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# Final Report of the Safety Assessment of L-Ascorbic Acid, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate as Used in Cosmetics[1]

**L-Ascorbic Acid, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate function in cosmetic formulations primarily as antioxidants. Ascorbic Acid is commonly called Vitamin C. Ascorbic Acid is used as an antioxidant and pH adjuster in a large variety of cosmetic formulations, over 3/4 of which were hair dyes and colors at concentrations between 0.3% and 0.6%. For other uses, the reported concentrations were either very low (<0.01%) or in the 5% to 10% range. Calcium Ascorbate and Magnesium Ascorbate are described as antioxidants and skin conditioning agents—miscellaneous for use in cosmetics, but are not currently used. Sodium Ascorbyl Phosphate functions as an antioxidant in cosmetic products and is used at concentrations ranging from 0.01% to 3%. Magnesium Ascorbyl Phosphate functions as an antioxidant in cosmetics and was reported being used at concentrations from 0.001% to 3%. Sodium Ascorbate also functions as an antioxidant in cosmetics at concentrations from 0.0003% to 0.3%. Related ingredients (Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate) have been previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel and found "to be safe for use as cosmetic ingredients in the present practices of good use." Ascorbic Acid is a generally recognized as safe (GRAS) substance for use as a chemical preservative in foods and as a nutrient and/or dietary supplement. Calcium Ascorbate and Sodium Ascorbate are listed as GRAS substances for use as chemical preservatives. L-Ascorbic Acid is readily and reversibly oxidized to L-dehydroascorbic acid and both forms exist in equilibrium in the body. Permeation rates of Ascorbic Acid through whole and stripped mouse skin were $3.43 \pm 0.74$ $\mu$g/cm$^2$/h and $33.2 \pm 5.2$ $\mu$g/cm$^2$/h. Acute oral and parenteral studies in mice, rats, rabbits, guinea pigs, dogs, and cats demonstrated little toxicity. Ascorbic Acid and Sodium Ascorbate acted as a nitrosation inhibitor in several food and cosmetic product studies. No compound-related clinical signs or gross or microscopic pathological effects were observed in either mice, rats, or guinea pigs in short-term studies. Male guinea pigs fed a control basal diet and given up to 250 mg Ascorbic Acid orally for 20 weeks had similar hemoglobin, blood glucose, serum iron, liver iron, and liver glycogen levels compared to control values. Male and female F344/N rats and B6C3F$_1$ mice were fed diets containing up to 100,000 ppm Ascorbic Acid for 13 weeks with little toxicity. Chronic Ascorbic Acid feeding studies showed toxic effects at dosages above 25 mg/kg body weight (bw) in rats and guinea pigs. Groups of male and female rats given daily doses up to 2000 mg/kg bw Ascorbic Acid for 2 years had no macro- or microscopically detectable toxic lesions. Mice given Ascorbic Acid subcutaneous and intravenous daily doses (500 to 1000 mg/kg bw) for 7 days had no changes in appetite, weight gain, and general behavior; and histological examination of various organs showed no changes. Ascorbic Acid was a photoprotectant when applied to mice and pig skin before exposure to ultraviolet (UV) radiation. The inhibition of UV-induced suppression of contact hypersensitivity was also noted. Magnesium Ascorbyl Phosphate administration immediately after exposure in hairless mice significantly delayed skin tumor formation and hyperplasia induced by chronic exposure to UV radiation. Pregnant mice and rats were given daily oral doses of Ascorbic Acid up to 1000 mg/kg bw with no indications of adult-toxic, teratogenic, or fetotoxic effects. Ascorbic Acid and Sodium Ascorbate were not genotoxic in several bacterial and mammalian test systems, consistent with the antioxidant properties of these chemicals. In the presence of certain enzyme systems or metal ions, evidence of genotoxicity was seen. The National Toxicology Program (NTP) conducted a 2-year oral carcinogenesis bioassay of Ascorbic Acid (25,000 and 50,000 ppm) in F344/N rats and B6C3F$_1$ mice. Ascorbic Acid was not carcinogenic in either sex of both rats and mice. Inhibition of carcinogenesis and tumor growth related to Ascorbic Acid's antioxidant properties has been reported. Sodium Ascorbate has been shown to promote the development of urinary carcinomas in two-stage carcinogenesis studies. Dermal application of Ascorbic Acid to patients with radiation dermatitis and burn victims had no adverse effects. Ascorbic Acid was a photoprotectant in clinical human UV studies at doses well above the minimal erythema dose (MED). An opaque cream containing 5% Ascorbic Acid did not induce dermal sensitization in 103 human subjects. A product containing 10% Ascorbic Acid was nonirritant in a 4-day minicumulative patch assay on human skin and a facial treatment containing 10% Ascorbic Acid was not a contact sensitizer in a maximization assay on 26 humans. Because of the structural and functional similarities of these ingredients, the Panel believes that the data on one ingredient can be extrapolated to all of them. The Expert Panel attributed the finding that Ascorbic Acid was genotoxic in these few assay systems due to the presence of other chemicals, e.g., metals, or certain enzyme systems, which effectively convert Ascorbic Acid's antioxidant action to that of a pro-oxidant. When Ascorbic Acid acts as an antioxidant, the Panel concluded that Ascorbic Acid is not genotoxic. Supporting this view were the carcinogenicity studies conducted by the NTP, which demonstrated no evidence of carcinogenicity. Ascorbic Acid**

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Amy R. Elmore, former CIR Scientific Analyst and Writer.

**was found to effectively inhibit nitrosamine yield in several test systems. The Panel did review studies in which Sodium Ascorbate acted as a tumor promoter in animals. These results were considered to be related to the concentration of sodium ions and the pH of urine in the test animals. Similar effects were seen with sodium bicarbonate. Because of the concern that certain metal ions may combine with these ingredients to produce pro-oxidant activity, the Panel cautioned formulators to be certain that these ingredients are acting as antioxidants in cosmetic formulations. The Panel believed that the clinical experience in which Ascorbic Acid was used on damaged skin with no adverse effects and the repeat-insult patch test (RIPT) using 5% Ascorbic Acid with negative results supports the finding that this group of ingredients does not present a risk of skin sensitization. These data coupled with an absence of reports in the clinical literature of Ascorbic Acid sensitization strongly support the safety of these ingredients.**

## INTRODUCTION

This report reviews the safety of Ascorbic Acid (L-form [CAS no. 50-81-7]), commonly known as Vitamin C, Calcium Ascorbate (CAS No. 5743-27-1), Magnesium Ascorbate, Magnesium Ascorbyl Phosphate (CAS No. 114040-31-2), Sodium Ascorbate (CAS No. 134-03-2), and Sodium Ascorbyl Phosphate (CAS No. 66170-10-3) in cosmetic formulations. These ingredients function primarily as antioxidants in cosmetics.

Related ingredients, Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate, have been previously reviewed and were found "to be safe for use as cosmetic ingredients in the present practices of good use" (Cosmetic Ingredient Review [CIR] 1997).

In a review article, Bates (1997) describes Ascorbic Acid as an acidic molecule with strong reducing activity, derived from hexose sugars, and essential to most living tissue. D-Isoascorbic acid is a structural analogue but with only 5% of the antioxidant activity of L-Ascorbic Acid in vivo. The enantiomer D-ascorbic acid has no vitamin activity.

This author also notes that many living species are able to synthesize Vitamin C from hexose sugars such as glucose. The final enzyme used in this pathway is L-glucuronolactone oxidase. This enzyme is missing in species not able to synthesize Vitamin C. Such species, including humans, higher primates, guinea pigs, and birds require a dietary source. Without the required dietary Vitamin C, the body stores become depleted and the fatal deficiency disease, scurvy, manifests. Clinical scurvy is characterized by failure of wound healing, bleeding gums, bone and joint lesions, and other signs of connective tissue failure culminating in death. Although contending that there are many functions of Ascorbic Acid yet to be defined, this author states that the Ascorbic Acid reducing potential and conversion to AFR (ascorbate free radical) are key to its biological activity, including its free radical scavenging and its relationship to the oxidation of transition metals such as iron and copper at enzyme active sites and in food (Bates 1997).

## CHEMISTRY

### Chemical and Physical Properties

The chemical descriptions of Ascorbic Acid the L-form, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate are presented in tabular form in Table 1.

The chemical properties, synonyms, and specifications of these ingredients are presented in Table 2.

### Impurities and Specifications

*Ascorbic Acid*

Hoffman-La Roche Inc. (1998) provided maximum allowable impurity data for Ascorbic Acid as shown in Table 3. The official monograph of Ascorbic Acid in the *United States Pharmacopeia* (USP) (2000) states that pharmaceutical preparations contain not less than 99% and more than 100.5% $C_6H_8O_6$. The limit of heavy metals (as Pb) is 0.002% and the specific rotation between $+20.5^\circ$ and $+21.5^\circ$, which is measured immediately following preparation.

*Calcium Ascorbate*

The USP official monograph (USP 2000) states that Calcium Ascorbate pharmaceutical preparations contain not less than 98% and not more than 101% of $C_{12}H_{14}CaO_{12}\cdot 2H_2O$, calculated on as-is basis. Specific limitation for heavy metals (as Pb) is 0.001%, 0.003% arsenic, and 10 ppm fluoride. The pH is between 6.8 and 7.4 in a solution (1 in 10) and the specific rotation is measured between $+95^\circ$ and $+97^\circ$ immediately following preparation.

*Sodium Ascorbate*

The USP official monograph (USP 2000) states that Sodium Ascorbate pharmaceutical preparations contain not less than 99% and not more than 101% $C_6H_7NaO_6$, calculated on dried basis. The limit for heavy metals (as Pb) is 0.002%. The pH is between 7 and 8 in a solution (1 in 10) and the specific rotation is measured between $+103^\circ$ and $+108^\circ$ immediately following preparation.

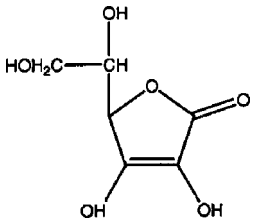
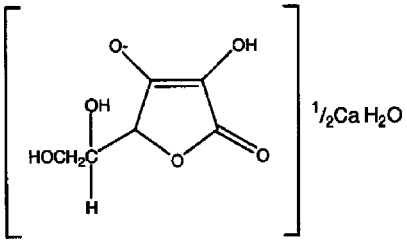
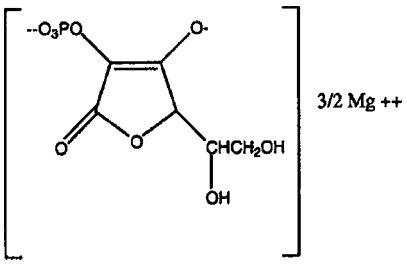
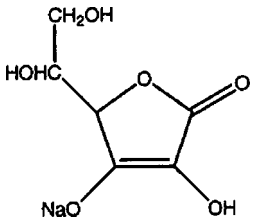
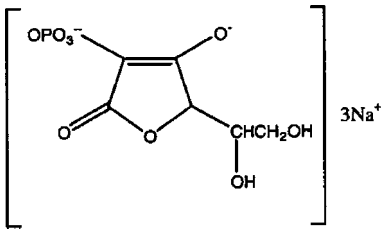
*Analytical Methods*

According to Ivanov et al. (1995), Ascorbic Acid may be detected by water-extraction, lipid removal, reaction with 2,4-dinitrophenylhydrazine, and absorption measured at 515 nm.

Bates (1997) stated that the analytical procedures used for Vitamin C analysis in food, tissues, and body fluids include redox dye reduction or ferric iron reduction, linked to optical density monitoring; formation of a colored osazone between dehydroascorbate and 2,4-dinitrophenyl-hydrazine; formation of a fluorescent quinoxaline between dehydroascorbate and *o*-phenylene diamine; high-performance liquid chromatographic (HPLC) separation linked to optical density, electro-chemical, or fluorescence detection.

Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate have all been analyzed by infrared spectroscopy (USP 2000).

**TABLE 1**
Descriptions of Ascorbic Acid the L-form, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate (Wenninger et al. 2000)

| Ingredient | Description | Structure |
|---|---|---|
| Ascorbic Acid | Ascorbic Acid is the organic compound that conforms to the formula: $C_6H_8O_6$ |  |
| Calcium Ascorbate | Calcium Ascorbate is the calcium salt of Ascorbic Acid (q.v.) $C_6H_8O_6 \cdot 1/2Ca \cdot H_2O$ |  |
| Magnesium Ascorbate | Magnesium Ascorbate is the magnesium salt of Ascorbic Acid (q.v.) $C_6H_8O_6 \cdot 1/2$ Mg | Not available |
| Magnesium Ascorbyl Phosphate | The organic compound that conforms to the formula: $C_6H_8O_9P \cdot 3/2$ Mg |  |
| Sodium Ascorbate | Sodium Ascorbate is the sodium salt of ascorbic acid that conforms to the formula: $C_6H_8O_6 \cdot Na$ |  |
| Sodium Ascorbyl Phosphate | Sodium Ascorbyl Phosphate is the organic compound that conforms to the formula: $C_6H_8O_9P \cdot 3Na$ |  |

## USE

### Cosmetic

Ascorbic Acid functions as an antioxidant and pH adjuster in cosmetic formulations (Wenninger and McEwen 2000). Of the 431 formulations reported by the Food and Drug Administration (FDA) (2001), 345 were used in hair dyes and colors (see Table 4). Westco Chemicals, Inc. (1998) reported Ascorbic Acid being used as an antioxidant, a preservative, a skin protectant, and a sunscreen agent/UV (ultraviolet) filter.

Calcium Ascorbate and Magnesium Ascorbate function as antioxidants and skin conditioning agents—miscellaneous (Wenninger et al. 2000), but there were no uses of either ingredient reported to the FDA in 2001 (FDA 2001).

