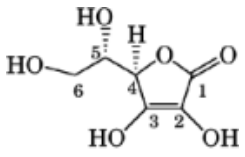
Kirk-Othmer Encyclopedia of Chemical Technology.

ASCORBIC ACID

Ascorbic Acid [50-81-1] (**1**) is the name recognized by the IUPAC-IUB Commission on Biochemical Nomenclature for Vitamin C (1). Other names are: L-ascorbic acid, L-xyloascorbic acid, and L-*threo*-hex-2-enoic acid γ-lactone. The name



(**1**) L-Ascorbic acid

implies the vitamin's antiscorbutic properties, *e.g.*, the prevention and treatment of scurvy. L-Ascorbic acid is widely distributed in plants and animals. The pure vitamin, $C_6H_8O_6$, mol wt 176.13, is a water-soluble, strongly reducing, optically active (chiral) colorless crystalline substance.

L-Ascorbic acid biosynthesis in plants and animals as well as the chemical synthesis starts from D-glucose. The vitamin and its main derivatives, sodium ascorbate, calcium ascorbate, and ascorbyl palmitate, are officially recognized by regulatory agencies and included in compendia such as the *United States Pharmacopeia/National Formulary* (*USP/NF*) and the *Food Chemicals Codex* (*FCC*).

The most significant chemical characteristic of L-ascorbic acid (**1**) is its oxidation to dehydro-L-ascorbic acid (L-*threo*-2,3-hexodiulosonic acid γ-lactone) 1 (Fig. 1). Vitamin C is a redox system containing at least three substances: L-ascorbic acid, monodehydro-L-ascorbic acid, and dehydro-L-ascorbic acid. Dehydro-L-ascorbic acid and the intermediate product of the oxidation, the monodehydro-L-ascorbic acid free radical 1, have antiscorbutic activity equal to L-ascorbic acid.

The reversible oxidation of L-ascorbic acid to dehydro-L-ascorbic acid is the basis for its known physiological activities, stabilities, and technical applications (2). L-ascorbic acid is by far the most predominant form found in blood plasma and tissues. Dehydroascorbic acid is rapidly reduced intracellularly to L-Ascorbic acid by enzymatic and/or chemical mechanisms. The importance of vitamin C in nutrition and the maintenance of good health is well documented. Over 22,000 references relating only to L-ascorbic acid have appeared since 1966. A good review article about chemistry and biochemistry is found in Ref. (3).

L-Ascorbic acid was isolated first by Albert Szent-Györgyi in 1928. However, its early history is associated with the etiology, treatment, and prevention of scurvy (4–6). Scurvy is one of the oldest diseases known to humankind. It affected many people in ancient times in Egypt, Greece, and Rome and influenced the course of history. Outbreaks of the disease resulting from inadequate amounts of vitamin C in rations spontaneously ended many military campaigns and long ocean voyages (7). During the Middle Ages, scurvy was endemic in northern Europe, occurring mostly in the winter season when fresh fruits and vegetables were unavailable.

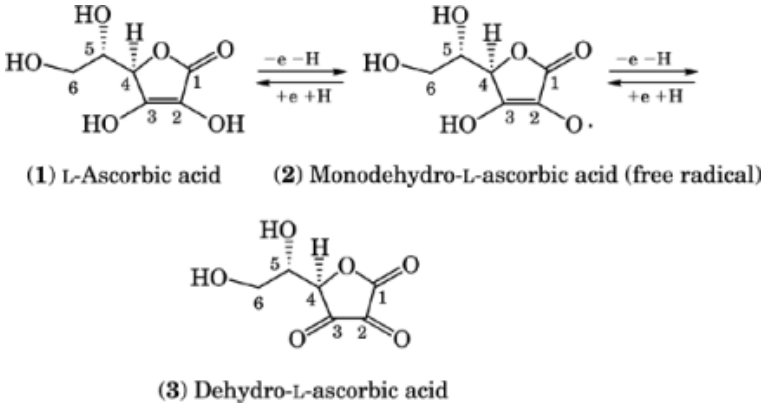
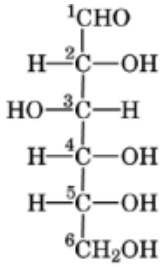


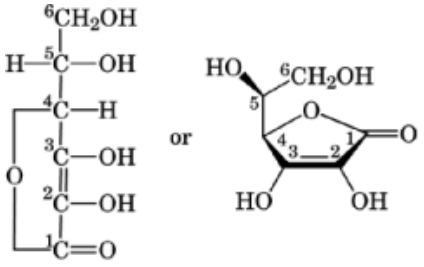
Fig. 1. Vitamin C redox system.

By the end of the seventeenth century, scurvy became a severe problem among sailors on long voyages. It was treated as a venereal disease, with disastrous results.

The first clues to the treatment of scurvy occurred in 1535–1536 when Jacques Cartier, on advice from Newfoundland Indians, fed his crew an extract from spruce tree needles to cure an epidemic. Various physicians were recommending the use of citrus fruits to cure scurvy in the mid-sixteenth century. Two hundred years later, in 1753, it was proved by James Lind, in his famous clinical experiment, that scurvy was associated with a dietary lack of fresh vegetables. He also demonstrated that oranges and lemons were the most effective cure against this disease. In 1753, in *A Treatise on the Scurvy*, Lind published his results and recommendations (8). Forty-two years later, in 1795, the British Navy included lemon juice in seamen's diets, resulting in the familiar nickname "limeys" for British seamen. Evidence has shown that even with undefined scorbutic symptoms, vitamin C levels can be low, and can cause marked diminution in resistance to infections and slow healing of wounds. Research leading to the discovery of vitamin C began in 1907 Axel Holst and Theodor Fröhlich observed that guinea pigs were as susceptible to scurvy as humans and that the disease could be produced experimentally in these animals (9). These findings led to the development of an assay for the biological determination of antiscorbutic activity of food products (10). Between 1910 and 1921, the vitamin was obtained in almost pure form from lemons and some of the physical and chemical properties were determined (11). It was discovered that vitamin C is easily destroyed by oxidation and best protected by reducing agents, and that 2,6-dichlorophenolindophenol is reduced by solutions of the vitamin. Subsequent studies showed that the dichlorophenolindophenol test measured only the vitamin and not dehydroascorbic acid, which also has antiscorbutic activity. The isolation of vitamin C was first accomplished by Szent-Györgyi in 1928 from cabbage, paprika, and the adrenal glands of animals (12). The relationship between vitamin C and the antiscorbutic factor was established in 1932 by Szent-Györgyi and, at the same time, by King and Waugh (13). Also in 1932, the chemical structure of ascorbic acid was determined independently by Haworth and Hirst (14). One year later, in 1933, Reichstein synthesized D-ascorbic acid and L-ascorbic acid (15). L-Ascorbic acid was synthesized from D-glucose (**4**) because the chiral centers of C-2 and C-3 were in the correct configuration to become C-4 and C-5, respectively, of L-ascorbic acid (16).



(**4**) D-Glucose



(**1**) L-Ascorbic acid

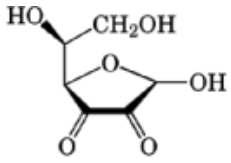
This synthesis was the first step toward industrial vitamin production, which began in 1936. The synthetic product was shown to have the same biological activity as the natural substance. It is reversibly oxidized in the body to dehydro-L-ascorbic acid 1 (L-*threo*-2,3-hexodiulosonic acid γ-lactone), a potent antiscorbutic agent with full vitamin activity. In 1937, Haworth and Szent-Györgyi received the Nobel Prize for their work on vitamin C.

In 1970 Linus Pauling claimed that vitamin C alleviates the symptoms of the common cold or even may prevent the cold. His conclusion was based on a single placebo-controlled trial on children in the Swiss Alps. Sudies carried out since then, administering 1 gr Ascorbic acid per day, indicating that the vitamin does indeed have physiological effects on the common cold. This finding is of major importance, because the prevailing opinion was that the only physiological effect vitamin C has is to prevent scurvy (17,18). However, there is still a difference of opinion between scientists that the vitamin has no proven effects on the common cold. In general, vitamin C enhances immune response in men, speeds up the healing of wounds, and contributes to our well-being overall.

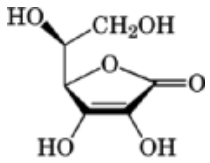
1. Structure Determination

Albert Szent-Györgyi demonstrated that "hexuronic acid," $C_6H_8O_6$, which was first isolated from sources such as cabbage juice, was identical to vitamin C (L-ascorbic acid). Ultraviolet absorption studies carried out in the mid-1930s led to the proposal that L-ascorbic acid was 3-oxo-L-sorbosone (**5**), which exists in a variety of tautomeric forms, including (**6**), (**7**), and L-ascorbic acid (**1**). The chemical structure of L-ascorbic acid was determined independently by Haworth and Hirst using x-ray crystallographic techniques (14). Soon afterwards, additional data supporting the structure **1** were published by other authors (19–21). Excellent review articles summarizing the early work on vitamin C have appeared (22, 23). Years later, with improved x-ray techniques (24, 25) and neutron-diffraction methods (26), the structure of L-ascorbic acid was refined (27, 28). The

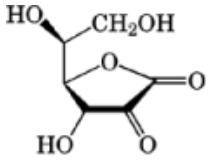
five-membered ring containing the enediol group is almost planar. The conformation of the side chain in the crystal is as shown by structure **1**. The chiral center at position C-4 has the R- or D-configuration, whereas C-5 has the S- or L-configuration.



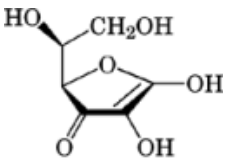
(**5**) 3-oxo-L-sorbosone



(**1**)



(**6**)



(**7**)

As a result of having two chiral centers, four stereoisomers of ascorbic acid are possible (Table 1) (Fig. 2). Besides L-ascorbic acid (Activity = 1), only D-araboascorbic acid (erythorbic acid 2) shows vitamin C activity (Activity = 0.025 − 0.05). The L-ascorbic acid structure (**1**) in solution and the solid state are almost identical. Ascorbic acid crystallizes in the space group $P2_1$ with four molecules in the unit cell. The crystal data are summarized in Table 2.

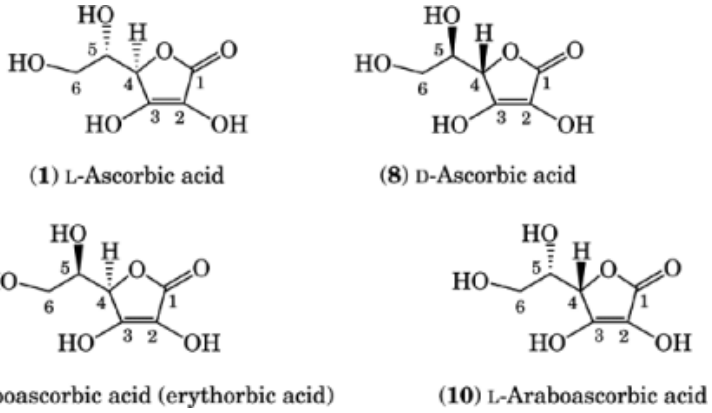


Fig. 2. Isomers of ascorbic acid.

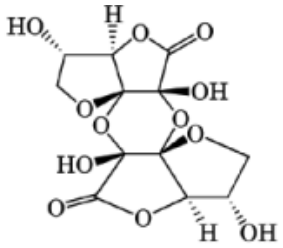
Table 1. Isomeric Ascorbic Acids

Substance	Structure	Mp, °C	$[\alpha]_D$ (H_2O), °	Activity
L-ascorbic acid	(**1**)	192	+24	1
D-xyloascorbic acid (D-ascorbic acid, D-*threo*-hex-2-enonic acid γ-lactone)	2	192	−23	0
D-araboascorbic acid (erythorbic acid, D-*erythro*-hex-2-enonic acid γ-lactone)	2	174	−18.5	0.025–0.05
L-araboascorbic acid (L-*erythro*-hex-2-enonic acid γ-lactone)	2	170	+17	0

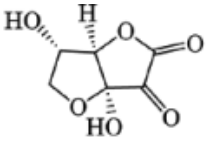
Table 2. X-Ray Crystal Data of L-Ascorbic Acid Ref. 27.

Space group	$P2_1$
a	1.7299 nm
b	0.6353 nm
c	0.6411 nm
β	102° 11′
V	0.68859 nm^3
Z	4
d_{calcd}	1.699 g/cm^3
$\mu(CuK_a)$	13.9 cm^{-1}

Crystalline dehydro-L-ascorbic acid 1 is reported to exist as the dimer (**11**) (29). In water, it is present as the hemiacetal monomer (**12**). An equivocal proof of its presence using today's physical techniques is still not possible (30).



(**11**) Dehydro-L-ascorbic acid dimer



(**12**) Dehydro-L-ascorbic acid hemiacetal

2. Properties

2.1. Physical Properties

Table 3 contains a summary of the physical properties of L-ascorbic acid. Properties relating to the structure of vitamin C have been reviewed and summarized (36). Stabilization of the molecule is a consequence of delocalization of the π-electrons over the conjugated enediol system. The highly acidic nature of the H-atom on C-3 has been confirmed by neutron diffraction studies (26).

2.2. Chemical Properties

The most significant chemical property of L-ascorbic acid is its reversible oxidation to dehydro-L-ascorbic acid. Dehydro-L-ascorbic acid has been prepared by uv irradiation and by oxidation with air and charcoal, halogens, ferric chloride, hydrogen peroxide, 2,6-dichlorophenolindophenol, neutral potassium permanganate, selenium oxide, and many other compounds. Dehydro-L-ascorbic acid has been reduced to L-ascorbic acid by hydrogen iodide, hydrogen sulfide, 1,4-dithiothreitol (1,4-dimercapto-2,3-butanediol), and the like (37).

The degradation of L-ascorbic acid in aqueous solution depends on several factors, *e.g.*, pH, temperature, presence of oxygen, or metals. Comprehensive reviews of degradation reactions and mechanisms have been published (38–41). In aqueous solution, L-ascorbic acid is more sensitive in the presence of oxygen to bases than to acids. The pH range with the highest stability is between 4 and 6. L-Ascorbic acid is sensitive to heat. In the presence of oxygen and heat, it is oxidized at a rate proportional to the temperature rise. On standing, dehydro-L-ascorbic acid 1, the initial oxidation product, undergoes irreversible hydrolysis to 2,3-dioxo-L-gulonic acid (*threo*-2,3-hexodiulosonic acid 3), which is further oxidized to oxalic acid 3 and L-threonic acid ((*R*-(*R**,*S**)-2,3,4-trihydroxybutanoic acid)) 3 (Fig. 3).

