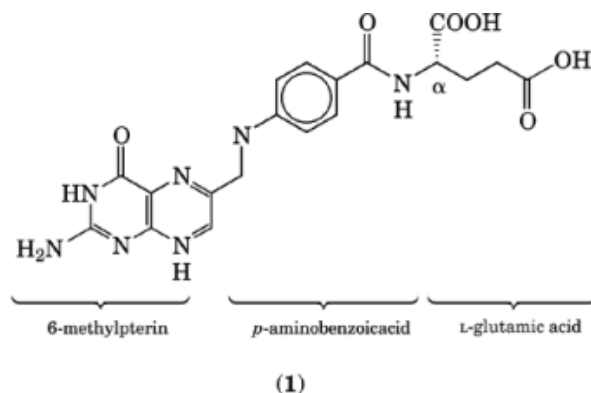


## FOLIC ACID

Folic acid [59-30-3] **1** belongs to the group of B vitamins. The term folate is used to designate all members of the family of compounds based on the *N*-[(6-pteridiny)methyl]-*p*-aminobenzoic acid skeleton conjugated with one or more L-glutamic acid units. In 1930, a dietary factor in yeast and crude liver extract was found to cure megaloblastic anemia in pregnant women (1). Purified folate was isolated by using two bioassay procedures, the microbiological growth assay and the assay for the antianemic factor for chickens (2–4). The growth factor from yeast and the antianemic factor (vitamin B<sub>9</sub>) were later shown to be different entities. A crystalline compound was isolated from spinach, which was called folic acid (5, 6). It was later shown that several of the above-mentioned factors belonged to the nutritionally and chemically related family of pteroylglutamic acid compounds. The structure of pteroylglutamic acid was elucidated in 1946 (7). The metabolically active forms of folic acid have a reduced pteridine ring and several glutamic acids residues. A detailed account on the discovery and early development of folic acid is available (8).



### 1. Occurrence, Source, and Bioavailability

Good food sources of folate are liver; fresh, dark green, leafy vegetables; beans; wheat germ; and yeasts (qv) (Table 1). Folic acid is synthesized only by microorganisms and plants (10–14). Most dietary folates exist in the polyglutamate form, which are converted to the more readily bioavailable monoglutamate form in the small intestine by the jejunal brush border folate conjugase. Certain foods such as cabbage and legumes contain conjugase inhibitors, which can decrease folate absorption.

The total folate content of food varies, based on the method of preparation and length of storage (15–17). Different forms of folates occur in nature and the stability or bioavailability of each form varies. Most folates in food are easily oxidizable and therefore are susceptible to oxidation under aerobic conditions during storage and processing. Folic acid (commercial form) has superior bioavailability because it is more readily absorbed

## 2 FOLIC ACID

**Table 1. Select Contributors of Folate in the U.S. Diet<sup>a</sup>**

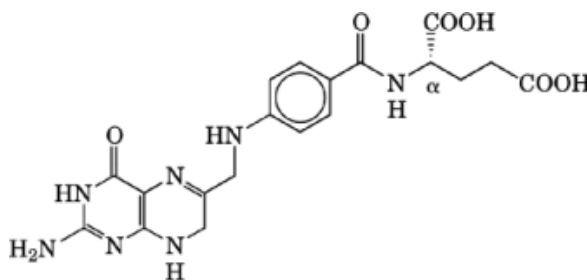
Ranking	Description	Total folate, %
1	orange juice	9.7
2	white bread, rolls, crackers	8.6
3	pinto, navy, other dried beans	7.1
4	green salad	6.8
5	cold cereals	5.0
6	eggs	4.6
9	liver	3.1
23	hamburger	1.2
25	spinach	1.0
30	green beans	0.8
34	broccoli	0.7

<sup>a</sup>Ref. 9.

when compared to the tri- or heptaconjugates. The factors affecting the bioavailability of food folates are not well understood, but seem to include iron and vitamin C (qv) status. Deficiencies of both of these nutrients in humans is associated with impaired utilization of dietary folate. Improved research techniques such as use of radiolabeled folates has provided a powerful tool for determining bioavailability (18). Under fasting conditions, folic acid is almost completely absorbed, whereas only 50–80% of folyl polyglutamate is absorbed, as determined by urinary excretion measurements (19).

## 2. Chemical and Physical Properties

L-Folic acid **1** contains three subunits: 6-methylpterin, *p*-aminobenzoic acid, and L-glutamic acid. The *Chemical Abstracts* name is *N*-[4-[(2-amino-1,4-dihydro-4-oxo-6-pteridiny)methyl]amino]benzoyl]-L-glutamic acid.



(2)

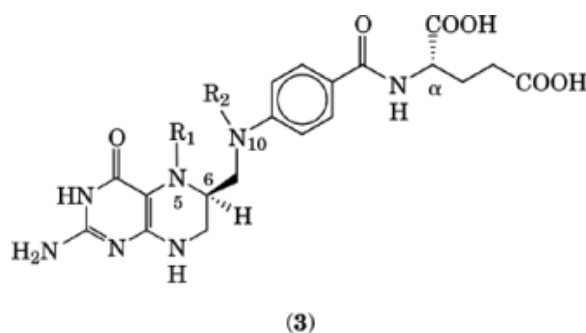
Enzymatic reduction of folic acid leads to the 7,8-dihydrofolic acid ( $H_2$  folate) **2**, a key substance in biosynthesis. Further reduction, catalyzed by the enzyme dihydrofolic acid reductase, provides (6*S*)-5,6,7,8-tetrahydrofolic acid ( $H_4$  folate) **3**. The  $H_4$  folate **3** is the key biological intermediate for the formation of other folates (**4–8**) (Table 2).

**Table 2. H<sub>4</sub> Folate Cofactors<sup>a, b</sup>**

Structure	R <sub>1</sub>	R <sub>2</sub>	Nomenclature	Configuration
<b>3</b>	H	H	tetrahydrofolic acid	6 <i>S</i> , α( <i>S</i> )
<b>4</b>	CH <sub>3</sub>	H	5-methyltetrahydrofolic acid	6 <i>S</i> , α( <i>S</i> )
<b>5</b>	CH <sub>2</sub>		5,10-methylenetetrahydrofolic acid	6 <i>R</i> , α( <i>S</i> )
<b>6</b>	HC=O	H	5-formyltetrahydrofolic acid	6 <i>S</i> , α( <i>S</i> )
<b>7</b>	H	HC=O	10-formyltetrahydrofolic acid	6 <i>R</i> , α( <i>S</i> )
<b>8</b>	HC=NH	H	5-formiminotetrahydrofolic acid	6 <i>S</i> , α( <i>S</i> )
<b>9</b>	CH <sup>+</sup>		5,10-methenyltetrahydrofolic acid	6 <i>R</i> , α( <i>S</i> )

<sup>a</sup>Nomenclature and symbols for folic acid assigned based on recommendation published by IUPAC-IUB joint commission on Biochemical Nomenclature 1986 (20, 21).

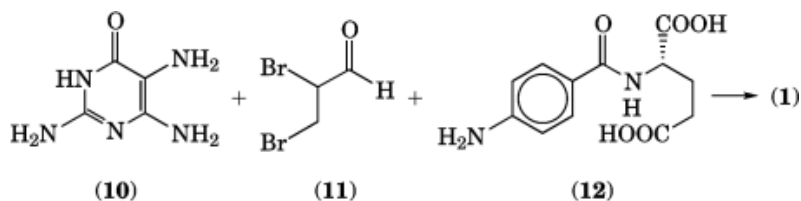
<sup>b</sup>See structure **3**.