**TABLE 2**
Chemical and physical properties and synonyms

| Property | Description | Reference |
|---|---|---|
| | *Ascorbic Acid* | |
| Molecular weight | 176.13 | Budavari (1989) |
| Description | White crystals with a sharp acidic taste | Budavari (1989) |
| Density | 1.65 | Budavari (1989) |
| Octanol/water partition coefficient | $0.02 \pm 0.002$ | Lee and Tojo (1998) |
| Solubility | Soluble in water and slightly soluble in acid, insoluble in ether, chloroform, benzene, petroleum ether, fixed oils and fats | Budavari (1989) |
| Melting point | 192°C | Lide (1993) |
| UV max | 245 nm (acidic); 265 nm (neutral soln.) | Lide (1993) |
| Specific rotation | $+20.5^\circ$ to $+21.5^\circ$ | US Pharmacopeial Convention (2000) |
| Synonyms | Vitamin C | Wenninger et al. (2000) |
| | L-Ascorbic acid, cevitamin, vitamin xyloascorbic acid, 3-oxo-L-gulofuranolactone, cevatine, Cescorbate, Cetamid, cevitamic acid | Budavari (1989) |
| | L-Threo-2,3,4,5,6-pentahydroxy-1-hexenoicacid-4-lactone | Hornig (1975a) |
| | 3-Keto-L-gulofuranlactone | Shamberger (1984) |
| | *Calcium Ascorbate* | |
| Molecular weight | 213.2 | National Academy of Sciences (1996) |
| Description | White to slightly yellowish, odorless, crystalline powder | National Academy of Sciences (1996) |
| Solubility | Soluble in water, slightly soluble in alcohol, insoluble in ether | National Academy of Sciences (1996) |
| pH (1:10 solution) | 6.8–7.4 | National Academy of Sciences (1996) |
| Synonyms | L-Ascorbic Acid, Calcium Salt (2:1), Dihydrate | Wenninger et al. (2000) |
| | Ascorbic Acid calcium salt, Calci-C, Calcium L-ascorbate, hemicalcium ascorbate, calcium diascorbate, calcium ascorbate anhydrous | ChemID (2000) |
| | *Magnesium Ascorbate* | |
| Synonyms | Magnesium L-Ascorbyl-2-phosphate, Ascorbyl Phosphate Magnesium, Ascorbyl PM | Wenninger et al. (2000) |
| | *Magnesium Ascorbyl Phosphate* | |
| Synonyms | Ascorbic Acid, Magnesium Salt | Wenninger et al. (2000) |
| | Ascorbyl monophosphate magnesium salt | ChemID (2000) |
| | *Sodium Ascorbate* | |
| Molecular weight | 198.12 | Budavari (1989) |
| Description | Minute white to yellowish crystals; odorless | Lewis (1993) |
| Solubility | Freely soluble in water, very slightly sol in alcohol, insoluble in chloroform and ether | Lewis (1993) |
| Decomposition | 218°C | Budavari (1989) |
| pH (aqueous) | 5.6–7.0 | Budavari (1989) |
| Synonyms | L-Ascorbic acid, monosodium salt | Wenninger et al. (2000) |
| | Sodium L-ascorbate, 3-keto-L-gulofuranlactone sodium enolate, 2,3-didehydro-L-theo-hexono-1,4-lactone sodium enolate | FAO/WHO (1974) |
| | L-Ascorbic acid sodium salt, ascorbicin, sodium-l-ascorbate, vitamin C, vitamin C sodium | Lewis (1993) |
| | *Sodium Ascorbyl Phosphate* | |
| Synonyms | L-Ascorbic Acid, 2-(dihydrogen phosphate), Trisodium Salt, Sodium L-Ascorbyl Phosphate Sodium | Wenninger et al. (2000) |

**TABLE 3**
Maximum allowable Ascorbic Acid impurities (Hoffman-La Roche Inc. 1998)

| Impurity | Allowable level |
|---|---|
| Sulphated Ash | max. 0.1% |
| Heavy Metals | max. 0.10 ppm |
| Lead | max. 10 ppm |
| Zinc | max. 25 ppm |
| Copper | max. 5 ppm |
| Iron | max. 2 ppm |
| Arsenic | max. 3 ppm |
| Oxalic Acid | max. 0.2% |

Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate function as an antioxidant in cosmetics (Wenninger et al. 2000), with 25, 5, and no uses reported to the FDA in 2001, respectively (FDA 2001).

Concentrations of use are no longer reported by industry to FDA (FDA 1992). Concentration of use data provided by Industry (Cosmetic, Toiletry, and Fragrance Association [CTFA] 2000), however, has provided current concentration of use information to CIR and these data are included in Table 4.

There are cases where a current concentration of use is given, but there were no uses reported to FDA. For example, no uses of Magnesium Ascorbyl Phosphate were reported to FDA, but industry reports current concentration of use in five product categories, at concentrations up to 3% in two categories. The CIR Expert Panel assumes that Magnesium Ascorbyl Phosphate is in current use in those five product categories at the concentrations given. Likewise, Ascorbic Acid is assumed to be in use in at least one eyebrow pencil at the low concentration of 0.0005%. For comparison purposes, historical concentrations of use (where available) reported to FDA in 1984 are provided in Table 4 (FDA 1984).

The Ministry of Health, Labor, and Welfare (2000) does not list any of the ingredients in this report as having restrictions for use in Japan. None of the ingredients in this safety assessment appear in Annex II (list of substances that must not form part of the composition of cosmetic products), Annex III (list of substances that cosmetic products must not contain except subject to the restrictions and conditions laid down), or Annex VI (list of preservatives which cosmetic products may contain) of the Cosmetics Directive of the European Union (European Economic Community 1999).

### Noncosmetic

The recommended daily dietary allowance (RDA) for ascorbate was determined by the Food and Nutrition Board of the National Research Council on the basis of several estimates that are directly relevant to scurvy. In the United States the RDA is 90 mg/day for men and 75 mg/day for women. These figures are set based on Vitamin C intake to maintain near maximal neutrophil concentration with minimal urinary excretion of ascorbate. Estimates of median dietary intakes of Vitamin C for adults are 102 mg/day in the United States. The tolerable upper intake level (UL) for adults is set at 2 g/day; the adverse effects upon which the UL is based are osmotic diarrhea and gastrointestinal disturbances. (National Academy of Sciences 2000).

The Code of Federal Regulations (CFR) includes the US FDA list of generally recognized as safe (GRAS) food additives. Ascorbic Acid is listed as a GRAS substance for use as a chemical preservative in foods (21 CFR 182.3013) and as a nutrient and/or dietary supplement (21 CFR 182.5013). The CFR lists Calcium Ascorbate and Sodium Ascorbate as GRAS substances for use as a chemical preservative in foods (21 CFR 182.3189 and 21 CFR 182.3731).

Ascorbic Acid is listed as a corn and callus remover, hair grower, wart remover, and an anorectic in the OTC Active Ingredient Status Report (FDA 1994). All these uses were ruled category II (conditions under which OTC drug products are not generally recognized as safe or effective or are misbranded). The CFR includes the U.S. Department of Agriculture regulations approving the use of Ascorbic Acid and Sodium Ascorbate as curing accelerators for pork, beef, and comminuted (finely chopped or pulverized) meat and requiring that Sodium Ascorbate be used in certain bacon preparation methods (9 CFR 318.7).

Specifications for food grade Ascorbic Acid and the other ascorbates are listed in Table 5 (National Academy of Sciences 1996).

## GENERAL BIOLOGY

As noted earlier, primates and guinea pigs, unlike other mammals, are unable to synthesize ascorbate and require a dietary source of Vitamin C. For this reason, studies have been separated according to the species used. Several studies used in vitro systems to assess specific cells/tissues and are presented in a separate section.

A review of the limited topical application data are given at the end of this section. Additional information may be found under "Clinical Pharmacokinetics."

According to the Select Committee on GRAS Substances, data from Ascorbic Acid ingestion studies are broadly applicable to ascorbates. The Committee concluded, "because ingested ascorbic acid forms both calcium and sodium ascorbates in the gastrointestinal tract . . . , the absorption and metabolism of their ascorbyl moiety would be expected to be essentially the same as for ascorbic acid" (Federation of American Societies for Experimental Biology 1979).

In a review article, Bates (1997) provided an overview of the metabolism of Ascorbic Acid. This author stated that the two forms of Vitamin C (L-Ascorbic Acid and L-dehydroascorbic acid) are interconvertible via an intermediate free radical form called ascorbate free radical (AFR) or semi- or mono-dehydroascorbate. L-Ascorbic Acid is readily and reversibly oxidized to L-dehydroascorbic acid and both forms exist in an equilibrium in the body. In alkaline solution, L-dehydroascorbic acid is

**TABLE 4**
Product formulation data

| Product category (total formulations reported to FDA) (FDA 2001) | Number of formulations containing ingredient (FDA 2001) | Current concentrations of use (CTFA 2000) (%) | Historical concentrations of use (FDA 1984) (%) |
|---|---|---|---|
| | | *Ascorbic Acid* | |
| Bubble baths (209) | 1 | — | — |
| Powders (272) | 9 | — | — |
| Hair conditioners (630) | 15 | 0.001–0.05 | ≤1 |
| Hair sprays (aerosol fixatives) (276) | 4 | — | ≤0.1 |
| Permanent waves* | — | — | ≤1 |
| Rinses (noncoloring) (41) | 1 | — | — |
| Shampoos (noncoloring) (851) | 17 | 0.0001–0.01 | ≤0.1 |
| Tonics, dressings, and other hair-grooming aids (577) | 7 | — | ≤0.1 |
| Wave sets* | — | — | ≤1 |
| Other hair preparations (276) | 6 | — | — |
| Hair dyes and colors (1588) | 345 | 0.3–0.6 | — |
| Hair tints (49) | 3 | — | — |
| Lipstick (942) | 1 | 0.001 | — |
| Makeup bases (136) | 1 | — | ≤0.1 |
| Other makeup preparations (186) | 1 | — | ≤0.1 |
| Mouthwashes and breath fresheners | 1 | — | — |
| Other manicuring preparations* | — | — | ≤1 |
| Mouthwashes and breath fresheners (46) | 1 | — | — |
| Bath soaps and detergents (405) | 2 | 0.001 | ≤0.1 |
| Cleansing (733) | 3 | 0.001–5 | ≤0.1 |
| Face and neck (excluding shaving) (304) | 3 | 0.001–10 | ≤1 |
| Body and hand (excluding shaving) (827) | 3 | 0.0001–10 | — |
| Foot powders and sprays (35) | 1 | 0.1–5 | — |
| Night preparations* | — | — | ≤1 |
| Paste masks* | — | — | ≤5 |
| Moisturizing (881) | 2 | 0.001–0.05 | ≤1 |
| Skin lighteners* | — | — | ≤1 |
| Other skin care preparations (715) | 4 | 0.01 | ≤1 |
| Eyebrow pencil (91) | — | 0.0005 | — |
| Eyeliner (514) | — | 0.001 | — |
| Eye lotion (18) | — | 0.00001 | — |
| Foundations (287) | — | 0.1 | — |
| Douches (5) | — | 0.001 | — |
| Shaving cream (aerosol, brushless, and lather) (139) | — | 0.001 | — |
| **2001 Total for Ascorbic Acid** | **431** | **0.00001–10** | **≤1–5** |
| | | *Magnesium Ascorbyl Phosphate* | |
| Other eye makeup preparations (151) | 1 | 0.001 | — |
| Other makeup preparations (186) | 1 | — | — |
| Cleansing (733) | 4 | 0.01–0.5 | — |
| Face and neck (excluding shaving) (304) | 6 | 0.05–3 | — |
| Foot powders and sprays (35) | 2 | — | — |
| Moisturizing (881) | 18 | 0.03–3 | — |
| Night (200) | 2 | — | — |
| Other skin care preparations (715) | 1 | 0.5–3 | — |

**TABLE 4**
Product formulation data *(Continued)*

| Product category (total formulations reported to FDA) (FDA 2001) | Number of formulations containing ingredient (FDA 2001) | Current concentrations of use (CTFA 2000) (%) | Historical concentrations of use (FDA 1984) (%) |
|---|---|---|---|
| Suntan gels, creams, and liquids (131) | 1 | 0.05–3 | — |
| Eye lotion (18) | — | 0.04–0.1 | — |
| Mascara (167) | — | 0.05 | — |
| Hair conditioners (636) | — | 0.001 | — |
| Shampoos (noncoloring) (860) | — | 0.001 | — |
| Tonics, dressings, and other hair-grooming aids (549) | — | 0.001 | — |
| Face powders (250) | — | 0.1–3 | — |
| Foundations (287) | — | 0.02–3 | — |
| Makeup bases (132) | — | 0.02 | — |
| Makeup fixatives (11) | — | 0.02 | — |
| Cuticle softeners (19) | — | 0.05 | — |
| Body and hand creams, lotions, powders, and sprays (excluding shaving preparations) (796) | 1 | 0.02–0.2 | — |
| Night creams, lotions, powders, and sprays (excluding shaving preparations) (188) | — | 0.04 | — |
| Paste masks (mud packs) (255) | — | 0.02 | — |
| Skin fresheners (184) | — | 0.001 | — |
| **2001 Total for Magnesium Ascorbyl Phosphate** | **37** | **0.001–3** | **—** |
| | | *Sodium Ascorbate* | |
| Lipstick (942) | 1 | 0.0003 | — |
| Body and hand (excluding shaving) (827) | 1 | — | — |
| Moisturizing (881) | 2 | — | — |
| Night (200) | 2 | — | — |
| Hair dyes and colors (all requiring caution statements and patch tests) (1572) | — | 0.3 | — |
| **2001 Total for Sodium Ascorbate** | **6** | **0.0003–0.3** | **—** |
| | | *Sodium Ascorbyl Phosphate* | |
| Eye lotion (18) | — | 0.01 | — |
| Hair sprays (aerosol fixatives) (261) | — | 0.05 | — |
| Body and hand creams, lotions, powders, and sprays (796) | — | 0.1–1 | — |
| Moisturizing creams, lotions, powders, and sprays (769) | — | 3 | — |
| Night creams, lotions, powders, and sprays (188) | — | 3 | — |
| **2001 Total for Sodium Ascorbyl Phosphate** | **—** | **0.01–3** | **—** |

*Historical use only, not in current use.

hydrolyzed to L-diketogulonic acid; this reaction is not reversible in the body.

## Ascorbic Acid Absorption, Distribution, Metabolism, Excretion

*Rats*

Four male albino Wistar rats were injected with radioactive Ascorbic Acid (6.23, 1.45, 1.46, and 0.49 mg) intraperitoneally and placed in a metabolic chamber. Urine samples and respiratory $CO_2$ were collected. The body pool of Ascorbic Acid for the rats averaged 10.7 mg/100 g bw (body weight). The amount of Ascorbic Acid synthesized per day averaged 2.6 mg/100 g bw. Urinary excretion only accounted for a fraction (15%) of the Ascorbic Acid synthesized each day. The remaining Ascorbic Acid was in part degraded to $CO_2$, and the remainder appeared as transformation products in the urine (Burns et al. 1954).