In acidic solution, the degradation results in the formation of furfural, furfuryl alcohol, 2-furoic acid, 3-hydroxyfurfural, furoin, 2-methyl-3,8-dihydroxychroman, ethylglyoxal, and several condensation products (40). Many metals, especially copper, catalyze the oxidation of L-ascorbic acid. Oxalic acid and copper form a chelate complex which prevents the ascorbic acid-copper-complex formation and therefore oxalic acid inhibits effectively the oxidation of L-ascorbic acid. L-Ascorbic acid can also be stabilized with metaphosphoric acid, amino acids, 8-hydroxyquinoline, glycols, sugars, and trichloracetic acid (42). Another catalytic reaction which

Table 3. Physical Properties of L-Ascorbic Acid

Property	Characteristic(s)	Reference
appearance	white, odorless, crystalline solid with a sharp acidic taste	
formula; mol. wt.	$C_6H_8O_6$; 176.13	
crystalline form	monoclinic; usually plates, sometimes needles	
mp °C	190–192 (dec)	27
density, g/cm^3	1.65	27
optical rotation	$[\alpha]^{25}$ +20.5° to +21.5° (c = 1 in water) $[\alpha]^{23}$ +48 (c = 1 in methanol)	27
pH		27
5 mg/mL	3	
50 mg/mL	2	
pK_1	4.17	27
pK_2	11.57	27
redox potential	first stage: E + 0.166 V (pH 4)	27
solubility, g/mL		27
water	0.33	
95 wt. % ethanol	0.033	
absolute ethanol	0.02	
glycerol USP	0.01	
propylene glycol	0.05	
ether	insoluble	
chloroform	insoluble	
benzene	insoluble	
petroleum ether	insoluble	
oils	insoluble	
fats	insoluble	
fat solvents	insoluble	
Spectral Properties		
uv	pH 2: E_{max} (1%, 1 cm) 695 at 245 nm (nondissociated form)	28
	pH 6.4: E_{max} (1%, 1 cm) 940 at 265 nm (monodissociated form)	
ir (KBr)	characteristic wavelengths, cm^{-1}	29
	3455, 3405, 3155 ν OH groups	
	2570 associated OH groups	
	1770, 1670 carbonyl lactone	
	1254 C–O–C lactone	
	1057 δ OH groups	
nmr[a]	^{1}H nmr (D_2O)	30
	δ 4.97 (d, 1 H, $J_{4.5}$ = 2 Hz, H-4), 4.10 (ddd, 1 H, $J_{5.6}$ = 5 and 7 Hz, $J_{4.5}$ = 2 Hz, H-5), 3.78 (m, 2 H, C-6)	
	^{13}C nmr (D_2O)	31
	δ 174.0 (C-1), 118.8 (C-2), 156.3 (C-3)	
	77.1 (d, $J_{c-4,H-4}$ = 158 Hz, C-4)	
	69.9 (d, $J_{c-5,H-5}$ = 145.0 Hz, C-5),	
	63.2 (t, $J_{C-6,H-6}$ = 145.0 Hz, C-6)	

[a] d = Doublet; ddd = doublet of doublet of doublet; m = multiplet; and t = triplet.

accounts for loss of L-ascorbic acid occurs with enzymes, eg, L-ascorbic acid oxidase, a copper protein-containing enzyme.

2.3. Stability

Ascorbic acid, a colorless crystalline compound, is very soluble in water and has a sharp, acidic taste. In solution, the vitamin oxidizes on exposure to air, light, and elevated temperatures. Solutions of ascorbic acid turn yellowish, followed by development of a tan color. This browning reaction of ascorbic acid leads to the

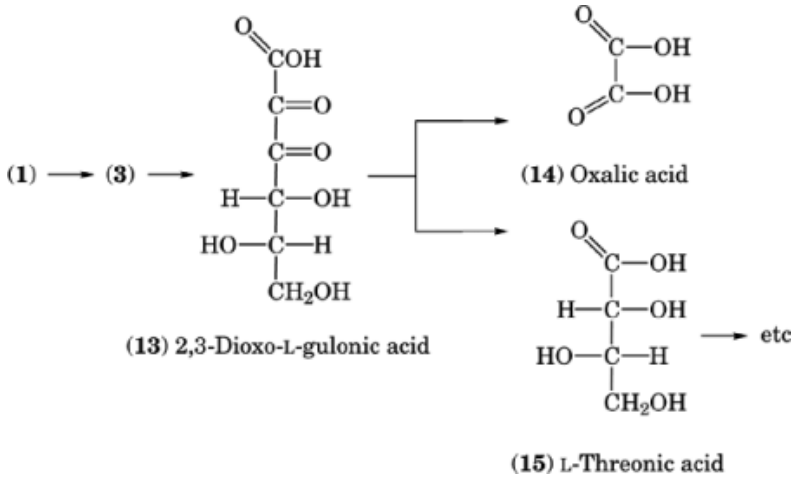


Fig. 3. Degradation of L-ascorbic acid.

formation of many degradation products (43) Ascorbic acid is stable to air when dry but gradually darkens on exposure to light.

3. Synthesis

3.1. Chemical Synthesis

The first synthesis of ascorbic acid was reported in 1933 by Reichstein and co-workers (14, 44–47) (Fig. 4). Similar, independent reports published by Haworth and co-workers followed shortly after this work (14, 48–50). L-Xylose 4 was converted by way of its osazone 4 into L-xylosone 4, which reacted with hydrogen cyanide forming L-xylonitrile 4. L-Xylonitrile cyclized under mild conditions to the cycloimine of L-ascorbic acid. Hydrolysis of the cycloimine yielded L-ascorbic acid. The yield for the conversion of L-xylosone to L-ascorbic acid was ca 40%.

The L-xylosone pathway to L-ascorbic acid was never commercialized because L-xylosone was not readily available and was too expensive to prepare. The route, however, was valuable for L-ascorbic acid structure determination and for the preparation of derivatives.

Most current industrial vitamin C production is based on the efficient second synthesis developed by Reichstein and Grüssner in 1934 (16). Various attempts to develop a superior, more economical L-ascorbic acid process have been reported since 1934. These approaches, which have met with little success, are summarized in Crawford's comprehensive review (51). Currently, all chemical syntheses of vitamin C involve modifications of the Reichstein and Grüssner approach (Fig. 5). In the first step, D-glucose (**4**) is catalytically (Ni-catalyst) hydrogenated to D-sorbitol 5. Oxidation to L-sorbose 5 occurs microbiologically with *Acetobacter suboxydans*. The isolated L-sorbose is reacted with acetone and sulfuric acid to yield 2,3:4,6 diacetone-L-sorbose, (DAS) (2,3:4,6-bis-*O*-isopropylidene-α-L-sorbofuranose) 5. The remaining unprotected primary hydroxyl group of DAS is oxidized, either catalytically (O_2, Pd), electrochemically, or by sodium hypochlorite with nickel chloride as catalyst to the corresponding carboxylic acid. The resulting 2,3:4,6-diacetone-2-keto-L-gulonic acid (DAG) (2,3:4,6-bis-*O*-isopropylidene-2-oxo-L-gulonic acid) 5 is treated with hydrogen chloride in an inert solvent system. Under these conditions, acetone removal occurs followed by consecutive lactonization and enolization to form L-ascorbic acid (**1**).

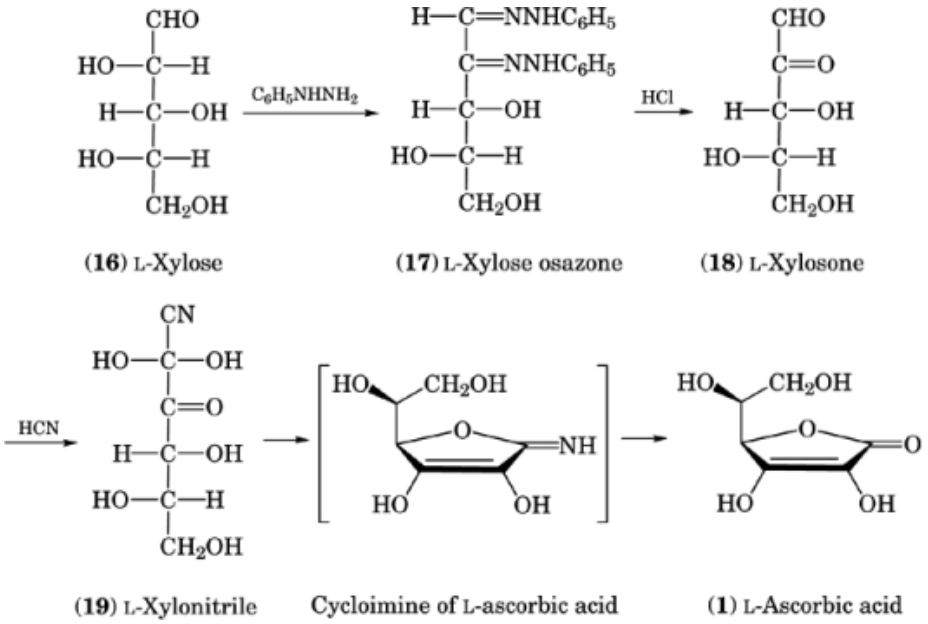


Fig. 4. First syntheses of L-ascorbic acid.

3.2. Fermentation

Much time and effort has been spent in undertaking to find fermentation processes for vitamin C (52). One such approach is now practiced on an industrial scale, primarily in China. It is not certain, however, whether these processes will ultimately supplant the optimized Reichstein synthesis. One important problem is the instability of ascorbic acid in water in the presence of oxygen; it is thus highly unlikely that direct fermentation to ascorbic acid will be economically viable. The successful approaches to date involve fermentative preparation of an intermediate, which is then converted chemically to ascorbic acid.

3.2.1. L-Sorbose to 2-KGA Fermentation

In China, a variant of the Reichstein-Grüssner synthesis has been developed on an industrial scale (see Fig. 5). L-Sorbose is oxidized directly to 2-ketogulonic acid (2-KGA) 5 in a mixed culture fermentation step (53). Acid-catalyzed lactonization and enolization of 2-KGA produces L-ascorbic acid (**1**).

A Chinese publication (52) with 17 references reviews the use of genetically engineered microorganisms for the production of L-ascorbic acid and its precursor, 2-KGA (54). For example, a 2-keto-L-gulonic acid fermentation process from sorbose has been published with reported yields over 80% (55).

3.2.2. D-Glucose to 2-KGA Fermentation

A different fermentative route to 2-KGA proceeds via 2,5-diketo-L-gluconic acid (56, 57). In a two-stage fermentation (Shionogi-Process), D-glucose (**4**) is oxidized to 2,5-diketo-D-gluconic acid (2,5-DKGA) 6 with *Erwinia* sp., followed by stereospecific reduction at C-5 by *Corynebacter* sp., forming 2-ketogulonic acid 5 (Fig. 6). The 2-KGA is rearranged upon treatment with acid to give ascorbic acid. A production of ascorbic acid by this route is reportedly being developed, ca 1997. An analogous process using a recombinant strain of *Erwinia citreus* was developed at Biogen (58).

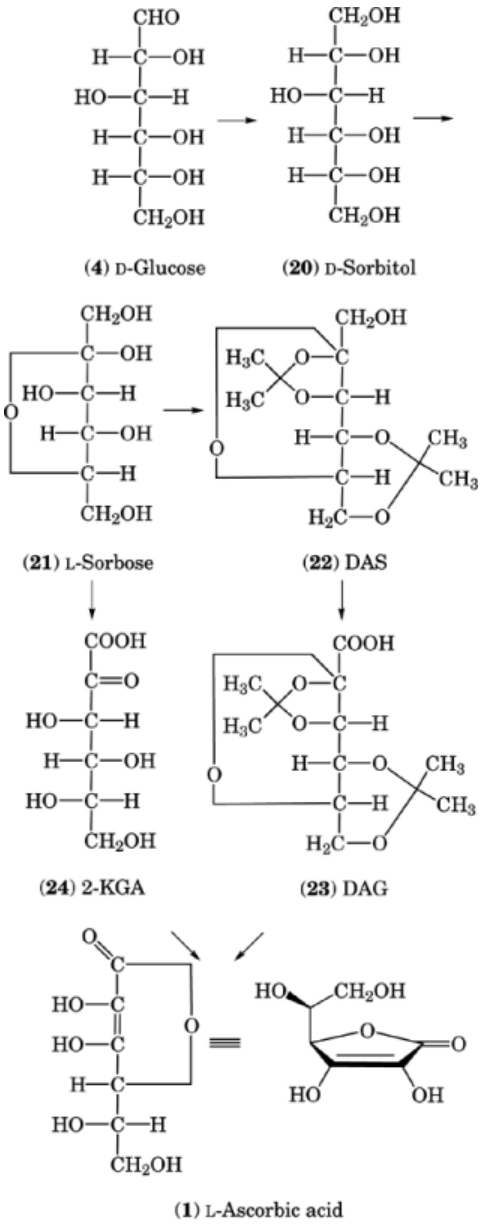


Fig. 5. Syntheses of L-ascorbic acid.

3.2.3. D-Glucose to L-Ascorbic Acid Fermentation

The direct heterotrophic fermentation of D-glucose to L-ascorbic acid with algae is disclosed in a European Patent Application (59). The overall yield of L-ascorbic acid is 4%.

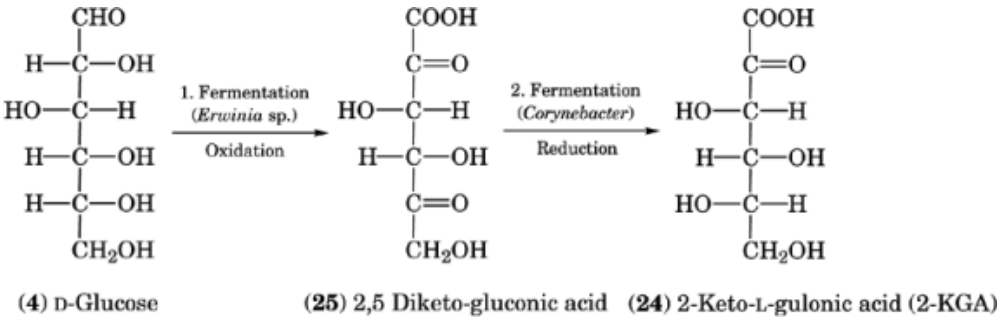


Fig. 6. D-Glucose to 2-KGA fermentation.

3.3. Conversion of 2-KGA to L-Ascorbic acid

The known fermentation processes leading to 2-ketogulonic acid (2-KGA) (24) as an intermediate with either glucose (**4**), sorbitol (20), or sorbose (21) as starting material. A process is disclosed to produce L-ascorbic acid through reaction of 2-ketogulonic acid with concentrated hydrochloric acid at temperatures between 40°C and 80°C without using an organic solvent (60).

2-ketogulonic acid is converted to L ascorbic acid with aqueous mineral acid in a solvent mixture containing an inert organic solvent, an aliphatic ketone, and an acid chloride. L-ascorbic acid yields > 90% and in good quality are claimed (61).

A different process for producing L-Ascorbic acid is patented in which 2-ketogulonic acid is rearranged using an acid (HCl gas) in an ether (or an inert organic solvent containing an ether) in the presence of water and a surfactant. L-ascorbic acid has been obtained in high yield (> 90%) and purity (62).

3.4. Purification of L-Ascorbic acid

L-Ascorbic acid can effectively and effiently be separated from other organic acids such as 2-ketogulonic acid etc. using a strong acidic cation exchange resin as adsorbant and water as eluant. A simulated moving bed process is the industrial chromatography method of choice. Recovery rate of L-ascorbic acid: 97.4% (63).

A process is disclosed to recover L-ascorbic acid from diluted aqueous solutions (i.e., fermentation broth) through adsorption on basic resins carrying a pyridine function. Desorption takes place with a neutral solvent at higher temperature than the adsorption took place. Advantages claimed: No acids are required , no salts are produced (64).

The recovery of L-ascorbic acid from a feed containing at least one ascorbic acid precurser, i.e., sodium ascorbate - using an organic extractant followed by stripping has been disclosed. The process can be applied to extract L-ascorbic acid from rose hips, citrus fruits, and persimmon (65).