Folic acid **1** is found as yellow, thin platelets which char above 250°C. The uv spectrum of L-folic acid at pH 13 shows absorptions at  $\lambda = 256$  nm ( $\epsilon = 30,000$ ), 282 nm ( $\epsilon = 26,000$ ), and 365 nm ( $\epsilon = 9800$ ). Folic acid has a specific rotation of  $[\alpha]_D^{27} = +19.9^\circ$  ( $c = 1$ , 0.1 *N* NaOH). Solutions of folic acid are stable at room temperature and in the absence of light. It is slightly soluble in aqueous alkali hydroxides and carbonates but is insoluble in cold water, acetone, and chloroform. Table 3 lists some physical properties of selected folic acid derivatives.

### 3. Synthesis

The first L-folic acid synthesis was based on the concept of a three-component, one-pot reaction (7, 22). Triamino-4(3*H*)-pyrimidinone [1004-45-7] **10** was reacted simultaneously with C<sub>3</sub>-dibromo aldehyde [5221-17-0] **11** and *p*-aminobenzoyl-L-glutamic acid [4271-30-1] **12** to yield folic acid **1**.



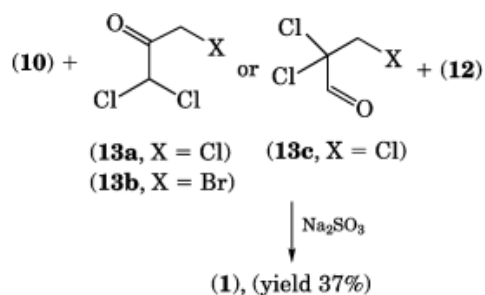
All known commercial syntheses are based on this approach with improvements in preparations of the three components (23). Shortly after the first synthesis, similar methods were published employing other

## 4 FOLIC ACID

**Table 3. Physical Properties of Folic Acid Derivatives**

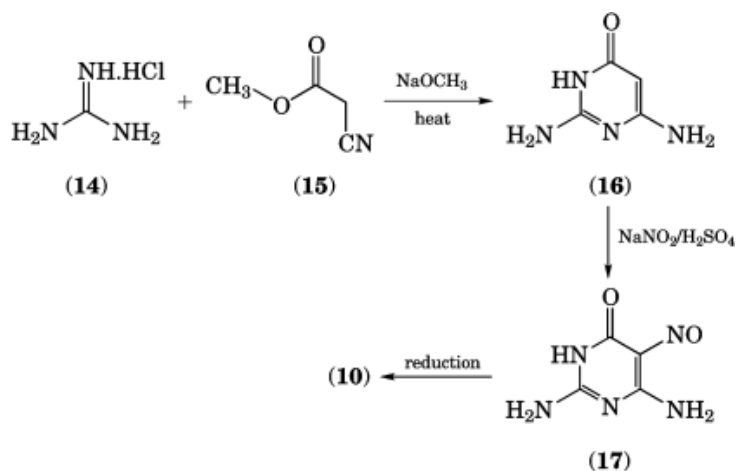
Compound	Structure number	CAS Registry Number	uv $\lambda_{\max}$ , nm	$\epsilon$	Stability	Molecular formula (mol wt)
folic acid pteroylglutamic acid PteGlu	<b>1</b>	[59-30-3]	(pH 13) 256	30,000	unstable in alkaline and acidic solutions	C <sub>19</sub> H <sub>19</sub> N <sub>7</sub> O <sub>6</sub> (441.41)
dihydrofolic acid H <sub>2</sub> folate	<b>2</b>	[4033-27-6]	(pH 7.2) 282	28,600	highly air-sensitive	C <sub>19</sub> H <sub>21</sub> N <sub>7</sub> O <sub>6</sub> (443.42)
tetrahydrofolic acid H <sub>4</sub> folate	<b>3</b>	[135-16-0]	(pH 7.8) 296	28,000	sensitive to oxygen	C <sub>19</sub> H <sub>23</sub> N <sub>7</sub> O <sub>6</sub> (445.44)
5-methyltetrahydrofolic acid 5-CH <sub>3</sub> -H <sub>4</sub> folate	<b>4</b>	[134-35-0]	(pH 7) 290	32,000	stable to oxygen	C <sub>20</sub> H <sub>25</sub> N <sub>7</sub> O <sub>6</sub> (459.46)
5,10-methylenetetrahydrofolic acid	<b>5</b>	[3432-99-3]	(pH 7.2) 294	32,000	sensitive to hydrolysis	C <sub>20</sub> H <sub>23</sub> N <sub>7</sub> O <sub>6</sub> (457.45)
5,10-CH <sub>2</sub> -H <sub>4</sub> folate	<b>6</b>	[2800-34-2]	(pH 7.5) 260	17,000	quite unstable	C <sub>20</sub> H <sub>23</sub> N <sub>7</sub> O <sub>7</sub> (473.45)
10-formyltetrahydrofolic acid 10-CHO-H <sub>4</sub> folate						C <sub>20</sub> H <sub>23</sub> N <sub>7</sub> O <sub>7</sub> (473.45)
5-formyltetrahydrofolic acid (6 <i>R,S</i> )-5-CHO-H <sub>4</sub> folate	<b>7</b>	[58-05-9] [68538-85-2]	(pH 13) 282	32,600	most stable	C <sub>20</sub> H <sub>23</sub> N <sub>7</sub> O <sub>7</sub> (473.45)
(6 <i>S</i> )-5-CHO-H <sub>4</sub> folate						C <sub>20</sub> H <sub>23</sub> N <sub>7</sub> O <sub>7</sub> (473.45)
5-formiminotetrahydrofolic acid	<b>8</b>	[2311-81]	(pH 7) 285	35,400	hydrolyzed in aqueous solution	C <sub>20</sub> H <sub>24</sub> N <sub>8</sub> O <sub>6</sub> (472.46)
5-NHCH-H <sub>4</sub> folate	<b>9</b>	[65981-89-7]	(pH 1) 352	23,900		C <sub>20</sub> H <sub>22</sub> N <sub>7</sub> O <sup>+</sup> <sub>6</sub> (cation)
5,10-methenyltetrahydrofolic acid 5,10-CH <sup>+</sup> -H <sub>4</sub> folate						

C<sub>3</sub>-halo compounds, such as 1,1,3-tribromo-2-propanone, 2,2,3-tribromopropanal (24), 2,2,3-trichloropropanal, and 1,1,3-trichloro-2-propanone (23).

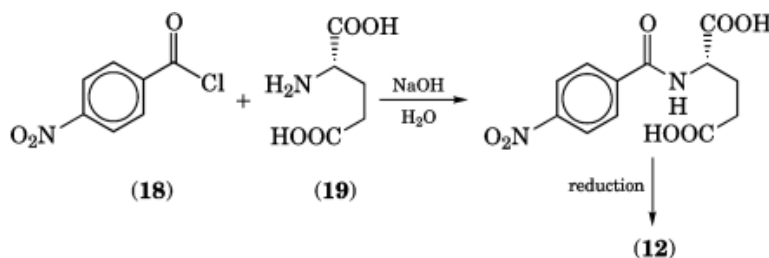


Yields were improved to  $\geq 37\%$  by the addition of sodium sulfite to the reaction mixture. Apart from the sulfite, the C<sub>3</sub>-component unit has the greatest influence on the yield of folic acid. The use of nickel(II) chloride as an additive has been claimed to give higher yields (25).