**TABLE 5**
Food grade specifications (National Academy of Sciences 1996)

| Compound | Assay % | Arsenic (mg/kg) | Heavy metals (as Pb) (mg/kg) | Fluoride (mg/kg) | Other impurities |
|---|---|---|---|---|---|
| Ascorbic Acid | Not <99 and not >100.5 $C_6H_8O_6$ | — | Not >10 | — | — |
| Calcium Ascorbate | Not <98 and not >100.5 $C_{12}H_{14}Ca_{12}O_{12}\cdot 2H_2O$ | Not >3 | Not >10 | Not >10 | Oxalate, passes test |
| Sodium Ascorbate | Not <99 and not >101 $C_6H_7NaO_6$ | — | Not >10 | — | — |

After an intraperitoneal injection of 1.5 to 5.9 mg of $^{14}C$-labeled Ascorbic Acid into rats, 19% to 29% was converted to $CO_2$ and only 0.4% was excreted as oxalic acid in the urine within 24 h (Curtain and King 1955).

*Rats and Guinea Pigs*

In a study of the metabolism of Ascorbic Acid to oxalic acid and the role of oxalic acid in formation of urinary calculi, Takenouchi et al. (1966) administered Ascorbic Acid-$^{14}C$ intraperitoneally to two rats and a guinea pig. The amount of $^{14}C$ recovered from the guinea pig was 86.8%; and 85.7% and 77.9% from rats 1 and 2, respectively. The $^{14}C$ eliminated in the feces and urine was in the range of 48% to 63% (urine) and 0.2% and 0.43% (feces) of the amount administered. In the expired air of the guinea pig, 5.5% (in 24 h) of the $^{14}C$ administered was detected, whereas 3.49% and 1.2% were found in the expired air of rat 1 and rat 2, respectively.

Proportionally, the incorporation of $^{14}C$ was markedly greater in the adrenals, lungs, and bones, 6.27, 2.80, and 2.34 times higher, respectively, than those in the liver. The authors stated that much Ascorbic Acid seems to be metabolized in the lungs serving as an excretory organ and in the bones for producing collagen. The bones gave the highest value of $^{14}C$ with 6.8%, followed by the adrenals and kidneys at 4.0%. All values for $^{14}C$ in the organs were from the guinea pig experiments (Takenouchi et al. 1966).

Schmidt et al. (1983) gave labeled Ascorbic Acid (25 $\mu$Ci) orally to male guinea pigs and male rats. Expired [$^{14}C$]carbon dioxide was collected. The radioactivity in the trapped $CO_2$, urine, feces, and tissues was measured. In both species, Ascorbic Acid was immediately metabolized to $CO_2$. The peak excretion time in rats was 2 to 3 h following oral administration and was 30 min in guinea pigs.

Of the administered labeled Ascorbic Acid, 43.2% and 46.7% could be recovered 48 h after dosage in the rat and guinea pig, respectively. Excretion pathways in the species were comparable except for the retention capacity of the liver; the guinea pig had a much larger retention compared to the rat. In separate studies, the homogenates of the liver, stomach, and small intestine of both rats and guinea pigs were incubated with labeled Ascorbic Acid; the determination of Ascorbic Acid and metabolites were performed by analytical isotachophoresis. Homogenate preparations of rat stomach, small intestine, or liver did not cause metabolic degradation on incubation with labeled Ascorbic Acid to [$^{14}C$]$CO_2$ (Schmidt et al. 1983).

*Guinea Pigs*

Reid (1969) fed 41 adult male and female guinea pigs a commercial diet devoid of Ascorbic Acid. Each animal was injected intraperitoneally each day with Ascorbic Acid (5 mg per 100 g bw). The animals remained on this regime for one month.

Ascorbic Acid was found in the stomach and small intestine of adult guinea pigs. The amount present varied with the length of time after the intraperitoneal injection of the substance, the maximum occurring at 4 h. Only small amounts of Ascorbic Acid were found in the cecum and large intestine. The maximum content of Ascorbic Acid was found in the blood and kidneys 1 h after injection, in the liver 2 h after injection, and the maximum excretion in the urine occurred between the second and third hours after injection (Reid 1969).

Ginter and Zloch (1972) divided adult male guinea pigs into a control group given a basic scorbutigenic (scurvy inducing) diet and a cholesterol-fed group given the same diet plus 0.3% cholesterol. All animals received Ascorbic Acid in a dose of 10 mg/24 h. After 18 weeks, Ascorbic Acid-*l*-$^{14}C$ was administered intraperitoneally to eight control and six cholesterol-fed guinea pigs. In all the animals, $^{14}CO_2$ output and excretion in the urine were studied for 48 h. The guinea pigs were killed and tissue Ascorbic Acid levels were determined.

The labeled Ascorbic Acid was distributed homogeneously in various tissues (liver, spleen, kidneys, adrenals, and small intestine). A marked accumulation of cholesterol occurred in the liver of cholesterol-fed animals ($\sim$10 times greater than controls). $^{14}CO_2$ output in the two groups was practically the same, but tissue Ascorbic Acid levels were significantly lower in cholesterol-fed animals (Ginter and Zloch 1972).

Sorensen et al. (1974) fed young male guinea pigs diets containing either 2 g/kg (18 control animals) or 86 g/kg (29 treatment animals) of Ascorbic Acid for 275 days. The average weight gain was significantly higher in the control group. Eight control and eight treatment animals, chosen to maintain comparable weights between the groups, were then given a totally deficient Ascorbic Acid diet 24 h before a metabolic study was initiated. In the metabolic study,$^{14}C$-labeled L-Ascorbic Acid (628 g) was then injected intraperitoneally into both treatment and control guinea

pigs to study the catabolism and excretion of the Ascorbic Acid. Catabolism of the labeled Ascorbic Acid to respiratory $^{14}CO_2$ was increased in treatment guinea pigs.

The control and treatment animals were then divided into two groups. One group received 3 mg/kg Ascorbic Acid (chronic deficiency) for 68 days. The other received a diet devoid of Ascorbic Acid (acute deficiency) for 44 days. Four control and three treatment animals from the chronic deficiency group and three control and four treatment animals from the acute deficiency group were given a totally deficient Ascorbic Acid diet 24 h before a second metabolic study was initiated. $^{14}$C-labeled L-Ascorbic Acid (628 g) was injected intraperitoneally as above. Treatment animals in the chronic deficiency and the acute deficiency groups had increased catabolism of the labeled Ascorbic Acid to respiratory $^{14}CO_2$ compared to control animals in the chronic and acute deficiency groups.

The amount of radioactivity recovered in the urine and feces was similar for both groups except for an increased urinary excretion of the label in treated animals exposed to the totally deficient diet. The treatment animals maintained higher tissue stores of Ascorbic Acid than the control animals. However, this difference was significant only in the testes. When subjected to a totally deficient diet the treatment animals were depleted of Ascorbic Acid at a faster rate than the control animals. The accelerated catabolism was not reversible by subnormal intakes of the vitamin (Sorensen et al. 1974).

Norkus and Rosso (1975) divided Hartley guinea pigs approximately 30 days pregnant into a control group receiving 25 mg Ascorbic Acid and a treated group receiving 300 mg/kg/day Ascorbic Acid daily. All animals were fed a 0.05% Ascorbic Acid diet. The groups were maintained for 10 days on their respective diets. Pups (both sexes) were randomly chosen on either day 5 or day 10 for the metabolic study. L-[*l*-$^{14}$C]Ascorbic Acid (10 $\mu$Ci/mM) was injected intraperitoneally into the pups and they were placed in a metabolic chamber for five hours to collect expired $^{14}CO_2$. From day 11 all pups were caged individually and weaned to a diet containing only traces of Ascorbic Acid. Every third day the animals were examined for physical signs of scurvy. Once signs appeared, the animals were examined daily until death. Necropsies were performed on all animals.

Pups from the treated group demonstrated a marked increase in $^{14}CO_2$ excretion following the intraperitoneal injection. Signs of scurvy appeared 4 days earlier in the treated group and mortality of the treated pups occurred approximately one week earlier. When excretion of labeled $CO_2$ in both groups was correlated with the day of onset of scurvy signs, a linear correlation was found between the two parameters, suggesting that the earlier appearance of signs of scurvy on the experimental pups is secondary to an increased rate of Ascorbic Acid catabolism (Norkus and Rosso 1975).

Nelson et al. (1978) perfused six male guinea pigs with 1.42, 4.26, and 14.2 mM concentrations of Ascorbic Acid; another six were perfused with 0.28 and 56.8 mM of Ascorbic Acid. In this study, the method of triple lumen intestinal perfusion was used. The perfusate was collected and frozen after 20 minutes. Tissue samples were taken from the proximal bowel transection site for cyclic nucleotide determination.

The rate of water secretion of the 4.26 mM ascorbate solution significantly exceeded that from all other sample concentrations. The percentage of absorption of Ascorbic Acid decreased as the amount of Ascorbic Acid entering the segment increased per unit time. Thus the highest concentration of Ascorbic Acid was the least efficiently absorbed. Significant changes in intracellular cyclic nucleotide levels were not observed in the small intestine (Nelson et al. 1978).

Norkus and Rosso (1981) fed 52 guinea pigs a 0.04% (control) Ascorbic Acid diet from day 3 of gestation. On day 31 of gestation, the animals were divided into the following groups: 16 guinea pigs continued on the control 0.04% (control) diet; 16 were fed 0.56% Ascorbic Acid diet; 5 were fed a 0.82% diet; and 15 animals were subdivided into five groups of three animals each.

These five subgroups were fed 0.56% Ascorbic Acid diet for a different week during the last 5 weeks of gestation (days 31 to 67). Before and after this week of Ascorbic Acid in the diet, these animals were maintained on the control diet.

Ten animals from the control group and six animals from the 0.56% group were killed on days 30, 45, or 60 of gestation for determinations of serum and tissue Ascorbic Acid concentrations. The remaining animals delivered at the expected time and together with their offspring were maintained on their respective diets for an additional 10 days.

Between days 5 and 10 after birth, the metabolism of a single intraperitoneal injection of $^{14}$C was determined in pups from all groups. From day 11 half of the pups were continued on the 0.56% diet and the other half plus all control pups were switched to a diet containing 0.006% Ascorbic Acid (the minimum daily requirement). Pups were randomly chosen for additional metabolic studies.

Food intake and maternal weight gain were similar in control, 0.56%, and 0.86% groups. Concentrations of total Ascorbic Acid (TAA) in serum and various organs of the mothers and fetuses were significantly higher in the 0.56% group animals compared with controls on days 45 and 60 of gestation.

Data from the control group indicated that Ascorbic Acid concentrations in maternal serum, liver and kidney remained unchanged during the last half of gestation while the tissues from the 0.56% dams accumulated progressively more TAA with time. Placental and fetal tissue in the 0.56% group contained significantly more Vitamin C than controls at days 45 and 60.

The cumulative $^{14}CO_2$ excretion from the offspring during 5 hours following the injection of [*l*-$^{14}$C]Ascorbic Acid demonstrated a marked increase in the catabolism of Ascorbic Acid in both treated groups compared with controls. After control pups were switched to the 0.06% diet, their catabolism of Ascorbic Acid decreased steadily until day 30 after birth and remained constant thereafter. Offspring from the 0.56% group switched to

the 0.06% diet demonstrated a more marked decrease in Ascorbic Acid catabolism reaching control values by 30 days after birth.

Experimental pups maintained on the 0.56% diet had a high Ascorbic Acid catabolism throughout the experiment. There was a rapid tissue depletion of excess Ascorbic Acid and a return to a consistent, low or basal catabolism of Ascorbic Acid within a short time of switching pups from a diet high in Ascorbic Acid to a diet containing 0.06% Ascorbic Acid. Prenatal exposure to high maternal intake of Ascorbic Acid for 1 week, preceded and followed by the control diet, did not affect the postnatal catabolism of Ascorbic Acid in the offspring (Norkus and Rosso 1981).

Tsao and Leung (1988) measured the urine and plasma from male Hartley guinea pigs (two groups with 12 animals each) colorimetrically for urinary and plasma levels of Ascorbic Acid. For the first four weeks both groups were fed a diet containing 0.1% Ascorbic Acid (40 mg/kg/day) and the basal levels of plasma and urinary Ascorbic Acid were measured. After the 4-week period, Sodium Ascorbate was administered by intraperitoneal injection for four weeks. Control animals received saline injections, whereas treated animals received 1.13 g/kg/day of Sodium Ascorbate (equivalent to 1 g of Ascorbic Acid). At the end of the 4-week treatment period, urine samples were taken and the injections were stopped. For the next 5 weeks the animals continued to receive 40 mg/kg/day of Ascorbic Acid and blood and urine collections continued. Basal and treatment values for urinary Ascorbic Acid, urinary dehydroascorbic acid (DHA) and plasma Ascorbic Acid values are given in Table 6.

Mean urinary Ascorbic Acid levels of control animals receiving saline injection were slightly lower than the basal value. After the saline injections were withdrawn, urinary Ascorbic Acid excretion in the controls returned to its basal value in the first week.

During the treatment period, urinary and plasma Ascorbic Acid levels rose markedly and when the Sodium Ascorbate injections were withdrawn, urinary Ascorbic Acid levels fell to pretreatment basal levels within 1 or 2 weeks. Among the 12 treated animals, 7 had abnormally low urinary Ascorbic Acid levels 2 to 4 weeks after the withdrawal. At both weeks 2 and 5 after withdrawal, the mean plasma Ascorbic Acid levels were below normal (Tsao and Leung 1988).

**TABLE 6**
Guinea pig urinary and plasma Ascorbic Acid values (Tsao and Leung 1988)

| Group | Basal | Treatment |
|---|---|---|
| | Urinary Ascorbic Acid (mg/day) | |
| Control | 0.71 ± 0.15 | 0.62 ± 0.14 |
| Treated | 0.68 ± 0.17 | 387 ± 83* |
| | Urinary DHA (mg/day) | |
| Control | 0.04 ± 0.06 | 0.07 ± 0.08 |
| Treated | 0.04 ± 0.07 | 17.44 ± 4.87* |
| | Plasma Ascorbic Acid (mg/100 ml) | |
| Control | 1.00 ± 0.07 | 1.00 ± 0.21 |
| Treated | 1.01 ± 0.14 | 2.44 ± 0.57* |

*Significant at $p < .0001$.