4. Industrial Technology

Vitamin C was the first vitamin to be manufactured by chemical synthesis on an industrial scale. Major suppliers of vitamin C are Hoffmann-La Roche, BASF, Takeda, E. Merck, and various companies in China. Additional production occurs in Eastern Europe and India.

Reichstein and Grüssner's second L-ascorbic acid synthesis became the basis for the industrial vitamin C production. Many chemical and technical modifications have improved the efficiency of each step, enabling this multistep synthesis to remain the principal, most economical process up to the present (ca 1997) (51).

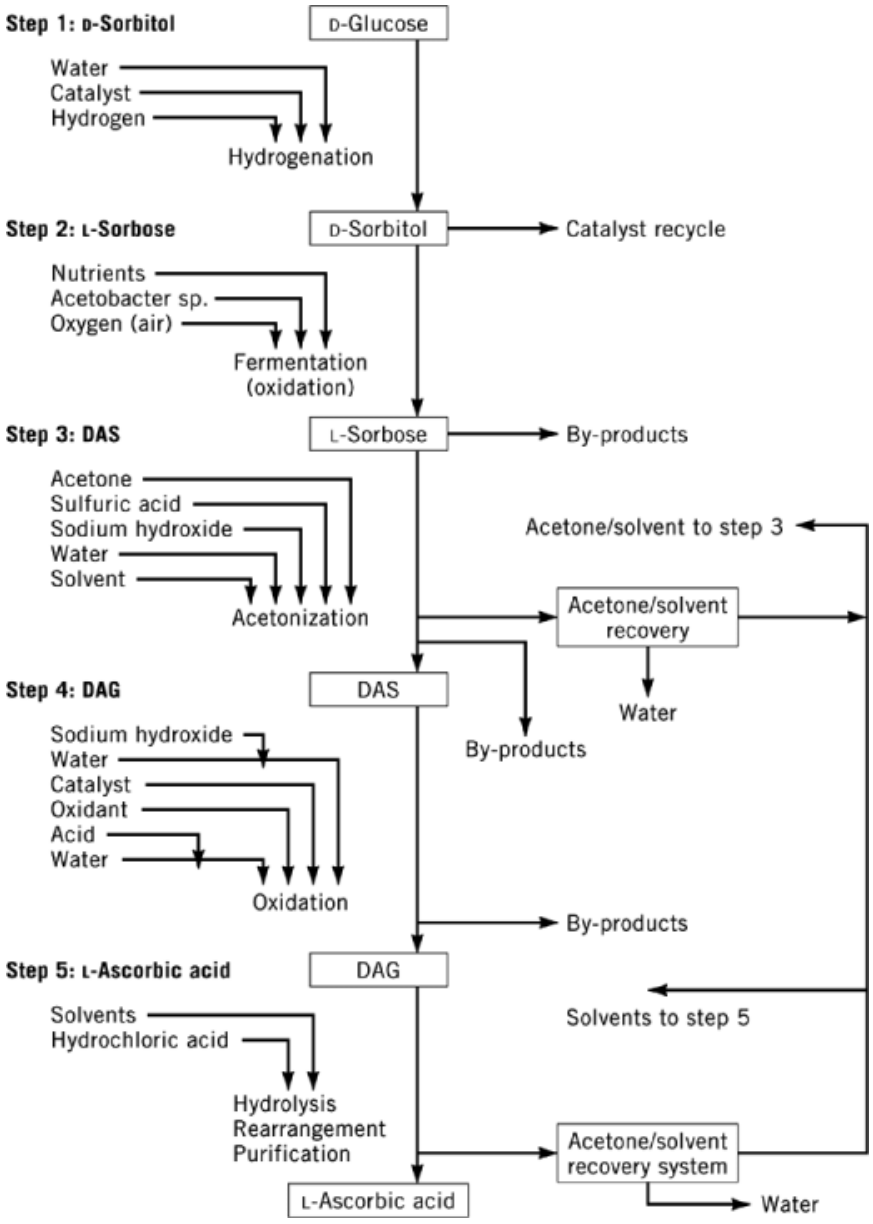


Fig. 7. L-Ascorbic acid manufactured by Reichstein and Grüssner Synthesis.

L-Ascorbic acid is produced in large, integrated, automated facilities, involving both continuous and batch operations. The process steps are outlined in Figure 7. Procedures require ca 1.7-kg L-sorbose/kg of L-ascorbic acid with ca 66% overall yield in 1977 (66). Since 1977, further continuous improvement of each vitamin C production step has taken place. Today's overall ascorbic acid yield from L-sorbose is ca 75%. In the mid-1930s, the overall yield from L-sorbose was ca 30%.

The catalytic hydrogenation of D-glucose to D-sorbitol is carried out at elevated temperature and pressure with hydrogen in the presence of nickel catalysts, in both batch and continuous operations, with >97% yield (67, 68). The cathodic reduction of D-glucose to L-sorbitol has been practiced (69). D-Mannitol is a by-product (70).

Sterile aqueous D-sorbitol solutions are fermented with *Acetobacter suboxydans* in the presence of large amounts of air to complete the microbiological oxidation. The L-sorbose is isolated by crystallization, filtration, and drying. Various methods for the fermentation of D-sorbitol have been reviewed (71). *Acetobacter suboxydans* is the organism of choice as it gives L-sorbose in >90% yield (72). Large-scale fermentations can be carried out in either batch or continuous modes. In either case, sterility is important to prevent contamination, with subsequent loss of product.

In the third step, L-sorbose is reacted with acetone and excess sulfuric acid at low temperatures. The sorbose dissolves on conversion into the 2,3-mono-*O*-isopropylidene-L-sorbose (2,3 monoacetone-L-sorbose) (MAS), and 2,3:4,6-bis-*O*-isopropylidene-*α*-L-sorbofuranose (2,3:4,6-diacetone-L-sorbose) (DAS). The equilibrium mixture consists of ca 65% DAS and 35% MAS. The sulfuric acid acts as a catalyst and a dehydrating agent. The reaction mixture is worked up by dilution, neutralization, and extraction to separate the DAS from the MAS. MAS is recycled and the mother liquors are distilled to recover acetone and solvents. The original Reichstein and Grüssner process has been optimized over decades by other workers, giving ca 85% yield (66). Other acidic catalysts, besides sulfuric acid, have been investigated, eg, zinc chloride–phosphoric acid, *p*-toluenesulfonic acid, copper(II) sulfate, and cationic exchange resins. Ferric chloride and perchloric acid are active catalysts that give >90% yields of DAS with azeotropic removal of the water (73). Other methods, including use of acetone dimethylacetal as a water removal reagent, have been reported (74). The use of other ketonic and aldehydic protective agents for L-sorbose, eg, cyclohexanone and its derivatives, formaldehyde, benzaldehyde, etc, has also been described (16, 75).

2,3:4,6-Diacetone-L-sorbose (DAS) is oxidized at elevated temperatures in dilute sodium hydroxide in the presence of a catalyst (nickel chloride for bleach or palladium on carbon for air) or by electrolytic methods. After completion of the reaction, the mixture is worked up by acidification to 2,3:4,6-bis-*O*-isopropylidene-2-oxo-L-gulonic acid (2,3:4,6-diacetone-2-keto-L-gulonic acid) (DAG), which is isolated through filtration, washing, and drying. With sodium hypochlorite/nickel chloride, the reported DAG yields are >90% (76). The oxidation with air has been reported, and a practical process was developed with palladium–carbon or platinum–carbon as catalyst (77, 78). The electrolytic oxidation with nickel salts as the catalyst has also been published (79).

DAG is treated with ethanol and hydrochloric acid in the presence of inert solvent, eg, chlorinated solvents, hydrocarbons, ketones, etc. The L-ascorbic acid precipitates from the mixture as it forms, minimizing its decomposition (80). Crude L-ascorbic acid is isolated through filtration and purified by recrystallization from water. The pure L-ascorbic acid is isolated, washed with ethanol, and dried. The mother liquor from the recrystallization step is treated in the usual manner to recover the L-ascorbic acid and ethanol contained in it. The crude L-ascorbic acid mother liquor contains solvents and acetone liberated in the DAG hydrolysis. The solvents are recovered by fractional distillation and recycled. Many solvent systems have been reported for the acid-catalyzed conversion of DAG to L-ascorbic acid (51). Rearrangement solvent systems are used which contain only the necessary amount of water required to give >80% yields of high purity crude L-ascorbic acid (81).

The DAG conversion to L-ascorbic acid also can occur by a base-catalyzed mechanism. Methyl 2-oxo-L-gulonate (methyl DAG) is converted, on treatment with sodium methoxide, to sodium-L-ascorbate, which is then acidified to L-ascorbic acid. Various solvent systems have been evaluated and reported (51).

A promising route to L-ascorbic acid is a combined biological/chemical process developed by ENCO Engineering, Switzerland. 2-KGA, key intermediate compound, either obtained from a biological or a chemical process is converted to 2-ketogulonic-acid-ester in a two stage esterification process, before further processed to ascorbate using preferably sodiumbicarbonate (alkaline lactonization) followed by sodium ascorbat conversion to L-ascorbic acid with ion exchange resins. The two-stage esterification warrants efficient removal of water

introduced by 2-KGA and liberated through esterification reaction. The anhydrous ester forms less by-products during lactonization to L-ascorbic acid which increases yield and improves product purity. Vitamin C has been obtained which fulfills all USP requirements (82).

A process for the preparation of L-ascorbic acid starting from an aqueous sodium ascorbate solution using an electric field is advantageous because no additional chemicals are required. Sodium ascorbate is cleaved under the influence of an electric field into sodium cations and ascorbate anions.The opposite charged ions are separated through ion-selective membranes and collected in acid and base compartments. Protons and hydroxide ions are generated simultaneously as well. L-ascorbic acid is formed in the acid compartment, sodium hydroxide in the base compartment. Both product solutions are removed separately. Currently, process is not commercialized (83).

Merck KGaA and BASF Aktiengesellschaft formed an joint venture with sorbitol producer Cerestar Deutschland GmbH (1998) to manufacture L-ascorbic acid. The three companies will use a modern fermentation process to produce 2-KGA from sorbitol. The plant will be built on Cerestar's site at Krefeld, Germany (84).

5. Packaging

L-Ascorbic acid is screened or pulverized into a variety of particle sizes. It is usually packaged in 25-kg and 50-kg quantities in standard, polyethylene-lined containers, eg, fiber drums, corrugated boxes, etc. The recommended storage conditions are low humidity and temperatures of $\leq 23^{\circ}C$.

6. Environmental Issues

The environmental concerns of an ascorbic acid manufacturing facility are those typical of a chemical processing plant. Its operating design must be patterned to conform to environmental protection regulations. Measures must be taken to contain solvents and to keep emissions within official guidelines. Special condensers, continuous instrumental monitoring, and emergency containment and cleanup systems are required. Wastewater-treatment facilities have to be provided to remove by-product organics and inorganics from effluent streams before disposal. The extent of these treatment facilities depends upon the location of the plant and the local tolerances. Usually, there are secondary treatment facilities for organic removal; at some plant sites, additional treatment may be required to remove inorganic salts and traces of residual metal catalyst.

7. Economic Aspects

Strong growth in demand during the period 1985–1995 from existing and new applications. Demand continues to increase. Annual growth is projected at 2 to 3 percent through to 2005 (85). Total world demand in 1995 was estimated to be approximately 60,000 metric tons.

The production in the US is at about 20,000 metric tons in 1999 (Hoffmann-La Roche, Inc. and Takeda, Inc.). There could be a capacity of about 30,000 to 40,000 metric tons in China. Chinese producers supply now coated material as well. Vitamin C prices in 1999 range from $ 5.50 to $ 6.50/kg for bulk amounts representing a drop of more than 50 percent in the last four years (85). About 55% of the U.S. consumption goes directly to pharmaceutical preparations. The remaining 45% is used in the manufacture of food and beverages or in animal feeds.

8. Specifications

Specifications for ascorbic acid, sodium ascorbate, calcium ascorbate, and ascorbyl palmitate are found in the *United States Pharmacopeia/National Formulary* (86) and the *Food Chemicals Codex* (87). The official assay for all four compounds is the iodimetric titration with 0.1 *N* iodine solution and starch as the indicator.

9. Analytical Methods

Many different analytical methods have been developed to determine L-ascorbic acid in feed, biological, and pharmaceutical samples. An excellent review article describes the methodology for finding the proper L-ascorbic acid assay method (88). Comprehensive reviews of all analytical methods, including the extraction of ascorbic acid from foods and biological samples, have been published (89–92). Ascorbic acid has been determined by a variety of methods, including uv absorption; redox and derivatization reactions; electrochemical and enzymatic oxidation reactions; chromatographic, eg, hplc methods; and biological methods with animals. The guinea pig, one of the few animal species requiring ascorbic acid, is used in the bioassay for ascorbic acid activity. The various methods practiced have been described in detail (93).

Because of the time and expense involved, biological assays are used primarily for research purposes. The first chemical method for assaying L-ascorbic acid was the titration with 2,6-dichlorophenolindophenol solution (94). This method is not applicable in the presence of a variety of interfering substances, eg, reduced metal ions, sulfites, tannins, or colored dyes. This 2,6-dichlorophenolindophenol method and other chemical and physiochemical methods are based on the reducing character of L-ascorbic acid (95). Colorimetric reactions with metal ions as well as other redox systems, eg, potassium hexacyanoferrate(III), methylene blue, chloramine, etc, have been used for the assay, but they are unspecific because of interferences from a large number of reducing substances contained in foods and natural products (96). These methods have been used extensively in fish research (97). A specific photometric method for the assay of vitamin C in biological samples is based on the oxidation of ascorbic acid to dehydroascorbic acid with 2,4-dinitrophenylhydrazine (98). In the microfluorometric method, ascorbic acid is oxidized to dehydroascorbic acid in the presence of charcoal. The oxidized form is reacted with *o*-phenylenediamine to produce a fluorescent compound that is detected with an excitation maximum of ca 350 nm and an emission maximum of ca 430 nm (99).

Another method that determines both ascorbic acid and dehydroascorbic acid first reduced the dehydroascorbic acid to ascorbic acid and then retains the ascorbic acid on an anionic Sephadex column (100). The ascorbic acid is oxidized on the column to dehyroascorbic acid by *p*-benzoquinone, which simultaneously elutes the dehydroascorbic acid. The dehydroascorbic acid is reacted with 4-nitro-1,2-phenylenediamine and absorbance of the resulting yellow solution produced is measured at 375 nm.

Chromatographic methods, notably hplc, are available for the simultaneous determination of ascorbic acid as well as dehydroascorbic acid. Some of these methods result in the separation of ascorbic acid from its isomers, eg, erythorbic acid and oxidation products such as diketogulonic acid. Detection has been by fluorescence, uv absorption, or electrochemical methods (101–104). Polarographic methods have been used because of their accuracy and their ease of operation. Ion exclusion (105) and ion suppression (106) chromatography methods have recently been reported. Other methods for ascorbic acid determination include enzymatic, spectroscopic, paper, thin layer, and gas chromatographic methods. Excellent reviews of these methods have been published (88, 107, 108). Gas chromatographic/mass spectrometric measurement of L-ascorbic acid and L-ascorbic acid degradation in solution has been studied (109, 110).

10. Uses

L-Ascorbic acid is used as a micronutrient additive in pharmaceutical, foods, feed, and beverage products, as well as in cosmetic applications. The over-the-counter (OTC) vitamin market is strong, growing in demand, and vitamin C is available in the form of pills and tablets to supplement the daily diet to maintain peak physical performance.