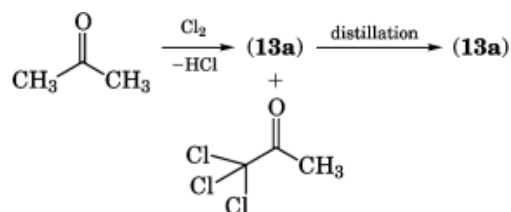
The required triamino-4(3*H*)-pyrimidinone is prepared in three steps starting from guanidine [50-01-1] **14** (26). Condensation with methylcyanoacetate [105-34-0] **15** under basic conditions, followed by nitrosation of the intermediate [56-06-4] **16**, gives 2,6-diamino-5-nitrosopyrimidinone [2387-48-6] **17**. Chemical reduction using sodium sulfite (27) or catalytic hydrogenation using Raney nickel (28) furnishes **10**.



*p*-Aminobenzoyl-L-glutamic acid **12** is obtained by condensation of *p*-nitrobenzoyl chloride [122-04-3] **18** with L-glutamic acid [56-86-0] **19** under Schotten-Baumann conditions. This is followed by reduction of the nitro group with either sodium hydrogen sulfide (29) or by electrochemical methods (30).



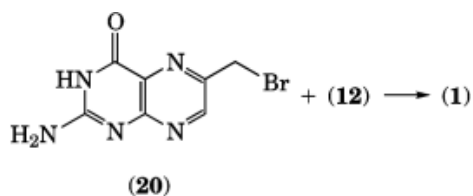
1,1,3-Trichloroacetone [921-03-9] (**13a**) is prepared by chlorination of acetone. The reaction is nonselective and the required compound is isolated by distillation. The selectivity has been improved by catalyzing the reaction with iodine (31).



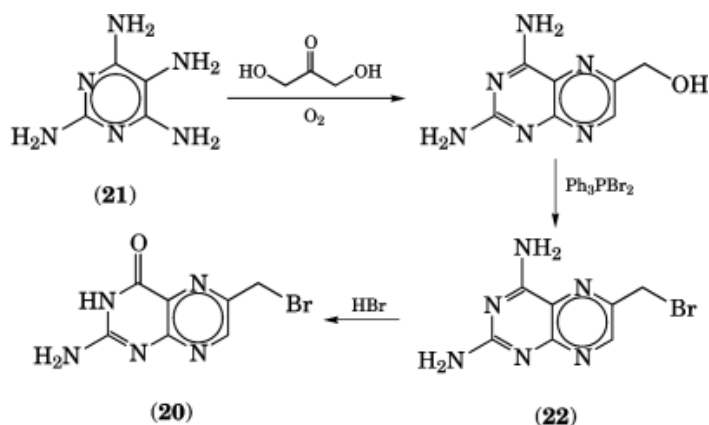
### 3.1. Alternative Approaches for Synthesis of L-Folic Acid

L-Folic acid **1** has been prepared in two steps by condensing 6-bromomethylpterin **20** with *p*-aminobenzoyl-L-glutamic acid **12** in 80% yield (32). Dissolved folic acid further reacts easily with one more equivalent of 6-bromomethylpterin to form the undesired dialkylated aminobenzoyl-L-glutamic acid.

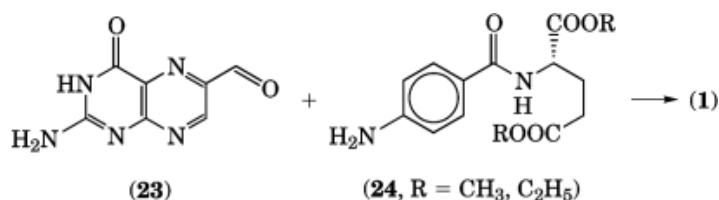
## 6 FOLIC ACID



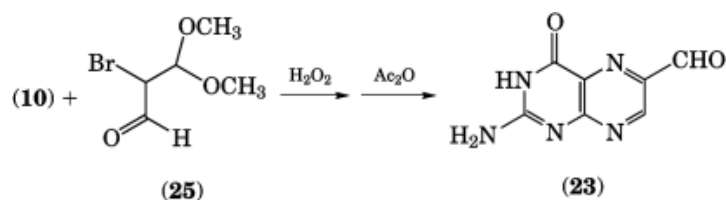
In spite of the good yields of L-folic acid obtained in this reaction, all of the published methods for the synthesis of 6-bromomethylpterin **20** are multistep procedures with low overall yields (33–36). For example, the route starting from 2,4,5,6-tetraaminopyrimidine [5392-28-9] **21** gave 6-bromomethylpterin **20** in three steps with an overall yield of only 18% (33, 35, 36). This synthesis is not economical because the intermediate 6-bromomethyl-2,4-diamino-4-pterin **22** has to be deaminated in an additional step to form 6-bromomethylpterin **20**.



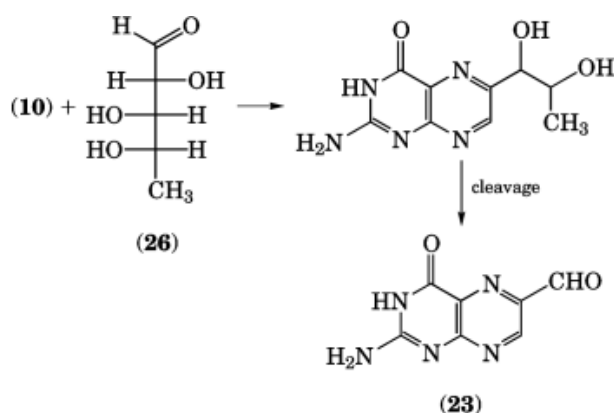
Another viable method for the synthesis of L-folic acid **1** starts from 6-formylpterin **23**. The diester of L-glutamic acid **24** is condensed with 6-formylpterin **23**. Reduction of the Schiff base with sodium borohydride is followed by hydrolysis to yield L-folic acid (37).



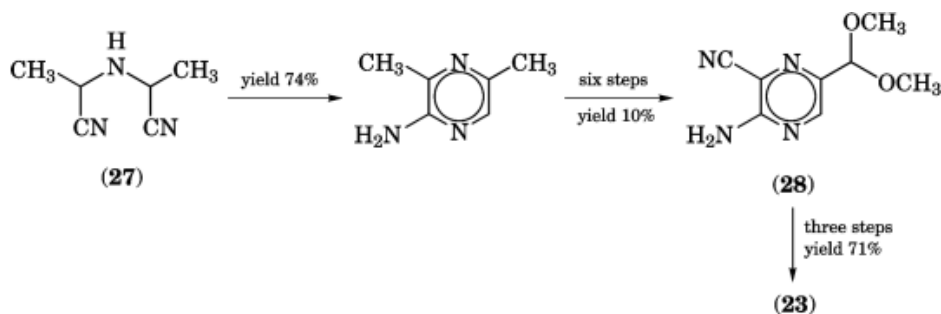
A cost-efficient synthesis of folic acid via Schiff base formation is feasible only if 6-formylpterin **23** is readily available. This compound is prepared by the reaction of 2-bromomalondialdehyde dimethylacetal [59453-00-8] **25** with triaminopyrimidinone **10**, followed by acetylation and cleavage of the acetal to give compound **23** in 51% overall yield (38).



A second approach for the synthesis of 6-formylpterin **23** involves the condensation of triaminopyrimidinone **10** with 5-deoxy-L-arabinose **26**. The key diol is obtained in four steps starting from compound **10**. Cleavage of the diol side chain is achieved either with periodate (39) or with lead(IV) (40) to furnish 6-formylpterin **23** in 45% overall yield.