*Primates*

Weight et al. (1974) placed a group of seven baboons on a scorbutigenic diet for 3 months, whereas the control group received 350 mg Ascorbic Acid daily for 3 months. The biosynthesis of [$^{14}$C]cholesterol from [2-$^{14}$C]acetate and [2-$^{14}$C]mevalonate in liver homogenates containing 0, 2, and 4 mg/100 ml of Ascorbic Acid was studied.

The addition of Ascorbic Acid to the incubation mixture had no effect on the incorporation of label into cholesterol from acetate or mevalonate. The incorporation of label from mevalonate into cholesterol in the case of the baboon liver homogenates receiving Ascorbic Acid was much higher than compared to that of baboons receiving no Ascorbic Acid. There was less effect when [2-$^{14}$C]acetate was used as a precursor (Weight et al. 1974).

*In Vitro Studies*

Rose and Nahrwold (1978) maintained guinea pigs for 14 to 28 days on either commercial feed, on an Ascorbic Acid–free diet, or on diets containing 5 to 25 times the content of Ascorbic Acid compared to that of the controls. All animals were killed and the ileum resected, opened along the mesenteric border, and washed of intestinal content. A segment of the intestine was mounted, mucosal side up, in a Lucite chamber; 0.3 cm$^2$ of mucosal surface was exposed to the bathing solution. A test solution containing [$^3$H]inulin and [$^{14}$C]Ascorbic Acid was introduced into the chamber. This solution bathed the mucosal surface for two minutes and then was removed. The exposed area of tissue was cut out of the port with a steel punch and washed. Aliquots of tissue and test solution were assayed for $^3$H and $^{14}$C activity by liquid scintillation spectrometry. The uptake of [$^{14}$C]Ascorbic Acid by the tissue was determined. Animals fed the Ascorbic Acid–free diet had a lower rate of Ascorbic Acid influx after 28 days, at which time they showed symptoms of scurvy; animals maintained longer on the Ascorbic Acid–free diet died of scurvy between 32 and 40 days.

Guinea pigs fed diets having greater levels of Ascorbic Acid for 14 or 28 days had rates of Ascorbic Acid uptake 32% to 52% lower than control animals. The animals given commercial feed with the standard Ascorbic Acid content for 14 days were injected IM (intramuscularly) during this period with 300 mg of Ascorbic Acid daily. Animals were killed and the influx rates of Ascorbic Acid were determined. The Ascorbic Acid influx measured was 16% lower than in control animals. It appears that high oral doses of Ascorbic Acid limit the transport of Ascorbic Acid into the ileal mucosa (Rose and Nahrwold 1978).

Castranova et al. (1983) conducted studies to measure intracellular ascorbate content and to characterize ascorbate uptake in three fractions of isolated rat pneumocytes (alveolar

macrophages, alveolar type II epithelial cells, and another fraction of small pneumocytes that contains neither macrophages or type II cells). Cell preparations were incubated with [$^{14}$C]Ascorbic Acid plus various concentrations of unlabeled Ascorbate (not further specified). When the cells were incubated in medium containing 0.1 mM Ascorbate the authors reported intracellular Ascorbate at 3.2 mM in alveolar macrophage and type II cells, and at 0.9 mM in the other lung cells.

The Ascorbate influx was 1.5 nmol/$10^7$ cell/h for alveolar macrophages and 0.24 nmol $10^7$ cells/h for type II cells. The Ascorbate influx was very slow in other pneumocytes. Ascorbate influx displayed saturation kinetics in both alveolar macrophages and type II cells. Ascorbate intake by alveolar macrophages and type II cells was dependent on metabolic activity and extracellular sodium. Ascorbate uptake in other lung cells did not exhibit saturation kinetics and was not dependent on metabolism or sodium (Castranova et al. 1983).

Patterson et al. (1984) investigated the cellular accumulation of Ascorbic Acid in vitro in distal intestinal mucosa of adult male guinea pigs. Muscle-free strips of guinea pig intestinal mucosa were incubated with [$^{14}$C]Ascorbic Acid (8 $\mu$m/L) in a sodium buffered bathing media. Tissue/medium concentration ratios of Ascorbic Acid of at least 5 were routinely achieved.

The Ascorbic Acid uptake was highly dependent on the presence of Na in the bathing medium and substitution of Tris buffer resulted in a 97% decrease in uptake. Metabolically depleted tissue did not accumulate Ascorbic Acid against a concentration gradient. Uptake of [$^{14}$C]Ascorbic Acid from the bathing solution (8 $\mu$M/L) was reduced 67% in the presence of 0.8 mM/L nonlabeled Ascorbic Acid. The recently absorbed [$^{14}$C]Ascorbic Acid moved more rapidly back into the lumen when the luminal solution contained nonlabeled Ascorbic Acid (5 mM) than when it contained mannitol (5 mM) (Patterson et al. 1984).

Rose (1986) incubated Sprague-Dawley rat and guinea pig kidneys as slices or as isolated tubules in the presence of low concentrations of [$^{14}$C]Ascorbic Acid. Ascorbic Acid accumulated in the renal tissue of both species to a concentration three to four times that of the bathing media. Renal slices and tubules of both species appeared to transport Ascorbic Acid across the brush-border membrane. The author expressed the view that, in the kidney, Ascorbic Acid is reabsorbed by a sodium-dependent active transport mechanism that operates by concentrating Ascorbic Acid in the cellular fluid.

Tojo and Lee (1989) described a donor/receptor chamber method of assessing the penetration of Ascorbic Acid through freshly excised abdominal skin from female hairless mice (5 to 7 weeks old, HRS/J strain). The donor chamber was charged with Ascorbic Acid at 12.11 $\pm$ 0.86 mg/ml and [$^{14}$C]Ascorbic Acid at 5.93 $\pm$ 0.25 $\times$ $10^5$ disintegrations per minute (DPM)/ml in 50:50 glycerin/water. The receptor cell contained 50:50 glycerin/water. The volume of each of the cells was 3.5 ml and the diffusion area was 0.64 cm$^2$. Samples (30 $\mu$l) were taken from the receptor chamber and the amount of Ascorbic Acid determined by HPLC or liquid scintillation counting.

The results of the HPLC determination agreed substantially with the detection of radioactive label, except that the lag time in the penetration profile assayed by HPLC (1.3 h) was appreciably shorter than that determined by radioactivity counting (3.9 h). The results also indicated that only a small amount of Ascorbic Acid is bioconverted to its metabolites in the hairless mouse skin (Tojo and Lee 1989).

Dreyer and Rose (1993) incubated sliced pig lacrimal glands with [$^{14}$C]Ascorbic Acid (6 to 20 mCi/mmol) or [$^{14}$C]dehydro-L-ascorbic acid at a final concentration of 12 $\mu$M. After 40 min incubations, the tissue slices were removed, blotted, and extracted to recover the radiolabeled compound absorbed by the gland. Bathing media samples were also collected and analyzed by HPLC.

Dehydro-L-ascorbic acid was taken up to a greater extent than the reduced compound. $^{14}$C label recovered from the tissue was at least 75% in the form of Ascorbic Acid after incubation with either substrate. Uptake of both the reduced and oxidized substrates proceeded to a tissue to medium ratio in excess of unity as shown in Table 7 (Dreyer and Rose 1993).

Lee and Tojo (1996) refined their model for the intrinsic skin permeation rate of Vitamin C by addressing the stability of Ascorbic Acid in the donor and receptor solutions. As above, a freshly excised full thickness of abdominal skin of a female hairless mouse was mounted between the half cells of their in vitro skin permeation system. The donor solution was charged with radiolabeled and unlabeled Ascorbic Acid. At predetermined time intervals 30 $\mu$l of receptor solution was withdrawn and assayed for the drug concentration with HPLC or liquid scintillation counting.

The authors reported that Ascorbic Acid at the high concentrations in the donor solution was stable over the 72 h of the study. At the low initial concentrations at which Ascorbic Acid appears in the receptor solution; however, Ascorbic Acid degraded quickly. The degradation of Vitamin C followed first-order kinetics with a degradation constant of 0.26 h$^{-1}$. Correcting the measured values using this degradation constant, the authors were able to use the difference between the initial appearance of radiolabeled and unlabeled Ascorbic Acid in the receptor solution to calculate an endogenous Ascorbic Acid concentration in mouse skin of 1.08 $\mu$mole/ml (Lee and Tojo 1996).

These authors extended their work still further (Lee and Tojo 1998) by postulating that the high skin penetration of Ascorbic

**TABLE 7**

Tissue to medium concentration ratio (T:M) in pig lacrimal glands exposed to Ascorbic Acid (AA) or Dehydro Ascorbic Acid (DHAA) (Dreyer and Rose 1993)

| Compound | Final tissue concentration | Final bath concentration | T:M |
|---|---|---|---|
| AA | 6.4 $\pm$ 0.6 $\mu$M | 4.1 $\pm$ 0.5 $\mu$M | 1.6 $\pm$ 0.2 |
| DHAA | 7.3 $\pm$ 0.3 $\mu$M | 2.5 $\pm$ 0.4 $\mu$M | 3.2 $\pm$ 0.6 |

Acid could be explained by its interaction with proteins in the stratum corneum. They again used freshly excised hairless mouse skin mounted between the half cells of their in vitro skin permeation system. Intact or stripped skin was used.

The authors reported a 10-fold increase in the permeation rate through stripped skin. The permeation rates through the whole skin and stripped skin were $3.43 \pm 0.74$ $\mu g/cm^2/h$ and $33.2 \pm 5.2$ $\mu g/cm^2/h$, respectively.

To address the possible interaction of Ascorbic Acid with proteins in the stratum corneum, a differential scanning calorimetry (DSC) anaylsis was done to characterize normal mouse stratum corneum and stratum corneum after Ascorbic Acid treatment. Additional DSC transition points in the region corresponding to proteins were found in the treated skin, suggesting to the authors that protein denaturation had occurred in the treated skin and that this is the reason for the relatively high permeation of Ascorbic Acid (Lee and Tojo 1998).

### Inhibition of Ascorbic Acid Absorption and Excretion

Chloretone, barbital, aminopyrine, antipyrine, orphenadrine, meprobamate, phenylbutazone, oxyphenbutazone, sulfinpyrazone, diphenhydramine, and chlorcyclizine markedly stimulated the urinary excretion of L-Ascorbic Acid in rats. 3-Methylcholanthrene, 1,2,5,6-dibenanthracene, and 3,4-benzpyrene had a potent stimulatory effect on Ascorbic Acid synthesis. The administration of adenosine triphosphate and lycorine inhibited the synthesis of L-Ascorbic Acid (Conney et al. 1961).

Kubo (1966) studied factors that influence absorption of Ascorbic Acid using the small intestine of the guinea pig, a species requiring dietary sources of Ascorbic Acid. The absorption of Ascorbic Acid was not proportional to the administered amount of Ascorbic Acid. The author stated that absorption is accelerated by aeration and, in the pH range of 6.5 to 7.7, absorption is almost constant and is partially inhibited by 2,4-dinitrophenol.

Iwamoto et al. (1976) performed a study in which male Hartley guinea pigs were fasted for about 15 h and the stomach or small intestine was cannulated for in situ recirculation. The stomach or small intestine was first perfused with 100 ml of 0.9% NaCl solution and with 50 ml of 0.5 to 5 mM Ascorbic Acid in HCl solution. One of the following was added to the drug solution: 0.01 mM phlorizin, ouabain, and 2,4-dinitrophenol, 1 mM of L-dehydroascorbic acid (DAA), and 0.1 mM glucose.

Degradation of Ascorbic Acid in 1 h perfusates of the stomach and small intestine followed first order kinetics. The absorption rate of Ascorbic Acid from the small intestine was somewhat greater than that from the stomach especially at lower doses, and a type of saturation kinetics was observed in both sites. The $K_m$ for the stomach and small intestine was 1.30 mM and 1.27 mM, respectively. Phlorizin or glucose were considered to exert a competitively inhibiting effect on the small intestinal absorption of Ascorbic Acid, while the stomach absorption was affected neither remarkably nor competitively by the other chemicals (Iwamoto et al. 1976). This result for 2,4-dinitrophenol is in contrast to the partial inhibition found by Kubo (1966).

Siliprandi et al. (1979) determined the uptake of L-Ascorbic Acid and D- and L-glucose from the vesicles of small intestinal brush border membranes of guinea pigs from fresh intestine. Ascorbic Acid was transported into brush border membrane vesicles from the guinea pig small intestine apparently by a single, saturable carrier with an apparent $K_m$ value of approximately 0.3 mM. The system was activated by $Na^+$; in the presence of this cation, transport of Ascorbic Acid was electrically neutral. L-Ascorbate uptake was subjected to heterologous inhibition by D-glucose, in all likelihood via increased intravesicular $Na^+$ concentration. D-Isoascorbate is a fully competitive inhibitor and an efficient elicitor of L-ascorbate transport.

Das and Nebioglu (1992) determined Ascorbic Acid concentrations in samples of plasma, leukocytes, urine, feces, and adrenal glands of guinea pigs after the oral administration of (1) 10, 25, or 100 mg of Ascorbic Acid; (2) 10 mg of Ascorbic Acid and 10, 25, or 50 mg of aspirin; or (3) 25 mg of aspirin and 25, 50, and 100 mg of Ascorbic Acid.

The transport of Ascorbic Acid into leukocytes was inhibited, the plasma concentration of Ascorbic Acid was elevated significantly, and the excretion of Ascorbic Acid in the urine was increased in direct proportion to the aspirin dose when the dose of aspirin was 25 mg or greater. The concentration of Ascorbic Acid was not significantly increased after 3 h.

When a constant dose of 25 mg of aspirin was given with increasing doses of Ascorbic Acid, both plasma and leukocyte Ascorbic Acid levels were increased but not significantly after 2, 3, and 24 h. Urine Ascorbic Acid levels did not show any changes with the same doses (Das and Nebioglu 1992).

