# 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 $B_{12}$. 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 $B_{12}$, $B_6$), genetic abnormalities and renal insufficiency.

## Stability

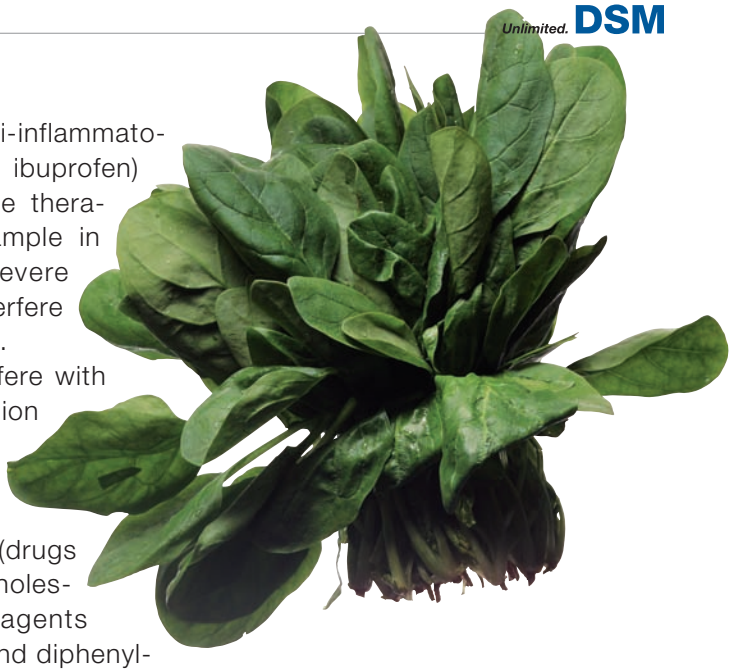
Most forms of folate in food are unstable. Fresh leafy vegetables stored at room temperature may lose up to 70% of their folate activity within three days. Considerable losses also occur through leaching into cooking water (up to 95%) and through heating.

## Interactions

**Positive interactions**
Proper folate utilisation depends on an adequate supply of other vitamins of the B group such as vitamin $B_{12}$ and $B_6$ 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 deficiences 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 $B_{12}$ or folate deficiency) has been made. To avoid complications that may arise by treating a $B_{12}$ deficiency with folic acid in such circumstances (see below), both folic acid and vitamin $B_{12}$ 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 $B_{12}$ deficiency. It should therefore not be used indiscriminately in patients with anaemia because of the risk of damage to the nervous system due to $B_{12}$ 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