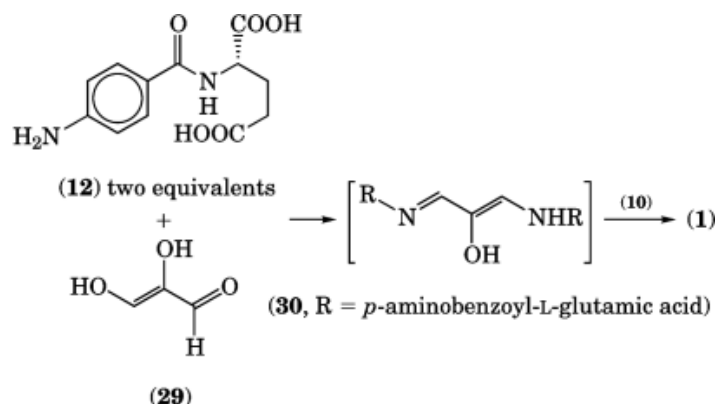


A third approach for the synthesis of 6-formylpterin **23** starts from iminodipropionitrile [2869-25-2] **27**. The intermediate pyrazine **28** is also prepared starting from chloropyruvaldehyde oxime (41, 42). The required formylpterin **23** is obtained in three steps in 71% yield, starting from the intermediate pyrazine **28**. A few other routes for the synthesis of 6-formylpterin **23** are described in the literature. All are multistep procedures with only moderate overall yields (43–45).



A new variant of the three-component, one-pot synthesis of L-folic acid has been reported by Hoffmann-La Roche Inc.

## 8 FOLIC ACID



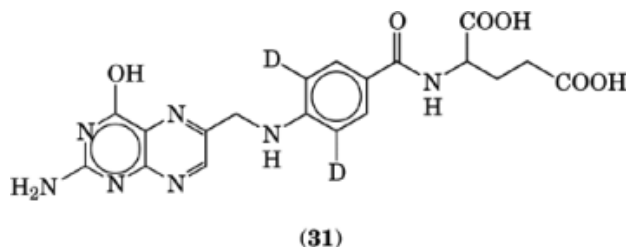
One equivalent of 2-hydroxymalondialdehyde [497-15-4] **29** is condensed with two equivalents of *p*-aminobenzoyl-L-glutamic acid **12**. The intermediate dimine **30** is treated with one equivalent of triaminopyrimidinone **10** to obtain L-folic acid in 84% yield (46).

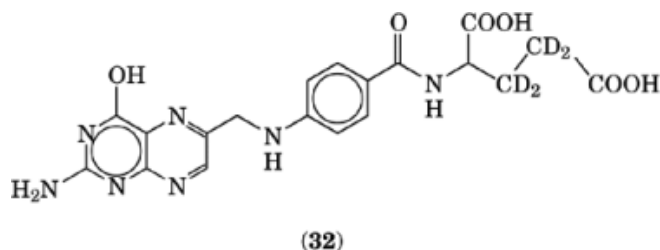
## 4. Fermentation

Development of an economically viable production process for folic acid either by genetically engineered microorganisms or by extraction from natural sources is not yet feasible.

## 5. Labeled Compounds

Radiolabeled folate provides a powerful tool for folate bioavailability studies in animals and for diagnostic procedures in humans. Deuteration at the 3- and 5-positions of the central benzene ring of folic acid **31** was accomplished by catalytic debromination (47, 48) or acid-catalyzed exchange reaction (49). Alternatively, deuterium-labeled folic acid **32** was prepared by condensing pteronic acid with commercially available labeled glutamic acid (50).





## 6. Derivatives and Analogues

The metabolically active  $H_4$  folate cofactors (see Table 2) are prepared synthetically as follows. 7,8-Dihydrofolic acid **2** and 5,6,7,8-tetrahydrofolic acid **3** are prepared via catalytic hydrogenation of folic acid under controlled reaction conditions (51, 52). Optical rotation of (6*S,R*)- $H_4$  folate **3** is  $[\alpha]_D^{27} = +14.9^\circ$  (0.1 *N* NaOH) and the natural (6*S*)- $H_4$  folate **3** is  $[\alpha]_D^{27} = -16.9^\circ$  (0.1 *N* NaOH).

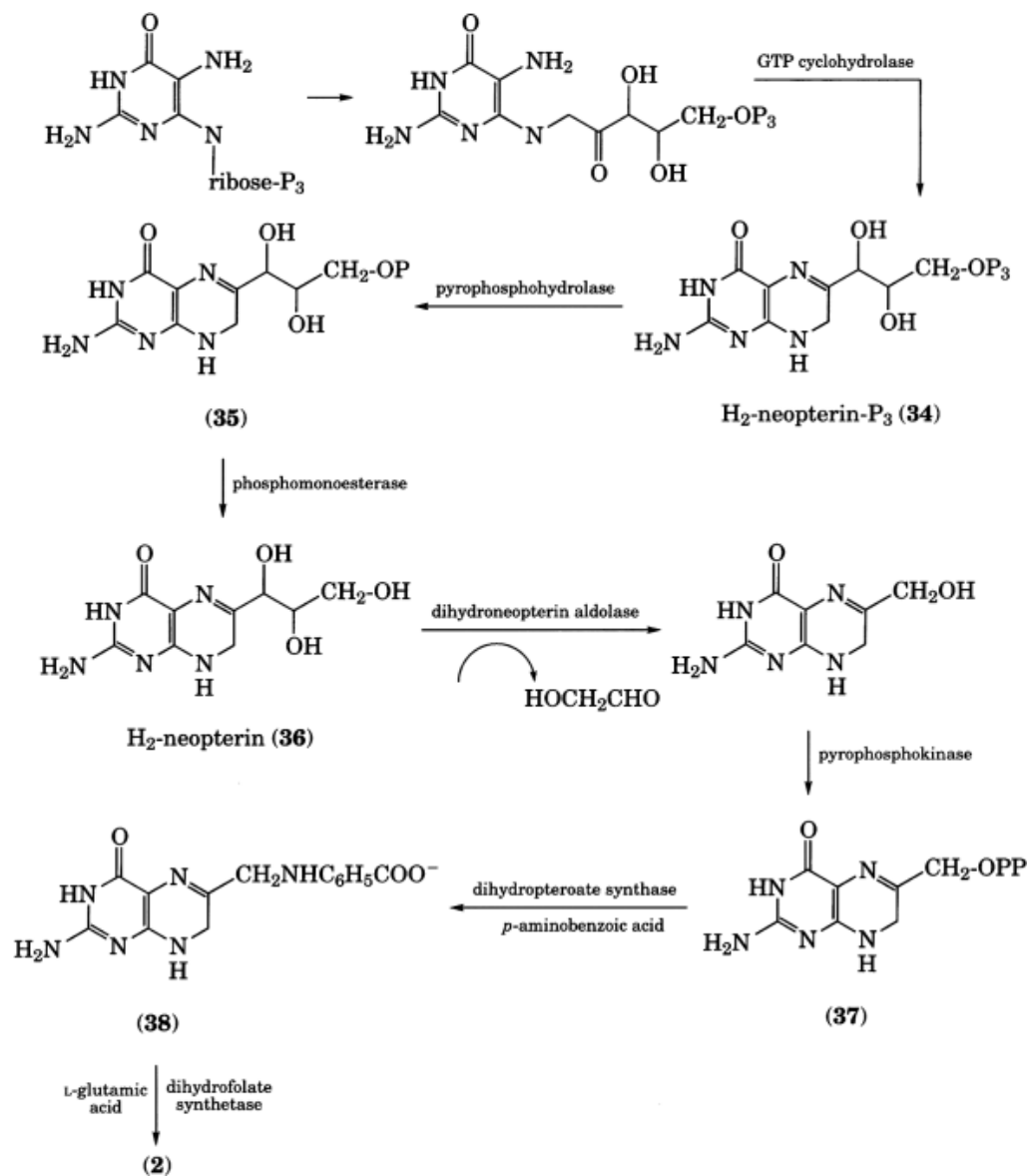
5-Methyltetrahydrofolic acid (5- $CH_3$ - $H_4$  folate) **4** is involved in methionine biosynthesis. Condensation of formaldehyde with  $H_4$  folate **3**, followed by the reduction of the intermediate 5,10- $CH_2$ - $H_4$  folate **5** with sodium borohydride gave 5- $CH_3$ - $H_4$  folate **4** (53). 5,10-Methylenetetrahydrofolic acid (5,10- $CH_2$ - $H_4$  folate) **5** is a coenzyme in thymidylate biosynthesis; the natural (6*R*)-stereoisomer is prepared by enzymatic reduction of  $H_2$  folate **2**, followed by condensation with formaldehyde (54).