Oral administration of benzanthrone (BA) (50 mg/kg bw per day) to guinea pigs for 30 days resulted in depletion of Ascorbic Acid in the liver, adrenals, and serum, and resulted in growth retardation (36%) and an increase (18%) in relative liver weight when compared with non-treated animals. Prior daily oral administration with Ascorbic Acid (50 mg/kg bw per day) to BA-administered guinea pigs resulted in marked improvement of histopathological and biochemical changes observed in the liver, testis, kidney, and urinary bladder of BA-exposed animals (Das et al. 1994).

### Effects of Ascorbic Acid on Mineral Absorption

Sahagian et al. (1967) reported a decreased intestinal manganese, cadmium, and zinc uptake, and an increased cadmium and zinc (but not manganese) transport in vitro in rat intestine treated with $10^{-2}$ M ascorbic acid. Solomons et al. (1979) reported that Ascorbic Acid failed to improve the absorption of zinc in humans.

Albino rats in groups of five were given calcium phosphate, calcium phosphate with 250 mg of Ascorbic Acid, or calcium phosphate with 5 ml of orange, parsley, or pepper juice. Blood hemoglobin and plasma total protein concentration were determined. The percentage of Ascorbic Acid was determined in the fruits and vegetables. The data showed an enhanced rate of

calcium intestinal absorption due to the addition of Ascorbic Acid. The juices of peppers and oranges also enhanced intestinal absorption of calcium (Morcos et al. 1976).

Van den Berg (1994a, 1994b) reported that Ascorbic Acid reduced the concentration of soluble copper in the small intestine of the rat and depressed biliary copper excretion.

## Topical Application of Ascorbic Acid

In a review of topical Vitamin C and aging, Colvin and Pinnell (1996) suggested that high cutaneous levels of Vitamin C, not obtainable by ingestion, may result from topical application.

## Magnesium Ascorbyl Palmitate Absorption, Distribution, Metabolism, Excretion

*Guinea Pigs*

Imai et al. (1967) reported a study in which male guinea pigs fed a stock diet were fasted for 16 h prior to use, at which time L-Ascorbic Acid (25 mg/animal) or Magnesium Ascorbyl Phosphate (54 mg/animal) dissolved in 1 ml $H_2O$ was orally administered. A control group received 1 ml $H_2O$. About 0.6 ml of blood was taken from the heart and the blood Ascorbic Acid concentration showed rapid elevation within 1 h and began to decline thereafter. No significant differences in the change of the blood Ascorbic Acid level between the L-Ascorbic Acid and Magnesium Ascorbyl Phosphate groups were measured throughout the experiment.

*In Vitro*

Kameyama et al. (1996) studied the percutaneous absorption of Magnesium Ascorbyl Phosphate in a cream base given the designation VC-PMG by the authors. A 3% VC-PMG cream was spiked with [$^{14}$C]-labeled VC-PMG and the activity of each cream formulation was determined by liquid scintillation spectrometry.

At time 0, approximately 10 mg of each vehicle was pipetted onto dermatomed human cadaver skin that was clamped to flow-through diffusion solution cells with an exposed area of 0.64 cm$^2$. Radiolabel in the epidermis and dermis, in the skin surface wash, and in the receptor phase were measured. The percentage of the applied dose recovered was determined.

Table 8 presents the findings. The percutaneous penetration of the radiolabeled VC-PMG was low, ranging from 0.09% to 0.51% of the applied dose. The amount of the radiolabel in the entire skin after topical application of the cream was 0.58%, obtained by the addition of the amount found in the epidermis and dermis (Kameyama et al. 1996).

**TABLE 8**
Percutaneous absorption of Magnesium Ascorbyl Phosphate in a cream base (Kameyama et al. 1996)

| Location | Percent of applied dose |
|---|---|
| Epidermis | $0.70 \pm 0.23$ |
| Dermis | $0.88 \pm 0.69$ |
| Wash | $86 \pm 5$ |
| Receptor | $0.19 \pm 0.12$ |
| Total % Recovered | $88 \pm 5$ |

## Sodium Ascorbate Absorption, Distribution, Metabolism, Excretion

*Rats*

The dorsal skin including subcutaneous tissue of Wistar male rats was excised and mounted in a penetration cell. The $k_{ss}$ ($\mu g \cdot h/cm^2$) and protein thiol content were measured as diclofenac passed throughout the skin from the donor solution to the receptor solution. Diclofenac is an anti-inflammatory agent and analgesic. The $k_{ss}$ for diclofenac was 0.47 $\mu g \cdot h/cm^2$ and the protein thiol content was 6.1. When Sodium Ascorbate (1.25 to 25 mM) was added to the donor solution, the diclofenac $k_{ss}$ increased significantly, as did the protein thiol content. The $k_{ss}$ for Sodium Ascorbate (25 mM) and diclofenac was 5.09 $\mu g \cdot h/cm^2$ and the protein thiol content was 9.5 (Nishihata et al. 1988).

*Guinea Pigs*

Male guinea pigs received Sodium Ascorbate (equivalent to 1 g/kg bw/day Ascorbic Acid) solution by intraperitoneal injection for 4 weeks. Control animals received daily 2 ml of saline solution. Measurements of eight minerals (calcium, copper, iron, potassium, magnesium, manganese, sodium, and zinc) in serum and urine were made. Serum copper and zinc levels of the experimental group were significantly lower than their respective control values. With the exception of sodium, derived from injected Sodium Ascorbate, no differences were seen in daily urinary excretion of the other seven minerals (Tsao et al. 1990).

## Effects of Ascorbic Acid on Lipids and Cholesterol

*Rats*

Soni et al. (1984) studied the effects of megadoses of Ascorbic Acid on lipid levels of plasma and tissues and lipid peroxidation of tissue homogenates from Wistar rats. The rats were divided into 4 groups: control animals fed a stock diet; rats fed the stock diet and 0.5% Ascorbic Acid; rats fed the stock diet and 1% cholesterol; and rats fed the stock diet, 1% cholesterol, and 0.5% Ascorbic Acid. At the end of 4 weeks the rats were killed and blood, liver, spleen, heart, and kidneys were removed. The measurements of total fat, cholesterol, phospholipids, glycerides + free fatty acids, and Ascorbic Acid were made. The in vitro lipid peroxidation of the tissue homogenates was determined as well.

Ascorbic Acid at 0.5% in the diet did not produce any toxic effect but resulted in a significant increase in the tissue ascorbic acid content. The total lipid, glycerides + free fatty acid, and cholesterol concentrations in the plasma and liver of the ascorbate fed groups were significantly lower only under

hypercholesterolemic dietary conditions. The ratio of cholesterol to phospholipid was significantly lowered both under normal and hypercholesterolemic dietary conditions. No changes in the measured parameters were reported in the kidney, spleen, or heart of animals treated with Ascorbic Acid. A significant decrease in the in vitro lipid peroxidation of tissue homogenates was also reported (Soni et al. 1984).

*Guinea Pigs*

Becker et al. (1953) reported severely scorbutic guinea pigs (21 to 28 days depletion), when compared to normal animals fed ad libitum, incorporated 6 times as much $C^{14}$ from acetate-*l*-$C^{14}$ into cholesterol isolated from the adrenals.

Banerjee and Singh (1958) stated that female guinea pigs fed a scorbutic diet for 1 month had an increased total body cholesterol content in comparison with normal controls. Prolonged treatment of the scorbutic animals with insulin lowered the cholesterol content to a normal concentration. Although both total and esterified cholesterol content of the adrenals and spleen diminished in scorbutic guinea pigs, cholesterol content of the intestine increased and there was no change in that of blood, liver, and kidney.

Ginter et al. (1973) conducted a study in which fifty-two male guinea pigs fed a scorbutigenic diet were divided into a control group (10 mg/animal/day Ascorbic Acid) and a treated group with latent vitamin C deficiency (2 weeks on scorbutigenic diet only, followed by a maintaining dose of 0.5 mg/animal/day Ascorbic Acid). After 13 weeks, [26-$^{14}$C]cholesterol was administered intraperitoneally to all animals. The $^{14}$C excretion in expired $CO_2$ and in urine and cholesterol specific activity in the blood serum and liver were then studied at intervals of 24 h and 1, 3, 5, 7, 9, and 11 weeks.

The Ascorbic Acid concentration in the liver and spleen of control animals was 5 times greater than those of the Vitamin C–deficient animals. The total cholesterol concentration in serum and liver was significantly greater in the treated animals; analysis of serum cholesterol specific activity showed the size of the cholesterol pool A (blood and tissues with rapid cholesterol exchange) was created in treated animals. Latent Vitamin C deficiency caused a significant decrease in the rate of the transformation of cholesterol to bile acids. The researchers concluded that in the guinea pig model, latent Vitamin C deficiency decreases the rate of cholesterol catabolism with the result that cholesterol accumulates in the blood and liver (Ginter et al. 1973).

In a review of his work, Ginter (1975) stated that latent chronic Ascorbic Acid deficiency in guinea pigs provoked a metabolic disorder in the liver, causing an impaired cholesterol transformation to its principle catabolic product, bile acids. This metabolic disorder induces hypercholesterolemia and accumulation of cholesterol in the liver and slows the release of cholesterol from the circulation. High doses of Ascorbic Acid significantly stimulate cholesterol transformation to bile acids in guinea pigs and decrease plasma cholesterol concentration in humans.

*Swine*

Weinberger and Hayes (1999) divided 43 male miniature swine into four experimental groups: group I, stock diet; group II, stock diet and 200 mg/kg/day Ascorbic Acid; group III, high-cholesterol-fat diet; group IV, high-cholesterol-fat diet and 200 mg/kg/day Ascorbic Acid. The animals were treated for 16.5 weeks and then killed.

The principal target organs for cholesterol were the abdominal aorta and coronary arteries. The most severe lesions occurred in the target organs of groups III and IV. The effects of Ascorbic Acid included a significant increase in blood cholesterol and an accompanying exacerbation of the atherosclerotic process in target organs in group II. In group IV Ascorbic Acid significantly decreased blood cholesterol which was accompanied by a mild reduction in severity of altherosclerotic lesions and deposition of stainable fat in target organs when compared to group III (Weinberger and Hayes 1999).

Ginter et al. (1969) reported that chronic hypovitaminosis C (2-week scorbutigenic regime followed by administration of a maintenance dose of 0.5 mg Ascorbic Acid for 24 h) in male guinea pigs produced hypercholesterolemia and an increased accumulation of cholesterol in the liver. The Ascorbic Acid deficiency significantly increased the content of saturated fatty acids ( up to length $C_{16}$) and decreased the content of mono- and polyunsaturated fatty acids in cholesterol esters of the liver.

*Humans*

Ginter et al. (1970) studied the effect of Vitamin C on cholesterolemia in a selected group of people above the age of 40 with a seasonal deficit of Ascorbic Acid and with hypercholesterolemia (initial level of serum cholesterol in the upper limit). The group was characterized by a high consumption of animal fats and sucrose, all-the-year-round deficit of Vitamins A, $B_2$, and iron, and a seasonal deficit of Vitamin C. The experimental group received 300 mg daily of Ascorbic Acid for 47 days. After 7 weeks of Vitamin C administration, blood samples were taken and saturation tests were performed. After Ascorbic Acid administration, cholesterolemia was significantly decreased and was more pronounced in persons with hypercholesterolemia. In the control group, the cholesterol level showed no significant changes throughout the examined period.

Peterson et al. (1975) gave nine hypercholesterolemic adults, six men and three women, 4 g of Ascorbic Acid/day for 8 weeks. The Ascorbic Acid was taken in divided doses of 1 g, four times a day. Plasma cholesterol, plasma triglycerides, and weight were determined after of 2, 4, 6, 7, and 8 weeks; plasma Ascorbic Acid determinations were determined after 4 and 8 weeks after the intake of Ascorbic Acid.

There was no apparent change in plasma cholesterol or triglyceride concentrations compared with baseline levels. No significant changes in the plasma cholesterol, plasma triglyceride, or body weight occurred throughout the study. Baseline Ascorbic Acid concentrations were normal and significant increases above baseline levels were achieved at 4 and 8 weeks. No significant

difference was measured between the cholesterol and triglyceride content of any of the lipoprotein fractions before or after Ascorbic Acid treatment. There was an unexpected appearance of extra pre-bands on lipoprotein electrophoresis by the end of the treatment period (Peterson et al. 1975).

*Multiple Species*

Ginter et al. (1970) fed male guinea pigs, male Wistar rats, and male albino rabbits vitamin C deficient diets for various lengths of time up to 196 days. The guinea pigs and rabbits were administered 0.3% cholesterol and rats received 2% cholesterol supplementation. Vitamin C was withheld from the animal species capable of Ascorbic Acid synthesis (rabbit and rat) while the guinea pig was administered by intubation as a solution of 5 mg Ascorbic Acid three times per week. At the end of the experiment the animals were killed.

The cholesterol supplemented diet increased the consumption of Vitamin C in the guinea pig, as reflected by a reduction of Vitamin C concentration in the tissues and increase of its urinary output. In the rabbits and rats, cholesterol feeding resulted in an accumulation of Vitamin C in the tissues and an increased elimination in the urine (Ginter 1970).

*Effect of Ascorbic Acid on Collagen Synthesis*

Murad et al. (1981) studied collagen synthesis in human skin fibroblasts in culture. Cells were grown to confluence in growth medium (20% fetal calf serum), growth was arrested in minimal medium (5% fetal calf serum), and cells were treated with L-Ascorbic Acid or D-Isoascorbate for 0, 24, or 96 h prior to harvest and analysis of collagen and other protein. The authors reported an 8-fold increase in collagen synthesis with prolonged exposure to L-Ascorbic Acid and D-Isoascorbate, but no change in noncollagen protein. Cell growth was not affected.

Davidson et al. (1996) reported that ascorbate doses (10 to 50 $\mu$g/ml) maximally stimulated collagen production and antagonized elastin biosynthesis in pig vascular smooth muscle cells and skin fibroblasts, depending on concentration and time. Reduced elastin mRNA levels and increased collagen I and III mRNA levels were noted as well. Ascorbic Acid decreased elastin mRNA stability and Ascorbic Acid withdrawal decreased collagen I mRNA stability. Transcription of elastin was reduced by 72% by Ascorbic Acid exposure.

Dumas et al. (1996) collected normal adult fibroblasts from mammary skin and periauricular facial skin from healthy Caucasian women between the ages of 19 and 70. The primary fibroblast cultures were grown to confluence then harvested and reseeded with or without 0.15 mM Ascorbic Acid. Collagen was quantitatively determined by enzyme-linked immunosorbent assay (ELISA) type I and III collagen assay.