Industrial uses of L-ascorbic acid relate to its antioxidant and reducing properties. It is used as an antioxidant in the commercial preparation of beer, fruit juices, cereals, and canned and frozen foods, etc.

A proposal was made to use L-ascorbic acid as an antioxidant replacement for sulfites in the food industries (111, 112). Ascorbic acid's antioxidant property also inhibits nitrosamine formation in cured meat. The addition of ascorbic acid in flour improves baking qualities of dough and appearance of baked goods (113). Vitamin C also prevents discoloration of food during cooking and storage. Its fatty acid esters, *e.g.*, L-ascorbyl palmitate, are used to stabilize fats and oils (111). Ascorbic acid and its more stable derivatives L-ascorbyl-2-sulfate, ascorbyl polyphosphate, L-ascorbyl 2-monophosphate, etc, are added to fish feed to improve feed utilization and decrease the rate of infection (114–116). The biological and pharmacological activities of L-ascorbyl-2-sulfate have been reviewed (117, 118). Ascorbic acid is also used in agriculture as an abscission agent for fruit, in photography as a developing agent, in metallurgy as a reducing agent, in the polymer industry as a catalyst, in cosmetic formulations, in the manufacture of inks, in explosives, and in a variety of other applications (113, 119).

In applications (eg, as food preservative) where vitamin C untiscurvy activity is unimportant, often D-erythorbic acid (D-araboascorbic acid) can also be used, providing the same antioxidant and reducing properties as L-ascorbic acid.

11. Derivatives

Ascorbic acid has a variety of reactive positions that can be used to synthesize derivatives (120). Various derivatives and analogues have been prepared in attempts to find substances with increased biological activity (121, 122).

Only L-ascorbic acid and its salts and C-6-substituted esters have full vitamin activity; sodium L-ascorbate, calcium L-ascorbate, and L-ascorbyl palmitate are commercially significant. The lipophilic 6-0-ascorbyl-palmitate is a good antioxidant in model systems and is also effective in cellular system. L-ascorbic acid 2-sulfate is bioavailable to fish. 6-Chloro-6-deoxy-L-ascorbic acid was prepared in 1977 and its antioxidant activity, compared to L-ascorbic acid, is 80%. Derivatives, such as 6-deoxy-L-ascorbic acid, L-ascorbic acid 3-*O*-methylether, and 2-amino-2-deoxy-L-ascorbic acid have been prepared; their respective antioxidant activities compared to L-ascorbic acid are 30%, 4–2%, and 0%. Many more derivatives with and without antioxidant and antiscorbutic activity have been synthesized (123, 124). The highest vitamin C antioxidant activity correlates with the enediol lactone group, D-configuration for the C-4 hydrogen group, at least a two-carbon substituent on C-4, and L-configuration for the C-5 hydroxyl group. The primary C-6 hydroxyl group has minor impact on the biological activity.

A wide variety of different hydrophobic compounds that possess the vitamin C ring as the polar group and one or more hydrocarbon side chains (basic molecular architecture of amphiphiles) have been synthesized recently. These molecules behave as surfactants in aqueous dispersions, produce supramolecular aggregates such as micelles, vesicles, or spreading monolayers, depending on their structure, and hold the same antioxidant properties. Large amounts of hydrophobic ascorbic acid-based chemicals are produced for the food, drug, or cosmetics industry (125). Research was conducted on vitamin C and its lipophilic derivatives, ascorbyl-6-caprylate, 6-laurate, and 6-palmitate in membrane mimetic systems to study vitamin C derivative antioxidant properties in hydrophobic systems (126).

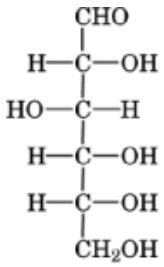
A patent was granted recently for the manufacturing of water soluble phosphonoxy-L-ascorbic acid magnesium salt. This cosmetic material has a skin whitening effect (127).

Mono- and di-ester of cinnamic acid or its derivatives and vitamin C are antioxidants with use in cosmetic, and pharmaceutical compositions. Claims are made, that these compounds protect skin lipids from oxidation (128).

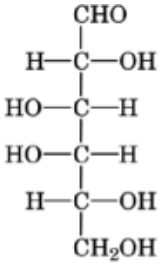
Methods for the preparation of L-ascorbic acids having isotopic C, H, O in various positions have been described and reviewed (129, 130). Labeled L-ascorbic acid has played an important role in the elucidation of the metabolic pathway of L-ascorbic acid in plants and animals.

12. Biosynthesis

In all plants and most animals, L-ascorbic acid is produced from D-glucose (**4**) and D-galactose (**26**). Ascorbic acid biosynthesis in animals starts with D-glucose (**4**). In plants, where the biosynthesis is more complicated, there are two postulated biosynthetic pathways for the conversion of D-glucose or D-galactose to ascorbic acid.



(**4**) D-Glucose



(**26**) D-Galactose

12.1. Biosynthesis in Animals

Amphibians and reptiles carry the enzyme L-gulono-γ-lactone oxidase which can transform a sugar-like glucose or galactose into ascorbic acid in the kidneys. In mammals (131) and birds (132) this enzyme system has been transferred from the kidneys to the liver. Humans, other primates, guinea pigs, fruit bats, and monkeys from the top of the evolutionary tree, as well as insects and other invertebrates from the bottom end of the evolutionary tree, cannot synthesize L-ascorbic acid. Thus, they must consume vitamin C from exogenous sources to survive (133). Animals that are able to produce ascorbic acid do so by the glucuronic acid pathway in the liver or kidneys. The reactions involved in rats are illustrated in Figure 8. In this pathway, the D-glucose chain remains intact, and the C-1 and C-6 of D-glucose become C-6 and C-1, respectively, of L-ascorbic acid as

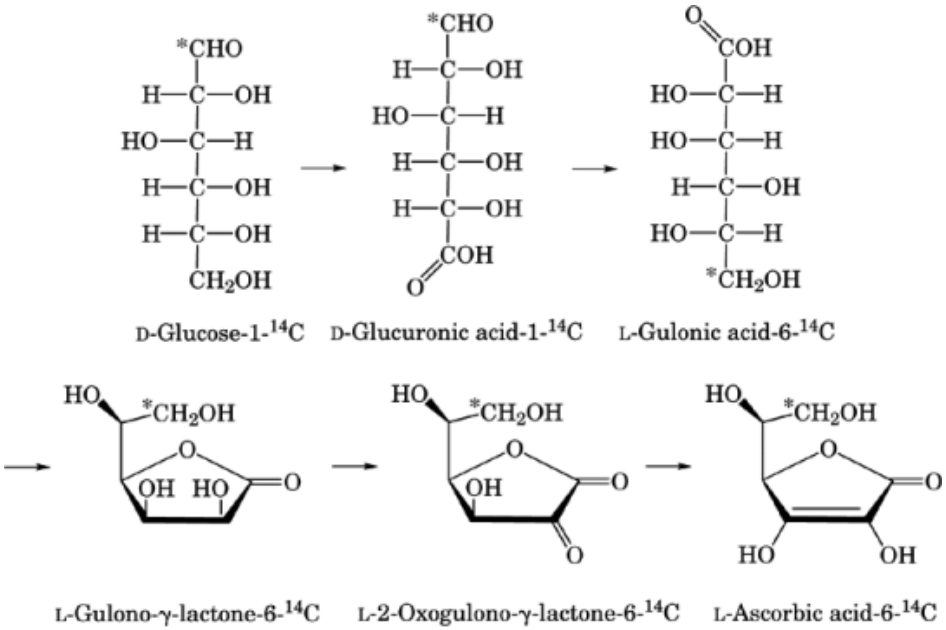


Fig. 8. Pathway for the biosynthesis of L-ascorbic acid in rats using C-1-labeled D-glucose; * indicates position of ^{14}C.

the sequence of carbon-chain numbering is inverted. By measuring the incorporation of ^{14}C from D-glucose-1-^{14}C into urinary L-ascorbic acid, it was determined that the label is found at the C-6 position of L-ascorbic acid (134).

In animals, the glucuronic pathway (Fig. 9) is an important route for glucose utilization leading to the formation of glucuronides and mucopolysaccharides. D-Glucose is first phosphorylated to D-glucose-6-phosphate, then isomerized to D-glucose-1-phosphate, which reacts with uridine-triphosphate (UTP) to form UDP-glucose. UDP-glucose is oxidized and hydrolyzed to D-glucuronic acid. D-Glucuronic acid is reduced to L-gulonic acid. L-Gulonic acid lactonizes and forms L-gulono-γ-lactone. Finally, oxidation of this intermediate is carried out by L-gulono-γ-lactone oxidase, the essential oxidizing enzyme, leading to L-ascorbic acid.

D-Galactose may also serve as a precursor of vitamin C because it can be converted to D-glucose. The pathway is important in the metabolism of sugars under normal and diseased conditions and in regulating physiological functions (135). The biosynthesis of L-ascorbic acid is inhibited by deficiencies of certain vitamins, eg, vitamin A, vitamin E, and biotin, but is stimulated by certain drugs, eg, barbiturates, chlorobutanol, aminopyrine, and antipyrine, and by carcinogens, eg, 3-methylcholanthrene and 3,4-benzpyrene (136–138). It has been proposed that the excretion of D-glucaric acid can be used to diagnose both the exposure to the body for foreign substances and the drug metabolic capacity of the liver (139).

12.2. Biosynthesis in Plants

As in animals, L-ascorbic acid is also the product of hexose phosphate metabolism in plants, but its biosynthesis is more complicated. There are two biosynthetic pathways for the conversion of D-glucose or D-galactose to L-ascorbic acid in plants (140). The main pathway is postulated as involving retention of configuration by oxidation at C-1 to yield D-gluconic-acid 10. Lactonization to yield D-glucono-γ-lactone 10 is followed by oxidation at C-2 or C-3 and epimerization at C-5 to afford L-2-oxogulono-γ-lactone 10 (Fig. 10). The other biosynthetic pathway from D-glucose and D-galactose may be postulated similarly to Figure 8 to account for configurational inversion

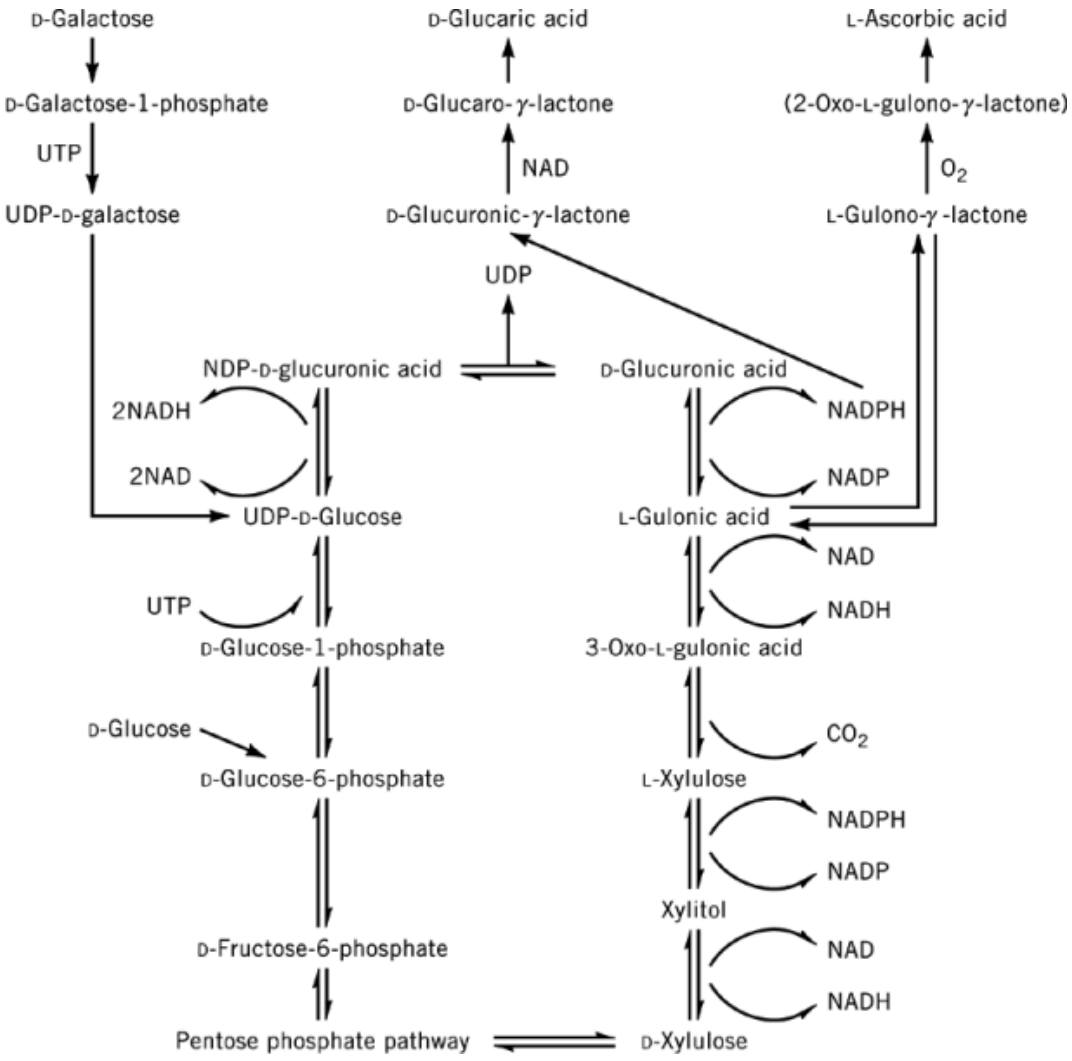


Fig. 9. Glucuronic acid pathway. NAD=nicotinamide–adenine dinucleotide; NADH=reduced nicotinamide–adenine dinucleotide; NADP=nicotinamide–adenine dinucleotide phosphate; NADPH=reduced nicotinamide–adenine dinucleotide phosphate; NDP=nucleoside diphosphate; UDP=uridine diphosphate; and UTP=uridine triphosphate.

(141). The main precursor of L-ascorbic acid is L-galactono-γ-lactone, rather than L-gulono-γ-lactone, which is less active (141, 142), and is thought to be epimerized to L-galactono-γ-lactone prior to oxidation to L-ascorbic acid (Fig. 11). Little is known about the functions of ascorbic acid in plants. It is involved in cellular respiration and may contribute to plant growth (143).