Formylation of  $H_4$  folate **3** or hydrolysis of 5, 10 -  $CH^+$  -  $H_4$  folate **9** gives (6*R,S*)-5-formyltetrahydrofolic acid **6** (5-HCO- $H_4$  folate) (55). On the other hand, (6*S*)-5-HCO- $H_4$  folate is obtained by selective crystallization in the form of its calcium salt from the diastereomeric mixture of (6*S,R*)-5-HCO- $H_4$  folate (56). 10-Formyltetrahydrofolic acid **7** is a coenzyme in purine synthesis which is synthesized by hydrolysis of 5, 10 -  $CH^+$  -  $H_4$  folate **9** or by hydrogenation of 10-CHO-folate (57).

Folic acid analogues containing amino acids other than glutamate, and also folate covalently bound to a protein for the purposes of antibody production, have been prepared (58–60). Methotrexate is an analogue of folic acid that is widely used in cancer chemotherapy (61) (see CHEMOTHERAPEUTICS, ANTICANCER). Other analogues such as trimethoprim and pyrimethamine are used in the treatment of malaria and protozoal diseases (62). These analogues bind extremely tightly to dihydrofolate reductase.

## 7. Biosynthesis

Folic acid is synthesized both in microorganisms and in plants. Guanosine-5-triphosphate (GTP) **33**, *p*-aminobenzoic acid (PABA), and L-glutamic acid are the precursors. Reviews are available for details (63, 64). The sequence of reactions responsible for the enzymatic conversion of GTP to 7,8-dihydrofolic acid **2** is shown.



In *E. coli*, GTP cyclohydrolase catalyzes the conversion of GTP **33** into 7,8-dihydroneopterin triphosphate **34** via a three-step sequence. Hydrolysis of the triphosphate group of **34** is achieved by a nonspecific pyrophosphatase to afford dihydroneopterin **35** (65). The free alcohol **36** is obtained by the removal of residual phosphate by an unknown phosphomonoesterase. The dihydroneopterin undergoes a retro-aldol reaction with the elimination of a hydroxy acetaldehyde moiety. Addition of a pyrophosphate group affords hydroxymethyl-7,8-dihydropterin pyrophosphate **37**. Dihydropteroate synthase catalyzes the condensation of hydroxymethyl-7,8-dihydropteroate pyrophosphate with PABA to furnish 7,8-dihydropteroate **38**. Finally, L-glutamic acid is condensed with 7,8-dihydropteroate in the presence of dihydrofolate synthetase.

## 8. Analytical Methods

Analysis of folic acid is difficult because most natural folates exist in the polyglutamate form and there is variation in the oxidation state of the single-carbon substituent. Determination of the individual folate vitamers is complicated; assay simplification is achieved by determining folate in the monoglutamyl or diglutamyl forms after enzymatic deconjugation. Methods for determining folic acid in food and feed include biological, microbiological, chemical, chromatographic, and radiometric assays. The microbiological assay using *Lactobacillus casei* is the official method of the Association of Official Analytical Chemists (AOAC). Folyl polyglutamates react very slowly with this organism compared to mono- and diglutamates. As a result, it is required to hydrolyze the polyglutamate chain using  $\gamma$ -glutamylhydrolase prior to microbiological analysis (66). The monoglutamate in the food and feed extract is separated using anion-exchange column chromatography, followed by differential assay of the separated fraction by a microbiological assay.

A radioassay procedure has been developed to determine folic acid in erythrocyte and blood samples. The method is based on competitive protein binding between radiolabeled and unlabeled folate compounds for folic acid binding protein. A very sensitive, nonisotopic microtitration plate, folate-binding protein assay (FBPA) was developed to measure down to the 6 pg level of folyl monoglutamate and vitamin-active folate (67). A hplc method is useful for analysis of high potency premix samples containing folic acid, along with other water-soluble vitamins. Ion-pair reverse-phase columns using uv detection at 280 nm or post-column derivatization techniques have been employed to determine free folic acid. Details on hplc applications are available (68–70).

## 9. Deficiency

Folic acid is a precursor of several important enzyme cofactors required for the synthesis of nucleic acids (qv) and the metabolism of certain amino acids. Folic acid deficiency results in an inability to produce deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and certain proteins (qv). Megaloblastic anemia is a common symptom of folate deficiency owing to rapid red blood cell turnover and the high metabolic requirement of hematopoietic tissue. One of the clinical signs of acute folate deficiency includes a red and painful tongue. Vitamin B<sub>12</sub> and folate share a common metabolic pathway, the methionine synthase reaction. Therefore a differential diagnosis is required to measure folic acid deficiency because both folic acid and vitamin B<sub>12</sub> deficiency cause megaloblastic anemia. Serum and red blood cell levels of folate are measured to confirm and diagnose the deficiency. Serum folate levels less than 3 ng/mL are diagnostic of a deficiency. Some forms of dietary folic acid are more poorly absorbed by the elderly than by younger individuals. However, vitamin supplements containing the monoglutamate form are well absorbed in all age groups (71).

Folate antagonists (eg, methotrexate and certain antiepileptics) are used in treatment for various diseases, but their administration can lead to a functional folate deficiency. Folate utilization can be impaired by a depletion of zinc (see ZINC COMPOUNDS). In humans, the intestinal brush border folate conjugase is a zinc metalloenzyme (72). One study indicates that the substantial consumption of alcohol, when combined with an inadequate intake of folate and methionine, may increase the risk of colon cancer (73). Based on this study, it is recommended to avoid excess alcohol consumption and increase folate intake to lower the risk of colon cancer.

Incomplete closure of neural tube during the embryo development in humans can lead to spina bifida. The condition is characterized by an opening in the spinal cord and results in physical disability in a child. Incomplete closure of the skull produces anencephaly. These and similar conditions are collectively called neural tube defects (NTD). Each year in the United States approximately 2500 infants are born with spina bifida and anencephaly and an estimated 1500 fetuses affected by these birth defects are spontaneously aborted (74). It has been shown that folic acid given at 400  $\mu$ g/day prevents the recurrence of NTD and that doses of 800  $\mu$ g/day prevent both the occurrence and recurrence of NTD in the majority of cases. Published studies also indicate that folic acid supplementation taken 6 weeks before conception may reduce the risk of neural tube

**Table 4. RDA and U.S. RDA for Folic Acid<sup>a</sup>**

Group	Age	Folic acid, $\mu\text{g}$
RDA		
infants	0–0.5	25
	0.5–1.0	35
children	1–3	50
	4–6	75
	7–10	100
males	11–14	150
	>15 – 51	200
females	11–14	150
	15–51	180
	pregnant, lactating	400
	1st six months	280
	2nd six months	260
U.S. RDA		
infants <13 months		100
children <4 yr		200
adults and children >4 yr		400
pregnant or lactating women		800

<sup>a</sup>Ref. 21.

defects by at least 50% (74, 75). Folic acid may be more effective in reducing neural tube defect incidence than conjugated food folate because free folic acid is more readily absorbed (76). A cost-benefit analysis, based on the U.S. population, of preventable neural tube defects indicates that folic acid fortification of grains in the United States may yield a substantial economic benefit (77). The U.S. Food and Drug Administration (FDA) has amended the standards of identity for several enriched grain products. The agency is requiring that these products be fortified with folic acid at levels ranging from 0.95 to 3.09 mg per kg of product (78). This is the first B-vitamin fortification requirement since 1943 when the U.S. government mandated fortification of flour with niacin, thiamin, and riboflavin. Incidence of cleft lip/cleft palate has been reported in animal studies due to folic acid deficiency. Poor folic acid status has been associated with megaloblastic changes in the cells of the uterine, cervix (79), and intestinal epithelium (80).