At 0.15 mM Ascorbic Acid, fibroblast cultures responded by an increase in collagen secretion, but to a lower extent for type III compared to type I, leading to an increase in the type I/III collagen ratio. Ascorbic Acid stimulation of both types of collagen secretion decreased in a linear manner with donor age. Also an age-related Ascorbic Acid stimulation of the cell associated collagen pool for type I collagen was reported. Analysis of Ascorbic Acid stimulation as a function of body site showed that during aging, the loss of Ascorbic Acid stimulation of type I and III collagen synthesis was more marked for periauricular than for mammary skin (Dumas et al. 1996).

The effects of Ascorbic Acid on type I and type III collagen synthesis and their mRNA levels were investigated by Tajima and Pinnell (1996). Nuclear run-on experiments demonstrated that Ascorbic Acid enhanced the transcription of type I and III collagen genes 4- and 3.4-fold, respectively, whereas transcription of type IV collagen was slightly stimulated (1.7-fold).

The effect of Ascorbic Acid on collagen subtype production was determined in Tenon's fibroblasts. Ascorbic Acid was added to cultures at concentrations of 0 to 250 $\mu$g/ml. Results from an ELISA-type dot blot revealed that Ascorbic Acid stimulated an increase in collagen production of both types that reached a maximum level at 100 $\mu$g/ml. No toxic effects were observed from 250 $\mu$g/ml Ascorbic Acid (Wendt et al. 1997).

## Effect of Sodium Ascorbate on Collagen Synthesis

To characterize the role of various cofactors in collagen synthesis in skin, Switzer and Summer (1972) focused on the hydroxylation of peptide bound proline to hydroxyproline in human diploid skin fibroblasts in culture. They measured the intracellular rate of proline hydroxylation and collagen-hydroxyproline formation as a function of Sodium Ascorbate, $\alpha$-ketoglutarate, and ferrous ion concentration in the supplemented growth medium. Cultures were treated with Sodium Ascorbate at concentrations from 0 to 200 $\mu$g/ml (Sodium Ascorbate is present in the growth medium at 5 $\mu$g/ml). The optimal concentration of Sodium Ascorbate was between 75 and 100 $\mu$g/ml. Toxic effects in cultures in logarithmic growth occurred at Sodium Ascorbate concentrations above 150 $\mu$g/ml, and at 350 $\mu$g/ml for cells in stationary growth. When Ascorbic Acid was used instead of Sodium Ascorbate, toxicity was seen only above 500 $\mu$g/ml.

## Antioxidant Activity

Husain et al. (1992) studied the effect of antioxidants on hyperoxia in rats. Adult male Wistar rats were divided into five groups. Group I served as control. Groups II to V were exposed to 80% oxygen and 20% nitrogen for 7 h a day for 5 days a week for 2 weeks in a whole body exposure chamber. Groups III, IV, and V were given (route not stated) daily Vitamin A ($60\ g \cdot kg^{-1}$), Vitamin C ($10\ mg \cdot kg^{-1}$), and Vitamin E ($10\ mg \cdot kg^{-1}$), respectively, 15 min prior to exposure. Groups I and II received normal saline. Twenty-four hours after the last exposure, blood was withdrawn from the ocular plexus and the animals were killed; the lungs and liver were removed.

As shown in Table 9, compared to control animals, group II animals had decreased blood hemoglobin and increased hydrogen peroxide induced red blood cell hemolysis. Reduced

**TABLE 9**
Effects of Vitamin C treatment on oxygen toxicity in rats (Husain et al. 1992)

| Group | Blood hemoglobin (g 100 ml$^{-1}$) | % $H_2O_2$-induced hemolysis | Reduced glutathione ($\mu$mole/ml/g) | | | Protein (mg/g) | |
|---|---|---|---|---|---|---|---|
| | | | Blood | Lung | Liver | Lung | Liver |
| I (control) | 13.29 ± 0.46 | 31.35 ± 4.02 | 20.80 ± 1.17 | 2.09 ± 0.06 | 5.70 ± 0.52 | 173.7 ± 8.3 | 252.8 ± 5.1 |
| II (oxygen exposed) | 11.22 ± 0.38 | 48.79 ± 4.93 | 17.61 ± 1.55 | 1.75 ± 0.08 | 2.77 ± 0.36 | 137.8 ± 8.8 | 215.0 ± 7.0 |
| IV (oxygen exposed + Vitamin C) | 11.92 ± 0.49 | 34.07 ± 4.95 | 22.41 ± 2.04 | 2.06 ± 0.08 | 5.14 ± 0.78 | 169.6 ± 6.5 | 240.6 ± 6.1 |

glutathione was not significantly reduced in the blood, but was reduced in the lung and liver. Treatment with Vitamin C reversed many of those toxic effects of hyperoxia (Husain et al. 1992).

Goode et al. (1995) investigated the effect of infusion of Ascorbic acid on ascorbyl radical concentrations in patients with sepsis syndrome. The baseline plasma total Ascorbic Acid, serum bleomycin-detectable 'free' Fe concentrations, and ascorbyl radical production using electron paramagnetic spectroscopsy were determined before and after intravenous infusion of Ascorbic Acid in seven patients with sepsis and nine healthy control patients.

Ascorbic Acid concentrations were markedly lower in patients (0.55 mg/ml) versus control subjects(1.71 mg/ml) and 'free' Fe was higher in patients (32.3 mmol/l) versus control subjects (4.7 mmol/l). Preinfusion ascorbyl radical concentrations were not significantly different between patients and controls. Postinfusion ascorbyl radical concentrations increased in both patients and controls. The postinfusion radical concentration in healthy subjects was significantly higher than in patients with sepsis, suggesting suboptimal basal Ascorbic Acid levels and increased scavenging of a constant oxidant pool by ascorbate in the control subjects (Goode et al. 1996).

## Effects on Visual Pigment

Organisciak et al. (1985) exposed cyclic light and dark-reared rats to intense visible light for various periods and rhodopsin was measured following recovery in darkness for up to 14 days. Twenty-four hours before and just before light exposure, animals were injected intraperitoneally with Ascorbic Acid or Sodium Ascorbate, at doses ranging from 0.062 to 1.0 g/kg bw. Control animals were injected with a water vehicle. All animals were fed ad libitum and were dark adapted for 16 to 18 h before exposure to intense light. Light intensity varied from 200 to 250 ft cd illumination. In most experiments, light exposures were for 24 h with cyclic light-reared rats and 12 h with dark-reared rats. Exposed animals remained in darkness for up to 14 days to optimize the rhodopsin concentration in the eyes.

Table 10 shows the rhodopsin retained after cyclic light-reared rats were exposed to intense light for 24 h with a full 14-days recovery. The results showed that Ascorbic Acid administration increased retinal ascorbate and reduced the loss of rhodopsin and photoreceptor nuclei resulting from intense light. Comparable doses of Ascorbic Acid and Sodium Ascorbate were equally effective in preserving rhodopsin.

The protective effect also was dose-dependent in both cyclic light- and dark-reared rats. Ascorbic Acid supplementation of cyclic light- and dark-reared rats resulted in greater retinal Ascorbic Acid levels than in the retinas of nonsupplemented animals.

Dark-reared rats had lower levels of retinal Ascorbic Acid than light-rearedones. In comparison to animals treated with Ascorbic Acid the day before and day of light exposure (day 0), Ascorbic Acid administered after the light exposure period (days 2 to 6) was not effective in reducing the loss of ocular rhopdopsin (Organisciak et al. 1985).

## Ascorbic Acid Photoprotection

Black and Chan (1975) subjected each of two groups of 100 female albino hairless mice daily (5 days/week) to suberythemic levels of UV light. The exposure level was increased every 2 weeks to compensate for epidermal thickening until 1.97 J/cm$^2$ was reached. This level was maintained through the end of 16 weeks. One group of mice was maintained on regular laboratory meal. A second group received the regular meal supplemented with 2% $(w/w)$ of the following additives: 1.2% Ascorbic Acid, 0.5% butylated hydroxytoluene, 0.2% DL-tocopherol, and 0.1% glutathione. Animals were evaluated weekly to evaluate actinic effects, including actinic keratoses, papillomas, and squamous cell carcinomas.

At the end of 22 weeks, 30% of the animals on the regular diet had frank squamous cell carcinomas compared to only 7% on the special diet. The authors also reported on their preliminary study

**TABLE 10**
Rhodopsin values in cyclic-reared rats after light exposure (Organisciak et al. 1985)

| Treatment | Rhodopsin (nmol/eye) | % of Control |
|---|---|---|
| Non–light exposed | 2.1 ± 0.1 (10 experiments) | 100 |
| Water vehicle | 0.8 ± 0.2 (15 experiments) | 38 |
| L-Ascorbic Acid | 0.3 ± 0.2 (13 experiments) | 62 |
| Sodium ascorbate | 1.2 ± 0.2 (7 experiments) | 57 |

of 13 animals receiving radiation and the treated diet (24 weeks) in which no carcinomas were observed and only 4 actinic lesions occurred in the entire group (Black and Chan 1975).

Bissett et al. (1992) exposed female albino hairless mice (Skh:HR-1) chronically to suberythemal doses of UV radiation. Mice in the treated group received a topical treatment (0.1 ml) of $\alpha$-tocopherol, Ascorbic Acid, or 2,4-hexadien-1-ol (all 5%) and an anti-inflammatory agent (hydrocortisone, naproxen, or ibuprofen) prior to each exposure. Control animals received the vehicle only. The mice were irradiated three times weekly with 30 mJ/cm$^2$ UVB radiation per exposure or five times weekly with 15 J/cm$^2$ UVA radiation per exposure to the dorsal surface.

The combination of an anti-inflammatory agent with an antioxidant was more effective against mouse skin wrinkling than were the individual test materials. UVA radiation-induced photodamage was inhibited effectively by the anti-inflammatory agent alone. Addition of the antioxidant did not increase the level of protection (Bissett et al. 1992).

Darr et al. (1992) conducted a study in which the backs of domestic Yorkshire pigs were shaved and exposed to UVB (0.45 to 0.5 mW/cm$^2$ and 0.8 mW/cm$^2$) and UVA light (2 mW/cm$^2$). Treated animals received a 10% Ascorbic Acid solution in 20% propylene glycol with 0.5% hydroxypropylcellulose and control animals received only the vehicle on shaved sites before irradiation. The experimental sites were biopsied with a 4 mm punch at 24 h for UVB experiments and sunburn cells, Ascorbic Acid levels in the skin, and cutaneous blood flow were measured. Skin was removed from non-treated animals to a depth of 500 $\mu$m and an estimation of penetration of $^{14}$C-labeled Ascorbic Acid through the skin was determined.

Percutaneous absorption studies with $^{14}$C-labeled Ascorbic Acid showed that 0.7% $\pm$ 0.1% passed through and 8.2% $\pm$ 1.1% of the applied dose was present 48 h after application. Additional experiments confirmed that skin concentrations of Ascorbic Acid increased 4- to 40-fold following multiple treatments. Ascorbic Acid concentrations in the skin were 3 to 4 mM. In UVB studies, the number of sunburn cells/4 mm biopsy were 33.1 and 20.5 cells/4 mm biopsy in control and Ascorbic Acid treated samples, respectively. Increased skin blood flow occurred 24 h after a 2 to 3 minimal erythemal dose (MED) of UVB radiation and was approximately doubled compared with adjacent nonirradiated control sites.

Pretreatment with topical Ascorbic Acid halved the increase in blood flow. In PUVA-mediated phototoxic reaction studies (8-methoxypsoralen plus UVA = PUVA), topical applications of Ascorbic Acid inhibited PUVA-mediated sunburn cell formation and in experiments using UVA doses greater than 500 mJ/cm$^2$, gross pathological changes were seen only in control sites. The Ascorbic Acid pretreated sites maintained normal histology. UV-mediated loss of cutaneous Ascorbic Acid measured 66% and nonirradiated samples measured 0% after UVB exposure (Darr et al. 1992).

Darr et al. (1996) studied the photoprotective effects of Ascorbic Acid in swine skin. Ascorbic Acid was tested in both UVB and UVA light source experiments and in combination with Vitamin E or other sunscreen agents. Sunburn cell studies were used in both UVB and UVA experiments; the protocol required punch-biopsies of experimental sites (4 mm) to be taken 24 h post exposure. These sites were then stained and analyzed for sunburn cells (basal keratinocytes having pyknotic nuclei as well as eosinophilic cytoplasm); the average number of cells per given condition were calculated.

In UVB-induced sunburn cell formation studies Ascorbic Acid reduced cell formation by 20%, while the combination of Ascorbic Acid (10% Ascorbic Acid $w/v$ and 0.1% PABA $w/v$) and PABA (a well-known photoprotectant) caused a reduction of $\sim$80%. The combination of Vitamin E and Ascorbic Acid provided protection from UVB insult, the bulk of protection attributable to Vitamin E. Ascorbic Acid was significantly better than Vitamin E in protecting against UVA-mediated phototoxic insult in sunburn cell formation studies. Additional protocols for UVA studies were required due to difficulty in sunburn cell analysis. The semiquantitative scoring scale shown in Table 11 was devised.

The authors concluded that the combination of Ascorbic Acid and Vitamin E with oxybenzone (UVA sunscreen) provided the greatest protection from UVA phototoxcity and was scored 0.5, whereas Ascorbic Acid treatment alone scored 2.9 (Darr et al. 1996).