13. Sources of Vitamin C

Fruits and vegetables that are good sources of vitamin C include peppers, greens, broccoli, cabbage, spinach, potatoes, tomatoes, strawberries, and citrus products. Meats, fish, poultry, eggs, and dairy products contain

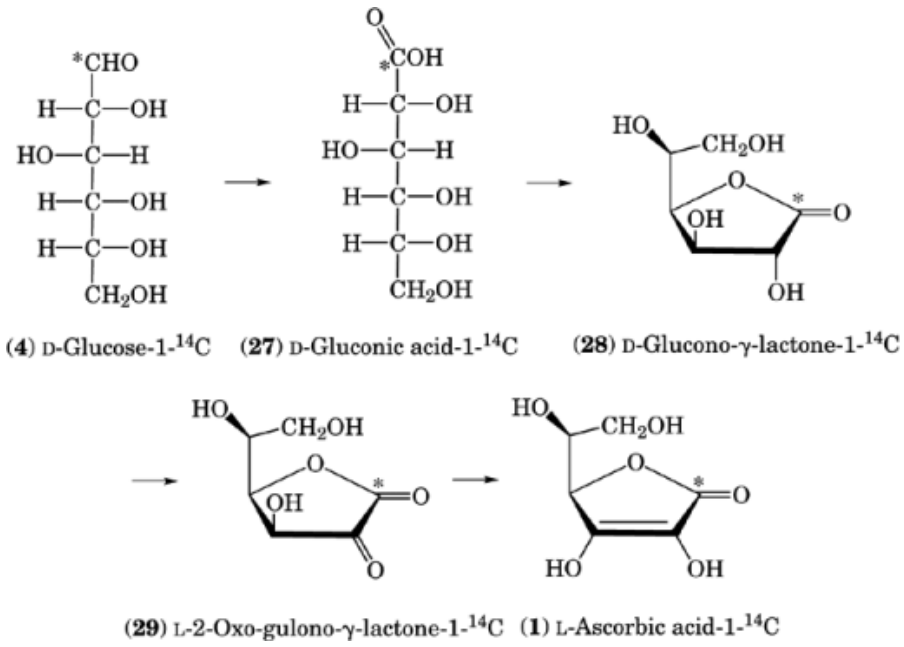


Fig. 10. Suggested pathway for the biosynthesis of L-ascorbic acid (with retention of configuration) in higher plants based on D-glucose-1-^{14}C experiments; * indicates position of the label.

small amounts and grain contains none (144–147). The vitamin C content of some representative foods is listed in Table 4. Potatoes and cabbage have traditionally been the most important sources of vitamin C for the majority of people in the Western World during the winter season.

The contribution of any fruit and vegetable to the vitamin C content of the diet varies depending on climate, soil, and freshness. In actuality, there is a 30% coefficient of variability for ascorbic acid in fresh food products (148). In the case of field-grown spinach, for example, transportation and storage losses have been reported to be as high as 90% (148). During storage after harvesting, the vitamin C content of fruit and vegetables will decrease depending on time and temperature of storage. Ascorbic acid readily oxidizes both enzymatically and chemically on exposure to oxygen. Based on its water solubility, losses are caused through leaching during washing and blanching operations. Heat sterilization of canned foods inactivates completely the enzyme ascorbic acid oxidase which destroys vitamin C. Freezing inactivates the enzymes and is likewise a good method for preserving the vitamin C content of foodstuffs.

Restoration means adding to the food the amount of vitamin C which has been lost during food processing. The term *enrichment* or *fortification* is used when a nutrient is added to a food in an amount in excess of that naturally present. Fortification of foods with vitamin C is widely practiced. Technologically, it is important to avoid oxygen, copper, iron, and heat as much as possible to minimize the overages necessary above label claim to ensure compliance throughout the shelf life of the product (149). In canned foods, breakfast drinks, and cereal products (131), enrichment by 25–50% is common.

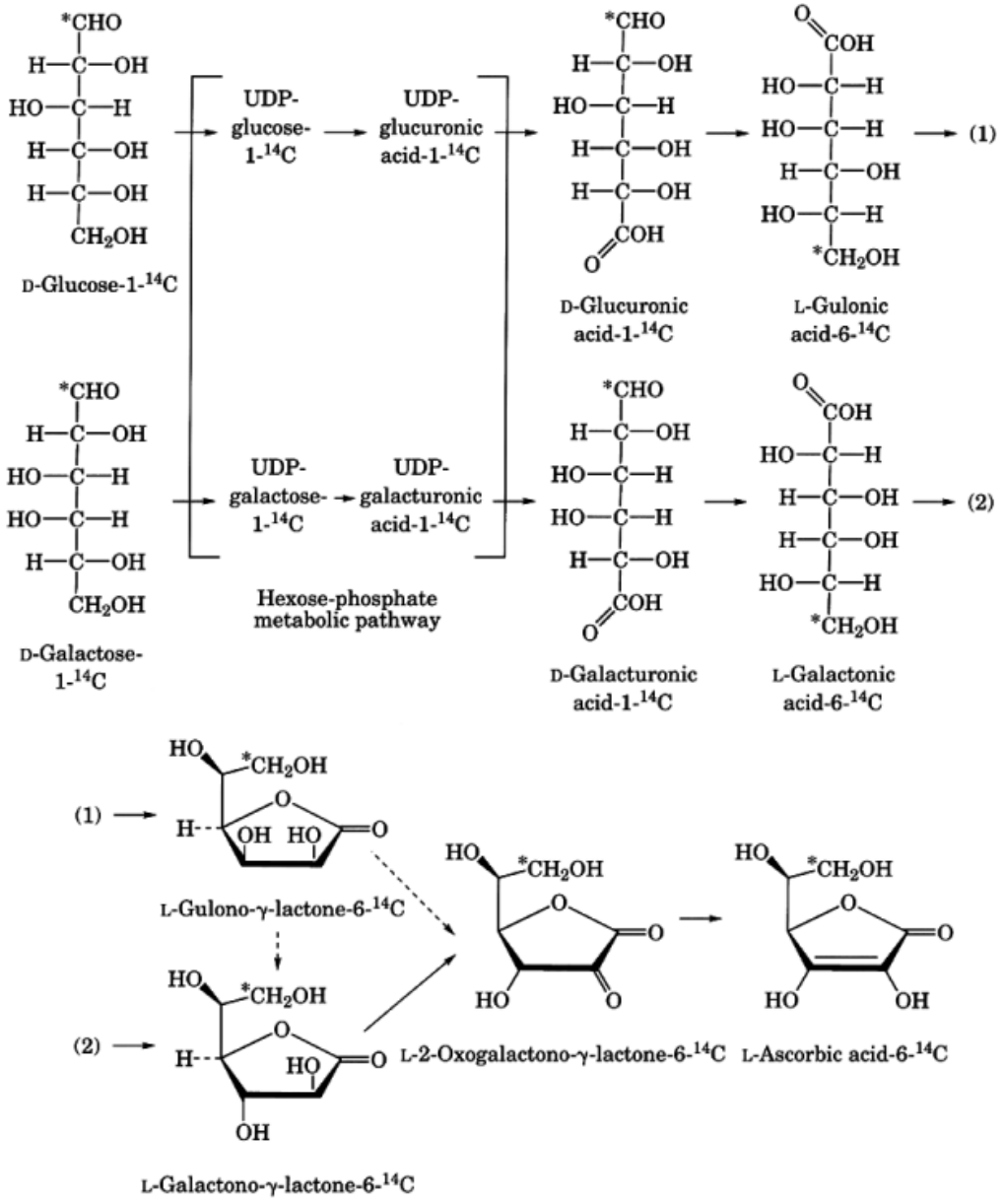


Fig. 11. Proposed pathway for the biosynthesis of L-ascorbic acid (with inversion of configuration) in plants using C-1-labeled D-glucose or C-1-labeled D-galactose; * indicates position of the ^{14}C.

14. Physiology and Biochemistry

14.1. Biochemical Functions

Ascorbic acid has various biochemical functions, involving, for example, collagen synthesis, immune function, drug metabolism, folate metabolism, cholesterol catabolism, iron metabolism, and carnitine biosynthesis.

Table 4. Content of L-Ascorbic Acid in Representative Foods

Food substances	L-Ascorbic acid, mg/100 g
Meat, fish, and milk	
beef, pork, fish	≤2
liver, kidney	10–40
cow's milk	1–2
Vegetables	
asparagus	15–30
brussel sprouts, broccoli	90–150
cabbage	30–60
carrots	9
cauliflower	60–80
kale	120–180
leek	15–30
onion	10–30
peas, beans	10–30
parsley	170
peppers	125–200
potatoes	10–30
spinach	50–90
tomatoes	20–33
Fruit	
apples	10–30
bananas	10
grapefruit	40
guava	300
hawthorne berries	160–800
oranges, lemons	50
peaches	7–14
pineapples	17
rose hips	1000
strawberries	40–90

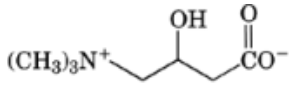
Clear-cut evidence for its biochemical role is available only with respect to collagen biosynthesis (hydroxylation of prolin and lysine). In addition, ascorbic acid can act as a reducing agent and as an effective antioxidant. Ascorbic acid also interferes with nitrosamine formation by reacting directly with nitrites, and consequently may potentially reduce cancer risk. The results of *in vitro* cancer cell studies demonstrated that L-ascorbic acid could exhibit a cytostatic or cytotoxic effect (150–154). New derivatives of N,N-disubstituted-amino-ascorbic acid-compounds have shown anticarcinogenic properties on some lines of tumor cells in vitro (155). First systematic review of epidemiological studies of vitamin C and blood pressure have been completed and published. Higher intakes or plasma levels of vitamin C have been associated with lower blood pressure in several studies (156). The oxidative modification of LDL (low-density lipoprotein) may be an early step in the pathogenesis of atherosclerosis. There is also evidence that vitamin C may protect against atherosclerosis by enhancing HDL(high-density lipoprotein)-cholesterol levels, and reduce LDL(low-density lipoprotein) levels. HDL-cholesterol is considered to be protective against heart disease (157). However more research is needed. A recently published review concluded that L-ascorbic acid might improve human fertility through various mechanisms. The results of controlled human studies support a possible role of vitamin C in human fertility as well (158).Vitamin C may delay or prevent cataract development (159), and might play a role in glucose metabolism and diabetes (160). A general review article covers vitamin C and nervous tissues—*in vivo* and *in vitro* aspects (161). Vitamin C is also involved in the hydroxylation reaction of cholesterol in the liver to

bile acids (cholesterol catabolism) (162). Impaired cholesterol transformation to bile acids causes cholesterol accumulation in the liver and blood, atherosclerotic changes in coronary arteries, and formation of cholesterol gallstone.

14.2. Enzymatic Reactions

The polypeptide collagens are the main component of skin and connective tissue, the organic substance of bones and teeth, and the ground substance between cells. The role ascorbic acid plays in collagen formation has been reviewed (163). The synthesis of collagens involves enzymatic hydroxylations of proline and of lysine. The former produces a stable extracellular matrix and the latter is needed for glycosylation and formation of cross-linkages in the fibers. Vitamin C's role in collagen formation is of importance in wound healing. Intake of 8–50 times the RDA level of 60 mg ascorbic acid per day before and after surgery increases the rate of wound healing considerably (164–166). Under conditions of L-ascorbic acid deficiency there is not only impaired wound healing, but increased wound susceptibility to infections, which are many times more severe (167). Many of the clinical signs of scurvy such as spongy and inflamed gums, loose teeth, swollen and tender joints, impaired wound healing, and so on are attributed to defects in collagen synthesis. The tentative mechanism of collagen degradation and protection by L-ascorbic acid is described by J.B. Chatterjee (168).

Ascorbic acid is involved in carnitine biosynthesis. Carnitine (γ-amino-β-hydroxybutyric acid, trimethylbetaine) (**30**) is a component of heart muscle, skeletal tissue, liver and other tissues. It is involved in the transport of fatty acids into mitochondria, where they are oxidized to provide energy for the cell and animal. Carnitine is synthesized in animals from lysine and methionine by two hydroxylases, both containing ferrous iron and L-ascorbic acid. Ascorbic acid involved as electron donor in the metabolism of L-tyrosine, cholesterol, and histamine (169).



(30) Carnitine

L-Tyrosine metabolism and catecholamine biosynthesis occur largely in the brain, central nervous tissue, and endocrine system, which have large pools of L-ascorbic acid (169). Catecholamine, (L-Dopa), a neurotransmitter, is the precursor in the formation of dopamine, which is converted to norepinephrine (noradrenaline) and epinephrin (adrenaline). The precise role of ascorbic acid has not been completely understood. Ascorbic acid is important biochemical functions with various hydroxylase enzymes in steroid, drug, and lipid metabolism. The cytochrome P-450 oxidase catalyzes the conversion of cholesterol to bile acids and the detoxification process of aromatic drugs and other xenobiotics, *e.g.*, carcinogens, pollutants, and pesticides, in the body (170–178). The effects of L-ascorbic acid on histamine metabolism related to scurvy and anaphylactic shock have been investigated (179). Ascorbic acid plays an essential role in the metabolism of folic acid. Folic acid is involved in many one-carbon transfer reactions in the formation of for example, choline, carnitine, creatine, adrenaline. Ascorbic acid is involved in the conversion of folate to tetrahydrofolate (180). Ascorbic acid has many biochemical functions which affect the immune system of the body (160) It plays an important role in the prevention of cancer, cataracts, and other disorders (181).

14.3. Antioxidant Activity

Ascorbic acid serves as an antioxidant to protect intracellular and extracellular components from free-radical damage. It scavenges free radicals and forms the less reactive ascorbyl radical. The ascorbate free radical is naturally detectable by EPR (electron paramagnetic resonance) at low steady state levels in biological samples,

such as plasma (182) and skin (183, 184). The ascorbyl radical can be either reduced to ascorbic acid or oxidized to dehydroascorbic acid (185). Vitamin E is the major fat-soluble antioxidant involved in protecting cells from free-radical impact. During the process of fatty acid oxidation, tocopherol (vitamin E) forms the tocopheroxyl radical. Ascorbic acid has been proven to protect membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E (186). This has been demonstrated multiple times *in vitro*, but little *in vivo* data have been obtained to support the common view that ascorbate regenerates tocopheroxyl radical (187). These free-radical scavenging reactions of vitamin C are important in protecting the intracellular and extracellular structure of the lung by quenching free radicals generated by smoke, ozone, and singlet oxygen. L-ascorbic acid has been shown to improve pulmonary function in some studies (188). One of the most predominant forms of oxidative damage through radicals is lipid peroxidation. *In vivo* oxygen free radicals and reactive oxygen species are produced by many aerobic cells and these leading to oxidative damage, degenerative diseases as well as aging; however mechanisms are not clear (173–180).

Increased levels of free radicals are found in such diseases as asthma, Alzheimer's disease, atheroscerosis, ischaemic heart disease, and multiple sclerosis (189). Free radicals and oxidative processes also are involved in both cancer initiation and tumor promotion (190). The possibility that free-radical damage is also involved in the pathogenesis of HIV is an area of intense interest (191).

Consumption of foods high in vitamin C (not necessarily ascorbic acid alone) is clearly associated with a decreased risk of certain cancer (192). Phytochemicals may be divided into health promoting compounds and toxicants (193). There is a need for more research focusing on the effects of multiple combinations of antioxidants and phytochemicals. In general, in disease prevention people with low fruit and vegetable intake have a cancer rate that is about twice that of individuals with high intakes of these goods. Fruits and vegetables contain a number of compounds including vitamin C that may contribute to cancer prevention.

14.4. Inhibition of Nitrosamine Formation

Nitrites can react with secondary amines and *N*-substituted amides under the acidic conditions of the stomach to form *N*-nitrosamines and *N*-nitrosamides. These compounds are collectively called *N*-nitroso compounds. There is strong circumstantial evidence that *in vivo* production of *N*-nitroso compounds contribute to the etiology of cancer of the stomach (194–196), esophagus (195, 197), and nasopharynx (194, 198). Ascorbic acid consumption is negatively correlated with the incidence of these cancers, due to ascorbic acid inhibition of *in vivo N*-nitroso compound formation (199). The concentration of *N*-nitroso compounds formed in the stomach depends on the nitrate and nitrite intake. Nitrite is part of the preserving process for cured meats. Cigarette smoke also contains high levels of nitrite.