Homocysteine arises from dietary methionine. High levels of homocysteine (hyperhomocysteinemia) are a risk factor for occlusive vascular diseases including atherosclerosis and thrombosis (81–84). In a controlled study, serum folate concentrations of  $\leq 9.2$  nmol/L were linked with elevated levels of plasma homocysteine. Elevated homocysteine levels have been associated also with ischemic stroke (9). The mechanism by which high levels of homocysteine produce vascular damage are, as of yet, not completely understood. Interaction of homocysteine with platelets or endothelial cells has been proposed as a possible mechanism. Clinically, homocysteine levels can be lowered by administration of vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and folic acid.

## 10. Requirements

The amount of folic acid required for daily intake is estimated based on the minimum amount required to maintain a certain level of serum folate. The recommended dietary allowance (RDA) for folic acid accounts for daily losses and makes allowances for variation in individual needs and bioavailability from food sources (85). The U.S. recommended daily allowance for adults is 400  $\mu\text{g}$  and for pregnant women is 800  $\mu\text{g}$  (Table 4).

## 11. Animal Nutrition

To obtain optimal performance of farm animals, folic acid supplementation is required (86) and as is the case with most of the vitamins, the majority of worldwide consumption is as feed supplements. The folic acid requirement for chickens and pigs is about 0.2–0.5 mg of folic acid/kg diet and 0.3 mg/kg diet, respectively. Increased amounts, 0.5–1.0 mg/kg feed for chickens and 0.5–2.0 mg/kg for swine, are recommended under commercial production conditions (87). The degree of intestinal folic acid synthesis and the utilization by the animal dictates the folic acid requirements for monogastric species. Also, the self-synthesis of folacin is dependent on dietary composition (88).

Folacin requirements are related to the type and level of production. The more rapid the growth or production rates, the greater the need for folacin owing to its role in DNA synthesis. In poultry, the requirement for egg hatchability is higher than for production (88). In swine, folic acid supplementation has been shown to increase fertility and growth rates (89).

## 12. Metabolism

The principal function of the folate coenzyme is to carry one-carbon units. Dietary folylpolyglutamate is hydrolyzed to the monoglutamate form by folyl polyglutamate hydrolyases (conjugases) prior to transport across the intestinal mucosa. Intestinal folate absorption occurs in the jejunum. A slightly acidic pH of 6 is optimum for intestinal absorption and transportation into the blood stream (90). It was shown by the competitive inhibition method (91) that a single protein seems to be responsible for transportation of the monoglutamate. Transportation may occur by an anion-exchange mechanism (92). Folate transportation may be partially dependent on sodium ions in the cell medium (93).

Three forms of folate appear to be transported in the blood: folic acid, folate loosely bound to low affinity binder serum proteins (such as albumin,  $\alpha$ -macroglobulin, and transferrin), and folate bound to high affinity protein binders. Approximately 5% of total serum folate is being transported by high affinity protein binders but the function of these proteins is not well understood (94, 95). Folic acid is stored in folyl polyglutamate form. The liver and other tissues (mitochondria) convert folic acid and methyltetrahydrofolate into folylpolyglutamate by employing polyglutamate synthetase. The total folate pool in adult humans is estimated to be around 5–10 mg, with half of this in the liver; folylpolyglutamate synthetase activity is highest in liver.

The metabolism of folic acid involves reduction of the pterin ring to different forms of tetrahydrofolylglutamate. The reduction is catalyzed by dihydrofolate reductase and NADPH functions as a hydrogen donor. The metabolic roles of the folate coenzymes are to serve as acceptors or donors of one-carbon units in a variety of reactions. These one-carbon units exist in different oxidation states and include methanol, formaldehyde, and formate. The resulting tetrahydrofolylglutamate is an enzyme cofactor in amino acid metabolism and in the biosynthesis of purine and pyrimidines (10, 96). The one-carbon unit is attached at either the N-5 or N-10 position. The activated one-carbon unit of 5,10-methylene- $H_4$  folate **5** is a substrate of T-synthase, an important enzyme of growing cells. 5-10-Methylene- $H_4$  folate **5** is reduced to 5-methyl- $H_4$  folate **4** and is used in methionine biosynthesis. Alternatively, it can be oxidized to 10-formyl- $H_4$  folate **7** for use in the purine biosynthetic pathway.

## 13. Toxicity

Folic acid is safe, even at levels of daily oral supplementation up to 5–10 mg (97). Gastrointestinal upset and an altered sleep pattern have been reported at 15 mg/day (98). A high intake of folic acid can mask the clinical signs of pernicious anemia which results from vitamin B<sub>12</sub> deficiency and recurrence of epilepsy in epileptics

## 14 FOLIC ACID

treated with drugs with antifolate activity (99). The acute toxicity ( $LD_{50}$ ) is approximately 500 and 600 mg per kg body weight for rats and mice, respectively (100).

### 14. Uses

L-Folic acid is available as a crystalline dihydrate containing 8% water. Approximately 80% of the commercial production is consumed for feed enrichment in animal nutrition. Folic acid is being offered by the pharmaceutical industry for therapeutic and prophylactic use (see PHARMACEUTICALS). Pharmacological doses of folic acid are commonly used as a rescue dose during cancer chemotherapy, in women using oral contraceptives, and alcoholics. Several studies have provided evidence that multivitamins or folic acid (0.8–4 mg/day) supplementation prevent the majority of neural tube defects (101).

### 15. Economic Aspects

The world production of synthetic L-folic acid 1996 was estimated at 400 metric tons per year. The total market is expected to grow with increasing recognition of need, especially during pregnancy and lactation. The principal producers of folic acid are Hoffmann-La Roche, Takeda, Sumika Fine Chemical (previously Yodogawa Pharmaceuticals), Kongo, and three Chinese companies, Changzhou Pharmaceuticals, Changshu Hugang Pharmaceuticals, and Zhejiang Jiangnan Pharmaceuticals. Smaller quantities are also produced by companies in India, China, and Russia. The 1996 sale price varied between \$50 to \$130/kg.

### 16. Conclusions

All known commercial syntheses of folic acid are based on the three-component process developed in the late 1940s. Industrial production of folic acid by genetically engineered microorganisms or extraction from natural sources is not yet economically viable. The mechanism governing folate turnover and excretion is still poorly understood. Improved research techniques will aid in the development of a better understanding of factors affecting intestinal absorption and *in vivo* kinetics in human beings. Folic acid and multivitamin supplement use are associated with a decreased occurrence of neural tube defects. Abnormalities in homocysteine metabolism are observed in many women who have given birth to children with neural tube defects and in individuals with cardiovascular disease. Folic acid is likely to be involved in overcoming this abnormality. The exact mechanism by which folic acid prevents these diseases is a current active area of research.

## BIBLIOGRAPHY

"Folic Acid," in *ECT* 2nd ed., Vol. 6, pp. 778–785, by M. E. Hultquist and T. H. Jukes.

