (5%-62%), and in cereals it appears to be lower.

Biotin content of foods

Food	Biotin (μ g/100g)
Brewer's yeast	115
Beef liver	100
Soya beans	60
Wheat bran	45
Peanuts	35
Egg	25
White mushrooms	16
Spinach	6.9
Bananas	6
Strawberries	4
Whole wheat bread	2
Asparagus	2

(Souci, Fachmann, Kraut)

Biotin-producing microorganisms exist in the large intestine, but the extent and significance of this enter-

al synthesis in the overall biotin turnover is difficult to calculate and thus remains a subject of controversy.

Absorption and body stores

In most foodstuffs biotin is bound to proteins from which it is released in the intestine by protein hydrolysis and a specific enzyme, biotinidase. Biotin is then absorbed unchanged in the upper part of the small intestine by an electron-neutral sodium (Na^+) gradient dependent carrier-mediated process and also by slow passive diffusion. The carrier is regulated by the availability of biotin, with up-regulation of the number of transporter molecules when biotin is deficient. The colon is also able to absorb biotin via an analogue transport mechanism. Once absorbed, biotin is distributed to all tissues. The presence of a specific biotin carrier protein in plasma is not yet conclusive. The liver and retinal tissues are the main storage places. Biotin metabolites are not active as vitamins and are excreted in the urine. Remarkable amounts of biotin appear in the faeces deriving from colonic bacteria.

Measurement

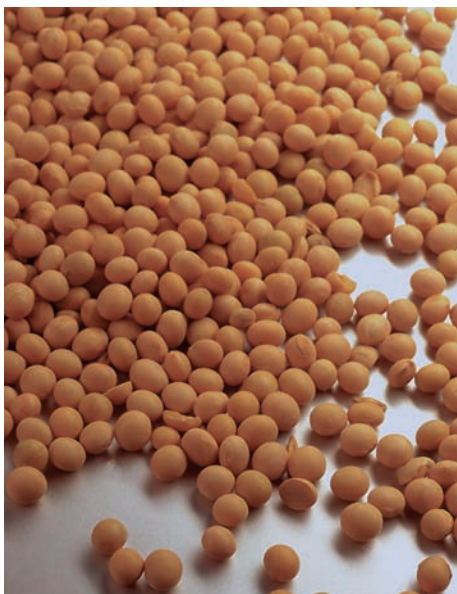
The body status of biotin can be determined by measuring its activity and/or activation of biotin dependent enzymes – predominately carboxylases – by added biotin. More convenient methods are direct determination of biotin in plasma or serum by microbiological methods or avidin binding assays, or determination of biotin excretion and 3-hydroisovaleric acid in urine. Measurement of biotin in plasma is not a reliable indicator of nutritional

status, because reported levels for biotin in the blood vary widely. Thus, a low plasma biotin concentration is not a sensitive indicator of inadequate intake.

Usual serum concentrations = 100 - 400 pmol/L.

Stability

Biotin is relatively stable when heated and so is not easily destroyed in the ordinary processes of cooking but it will leach into cooking water. Processing of food, e.g. canning, causes a moderate reduction in biotin content.



Groups at risk of deficiency

- patients maintained on total parenteral nutrition
- people who eat large amounts of raw egg white
- haemodialysis patients
- diabetes mellitus
- individuals receiving some forms of long-term anticonvulsant therapy
- individuals with biotinidase deficiency or holocarboxylase synthetase (HCS) deficiency (genetic defects)
- patients with malabsorption, including short-gut syndrome
- pregnancy may be associated with marginal biotin deficiency

Interactions

Negative interactions

Raw egg whites contain avidin, a glycoprotein that strongly binds with biotin and prevents its absorption. Thus, the ingestion of large quantities of raw egg white over a long period can result in a biotin deficiency. It has also been reported that antibiotics which damage the intestinal flora (thus decreasing bacterial synthesis) can reduce biotin levels. Interactions with certain anticonvulsant drugs and alcohol have also been reported, as they may inhibit intestinal carrier-mediated transport of biotin. Pantothenic acid ingested in large amounts competes with biotin for intestinal and cellular uptake because of their similar structures.