Nakamura et al. (1997) exposed the dry shaved abdominal skin of adult C3H/HeN mice (groups of five) to UVB from a bank of four FS-20 fluorescent lamps (290 to 320 nm). The mice were exposed for four consecutive days (400 J per m$^2$ per day). Within 1 h of the final exposure dinitrofluorobenzene (DNFB) was applied to the irradiated site. DNFB (185 $\mu$g) in acetone was applied epicutaneously to shaved abdominal skin on day 0. Contact hypersensitivity was elicited 5 days later by challenging one ear of each mouse with 20 $\mu$l of 0.05% DNFB (14.8 $\mu$g) in acetone. Panels of the mice were also treated epicutaneously with Vitamin C (10% solution Ascorbic Acid $w/v$ in 20% $v/v$ propylene glycol) or the vehicle (propylene glycol)

**TABLE 11**
Score definitions for UVA photoprotection (Darr et al. 1996)

| Score | End point description |
|---|---|
| 0 | No histopathological change |
| 1 | Several keratinocytes (more than 4) with brightly eosinophilic cytoplasm and dyskeratotic nuclei (sunburn cells) |
| 2 | Sunburn cells plus vaculated deratinocytes at the dermal-epidermal junction (D/E) |
| 3 | Same as 2 plus separation at the D/E junction as a blister with $<$1/2 of specimen width involved |
| 4 | Same as 3 plus $>$1/3 specimen width involved |
| 5 | Epidermal necrosis and neutrophilic leukocyte infiltrate |

for 3 h. DNFB (25 $\mu$l) was applied epicutaneously to Vitamin C or vehicle-treated skin within 1 h of the final Vitamin C or vehicle treatment. Ears were challenged 5 days later with 20 $\mu$l of 0.05% DNFB and measured 24 and 48 h after the challenge. The authors concluded that Vitamin C abrogated the deleterious effects of acute low-dose UV radiation induction of contact hypersensitivity and prevented the induction of tolerance.

Steenvoorden et al. (1999) estimated the absorption of UVB by the highest levels of Ascorbic Acid (122 nmol/cm$^2$) and $\alpha$-tocopherol (571 pmol/cm$^2$) in the epidermis after topical administration. UV spectra were taken after dissolving the compounds separately in ethanol at concentrations of (0.12 $\mu$mol/ml and 0.57 nmol/ml, respectively) using a conventional spectrometer.

The only significant Ascorbic Acid UV absorption was found at wavelengths below 290 nm. To determine the possible contribution of UV absorption to the protection against UVB immunosuppression, the relative contribution to the immune suppression of each wavelength (taken from the efficiency spectrum) was multiplied with the transmission at that wavelength. Based on the results of that analysis, the authors excluded UV absorption as the mechanism of protection against UVB immunosuppression.

These authors also treated the shaved backs of BALB/c mice topically with Ascorbic Acid or $\alpha$-tocopherol ($\alpha$-Toc) or a combination, followed by UVB exposure. The first group was treated with Ascorbic Acid (0.5, 1, 2, and 5 $\mu$l/cm$^2$), the second with $\alpha$-Toc (2.5, 5, and 10 nmol/cm$^2$), and the third group with a combination of Ascorbic Acid (0.5, 1, and 2 $\mu$mol/cm$^2$) and $\alpha$-Toc (2.5 nmol/cm$^2$). On day 1, the treatments for groups 1 to 3 were applied to the backs of the mice and after 45 min, the shaved area of the backs were irradiated with UVB (15 kJ/m$^2$). The positive and negative controls were treated with solvent only and sham-irradiated. On day 4, all mice were sensitized by applying 100 $\mu$l (1% $v/v$) DNFB in acetone to the shaved unexposed ventral skin. The negative controls received no DNFB, but were treated with the acetone vehicle. On day 9, all animals were challenged by topical application of 10 $\mu$l (0.2% $v/v$) DNFB in acetone to one ear, whereas the other ear was treated with acetone only. The thickness of both ears was measured before treatment on day 1 and was measured 24 h after the challenge.

Irradiation of mice with 15 kJ/m2 UVB caused considerable suppression of the systemic contact hypersensitivity (CHS) response to DNFB. Ascorbic Acid dose-dependently protected against the immunosuppression when topically applied to the skin prior to irradiation. The CHS response after irradiation was reduced to 29% in unprotected control mice, and returned to 97% in mice treated with 5 $\mu$mol/cm$^2$ Ascorbic Acid prior to irradiation. This value was not significantly different from the positive control, indicating complete protection. $\alpha$-Toc was also effective at preventing UV-induced CHS at concentrations of 2.5 to 10 nmol/cm$^2$. The combination of the two antioxidants at lower concentrations resulted in a 33% protection, similar to the lower concentrations of the chemicals alone. Again, the high concentrations together provided more protection, as high as 57%, but was less than the protection of the individual compounds alone ($\sim$87%).

To investigate the mechanism of the protection, these authors treated animals with *cis*-urocanic acid (*cis*-UCA). A model which measures local versus systemic immune suppression was used. On day 1, the back of each mouse was shaved and treated with Ascorbic Acid or $\alpha$-Toc alone, or with a combination (as described in above paragraph). After 45 min, 200 $\mu$g of *cis*-UCA in 100 $\mu$l (5% $v/v$) dimethylsulfoxide in ethanol was applied to the same area of the back. The positive- and negative-control groups were treated with solvent only. Twenty-four hours post application the mice were sensitized by applying 50 $\mu$l DNFB (0.5% $v/v$) in acetone to the same skin area. The negative control was treated with acetone only. On day 6, all animals were challenged by topical application of 10 $\mu$l (0.2% $v/v$) DNFB in acetone on one ear, whereas the other ear was treated with acetone only. Further detail of the procedures is described in the above paragraph.

The immune response was reduced to 23% by the *cis*-UCA treatment in unprotected mice, and only returned to 51% (36% protection) at the maximal dose of Ascorbic Acid (5.0 $\mu$mol/cm$^2$) (Steenvoorden et al. 1999).

Quevedo et al. (2000) exposed black hairless (C3HBYB/Wq) mice daily to 1 to 3 MED (24 mJ/cm$^2$) of UVR (ultraviolet radiation) for up to 15 days. At 30 min prior to irradiation, an antioxidant lotion and cream containing both Vitamins C and E were applied to the dorsal skin of mice. The lotion vehicle contained SD alcohol 40 (45%), purified water, laureth-4, isopropyl alcohol (4%), and propylene glycol. The cream vehicle contained emulsifying wax NF, isopropyl palmitate, glycerin, sorbitol solution, benzyl alcohol, lactic acid, and purified water. The antioxidant formulations contained 10%, $w/v$ Vitamin C and 5%, $v/v$ Vitamin E. The standard volume of formulation applied to the dorsal body skin was 0.1 ml. Control mice either received no treatment or one application of the vehicle without additives before each exposure to UVR. Additional control mice were treated with the experimental formulation or the vehicle, but were not exposed to UVR.

A preliminary study established that untreated control mice exposed daily (Saturdays excluded) for a total of 15 treatments with 1 MED of UVR develop a generally uniform dark tan generated by a greatly increased number of melanogenic epidermal melanocytes. On the day following the last UVR treatment, the mice were euthanized and representative skin samples were fixed and sectioned for examination by light microscopy. For each control and treated mouse, the activity of its pigmentary system was measured by assessing gross tanning, melanocyte confluence, and melanocyte population density.

Exposure of hairless mice to UVR for 15 days induced intense tanning of their dorsal skin. The gross changes in the skin color were matched initially by the appearance of scattered epidermal melanocytes that proliferated to form discrete, progressively expanding and abutting populations, resulting in a uniform melanocyte network throughout the basal layer of the

interfollicular epidermis. In contrast, when applied topically before each daily exposure to UVR, both the cream and lotion containing Vitamins C and E inhibited UVR-induced erythema and tanning. Application of the vitamins before and after irradiation, was no more effective in providing photoprotection than pretreatment only. At the tissue level, UVR-induced proliferation of melanocytes and melanogenesis were reduced compared with irradiated controls. The density of individual melanocyte populations was reduced, as was the number of melanocyte populations achieving confluence with others. Confluence grades and cell counts, which estimate the maximum density of melanocyte populations, in UVR-vitamin-treated mice were approximately two-thirds those of UVR-vehicle-treated controls. Tanning was one-fifth that of UVR-vehicle-treated controls.

The ability of Vitamins C and E to prevent UVR-induced suppression of contact hypersensitivity was determined using the method of Strickland et al. (1994). All groups of mice were exposed to UVR (1 MED) with the topical agents 30 min prior irradiation, treated with UVR for 4 consecutive days, and were sensitized by applying a solution of 0.5% fluorescein isothiocyanate (FITC) isomer 1 in acetone:dibutylphthalate to their abdominal skin 6 h after the last UV treatment. The sensitized mice were challenged 5 days later by applying 5 $\mu$l of 0.5% FITC on both the dorsal and ventral surfaces of each ear. Vitamins C and E also inhibited UVR-induced suppression of contact hypersensitivity (Quevedo et al. 2000).

### Magnesium Ascorbyl Phosphate Photoprotection

Hairless mice were given an intraperitoneal injection of 100 mg/kg of Magnesium Ascorbyl Phosphate (MAP). Immediately afterwards, the mice received to an acute exposure of 15 kJ/m$^2$ of UVB radiation. MAP significantly prevented increases of UVB-induced lipid peroxidation in skin and sialic acid (an inflammation marker) in the serum. The administration of 50 mg/kg of MAP immediately after the same exposure in hairless mice significantly delayed skin tumor formation and hyperplasia induced by chronic exposure to 2 kJ/m$^2$ of UVB (Kobayashi et al. 1998).

### Ascorbic Acid Cellular Effects

Ascorbic Acid (100 mg/ml) and acetylsalicylic acid were injected intraperitoneally into chow-fed and tocopherol-deficient mice. In vitro lysis of red blood cells by hydrogen peroxide increased from 40% to 64% in chow-fed and from 60% to 90% in tocopherol-deficient mice. A linear response between Ascorbic Acid dose and hydrogen peroxide lysis existed (Serrill et al. 1971).

Primary cultures of rat myocytes were exposed to various doses of L-isoproterenol for 4, 12, and 24 h. L-Ascorbic Acid ($5 \times 10^{-3}$, $1.5 \times 10^{-2}$, and $3 \times 10^{-2}$ M) was added to some cultures immediately after exposure to L-isoproterenol. Leakage of lactate dehydrogenase (LDH) and cell viability were measured. A significant increase in LDH release was found 24 h after exposure to $1 \times 10^{-4}$ L-isoproterenol alone. A dose-dependent decrease in cell viability was observed 12 and 24 h after L-isoproterenol exposure. L-Ascorbic Acid ($5 \times 10^{-3}$ and $1.5 \times 10^{-2}$ M) significantly reduced the LDH release caused by L-isoproterenol. Concentrations of $1.5 \times 10^{-2}$ and $3 \times 10^{-2}$ M Ascorbic Acid caused marked LDH release. The viability of cultures treated with L-isoproterenol in the presence of $5 \times 10^{-3}$ M Ascorbic Acid was similar to controls (Ramos and Ascosta 1982).

The effect of Ascorbic Acid on the growth of cultured rabbit keratocytes was examined. In 12-day cultures, 0.05 mM Ascorbic Acid enhanced the growth of the cells, whereas 0.1 to 1.0 mM was cytotoxic. Cultures with 0.1 to 1.0 mM Ascorbic Acid contained spheriod cells that adhered to the surface of the wells (Saika 1993).

Schimdt et al. (1993) tested concentrations of L-Ascorbic Acid between 0.5 mM and 11 mM on L929 mouse fibroblasts. At concentrations above 2 mM, Ascorbic Acid inhibited cell proliferation. Cell viability also decreased as the concentration of Ascorbic Acid increased. Superoxide dismutase or catalase protected the fibroblasts from the toxic effects of Ascorbic Acid. Assays of glutathione and glutathione disulfide were determined on 8-day-old cultures exposed to the same concentrations of Ascorbic Acid for 24 h.

With increasing concentrations of Ascorbic Acid, a depletion of reduced glutathione was observed while levels of glutathione disulfide remained constant. Ascorbic Acid also induced the activity of both glucose-6-phosphate dehydrogenase and lactate dehydrogenase. A dose-dependent increase in levels of NADP occurred while NADPH remained constant. The ratio of NADPH/NADPH + NADP decreased with increasing concentrations of Ascorbic Acid. Reduced levels of ATP were observed with high concentrations of Ascorbic Acid (8 mM) (Schimdt et al. 1993).

The cytotoxicity of Ascorbate on isolated mouse islet cells was investigated by Andersson and Grankvist (1995). Ascorbate (0.5 to 2.0 mmol/L) induced a concentration-dependent increase of trypan blue uptake by the cells. Trypan blue uptake induced by 2.0 mmol/L ascorbate was inhibited by concomitant incubation of the cells with 200 mg/L superoxide dismutase, 200 mg/L catalase, 3.0 mmol/L cytochrome *c* or 50 $\mu$mol/L diethylenetriaminepentacetic acid (DTPA), but not by 50 mmol/L D-mannitol. The results indicated that Ascorbate is cytotoxic to islet cells by metal-catalyzed free radical generation.

Ponec et al. (1997) added Ascorbic Acid (50 $\mu$g/ml) to cultures of normal human epidermal keratinocytes and dermal fibroblasts. Glucosylceramides and ceramides 6 and 7 content was markedly increased, both in the presence and absence of serum and irrespective of the substrate used in Ascorbic Acid supplemented media. An improvement of the lipid profile was accompanied by a marked improvement of the barrier formation as judged from extensive production of lamellar bodies, their complete extrusion at the stratum granulosum/stratum corneum interface, and the formation of multiple broad lipid lamellar

structures in the intercorneocyte space. The presence of well-ordered lipid lamellar phases was confirmed by small angle x-ray diffraction.

According to Wu et al. (1997), Ascorbic Acid (0.5 mM) significantly inhibited bromobenzene induced enhancement of fluorescence intensity (antioxidant effect) in Hep $G_2$ cells in culture.

### Sodium Ascorbate Cellular Effects

Sodium Ascorbate (0.05 to 0.25 mM) was cytotoxic to human, chick embryo, and mouse cultured fibroblasts at inoculation and after cells were attached. Ascorbate did not affect the attachment of cells to the substratum. Preincubation of the medium containing Sodium Ascorbate alone resulted in its degradation, and the degradation products were not lethal to the cells. The lethal effect of both Sodium Ascorbate and glutathione was prevented by the addition of catalase to the medium, suggesting that hydrogen peroxide was the cytotoxic agent. $H_2O_2$ (0.05 M) added to the fibroblasts was lethal (Peterkofsky and Prather 1977).