### Cited Publications

1. L. Wills, P. W. Clutterbuck, and P. D. F. Evans, *Biochem. J.* **31**, 2136 (1937).
2. A. G. Hogan and E. M. Parrott, *J. Biol. Chem.* **132**, 507 (1940).
3. E. E. Snell and W. H. Peterson, *J. Bacteriol.* **39**, 273 (1940).
4. H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Am. Chem. Soc.* **63**, 2284 (1941).
5. S. B. Binkley, O. D. Bird, E. S. Bloom, R. A. Brown, D. G. Calkins, C. J. Campbell, A. D. Emmett, and J. J. Pfiffner, *Science*, **100**, 36 (1944).
6. J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and L. B. O'Dell, *J. Am. Chem. Soc.* **68**, 1392 (1946).

7. R. B. Angier and co-workers, *Science*, **103**, 667 (1946).
8. A. D. Welch, *Perspect. Biol. Med.* **27**, 64 (1983).
9. W. H. Giles, S. J. Kittner, R. F. Anda, J. B. Croft, and M. L. Casper, *Stroke*, **26**, 1166 (1995).
10. W. Friedrich, *Handbuch der Vitamine*, Urban & Schwarzenberg, Baltimore, Md., 1987, p. 398.
11. B. Botticher and R. Kluthe, in K. Pietrzik, ed., *Folsäure-Mangel*, W. Zuckschwerdt Verlag, Germany, 1987, p. 15.
12. T. Brody, in L. J. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker Inc., New York, 1991, p. 453.
13. L. B. Bailey, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., New York, 1995, p. 123.
14. K. H. Bässler, E. Grün, D. Loew, and K. Pietrzik, *Vitamin-Lexikon*, G. Fischer Verlag, New York, 1992, p. 127.
15. J. Leichter, A. F. Landymore, and C. L. Krumdieck, *Am. J. Clin. Nutr.* **32**, 92 (1979).
16. S. P. Rothenberg, M. P. Iqbal, and M. Da Costa, *Anal. Biochem.* **103**, 152 (1980).
17. J. F. Gregory, *Adv. Food Nutr. Res.* **33**, 1 (1989).
18. J. F. Gregory, *Food Technol.* **42**, 230 (1988).
19. H. E. Sauberlich, M. J. Kretsch, J. H. Skala, H. L. Johnson, and P. C. Taylor, *Am. J. Clin. Nutr.* **46**, 1016 (1987); J. F. Gregory, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., 1995, p. 195.
20. IUPAC-IUB Joint commission on biochemical nomenclature (JCBN), *Eur. J. Biochem.* **168**, 251 (1987); *Pure Appl. Chem.* **59**, 834 (1987).
21. D. Bhatia, ed., in D. Bhatia, ed., *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, p. 2770.
22. C. W. Waller and co-workers, *J. Am. Chem. Soc.* **70**, 19 (1948).
23. F. Weygand and V. Schmied-Kowarzik, *Chem. Ber.* **82**, 333 (1949).
24. S. Uyeo, S. Mizukami, T. Kubota, and S. Takagi, *J. Am. Chem. Soc.* **72**, 5339 (1950).
25. Rus. Pat. 7405995 (1974), K. Ito, H. Fukushima, and K. Nakagawa (to Nisshin Flour Milling Co., Ltd.).
26. W. Traube, *Berliner Berichte* **33**, 1371 (1900).
27. Ger. Pat. 3403468 A1 (Aug. 8, 1985), H. Blum and G. Dreesmann (to Federal Republic of Germany).
28. Eur. Pat. 444266 A1 (Sept. 4, 1991), A. Hunds and W. Rogler (to Huls AG).
29. Rus. Pat. 59204158 (1974) (to Daicel Chem Ind., KK).
30. Ger. Pat. 3419817 A1 (Dec. 6, 1984), K. Yoshida, T. Niinobe, and T. Baba (to Takeda Chemical Industries Ltd.).
31. Ger. Pat. 3605484A (Aug. 8, 1987), W. Deinhammer and H. Petersen (to Walker-Chemie GmbH); Eur. Pat. 394968 A (Oct. 31, 1990), B. D. Dombek and T. T. Wenzel (to Union Carbide Chemical).
32. J. R. Piper, G. S. McCaleb, and J. A. Montgomery, *J. Heterocycl. Chem.* **24**, 279 (1987).
33. C. M. Baugh and E. Shaw, *J. Org. Chem.* **29**, 3610 (1964).
34. Ger. Pat. 2741383 (Feb. 22, 1979), E. Catalucci (to Lonza AG).
35. J. R. Piper and J. A. Montgomery, *J. Org. Chem.* **42**, 208 (1977).
36. J. R. Piper and J. A. Montgomery, *J. Heterocycl. Chem.* **11**, 279 (1974); U.S. Pat. 4,077,957A (Mar. 7, 1978), J. A. Montgomery and J. R. Piper (to U.S. Sec. Dept. Health); U.S. Pat. 4,079,056 (Mar. 13, 1978), J. A. Montgomery and J. R. Piper (to U.S. Sec. Dept. Health).
37. J. H. Bieri and M. Viscontini, *Helv. Chim. Acta* **56**, 2905 (1973); K. Khalifa, P. K. Sengupta, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta* **59**, 242 (1976).
38. M. Slettinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *J. Am. Chem. Soc.* **77**, 6365 (1955).
39. M. Viscontini and J. H. Bieri, *Helv. Chim. Acta* **54**, 2291 (1971).
40. Swiss Pat. 255409 (1949) (to Hoffmann-La Roche Inc.).
41. Ger. Pat. 3242193 A1 (May 17, 1984), F. Brunnmueller and M. Kroener (to BASF AG).
42. E. C. Taylor and T. Kobayashi, *J. Org. Chem.* **38**, 2817 (1973); E. C. Taylor, R. N. Henrie, and R. C. Portnoy, *J. Org. Chem.* **43**, 736 (1978).
43. B. Schirks, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta* **68**, 1639 (1985).
44. E. C. Taylor and K. Lenard, *Liebigs. Ann. Chem.* **726**, 100 (1969); E. C. Taylor and D. J. Dumas, *J. Org. Chem.* **46**, 1394 (1981).
45. Ger. Pat. 3242195 A1 (May 17, 1984), F. Brunnmueller and M. Kroener (to BASF AG); Eur. Pat. 175263 A2 (Mar. 26, 1986), H. Leninger, W. Littmann, J. Paust, and W. Trautmann (to BASF AG); Eur. Pat. 175264 A2 (Mar. 26, 1986), H. Leninger, W. Littmann, and J. Paust (to BASF AG).
46. Eur. Pat. Appl. 608 693 A2 (Aug. 3, 1994), C. Wehrli (to Hoffmann-La Roche AG).
47. J. F. Gregory and J. P. Toth, *J. Labelled Comp. Radiopharm.* **25**, 1349 (1988).