14.5. Iron Absorption

A very important effect of ascorbic acid is the enhancement of absorption of nonheme iron (Fe^3+) from foods. In individuals with iron deficiency, ingestion of vitamin C enhances the absorption of non-heme iron from food consumed at the same meal (200), while the absorption of heme iron is not affected by vitamin C intake (201). Ascorbic acid also enhances the reduction of ferric iron to ferrous iron. This is important in increasing iron absorption. Many hydroxylation reactions need ferrous iron (Fe^2+) as well (202, 203). In addition, ascorbic acid is involved in iron metabolism. It serves to transfer iron to the liver and to incorporate it into ferritin (204–206) [serum ferritin was first measured in 1972 (207)]. Ascorbic acid also forms soluble chelate complexes with iron (Fe^3+) (208–211). It seems ascorbic acid has no effect on high iron levels found in people with iron overload (212). Over 10% of nonblacks and up to 30% of blacks have a gene for iron overload (207). It is well known, in fact, that ascorbic acid in the presence of iron can exhibit either prooxidant or antioxidant effects, depending on the concentration used (213). Strong prooxidative nature of the iron-ascorbate couple has been demonstrated *in vitro* and warrants further study in *in vivo* reactions (214). Evaluation of age-related dietary

trends over time and factors involved in iron absorption led to the hypothesis that the combination of citric acid and ascorbic acid causes an increase in iron load in aging populations (215. Iron overload may be the most important common etiologic factor in the development of heart disease, cancer, diabetes, osteoporosis, arthritis, and possibly other disorders (215). The synergistic combination of citric acid and ascorbic acid needs further study, particularly because the iron overload produced may be correctable (213).

14.6. Deficiency

Scurvy is a vitamin C-specific disease. It is characterized by anemia and alteration of protein metabolism; weakening of collagenous structures in bone, teeth, and connective tissues; swollen, bleeding gums with loss of teeth; fatigue and lethargy; rheumatic pains in the legs and degeneration of the muscles, skin lesions, and capillary weakness, massive hematomas in the thighs; and hemorrhages in many organs, including the eyes. Small (10–60 mg/d) quantities of L-ascorbic acid are sufficient to reverse the trend of both subclinical and clinical scurvy and alleviate their symptoms. Plasma and leukocyte ascorbic acid levels are the most reliable markers of vitamin C intake. Leukocyte levels are more reliable, less sensitive to recent vitamin C intake, and better reflect tissue ascorbate. Normal leukocyte vitamin C levels are 20–40 $\mu g/10^8$ cells (214). Plasma concentrations of ascorbic acid less than 0.2 mg/dL and leukocyte concentrations below 2 $\mu g/10^8$ cells are seen in scurvy (216). Plasma ascorbic acid levels of 0.5 mg/dL are considered to prevent deficiency symptoms. Normal plasma levels are 0.8–1.4 mg/dL.

Vitamin C levels may be regulated and controlled tightly by both nonenzymatic and enzymatic recycling mechanisms that may become dysfunctional in certain disease states of diabetes, cataract, heart disease, cancer, and so on (217). Adequate vitamin C levels are essential in maintaining optimal health.

14.7. Absorption, Transport, and Excretion

The vitamin is absorbed through the mouth, the stomach, and predominantly through the distal portion of the small intestine and, hence, penetrates into the bloodstream. Ascorbic acid is widely distributed to the cells of the body and is mainly present in the white blood cells (leukocytes) which are involved in the destruction of bacteria (218–221). The ascorbic acid concentration in these cells is about 150 times its concentration in the plasma (222, 223). Dehydroascorbic acid is the main form in the red blood cells (erythrocytes).

Up to 80% of oral doses of ascorbic acid are absorbed in humans with intakes of less than 0.2 g of vitamin C. Absorption of pharmacological doses ranging from 0.2 g to 12 g results in an inverse relationship, with less than 20% absorption at the higher doses. A single oral dose of 3 g has been reported to approach the absorptive capacity (tissue saturation) of the human intestine.

The adrenal glands and pituitary glands have the highest tissue concentration of ascorbic acid. The brain, liver, and spleen, however, represent the largest contribution to the body pool. Plasma and leukocyte ascorbic acid levels decrease with increasing age (224). Elderly people require higher ascorbic acid intakes than children to reach the same plasma and tissue concentration (225).

Ascorbic acid is very soluble in water and mainly excreted in the urine. No ascorbic acid is excreted during vitamin C deficiency. A minimum amount is lost in the feces, even after intake of gram dosages (226).

14.8. Mobilization and Metabolism

The total ascorbic acid body pool in healthy adults has been estimated to be approximately 1.5 g, which increases to 2.3–2.8 g with intakes of 200 mg/d (223–230).

Depletion of the body pool to 600 mg initiates physiological changes, and signs of clinical scurvy are reported when the body pool falls below 300 mg (216). Approximately 3–4% of the body pool turns over daily, representing 40–60 mg/d of metabolized, or consumed, vitamin C. Smokers have a higher metabolic turnover

Table 5. The 1989 RDA and US RDA for Vitamin C

Group	Age, yr	Vitamin C, mg
	RDA	
infants	0–0.5	30
	0.5–1.0	35
children	1–3	40
	4–10	45
males	11–14	50
	15 – 51+	60
females	11–14	50
	15 – 51+	60
pregnant		70
lactating		
1st 6 mo		95
2nd 6 mo		90
cigarette smokers		100
	U.S. RDA	
infants under 13 mo		35
children under 4 yr		40
adults and children over 4 yr		60
pregnant or lactating women		60

rate of vitamin C (approximately 100 mg/d) and a lower body pool than nonsmokers, unless compensated through increased daily intakes of vitamin C (231). The metabolism of ascorbic acid varies among different species.

In rats and guinea pigs, respiratory carbon dioxide is the major oxidation product of vitamin C (232). Given to humans in physiological doses, approximately 10% of a dose will be recovered in the urine, and urinary oxalic acid is the predominant metabolite. The formation of urinary metabolites is limited to 30–50 mg/d. Intake of up to 10 g/d ascorbic acid does not result in a considerable increase of urinary metabolites. The vitamin is excreted largely unmetabolized (233). In humans, the metabolites are dehydroascorbic acid, oxalate, 2,3-diketo-L-gulonic acid and derivatives of L-threose and L-threonic acid. L-Ascorbate-2-sulfate has been found in human urine as well (234). The feces are not a significant excretory route unless doses over 1 g are given.

The half-life of ascorbic acid is inversely related to the daily intake and is 13–40 d in humans and 3 d in guinea pigs, which is consistent with the longer time for humans to develop scurvy 216.

14.9. Requirements

The level of ascorbic acid intake required is dependent in part upon the body's handling of the nutrient 180, 190. The recommendations for the daily intake of vitamin C in various countries range from 30 to 100 mg/d. There is an extensive lack of knowledge about the biochemical and physiological functions of vitamin C. Although as little as 10 mg/d of ascorbic acid can prevent clinical scurvy, this intake is insufficient to maintain an adequate body pool of the vitamin for peak physical and mental health. The RDA (Recommended Dietary Allowances based on National Academy of Sciences' 1989) for vitamin C in the United States is currently 60 mg for men and women to maintain the body pool (Table 5). Vitamin C levels are higher for pregnant and lactating women to account for losses to the fetus and to breast milk.

The most recent RDA has included a vitamin C recommendation of 100 mg/day for cigarette smokers. An increasing number of investigators have concluded that the current RDA for vitamin C may not be adequate for elderly individuals. Plasma vitamin C level is generally accepted as an indicator of vitamin C status.

A recent study recommended that the current RDA be increased from 60 mg/d to 200 mg. The researchers indicated, however, that vitamin C daily doses above 400 mg have no value (235). The amount of L-ascorbic acid that needs to be consumed for optimum well-being is still uncertain. It seems that the current opinions are very diverse. Increased vitamin C may be needed under stress to maintain optimal physiologic function.

14.10. Toxicity

The acceptable daily allowance, which may be ingested without any risk of harm, is 1050 mg for a 70-kg healthy person (23). The literature documents that these and much higher intake levels of L-ascorbic acid are safe (mega doses of vitamin C are being administered as a cure of cancer by "alternative" practitioners.) Since 1993 many well controlled studies have been completed examining high doses of vitamin C intake for safety and efficacy (236). There is also no evidence in the literature that ingestion of up to 10 g vitamin C per day constitutes a serious health risk for humans.

BIBLIOGRAPHY

"Ascorbic Acid" in *ECT* 1st ed., Vol. 2, pp. 150–163, by P. Regna, Chas. Pfizer & Co., Inc.; in *ECT* 2nd ed., Vol. 2, pp. 747–762, by J. J. Burns, Burroughs Wellcome & Co. (U.S.A.), Inc.; "Ascorbic Acid" under "Vitamins" in *ECT* 3rd ed., Vol. 24, pp. 8–40, by G. M. Jaffe, Hoffmann-La Roche Inc.; "Vitamins (Ascorbic Acid)" in *ECT* 4th ed., Vol. 25, pp. 17–47, by Volker Kuellmer, Hoffmann-La Roche Inc.;"Ascorbic Acid" in *ECT* (online), posting date: December 4, 2000, by Volker Kuellmer, Hoffman-La Roche, Inc.

Cited Publications

1. *Biochem. Biophys. Acta* **107**, 4 (1965).
2. W. N. Haworth, *Chem. Ind. (London)* **52**, 482 (1993); W. N. Haworth and E. L. Hirst, *Ergeb. Vitamin-u: Hormonforsch.* **2**, 160 (1939).
3. G. R. Buettner, B. A. Jurkiewicz, *Antioxid. Health Dis.* (1996), **3** (Handbook of Antioidants), 91–115.
4. R. E. Hodges and E. M. Baker in R. S. Goodhart and M. E. Shils, eds., *Modern Nutrition in Health and Disease*, 5th ed., Lea & Febiger, Philadelphia, Pa., 1973, p. 245; U. Wintermeyer, H. Lahann, and R. Vogel, *Vitamin C: Entdeckung, Identifizierung und Synthese—Heutige Bedeutung in Medizin und Lebensmitteltechnologie*, Deutscher Apotheker Verlag, Stuttgart, Germany, 1981.
5. L. Stone, *The Healing Factor—Vitamin C Against Disease*, Grosset & Dunlap, Inc., New York, 1972, p. 7.
6. I. M. Sharman in G. G. Birch and K. J. Parker, eds., *Vitamin C: Recent Aspects of its Physiological and Technological Importance*, John Wiley & Sons, Inc., New York, 1974, p. 1.
7. A. F. Hess, *Scurvy, Past and Present*, J. B. Lippincott Co., Philadelphia, Pa., 1920.
8. J. Lind, *A Treatise on the Scurvy*, London, 1753 (republished by Edinburgh University Press, Edinburgh, Scotland, 1953).
9. A. Holst and T. Fröhlich, *J. Hyg.* **7**, 634 (1907).
10. H. Chick and E. M. Hume, *J. R. Army Med. Corps* **29**, 121 (1917); *J. Biol. Chem.* **39**, 203 (1919).
11. A. Harden and S. S. Zilva, *Biochem. J.* **12**, 259 (1918); S. S. Zilva and F. M. Wells, *Proc. R. Soc. London Ser. B* **90**, 505 (1917–1919); S. S. Zilva, *Biochem. J.* **21**, 689 (1927).
12. A. Szent-Györgyi, *Biochem. J.* **22**, 1387 (1928); J. L. Svirbely and A. Szent-Györgyi, *Biochem. J.* **26**, 865 (1932).
13. C. G. King and W. A. Waugh, *Science* **75**, 357 (1932).
14. W. N. Haworth and E. L. Hirst, *J. Soc. Chem. Ind.* **52**, 645–646 (1933).
15. T. Reichstein, A. Grüssner, and R. Oppenhauer, *Helv. Chim. Acta* **16**, 1019–1033 (1933).
16. T. Reichstein and A. Grüssner, *Helv. Chim. Acta* **17**, 311 (1934).
17. H. Hemilä, *Int. J. Vitam. Nutr. Res.* **67** (5), 329–335 (1997).
18. H. Hemilä, *Nutrition* (Tarrytown, NY.), **12** (11/12), 804–809 (1996).

19. P. Karrer, K. Schöpp, and F. Zehner, *Helv. Chim. Acta* **16**, 1161–1163 (1933).
20. F. Michael and K. Kraft, Hoppe-Seyler's *Z. Physiol. Chem.* **222**, 235–249 (1933).
21. H. von Euler and C. Martius, *Ann.* **505**, 73–87 (1933).
22. E. L. Hirst, *Fortsch. Chem. Org. Naturst.* **2**, 132–159 (1939).
23. *Wld. Hlth. Org. Techn. Rep. Ser.* 1972, No. 505; *Wld. Hlth. Org. Techn. Rep. Ser.* 1974, No. 539.
24. Armour Research Foundation, *Anal. Chem.* **20**, 986–987 (1948).
25. C. von Planta, *Helv. Chim. Acta* **44**, 1444–1446 (1961).
26. J. Hvoslef, *Acta Crystallogr. Sect. B* **24**, 1431–1440 (1968).
27. J. Hvoslef, *Acta Chem. Scand.* **18**, 841–842 (1964).
28. J. Hvoslef, *Acta Crystallogr. Sect. B* **24**, 23–35 (1968).
29. W. Müller-Mulot, Hoppe-Seyler's *Z. Physiol. Chem.* **351**, 52, 56 (1970); J. Hvoslef, *Acta Chem. Scand.* **24**, 2238 (1970); *Acta Crystallogr.* **B28**, 916 (1972); J. Hvoslef and B. Pedersen, *Acta Chem. Scand.* **B33**, 503 (1979); *Carbohydr. Res.* **92**, 9 (1981).
30. G. Fodor, *Trends in Organic Chemistry*, **4**, 661–667 (1993).
31. R. J. Kutsky, *Handbook of Vitamins and Hormones*, Van Nostrand Reinhold Co., Inc., New York, 1973, p. 71; *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, N.J., 1989, p. 130.
32. J. S. Koevendel, *Nature* **180**, 434 (1957); Y. Ogata and Y. Kosugi, *Tetrahedron* **26**, 4711 (1970).
33. Sadtler Standard Spectra, *Spectrum No. 5424*, Sadtler Research Laboratory, Inc., Philadelphia, Pa., 1972.
34. High Resolution NMR Spectra Catalog, Vol. **2**, *Spectrum No. 464*, Varian Assoc., Palo Alto, Calif., 1963.
35. V. F. Johnson and W. C. Jankowski, Carbon-13 NMR Spectra, *Spectrum No. 171*, Wiley-Interscience, New York, 1972; S. Berger, *Tetrahedron* **33**, 1587 (1977); T. Ogama, J. Uzawa, and M. Matsui, *Carbohydr. Res.* **59**, C32 (1977); T. Radford, J. G. Sweeny, and G. A. Jacobucci, *J. Org. Chem.* **44**, 658 (1979).
36. B. M. Tolbert and co-workers, *Ann. N.Y. Acad. Sci.* **258**, 44 (1975); B. M. Tolbert, *Int. J. Vit. Nutr. Res. Suppl.* **19**, 127 (1979).
37. B. Tolbert and J. B. Ward, in *Ascorbic Acid, Chemistry, Metabolism and Uses, Adv. Chem. Sci.* **200**, 101–123 (1982); M. Ohmori, M. Takage, *Agr. Biol. Chem.* **42**, 173–174 (1978).
38. F. E. Huelin, *Food Res.* **18**, 633 (1953).
39. J. Campbell and W. G. Tubb, *Can. J. Res.* **28E**, 19–32 (1950).
40. K. Mikova and J. Davidele, *Chem. Listy* **68**, 715 (1974).
41. F. E. Huelin, *Food Res.* **18**, 633 (1953); J. Campbell and W. G. Tubb, *Can. J. Res.* **28E**, 19 (1947).
42. D. W. Bradley, G. Emery, and J. E. Maynard, *Clin. Chim. Acta* **4**, 47–52 (1973).
43. J. H. Tatum, P. E. Shaw, R. E. Berry, *J. Agric. Food. Chem.* **17**, 38–40 (1969).
44. T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta* **16**, 561–565 (1933).
45. T. Reichstein, A. Grüssner, and R. Oppenauer, *Nature (London)* **132**, 280 (1932).
46. T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta* **17**, 510–520 (1934).
47. U.S. Pat. 2,056,126 (Sept. 29, 1936), T. Reichstein (to Hoffmann-La Roche Inc.).
48. W. N. Haworth, *Chem. Ind. (London)* **52**, 482–485 (1933).
49. R. G. Ault and co-workers, *J. Chem. Soc.* 1419–1423 (1933).
50. W. N. Haworth and E. L. Hirst, *Helv. Chim. Acta* **17**, 520–523 (1934).
51. T. C. Crawford and S. A. Crawford, *Adv. Carbohydr. Chem. Biochem.* **37**, 79 (1980).
52. M. Kulhanek, *Adv. Appl. Microbiol.* **12**, 11–33 (1970).
53. G. Xin, *Weishengwuxue Tongbao* **14**(2), 73 (1987).
54. Eur. Pat. Appl. 278 447 (Prior. Feb. 2, 1987) W. Ning, Z. Tao, C. Wang, S. Wang, Z. Yan, and G. Yin (to Institute of Microbiology Academia Sinica).
55. Eur. Pat. Appl. 221 707 (Prior. Oct. 22, 1985) I. Nogami, H. Shirafuji, M. Oka, and T. Yamaguchi (to Takeda Chemical Industries, Ltd.).
56. T. Sonoyama and co-workers, *Appl. Environ. Microbiol.* **43**, 1064 (1982).
57. S. Anderson and co-workers, *Science* **230**, 144–149 (1985).
58. J. F. Grindley, M. A. Payton, H. van do Pol, and K. G. Hardy, *Appl. Environ. Microbiol.* **54**, 1770 (1988).
59. Eur. Pat. Appl. No. 207 763 (1985), T. J. Skatrud and R. J. Huss (to Bio-Technical Resources).
60. Pat. WO 99/07691 (Feb. 18, 1999), U. Fechtel, K. Beschmann, W. Heinz and W. Kuhn (to Merck GMBH).
61. U.S. Pat. 5,744,618 (April 24, 1998), U. Fechtel, K. Wembacher, (to Merck GMBH).