Deficiency

Human biotin deficiency is extremely rare. This is probably due to the fact that biotin is synthesised by beneficial bacteria in the human intestinal tract. Potential deficiency symptoms include anorexia, nausea, vomiting, glossitis, depression, dry scaly dermatitis, conjunctivitis and ataxia, and after long-lasting, severe biotin deficiency, loss of hair colour and hair loss (alopecia). Signs of biotin deficiency in humans have been demonstrated in volunteers consuming a biotin-deficient diet together with large amounts of raw egg white. After 3-4 weeks they developed a fine dry scaly desquamating dermatitis, frequently around the eyes, nose, and mouth. After ten weeks on the diet, they were fatigued, depressed and sleepy, with nausea and loss of appetite. Muscular pains, hyperesthesia and paresthesia occurred, without reflex changes or other objective signs of neuropathy. Volunteers also developed anaemia and hypercholesterolaemia. Liver biopsies in sudden infant death syndrome babies reveal low biotin levels. Most of the affected infants were bottle-fed.

Disease prevention and therapeutic use

There is no direct evidence that marginal biotin deficiency causes birth defects in humans, but an adequate biotin intake/supplementation during pregnancy is advisable.

Biotin is used clinically to treat the biotin-responsive inborn errors of metabolism, holocarboxylase synthetase deficiency and biotinidase deficiency.

Large doses of biotin may be given to babies with a condition called infantile seborrhea or to patients with genetic abnormalities in biotin metabolism. A large number of reports have shown a beneficial effect of biotin in infant seborrheic dermatitis and Leiner's disease (a generalised form of seborrheic dermatitis).

Biotin supplements are sometimes given to help reduce blood sugar in diabetes patients. People with type 2 diabetes often have low levels of biotin. Some patients with diabetes may have an abnormality in the biotin-dependent enzyme pyruvate carboxylase, which can lead to dysfunction of the nervous system.

The main benefit of biotin as a

dietary supplement is in strengthening hair and nails. Biotin supplements may improve thin or splitting toenails or fingernails and improve hair health. Uncomable hair syndrome in children also improves with biotin supplementation, as do certain skin disorders, such as “cradle cap”. Biotin has also been used to combat premature graying of hair, though it is likely to be useful only for those with a low biotin status. In orthomolecular medicine biotin is used to treat hair loss, but scientific evidence is not conclusive.

Biotin has been used for people in weight loss programs to help them metabolise fat more efficiently.

Recommended Dietary Allowance (RDA)

In 1998 the Food and Nutrition Board of the Institute of Medicine felt the existing scientific evidence was insufficient to calculate an EAR,

and thus an RDA, for biotin. Instead an Adequate Intake level (AI) has been defined. The AI for biotin assumes that current average intakes of biotin (35 µg to 60 µg/day) are meeting the dietary requirement. An estimation of the safe and adequate daily dietary intake for biotin was made for the first time in 1980 by the Food and Nutrition Board of the United States National Research Council. The present recommendations in the USA are 20-30 µg daily for adults and children over 11 years, and 5-12 µg daily for infants and younger children. France and South Africa recommend a daily intake of up to 300 µg, and Singapore up to 400 µg biotin. Others, including the Federal Republic of Germany, assume that diet and intestinal synthesis provide sufficient amounts.

Safety

No known toxicity has been associated with biotin. Biotin has been administered in doses as high as 40

mg per day without objectionable effects. Due to the lack of reports of adverse effects, no major regulatory authorities have established a tolerable upper level of intake (UL) for biotin.

Supplements and food fortification

Biotin, usually either in the form of crystalline D-biotin or brewer's yeast, is added to many dietary supplements, infant milk formulas and baby foods, as well as various dietetic products. As a supplement, biotin is often included in combinations of the B vitamins. Mono-preparations of biotin are available in some countries as oral and parenteral formulations.