48. J. F. Gregory, *J. Agric. Food Chem.* **38**, 1073 (1990).
49. D. L. Hachey, L. Palladino, J. A. Blair, I. H. Rosenberg, and P. D. Klein, *J. Labelled Comp. Radiopharm.* **14**, 479 (1978).
50. J. F. Gregory and J. P. Toth, *Anal. Biochem.* **170**, 94 (1988).
51. P. A. Charlton, D. W. Young, B. Birdsall, J. Feeney, and G. C. K. Roberts, *J. Chem. Soc. Perkin Trans. I*, 1349 (1985).
52. C. M. Tatum, M. G. Fernald, and J. P. Schimel, *Anal. Biochem.* **103**, 255 (1980).
53. J. A. Blair and K. J. Saunders, *Anal. Biochem.* **34**, 376 (1970).
54. C. Zarow, A. M. Pellino, and P. V. Danenberg, *Prep. Biochem.* **12**, 381 (1983).
55. E. Khalifa, A. N. Ganguly, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta*, **63**, 2554 (1980).
56. D. B. Cosulich, J. M. Smith, and H. P. Broquist, *J. Am. Chem. Soc.* **74**, 4215 (1952).
57. J. E. Baggott and C. L. Krumdieck, *Biochemistry*, **18**, 1036 (1979).
58. C. M. Baugh, J. Stevens, and C. Krumdieck, *Biochim. Biophys. Acta*, **212**, 116 (1970).
59. B. L. Hutchings and co-workers, *J. Biol. Chem.* **170**, 323 (1947).
60. J. D. Cook, D. J. Cichowicz, S. George, A. Lawler, and B. Shane, *Biochemistry*, **26**, 530 (1987).
61. M. C. Li, R. Hertz, and D. M. Bergenstal, *New Engl. J. Med.* **259**, 66 (1958).
62. J. Burchanall and G. Hitchings, *Mol. Pharmacol.* **1**, 216 (1965).
63. G. M. Brown and H. Williamson, *Adv. Enzymol. Relat. Areas Mol. Biol.* **53**, 345 (1982).
64. G. M. Brown and H. Williamson, in F. C. Neidhart, ed., *Escherichia Coli and Salmonella Typhmuri*, Vol. 1, American Society for Microbiology, Washington, D.C., 1987, p. 521.
65. Y. Suzuki and G. M. Brown, *J. Biol. Chem.* **249**, 2405 (1974).
66. J. Kas and J. Cerna, *Methods Enzymol.* **66E**, 443 (1980).
67. P. M. Finglas, R. M. Faulks, and M. R. A. Morgan, *J. Micronutrient Anal.* **4**, 295 (1988).
68. C. L. Krumdieck, T. Tamura, and I. Eto, *Vitamins Horm.* **40**, 45 (1983).
69. G. Brubacher, W. Müller-Mulot, and D. A. T. Southgate, *Methods for Determination of Vitamins in Food*, Elsevier Applied Science Publisher, New York, 1985, p. 158.
70. D. B. McCormick and L. D. Wright, ed., *Methods Enzymol.* **66E**, 429 (1980).
71. H. Baker, S. P. Jaslow, and O. Frank, *J. Am. Geriatr. Soc.* **26**, 218 (1978).
72. S. A. Anderson and J. M. Talbot, *FDA Technical Report* FDA/RF-82/13, Washington, D.C., 1981.
73. E. Giovannucci, E. B. Rimm, A. Ascherio, M. J. Stampfer, G. A. Colditz, and W. C. Willett, *J. Natl. Cancer Inst.* **87**, 265 (1995).
74. *MMWR Morb Mortal Wkly Rep.* **44**, 716 (1995).
75. A. Milunsky, J. Herschel, S. S. Jick, C. L. Bruell, D. S. Maclaughlin, K. J. Rothman, and W. Willett, *J. Am. Med. Assoc.* **262**, 2847 (1989).
76. C. Bower and J. F. Stanley, *Med. J. Austral.* **150**, 613 (1989).
77. P. S. Romano, N. J. Waitzman, R. M. Scheffler, and R. D. Pi, *Am. J. Public Health*, **85**, 667 (1995).
78. B. F. Satchell, *FDA Technical Report*, FDA/61RF-8781, Washington, D.C., 1996.
79. N. Whitehead, F. Reyner, and J. Lindenbaum, *J. Am. Med. Assoc.* **226**, 1421 (1973).
80. A. Bianchi, D. W. Chipman, A. Dreskin, and N. S. Rosensweig, *New Engl. J. Med.* **282**, 859 (1970).
81. K. S. McCully, *Am. J. Pathol.* **56**, 111 (1969).
82. C. S. Berwanger, J. Y. Jeremy, and G. Stansby, *Br. J. Surg.* **82**, 726 (1995).
83. J. Selhub, P. F. Jacques, A. G. Bostom, R. B. D'Agostino, P. W. Wilson, A. J. Belanger, D. H. O'Leary, P. A. Wolf, E. J. Schaefer, and I. H. Rosenberg, *N. Engl. J. Med.* **332**, 286 (1995).
84. J. L. Mills, J. M. McPartlin, P. N. Kirke, Y. J. Lee, M. R. Conley, D. G. Weir, and J. M. Scott, *Lancet*, **345**, 149 (1995).
85. A. F. Subar, G. Block, and L. D. James, *Am. J. Clin. Nutr.* **50**, 508 (1989).
86. *Nutrient Requirements of Poultry*, 9th. ed., National Research Council, National Academy Press, Washington, D.C., 1994; *Nutrient Requirements of Swine*, 9th ed., National Research Council, National Academy Press, Washington, D.C., 1988.
87. L. R. McDowell, *Vitamins in Animal Nutrition; Comparative Aspects to Human Nutrition*, Academic Press, Inc., San Diego, CA, 1989, 298–325.
88. *Vitamin Nutrition for Poultry*, Dept. of Animal Health and Nutrition, Hoffmann-La Roche, Inc., Nutley, N.J., 1991.
89. *Vitamin Nutrition for Swine*, Dept. of Animal Health and Nutrition, Hoffmann-La Roche, Inc., Nutley, N.J., 1991.
90. J. Zimmerman, Z. Gihula, J. Selhub, and I. H. Rosenberg, *Int. J. Vit. Nutr. Res.* **59**, 151 (1989).
91. J. Zimmerman, J. Selhub, and I. H. Rosenberg, *Am. J. Clin. Nutr.* **46**, 518 (1987).

92. C. H. Young, F. M. Sirtnak, and M. Dembo, *J. Membrane Biol.* **79**, 285 (1984).
93. D. W. Horne, W. Y. Briggs, and C. Wagner, *J. Biol. Chem.* **253**, 3529 (1978).
94. G. B. Henderson, *Ann. Rev. Nutr.* **10**, 319 (1990).
95. C. Wagner, *Ann. Rev. Nutr.* **2**, 229 (1982).
96. C. Wagner, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker, Inc., New York, 1995, p. 23.
97. C. E. Butterworth and T. Tamura, *Am. J. Clin. Nutr.* **50**, 353 (1989).
98. R. Hunter, J. Barnes, H. F. Oakeley, and D. M. Matthews, *Lancet*, **1**, 61 (1970).
99. *New Engl. J. Med.* **237**, 713 (1947).
100. A. Hanck, in *Spektrum Vitamine*, **42**, 81 (1986).
101. A. F. Czeisal and J. Dudas, *New Engl. J. Med.* **327**, 1832 (1992); MRC Vitamin Study Research Group, *Lancet*, **338**, 131 (1991).

### General References

102. D. Bhatia, ed., in D. Bhatia, ed., *Encyclopedia of Food Science and Technology*, John Wiley & Sons, Inc., New York, 1991, p. 2770.
103. J. F. Gregory, in L. B. Bailey, ed., *Folate in Health and Disease*, Marcel Dekker Inc., New York, 1995, p. 195.
104. T. Brody, in L. J. Machlin, ed., *Handbook of Vitamins*, Marcel Dekker Inc., New York, 1991, p. 453.
105. O. Isler and G. Brubacher, in O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, eds., *Vitamine II*, Georg Thieme Verlag, New York, 1982, p. 264.
106. S. K. Gaby and A. Bendich, in S. K. Gaby, A. Bendich, V. N. Singh, and L. J. Machlin, eds., *Vitamin Intake and Health*, Marcel Dekker Inc., New York, 1991, p. 175.

THIMMA R. RAWALPALLY  
Hoffmann-La Roche Inc.

### Related Articles

Vitamins, Survey; Ascorbic Acid; Biotin; Niacin, Nicotinamide, and Nicotinic Acid; Pantothenic Acid; Pyridoxine (B6); Riboflavin (B2); Thiamine (B1); Vitamin A; Vitamin B12