62. U.S. Pat. 5,637,734 (June 10, 1997), H. Honda, T. Yamano, M. Yamashita (to Takeda Chem. Ind., Ltd.).
63. U.S. Pat. 5,817,238 (Oct. 6, 1998), K. Kawai, K. Makino, M. Tamura, M. Tanimura (to Nippon Rensu Co.; Tokyo, Japan).
64. Int. Pat. WO 97/13761 (April, 1997), K. Whally (to Yissum Research Development Company).
65. Int. Pat. WO 98/24777 (June 11, 1998), A. M. Eyal, (to Yissum Research Development Company of the Hebrew University of Jerusalem; A. E. Staley Manufacturing Co., Decatur, IL).
66. I. T. Strukov and N. A. Kopylova, *Farmatsiya* **10**(3), 8 (1974); *Drug Cosmet. Ind.* 40 (Aug. 1977).
67. W. Ipatiew, *Ber.* **45**, 3218 (1912).
68. L. W. Cover, R. Connor, and H. Adkins, *J. Am. Chem. Soc.* **54**, 1651 (1932).
69. A. T. Kuhn, *J. Appl. Electrochem.* **11**, 261 (1981).
70. W. E. Cake, *J. Am. Chem. Soc.* **44**, 859 (1922).
71. M. Kulhanek, *Adv. Appl. Microbiol.* **12**, 11 (1970).
72. P. A. Wells, J. J. Stubbs, L. B. Lockwood, and E. T. Roe, *Ind. Eng. Chem.* **29**, 1385 (1937); E. J. Fulmer and L. A. Underkofler, Iowa State Coll. *J. Sci.* **21**, 251 (1974).
73. U.S. Pat. 3,622,560 (Nov. 23, 1971), N. Halder, M. J. O'Leary, and N. C. Hindley; U.S. Pat. 3,598,804 (Aug. 10, 1971), N. Halder, N. C. Hindley, G. M. Jaffe, M. J. O'Leary, and P. Weinert; U.S. Pat. 3,607,862 (Sept. 21, 1971), G. M. Jaffe, W. Szkrybalo, and P. Weinert (all to Hoffmann-La Roche Inc.).
74. R. S. Glass, S. Kwoh, and E. P. Oliveto, *Carbohydr. Res.* **26**, 181 (1973).
75. U.S. Pat. 2,039,929 (May 5, 1936), T. Reichstein, U.S. Pat. 2,301,811 (Nov. 10, 1942), T. Reichstein (both to Hoffmann-La Roche Inc.); V. F. Kazimirova, *J. Gen. Chem. (USSR)* **24**, 1559 (1955).
76. J. W. Weijlard, *J. Am. Chem. Soc.* **67**, 1031 (1945); U.S. Pat. 2,367,251 (Jan. 16, 1945), J. Weijlard and J. B. Ziegler (to Merck and Co.); K. Nakagawa, R. Konaka, and T. Nakata, *J. Org. Chem.* **27**, 1597 (1962).
77. L. O. Shnaidman and I. N. Kushehinskaya, *Tr. Vses. Nauchno-Issled. Vitam. Inst.* **8**, 13 (1961).
78. U.S. Pat. 3,832,355 (Aug. 27, 1972), G. M. Jaffe and E. Pleven (to Hoffmann-La Roche Inc.).
79. U.S. Pat. 3,453,191 (July 1, 1969), G. J. Fröhlich, A. J. Kratavil, and E. Zrike (to Hoffmann-La Roche Inc.); Eur. Pats. 40331 (Nov. 25, 1981) and 40709 (Dec. 2, 1981), R. Whittmann, W. Wintermeyer, and J. Butska (to Merck GMBH); P. M. Robertson, P. Berg, H. Reimann, K. Schleich, and P. Seiler, *Chimia* **36**, 305 (1982).
80. P. P. Regna and B. P. Caldwell, *J. Am. Chem. Soc.* **66**, 246 (1944).
81. F. Elger, *Festschr. Emil Barell*, 229 (1936); Brit. Pat. 428,815 (May 20, 1935); and Brit. Pat. 466,548 (May 31, 1937), T. Reichstein (to Hoffmann-La Roche, Inc.).
82. Int. Pat. WO 99/03853 (Jan. 28, 1999), M. Oklobdzija, M. Hohnjec, (to ENCO Engineering).
83. U.S. Pat. 5,702,579 (Dec. 30, 1997), J. Veits, (to Roche Vitamins, Inc.).
84. Chemical Market Reporter, July 7, 1999.
85. Chemical Market Reporter, July 12, 1999.
86. *The United States Pharmacopeia/National Formulary (USP XXIII/NFXVIII)*, United States Pharmacopeial Convention, Inc., Rockville, Md., 1995, pp. 130, 245, 1414, 2215.
87. Food and Nutrition Board, National Research Council, *Food Chemicals Codex*, 3rd ed., National Academy Press, Washington, D.C., 1981, pp. 27, 28, 277–278.
88. L. A. Pachla and D. L. Reynolds, *J. Assoc. Off. Anal. Chem.* **68**, 1–12 (1985).
89. J. R. Cooke and R. E. D. Moxon, in *Vitamin C (Ascorbic Acid)*, J. N. Counseil and D. H. Hornig, eds., Applied Science Publishers, London, 1981, 167–198; *Adv. Chem. Ser.* **200**, 199 (1982); R. C. Rose and D. L. Nahrwold, *Anal. Biochem.* **123**, 389 (1982).
90. R. R. Eitenmiller, W. O. Landen, Jr., *Vitamin Analysis for the Health and Food Sciences*, Boca Raton: CRC Press Boca Raton, 1999, *Vitamin C*, Chapt. 6, 221–270.
91. R. R. Eitenmiller and W. O. Landen, Jr., *Vitamins, in Analyzing Food for Nutrition Labeling and Hazardous Contaminants*, I. J. Jeon and W. G. Ikins, Eds., New York: Marcel Dekker, 1995, Chapt. 9.
92. G. F. M. Ball, *Chemical and biological nature of the water-soluble vitamins, in Water-Soluble Vitamin Assays in Human Nutrition*, New York: Chapman and Hall, 1994, Chapt. 2.
93. C. I. Bliss and P. György, in P. György, ed., *Vitamin Methods*, Vol. **II**, Academic Press, New York, 1951, 244–261.
94. J. Tillmans, *Z. Unters. Lebensmittel* **54**, 33 (1927).
95. R. Strohecker, *Wiss. Veroff. Dtsch. Ges. Ernahr.* **14**, 157 (1965).
96. T. Tono and S. Fujita, *Agric. Biol. Chem.* **45**, 2947 (1981).

97. K. Dabrowski and co-workers, *J. Aquaculture* **124**, 169–192 (1994).
98. J. H. Roe and C. A. Kuether, *J. Biol. Chem.* **147**, 399–407 (1943).
99. M. J. Deutsch and C. E. Weeks, *J. Assoc. Off. Anal. Chem.* **48**, 1248–1256 (1965).
100. G. Brubacher, W. Müller-Mulot, and D. A. T. Southgate, *Methods for the Determination of Vitamins in Food Recommended by Cost 91*, Elsevier Applied Science, New York, 1985, Chapt. 7.
101. H. Goldenberg, L. Jirovetz, P. Krajnik, et al., *Anal. Chem.* **66**, 1086–1089 (1994).
102. J. Geigert, D. S. Hirano, and S. L. Neidleman, *J. Chromatogr.* **206**, 396–399 (1981).
103. S. Garcia-Castineiras, V. D. Bonnet, R. Figueroa, and M. Miranda, *J. Liq. Chromatogr.* **4**, 1619–1640 (1981).
104. W. Lee, P. Hamernyik, M. Hutchinson, V. A. Raisys, and R. F. Labbe, *Clin. Chem.* **28**, 2165–2169 (1982).
105. H. J. Kim, *J. Assoc. Official Anal. Chem.* **72**, 681–686 (1989).
106. L. L. Lloyd, F. Warner, J. F. Kennedy, and C. A. White, *J. Chromatogr.* **437**, 453–456 (1988).
107. O. Pelletier in J. Augustin, B. P. Klein, D. A. Becker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., John Wiley & Sons, Inc., New York, 1985, Chapt. 12.
108. M. H. Bui-Nguyen, in A. P. DeLeenheer, eds., *Modern Chromatographic Analysis of the Vitamins*, Marcel Dekker, New York, 1985, Chapt. 5.
109. H. Goldenberg, L. Jirovetz, P. Krajnik et.al., *Anal. Chem.* **66**, 1086–1089 (1994).
110. J. C. Deutsch, *Methods Enzymol.*, **279** (1997), (Vitamins and Coenzymes, Part I), pp. 13–24.
111. B. Borenstein, *Food Tech.* **41**, 98–99 (1987).
112. M. Liao and P. A. Seib, *Food Tech.* **41**, 104–107 (1987).
113. J. C. Bauernfeind, in *Ascorbic Acid: Chemistry, Metabolism, and Uses*, P. A. Seib and B. M. Tolbert, eds., American Chemical Society, Washington, D.C., 1982, 395–497.
114. V. S. Durve and R. T. Lovell, *Can. J. Fish Aquat. Sci.* **39**, 948–951 (1982).
115. M. Matusiewicz, K. Dabrowski, L. Volker, and K. Matusiewicz, *J. Nutr.* **125**, 3055–3061 (1995).
116. R. T. Lovell and G. O. Elnaggar, *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, Toba, Japan, (Aug. 28–Sept. 1, 1989), 159–165.
117. G.-O. Toa, *Sheng Lik'o Hseuh Chin Chan* **12**, 26 (1981).
118. K. Dabrowski, M. Matusiewicz, and J. H. Blom, *Aquaculture* **124**, 169–190 (1994).
119. U.S. Pat. No. 4,728,376 (Mar. 1, 1988), E. F. Kurtz (to Golden Powder of Texas, Inc.).
120. O. Isler, G. Brubacher, S. Ghisla, and B. Kräutler, *Vitamin II*, Georg Thieme Verlag, Stuttgart, Germany, 1988, 396–444.
121. F. Smith, *Adv. Carbohydr. Chem. Biochem.* **2**, 79 (1946).
122. J. Stanek, M. Cerny, J. Kocourek, and J. Pacak, *The Monosaccharides*, Academic Press, Inc., New York, 1963, p. 735.
123. G. Mohn, in W. Dirscherl and W. Friedrich, eds., *Fermente, Hormone, Vitamine, und die Beziehungen dieser Wirkstoffe sueinander*, 3rd ed., Vol. **3**, Pt. 1 (Vitamine), Thieme, Stuttgart, Germany, 1982, 910–913.
124. R. S. Harris, *The Vitamins*, 2nd ed., Vol. **1**, Academic Press, New York, London, 1967, 383–385.
125. P. L. Nostro, *Biol. Chem. Internet J. SCI.*
126. Z. L. Liu, *Bioradicals Detected ESR Spectrosc*. (1955), 259–275, Editor(s): H. O. Nishiguchi, L. Packer, Basel, Switzerland: Birkhaeuser.
127. Pat. JP 10279423A (1999), (to CHISSO Corp.).
128. U.S. Pat. 5,536,500 (July 16, 1996), J.-B. Galey, E. Terranova, (to L'OREAL SA).
129. M. Williams and F. A. Loewus, *Carbohydr. Res.* **63**, 149 (1978).
130. S. L. von Schuching and A. F. Abt, *Methods Enzymol.* **18**, 1 (1970).
131. I. B. Chatterjee, *Science* **82**, 1271–1272 (1973).
132. I. B. Chatterjee, A. K. Majumder, B. K. Nandi, and N. Subramanian, in *Second Conference on Vitamin C*, Vol. **258**, C. G. King and J. J. Burns, eds., New York Academy of Sciences, New York, 1975, 24–47.
133. J. J. Burns, *Am. J. Med.* **26**, 740 (1959).
134. F. A. Isherwood, Y. T. Chen, and L. W. Mapson, *Biochem. J.* **56**, 11 (1954); J. J. Burns and C. Evans, *J. Biol. Chem.* **23**, 897 (1956).
135. G. J. Dutton, ed., *Glucuronic Acid–Free and Combined: Chemistry, Biochemistry, Pharmacology and Medicine*, Academic Press, Inc., New York 1966; B. L. Horecker in G. Ritzel and G. Brubacher, eds., *Monosaccharides and Polyols in Nutrition, Therapy and Dietetics*, Hans Huber, Bern, Switzerland, 1967, p. 1.
136. A. H. Conney, G. A. Bray, C. E. Evans, and J. J. Burns, *Ann. N.Y. Acad. Sci.* **92**, 115 (1961).