Therapeutic doses of biotin for patients with a biotin deficiency range between 5 and 20 mg daily. Seborrheic dermatitis and Leiner's disease in infants respond to daily doses of 5 mg. Patients with biotinidase deficiency require life-long

Current recommendations in the USA

RDA*

Infants	< 6 months	5 µg (Adequate Intake, AI)
Infants	7-12 months	6 µg (AI)
Children	1-3 years	8 µg (AI)
Children	4-8 years	12 µg (AI)
Children	9-13 years	20 µg (AI)
Children	14-18 years	25 µg (AI)
Adults	> 19 years	30 µg (AI)
Pregnancy		30 µg (AI)
Lactation		35 µg (AI)

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people



biotin therapy in milligram doses (5-10mg/day). Patients with HCS deficiency require supplementation of 40-100 mg/day. If biotin therapy is introduced in infancy, the prognosis for both these genetic defects are good.

A daily supplement of 60 µg biotin for adults and 20 µg for children has been recommended to maintain normal plasma levels in patients on total parenteral nutrition.

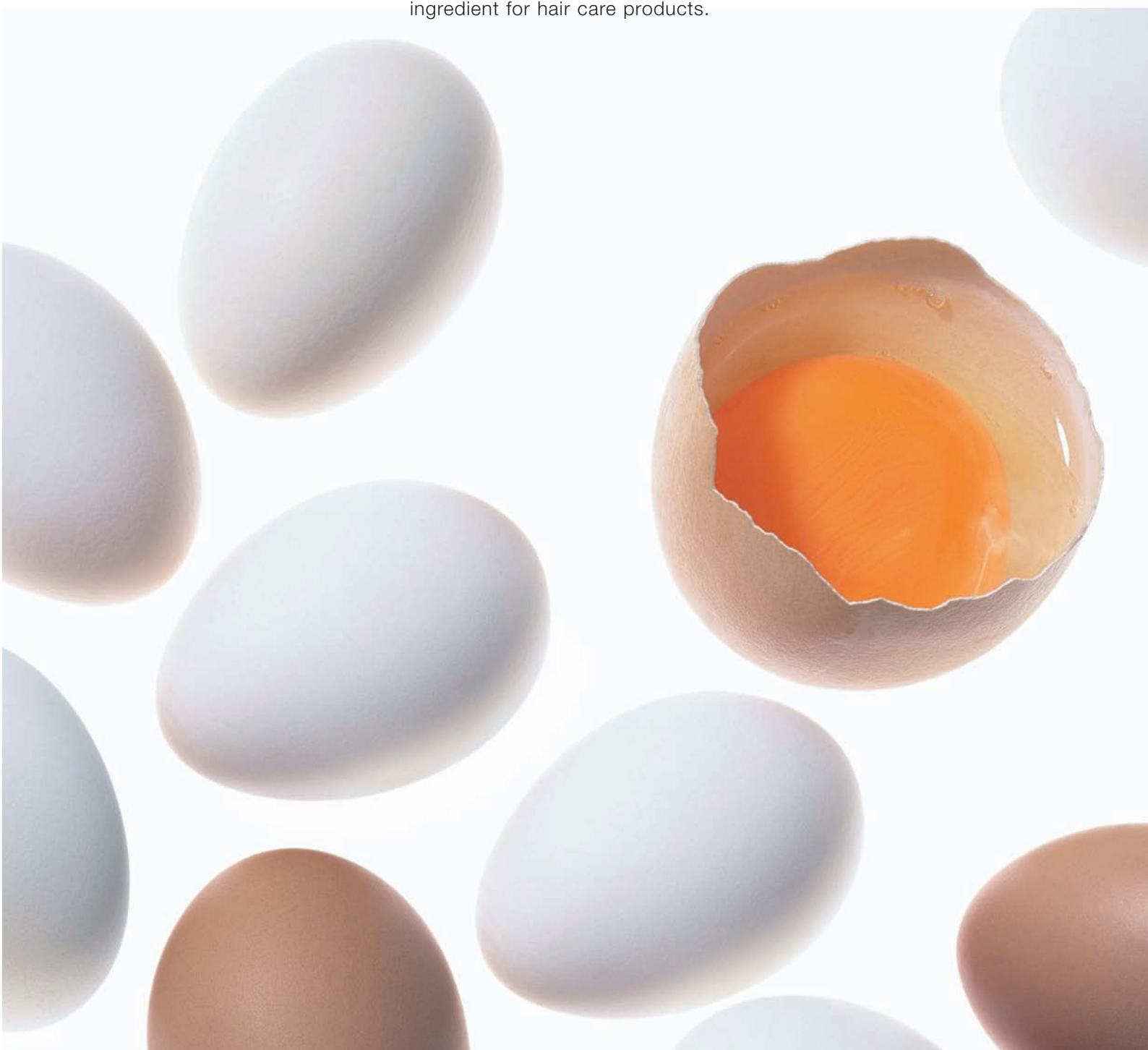
Other technical applications

Baker's yeast (*Saccharomyces cerevisiae*) is dependent on biotin for growth. Biotin is therefore added as a growth stimulant to the nutrient medium used in yeast fermentation. Also, many of the microorganisms used in modern biotechnology are biotin-dependent. Thus, biotin is added to the growth medium in such cases.

In cosmetics, biotin is used as an ingredient for hair care products.

Industrial production

Commercial synthesis of biotin is based on a method developed by Goldberg and Sternbach in 1949 and using fumaric acid as starting material. This technique produces a pure D-biotin which is identical to the natural product.

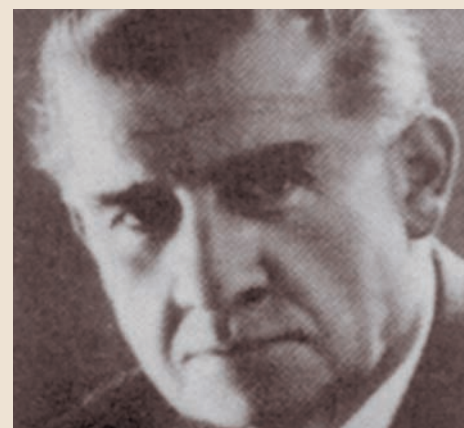


History

- 1901** Wildiers discovers that yeast requires a special growth factor which he names “bios”. Over the next 30 years, bios proves to be a mixture of essential factors, one of which – bios IIB – is biotin.