137. J. J. Burns, C. Evans, and N. Trousof, *J. Biol. Chem.* **227**, 785–794 (1957).
138. F. Horio and A. Yoshida, *J. Nutr.* **112**, 416–425 (1982).
139. W. R. F. Notten, Stichting Studentenpers, University of Nijmegen, Nijmegen, The Netherlands, 1975; W. R. F. Notten and P. T. Henderson, *Int. Arch. Occup. Environ. Health* **38**, 197, 209 (1977).
140. F. A. Loewus and M. M. Baig, *Methods Enzymol.* **18**, 22 (1970); F. A. Loewus, G. Wagner, and J. C. Yang, *Ann. N.Y. Acad. Sci.* **258**, 7 (1975); F. A. Loewus in Preiss, ed., *Biochemistry of Plants*, Vol. **3**, Academic Press, Inc., New York, 1980, p. 77; C. H. Foyer and B. Halliwell, *Phytochemistry* **16**, 1347 (1977).
141. F. A. Isherwood, Y. T. Chen, and L. W. Mapson, *Biochem. J.* **56**, 1 (1954).
142. M. M. Baig, S. Kelley, and F. A. Loewus, *Plant. Physiol.* **46**, 277 (1970).
143. N. Smirnoff, *Ann. Bot. (London)* **78** (6), 661–669 (1996).
144. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, 115–124.
145. W. H. Sebrell and R. S. Harris, eds., *The Vitamins, Chemistry, Physiology, Pathology, Methods*, 2nd ed., Vol. **1**, Academic Press, Inc., New York, 1967, p. 305.
146. J. Marks, *A Guide to the Vitamins, Their Role in Health and Disease*, Medical and Technical Publishing Co., Lancaster, U.K., 1975.
147. S. Nobile and J. M. Woodhill, *Vitamin C: The Mysterious Redox-System—A Trigger of Life*, MTP Press, Boston, Mass., 1981, p. 38.
148. P. A. La Chance and M. C. Fisher, in R. Karmas and R. S. Harris, eds., *Nutritional Evaluation of Food Processing*, 3rd ed., Van Nostrand Publishing Co., New York, 1988, p. 506.
149. B. Borenstein, in T. E. Furis, ed., *Handbook of Food Additives*, 2nd ed., CRC Press, Cleveland, Ohio, 1972, pp. 91, 101.
150. G. Shklar, J. L. Schwartz, *Subcell. Biochem.* **25**, (1996), (Ascorbic Acid: Biochemistry and Biomedical Cell Biology), 233–247.
151. T. Hovi, A. Hirvimies, M. Stenvik, et.al., *Antiviral Res.* **27**, 263–270 (1995).
152. B. Peterkofsky, W. Prather, *J. Cell. Physiol.* **90**, 61–70 (1977).
153. PY. Leung, K. Miyashita, M. Young, CS. Tsao, *Anticancer Res.* **13**, 475–480 (1993).
154. M. Anderson, K. Grankvist, *Int. J. Biol.* **27**, 493–498 (1995).
155. Pat. EP 0791588 A1 (Aug. 27, 1997), M. Buncic, V. Vela, B. Suskovic, (to Pliva farmacentska, Kemijska, prehrambena).
156. A.R. Ness, D. Chee, P. Elliott, *J. Hum. Hypertens*, **11** (6), 343–350 (1997).
157. I. Jialal, C. J. Fuller, *Can. J. Cardiol.* **11** (Suppl. G), 97G–103G (1995).
158. M. R. Luck, I. Jeyaseelan, R. A. Scholes, *Biol. Reprod.* **52**, 262–266 (1995).
159. A. Taylor, *J. Am. Coll. Nutr.* **12**, 138–146 (1993).
160. Lou Ann S. Brown, D.P. Jones, *Antioxid. Health Dis.* (1996), **3** (Handbook of Antioxidants), 117–154.
161. H. Katsuki, *Subcell. Biochem.* **25** (1996), (Ascorbic Acid: Biochemistry and Biomedical Cell Biology), 293–311.
162. C. L. Rock, R. A. Jacob, P. E. Bowen, *J. Am. Diet. Assoc.* **96** (7), 693–702 (1996).
163. B. Peterkofsky and S. Udenfriend, *Proc. Nat. Acad. Sci.* **53**, 335 (1965); M. J. Barnes and E. Kodicek, *Vitam. Horm.* **30**, 1 (1972).
164. A. H. Hunt, *Br. J. Surg.* **28**, 436–461 (1941).
165. S. P. Shukla, *Experientia* **25**, 704 (1969).
166. T. T. Irvin, D. K. Chattopadhyay, and A. Smythe, *Surg. Gynec. Obstet.* **147**, 49–55 (1978).
167. S. Lewenson, A. Demetrion, In: Cohen, Dioegelmann, Lindblad, eds. *Wound Healing: Biochemical and clinical Aspects*, Philadelphia: Saunders, 1992: 248–273.
168. J. B. Chatterjee, PINSA B64, No. 3+4, pp. 213–234 (1998).
169. C. J. Bates, in J. N. Counsell and D. H. Hornig, eds., *Vitamin C (Ascorbic Acid)*, Applied Science Publishers, London, 1981.
170. V. G. Zannoni, J. I. Brodfuehrer, R. C. Smart, and R. L. Susick, in J. J. Burns, J. M. Rivers, and L. J. Machlin, eds., *Third Conference on Vitamin C*, N.Y. Academy of Sciences, New York, 1987.
171. K. Shimpo, T. Nagatsu, K. Yamada, et.al., *Am. J. Clin. Nutr.* **54**, 1298S–1301S (1991).
172. W. Ficek, *Biochem. Arch.* **11**, 21–26 (1995).
173. A. J. Brown, *Nutr. Biochem.* **7**, 29–39 (1996).
174. G. Schectman, J. C. Byrd, R. Hoffmann, *Am. J. Clin. Nutr.* **53**, 1466–1470 (1991).
175. O. Pelletier, *Ann. NY Acad. Sci.* **258**, 156–168 (1975).

176. D. O. Overman, M. N. Graham, W. A. Roy, *Teratology* **13**, 85–94 (1976).
177. Z. Kovacikova, E. Ginter, *J. Appl. Toxicol.* **15**, 321–324 (1995).
178. J. P. Gonzalez, A. Valdivieso, R. Calvo, *Eur. J. Clin. Pharmacol*, **48**, 295–297 (1995).
179. W. Dawson and G. B. West, *Br. J. Pharmacol.* **24**, 75 (1965).
180. S. N. Gershoff, *Nutr. Rev.* **51**, 313–326 (1993).
181. A. Bendich, *Food Tech.* **41**, 112–114 (1987).
182. D. M. Miller, S. D. Aust, *Arch. Biochem. Biophys.* **271**, 113–119 (1989).
183. G. R. Buettner, A. G. Motten, R. D. Hall, C. F. Chignell, *Photochem. Photobiol*, **46** (2), 161–164 (1987).
184. B. A. Jurkiewicz, G. R. Buettner, *Photochem. Photobiol.* **59** (1), 1–4 (1994).
185. A. Bendich, L. J. Machlin, O. Scandurra, G. W. Burton, and D. D. M. Wayner, *Adv. in Free Radical Biol. Med.* **2**, 419–444 (1986).
186. R. E. Beyer, *J. Bioenerget. Biomembr.*, **26**(4), 349–358 (1994).
187. B. Halliwell, *Free Rad. Res.* **25** (5), 439–454 (1996).
188. R.A. Jacob, *Subcell. Biochem.* **25** (1996), (Ascorbic Acid: Biochemistry and Biomedical Cell Biology), 1–16.
189. W. Ficek, *Biochemical Archives* **13** (4), 207–213 (1997).
190. P. Weber, A. Bendich, W. Schalch, *Internat. J. Vit. Nutr. Res.* **66**, 19–30 (1996).
191. R. J. Jariwalla, S. Harakch, in R. R. Watson, ed., *Nutrition and AIDS*, CRC Press, Boca Raton, Fla., 1994, 117–139.
192. M. A. M. Rogers, D. B. Thomas, *Nutr. Cancer Prev.* 205–237 (1996), Editor(s): R. R. Watson, S. I. Mufti, Boca Raton, Fla: CRC.
193. S. T. Omaye, P. Zhang, *Phytochemicals*, Edited by W. R. Bidlack, S. T. Omaye, M. S. Meskin, D. Jahner, Lancaster, Basel: Technomic Publishing Co. Inc. .
194. S. S. Mirvish, *J. Natl. Cancer Inst.* **71**, 629–647 (1983).
195. P. N. Magee, *Cancer Surv.* **8**, 207–239 (1989).
196. M. Cohen, H. N. Bhagavan, *J. Am. Coll. Nutr.* **14** (6), 565–578 (1995).
197. V. M. Craddock, *Eur. J. Cancer Prev.* **1**, 89–103 (1992).
198. Y. Zeng and co-workers, in *Cancer Epidemiol. Biomarkers & Prev.* **2**, 195–200 (1993).
199. S. S. Mirvish, *Cancer Res.* (Suppl.), 1948–1951 (Apr. 1, 1994).
200. C. V. Moore, R. Dubach, *Trans. Assoc. Am. Physicians* **64**, 245–246 (1951).
201. A. Bendich, L. Langseth, *J. Am. Coll. Nutr.* **14**, 124–136 (1995).
202. L. Hallberg, in L. Hallberg, *Vitamin C (Ascorbic Acid)*, J. N. Counsell and D. H. Hornig, eds., Applied Science Publishers, London, 1981, 49–61.
203. L. Hallberg, M. Brune, and L. Rossander-Hulthen, in J. J. Burns, J. M. Rivers, and L. J. Machlin, eds., *Third Conference on Vitamin C*, Vol. **498**, New York Academy of Sciences, New York, 1987, 324–332.
204. B. Halliwell, M. Whiteman, *Antioxid. Health Dis.* (1997), **5** (Vitamin C in Health and Disease), 59–73.
205. Z. Iqbal, *Sci. Int.* (Lahore) **7** (3), 357–360 (1995).
206. V. Herbert, S. Shaw, E. Jayatilleke, T. Stopler-Kasdan, *STEM CELLS* **12**, 289–303 (1994).
207. V. Herbert, S. Shaw, E. Jayatilleke, *J. Nutr.* **126** (4S), 1213S–1220S (1996).
208. E. R. Monsen and J. F. Page, *J. Agric. Food Chem.* **26**, 223–226 (1978).
209. N. W. Solomons and F. E. Viteri, in P. A. Seib and B. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism, and Uses*, American Chemical Society, Washington, D.C., 1982, 551–569.
210. C. M. Plug, D. Decker, and A. Bult, *Pharm. Weekblad* **16**, 245–248 (1984).
211. J. J. M. Marx and J. Stiekema, *Eur. J. Clin. Pharmacol.* **23**, 335–338 (1982).
212. A. Bendich in L. Packer and J. Fuchs, eds., *Vitamin C in Health and Disease*, J. Marcel Dekker Inc., NY, 1997, 411–416.
213. R. D. Crawford, *Biochem. Molec. Med.* **54**, 1–11 (1995).
214. C. J. Schorah, in T. G. Tayler, ed., *The Importance of Vitamins to Health*, MTP Press, Lancaster, U.K., 1979, 61–72.
215. B. Halliwell, *Free Rad. Res.* **25** (5), 439–454 (1996).
216. R. E. Hodges, E. M. Baker, J. Hood, H. E. Sauberlich, and S. C. March, *Am. J. Clin. Nutr.* **22**, 535–548 (1969).
217. A. M. Bode, *Adv. Pharmacol* (San Diego) (1997), **38** (Antioxidants in Disease Mechanisms and Therapy), 21–47.
218. L. A. Boxer, B. Vanderbier, S. Bonsib, et.al., *J. Cell. Physiol.* **100**, 119–126 (1979).
219. B. D. Rawal, *Chemotherapy* **24**, 166–177 (1978).
220. M. C. Goldschmidt, *Am. Clin. Nutr.* **54**, 1214S–1220S (1991).
221. R. Anderson, P. T. Lukey, *Ann. NY Acad. Sci.* **498**, 229–247 (1987).

222. R. Anderson, in Ref. 128, 249–272.
223. A. Castelli, G. E. Martorana, E. Meucci, and G. Bonetti, *Acta Vitaminol. Enzymol.* **4**, 189–196 (1982).
224. C. J. Schorah and co-workers, *Am. J. Clin. Nutr.* **34**, 871–876 (1981).
225. P. J. Garry, J. S. Goodwin, W. C. Hunt, and B. A. Gilbert, *Am. J. Clin. Nutr.* **36**, 332–339 (1982).
226. D. Hornig, J. P. Vuileumier, and D. Hartmann, *Intern. J. Vitamin Nutr. Res.* **50**, 309–314 (1980).
227. E. M. Baker, J. C. Saari, and B. M. Tolbert, *Am. J. Clin. Nutr.* **19**, 371–378 (1966).
228. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and S. C. March, *Am. J. Clin. Nutr.* **22**, 549–558 (1969).
229. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 444–454 (1971).
230. R. E. Hodges, J. Hood, J. Canham, H. E. Sauberlich, and E. M. Baker, *Am. J. Clin. Nutr.* **24**, 432–443 (1971).
231. A. B. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr.* **34**, 1347–1355 (1981).
232. D. Hornig and D. Hartmann, in P. A. Seib and P. M. Tolbert, eds., *Ascorbic Acid: Chemistry, Metabolism and Uses*, American Chemical Society, Washington, D.C., 1982, 293–316.
233. A. Kallner, D. Hartmann, and D. Hornig, *Am. J. Clin. Nutr.* **32**, 530–539 (1979).
234. E. M. Baker, D. C. Hammer, S. C. March, B. M. Tolbert, and J. E. Canham, *Science* **173**, 826–827 (1971).
235. M. Levine and co-workers, *Proc. Natl. Acad. Sci. USA* **93**, 3704 (1996).
236. A. Bendich, *Antioxidants in Health and Disease* (1997), **5** (Vitamin C in Health and Disease), 367–379.

General References

237. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Dietary Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1989, 115–124.
238. D. H. Hornig, U. Moser, and B. E. Glatthaar, in M. E. Shils and V. R. Young, eds., *Modern Nutrition in Health and Diseases*, 7th ed., Lea & Febiger, Philadelphia, 1988, 417–435.
239. R. A. Jacob, J. H. Skala, and S. T. Omave, *Am. J. Clin. Nutrit.* **46**, 818–826 (1987).
240. R. Marcus and A. M. Coulston, in A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murari, eds., *Goodman and Gilman's Pharmacological Basis of Therapeutics*, 7th ed., Macmillan Publishing Co., New York, 1985, 1151–1573.
241. U. Moser and A. Bendich, in L. J. Machlin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, 195–232.
242. R. L. Pike and M. L. Brown, *Nutrition: An Integrated Approach*, 3rd ed., Macmillan Publishing Co., New York, 1986, 131–136.
243. H. E. Sauberlich, in M. L. Brown, ed., *Present Knowledge in Nutrition*, 6th ed., International Life Sciences Institute, Nutrition Foundation, Washington, D.C., 1990, 132–141.
244. C. S. Tsao, *Antioxid. Health Dis.* (1997), **5** (Vitamin C in Health and Disease), 25–58.

VOLKER KUELLMER
ADM Nutraceutical

Related Articles

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