- 1916** Bateman observes the detrimental effect of feeding high doses of raw egg white to animals.
- 1927** Boas confirms the findings of dermatosis and hair loss in rats fed with raw egg white. She shows that this egg white injury can be cured by a “protective factor X” found in the liver.
- 1931** György also discovers this factor in the liver and calls it vitamin H (from Haut, the German word for skin).
- 1933** Allison and coworkers isolate a respiratory coenzyme – coenzyme R – that is essential for the growth of *Rhizobium*, a nitrogen-fixing bacterium found in leguminous plants.
- 1935** Kögl and Tönnis extract a crystalline growth factor from dried egg yolk and suggest the name ‘biotin’.
- 1940** György and his associates conclude that biotin, vitamin H and coenzyme R are identical. They also succeed in isolating biotin from the liver.
- 1942** Kögl and his group in Europe and du Vigneaud and his associates in the USA establish the structure of biotin.
- 1942** Sydenstricker and colleagues demonstrate the need for biotin in the human diet.
- 1943** Total synthesis of biotin by Harris and colleagues in the USA.
- 1949** Goldberg and Sternbach develop a technique for the industrial production of biotin.
- 1956** Traub confirms the structure of biotin by X-ray analysis.
- 1959** Lynen's group describes the biological function of biotin and paves the way for further studies on the carboxylase enzymes.
- 1971** First description of an inborn error of biotin-dependent carboxylase metabolism by Gompertz and associates.
- 1981** Burri and her colleagues show that the early infantile form of multiple carboxylase deficiency is due to a mutation affecting holocarboxylase synthetase activity.
- 1983** Wolf and coworkers suggest that late-onset multiple carboxylase deficiency results from a deficiency in biotinidase activity.



Paul György



Fritz Kögl



Vincent du Vigneaud



Feodor Lynen

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