The effects of Sodium Ascorbate with or without Vitamin $K_3$ was studied in vitro using cultured human neoplastic cell lines MCF-7 (breast carcinoma), KB (oral epidermal carcinoma), and $AN_3$-CA (endometrial adenocarcinoma) at concentrations of 0.198 $\mu$g/ml to 1.98 mg/ml. Culture media without Sodium Ascorbate and the vitamin were used as a control. At 50% confluence, different combinations of Sodium Ascorbate and Vitamin $K_3$ were added to the cultures for a 1 h incubation. DNA determinations were made. Sodium Ascorbate supplemented media had a growth inhibiting action only at high concentrations ($5 \times 10^3$ mol/L). Combined administration demonstrated a synergisitic inhibition of cell growth at 10 to 50 times lower concentrations. These results are for all three cell types (Noto et al. 1989).

L-Ascorbic Acid and Sodium Ascorbate at concentrations of 1 to 10 mM induced apoptotic cell death characterized by cell shrinkage, nuclear fragmentation, and internucleosomal DNA cleavage in human promyelocytic leukemic HL-60 cells. L- Ascorbic Acid-2-phosphate magnesium salt did not produce any of these apoptosis-associated characteristics. Electron spin resonance measurements revealed that all the active compounds were progressively degraded, producing the ascorbyl radical in culture medium, whereas the inactive compounds were stable and did not produce the radical. Exposure of HL-60 cells to Ascorbic Acid or Sodium Ascorbate resulted in the rapid elevation of intracellular $Ca^{2+}$ concentration (Sakagami et al. 1996).

### Effects on Nitrosamine Formation

Sodium Ascorbate (550 or 5500 ppm) was added to frankfurter emulsions containing 1500 ppm sodium nitrite. The quantity of dimethylnitrosamine (DMNA) was measured after 2 and 4 h of processing time. Frankfurters prepared with either 550 or 5500 ppm Sodium Ascorbate and processed for 2 h had no DMNA present, in comparison with the approximate 10 ppb DMNA present in frankfurters made with sodium nitrite alone. When cooked an additional 2 h, the addition of Sodium Ascorbate significantly reduced the quantity of nitrosamine formed compared to the positive control (sodium nitrite) (Fiddler et al. 1973).

Cardesa et al. (1974) treated male Wistar rats with solutions of dimethylamine (DMA) (1500 mg/kg bw), Sodium Ascorbate (22.5 to 720 mg/kg bw), and sodium nitrite (125 mg/kg bw) by gastric intubation. Treatments were done singly and in combination. After 48 h, the rats were killed. Blood was collected and two lobes of the liver were evaluated microscopically.

DMA plus sodium nitrite produced severe liver necrosis and an elevation of serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase concentrations. These effects were attributed to the in vivo formation of dimethylnitrosamine. Sodium Ascorbate at doses of 90 to 720 mg/kg given simultaneously with DMA completely blocked liver damage and serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase concentrations remained normal.

An analysis of DMNA in the DMA plus sodium nitrile experiment indicated that the apparent DMNA yield was at least 40 mg/kg, and that Sodium Ascorbate reduced this yield to 10 mg/kg at most (Cardesa et al. 1974).

Methylurea (MU) and sodium nitrite (100 mg and 4 g/kg, respectively) were added to a control diet, with varying quantities of Sodium Ascorbate. Groups of three to seven male MRC-Wistar rats were fed the diet and were killed after 3 h. For Sodium Ascorbate concentrations of 11.6, 5.8, 2.9, and 1.45 g/kg, the nitrosomethylurea (NMU) yields were $0.07 \pm 0.02$, $0.54 \pm 0.11$, $1.75 \pm 0.08$, $2.87 \pm 0.74$, and $4.06 \pm 0.59$ mmol/kg, respectively. The concentration of 11.6 g/kg of Sodium Ascorbate was 58 mmol/kg, equivalent to the the standard sodium nitrite concentration of 4 g/kg. A 98% inhibition of NMU production occurred with the highest ascorbate concentration (11.6 g/kg). A 50% inhibition occurred with 2.9 g/kg Sodium Ascorbate (Mirvish et al. 1975).

Pensabene et al. (1976) carried out a study in a chemical model system for bacon consisting of 60% fat, 30% $H_2O$, 8% protein, and 2% ash (lipid-aqueous-protein system). To this system was added 71 ppm pyrrolidine, $2.52 \times 10^{-4}$ mole Sodium Ascorbate, $2.52 \times 10^{-4}$ mole of the test compounds, and $2.17 \times 10^{-4}$ mole sodium nitrite. The amount of nitrosopyrrolindine (NO-Pyr) in the model was determined. The test compounds included Sodium Ascorbate, several ascorbyl esters, and Magnesium Ascorbyl Phosphate.

Sodium Ascorbate reduced NO-Pyr formation by 43% in the aqueous phase, but had little effect on NO-Pyr formation in the lipid layer. The combination of several ascorbyl esters with Sodium Ascorbate increased the inhibitory effect to 70% in the aqueous phase and 40% in the lipid phase. Magnesium Ascorbyl Phosphate gave a 9.5% reduction in the lipid phase (Pensabene et al. 1976).

Benzene or pure corn oil (100 ml) containing dipropylamine (DPA) or pyrrolidine (PYR) (0.125 mmol) was mixed with an

aqueous solution of Sodium Ascorbate (0.5 mmol) in a sodium citrate–citric acid buffer. Sodium nitrite (0.25 mmol) in a similar buffer was also added. A control mixture contained no reductant. The quantitation of nitrosamines was determined. Sodium Ascorbate increased amine nitrosation in this two-phase simulated fat system by 5 to 25 times compared with the ascorbate-free controls (Mottram and Patterson 1977).

The effect of reducing agent on the nitrosation of methylguanidine (MG) and on the in vitro activation of dimethylnitrosamine (DMN) was examined by Lo and Stich (1978). In the MG nitrosation reaction, mixtures of 0.2 M MG, 0.6 M sodium nitrite, and, in some instances, 0.3 or 0.6 M Sodium Ascorbate were added to the DMN activation system. For the application of reducing agents to the activation system, reducing agent (Sodium Ascorbate 0.25 ml), DMN (25 ml), and 0.5 ml of S-9 activation mixture were added to human skin fibroblasts. The S-9 activation system was made from the livers of adult Swiss mice. DNA repair-synthesis, clone-forming capacity, and chromosomal aberrations, and shifts in alkaline sucrose gradients were measured.

The nitrosation of MG produces reactive products that are mutagenic. At higher starting concentrations of MG and sodium nitrite the addition of Sodium Ascorbate prevented the general toxic effect but did not completely abolish DNA synthesis, indicating that DNA damage did occur. The addition of Sodium Ascorbate virtually abolished the chromosomal damage when the starting dose of MG was $<10^{-4}$ M. A 4-h application of MG, sodium nitrite, and Sodium Ascorbate, respectively, had no detectable effect on the clone-forming capacity at the concentration range used in this experiment.

The addition of Sodium Ascorbate to the standard S-9 activation preparation and DMN seemed to inhibit the formation of the reactive metabolites or prevented their action. With increasing concentration of Sodium Ascorbate in the S-9 activation mixture the degree of DNA-repair synthesis in the target cells decreased. DNA-repair synthesis was examined by irradiating a monolayer of human fibroblasts with UV (100 erg/mm$^2$), and treating the UV-irradiated cells with Sodium Ascorbate ranging from $10^{-4}$ to $5 \times 10^{-2}$ M. Sodium Ascorbate at concentrations below $10^{-2}$ M had no detectable inhibitory effect on the UV-elicited DNA-repair synthesis of cultured fibroblasts.

In a second experiment, Sodium Ascorbate was added to the S-9 activation preparation and the precarcinogen, sterigmatocystin. The unscheduled incorporation of [$^3$H]TdR was not blocked by Sodium Ascorbate. The use of alkaline sucrose gradient procedure revealed a marked shift towards smaller DNA fragments when $1 \times 10^{-2}$ M was added to the S-9 preparations during DMN activation. Sodium Ascorbate ($10^{-2}$ M) inhibited DNA synthesis by 83.1% (Lo and Stich 1978).

Douglass et al. (1978) tested a number of effective nitrosation inhibitors in the methylene chloride-mediated nitrosating system. The relative effectiveness of Ascorbic Acid in inhibiting nitrosation is given in Table 12.

Kabacoff et al. (1981) reported similar results when the effect of Ascorbic Acid on the inhibition of the formation of N-nitrosodiethanolamine was studied in an oil in water anionic solution. Ascorbic Acid was an effective inhibitor (79% inhibition).

Dunnett and Telling (1983) studied the role of Ascorbate in preventing nitrosamine formation in shampoos and skin creams. The addition of 0.05% Ascorbate was effective in preventing nitrosamine formation in products preserved with 0.01% Bronopol. At 0.05% Bronopol, 0.2% of Ascorbate was effective but not at 0.1% Bronopol.

Six groups of female Wistar rats (eight rats in each group) received a basal diet and water ad libitum for 14 weeks. Sodium nitrate and sodium nitrite, dimethylamine chlorohydrate and Ascorbic Acid were supplemented in the drinking water. The daily doses of Ascorbic Acid were 4.2 and 39.9 mg/kg bw. Animals were killed at 14 weeks. A histopathological study of the liver was done. No significant differences were found among

**TABLE 12**
Relative effectiveness of nitrosamine inhibitors (Douglass et al. 1978)

| Reagent added | Reagent solubility in $CH_2Cl_2$ (mg/L at 23°C) | Nitrosamine yield (% formed in 10 days)[a] | Time required to reach given yield (h) | | | Result |
|---|---|---|---|---|---|---|
| | | | 10% | 25% | 50% | |
| None[b] | — | 60–71 | 40 | 67 | 84 | — |
| Sodium Ascorbate[b] | Trace | 72 | 41 | 62 | 84 | — |
| Ascorbic Acid[b] | Trace | 4–7 | >450 | — | — | Inhibition |
| Ascorbyl Palmitate[b] | 1710 | 11 | 89 | — | — | Inhibition |
| Dehydroascorbic Acid[b] | 49 | 32 | 18 | 45 | — | Initial catalysis, then inhibition |

[a] Yields determined by gas chromatography, based on starting amine.

[b] Reaction mixtures consisted of 0.24 mmoles pyrrolidine and 0.47 mmoles dry powdered sodium nitrite in 10 ml methylene chloride at 23°C. Solvent dried by filtering through neutral alumina.

groups, regarding weights of the liver, free and total biliribin, and the activity of alkaline phosphatase enzymes and pyruvic glutamic transaminase. Only 25% of the animals receiving nitrate and nitrite showed mild lesions and discrete cellular swelling. The nitrate, nitrite, and dimethylamine chlorohydrate supplemented diets resulted in moderate hepatic lesions and fat metamorphosis in 75% of the animals. No lesions were noted in any of the animals receiving the three supplements and Ascorbic Acid. Hepatotoxicity induced by the daily intake of nitrate, nitrite, and dimethylamine was largely reduced by the addition of Ascorbic Acid to the drinking water (Garcia Roche et al. 1987).

Adams (1997) proposed the reaction of Ascorbic Acid with nitrous acid as shown in Figure 1 as one mechanism of preventing nitrosation in cured meats.

Fellion et al. (2000) evaluated the effects of Ascorbic Acid on *N*-nitrosodiethanolamine (NDELA) formation. Ascorbic Acid was added to solutions of diethanolamine and triethanolamine. Ascorbic Acid had a marked inhibitory effect on NDELA formation shown by its low mg/kg (ppm) values (22 mg/kg in diethanolamine solution) and 5 and 3 mg/kg in triethanolamine solution.

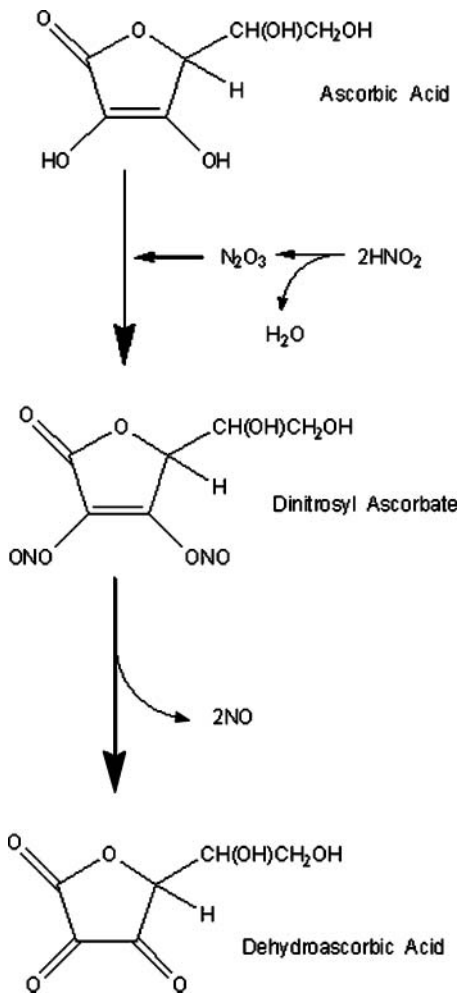



**FIGURE 1**
Reaction of Ascorbic Acid with nitrous acid (adapted from Adams 1997).

Kabacoff et al. (2000) studied the nitrosation of diethanolamine, dodecylmethylamine, and dicyclohexylamine in the absence and presence of inhibitors in model anionic and nonionic emulsions.

The authors concluded that Ascorbic Acid was an effective NDELA inhibitor in nonionic emulsions but less effective in anionic emulsions.

## Ocular Protective Effects

*Ascorbic Acid*

In a study of the effect of Ascorbic Acid on corneal ulceration and perforation following experimental alkali burns, Levinson et al. (1976) placed circular lucite wells (6- and 12-mm wells) on the cornea of the proptosed eyes of male and female New Zealand–Dutch albino rabbits and filled with 0.4 ml of 1 M sodium hydroxide (NaOH). Control animals received no treatment whereas the experimental group received a daily injection of 1.5 g Ascorbic Acid subcutaneously.

Treated corneas had persistent epithelial defects, occasional peripheral vascularization, but no ulceration or perforation. All burned corneas in the 12-mm-well group became markedly ulcerated in approximately 60% of animals and frequently perforated. In contrast, following 12-mm alkali burns and daily treatment with Ascorbic Acid, the rabbits rarely developed ulcerations and no perforations were seen (Levinson et al. 1976).

Pfister et al. (1980) conducted a study in which rabbit eyes were subjected to severe alkali burns (35 s, 12 mm, 1 M NaOH). In the first experiments, rabbits in the treated group received a daily subcutaneous injection of neutralized Ascorbic Acid solution (0.5 g/kg bw), while control animals received no treatment. At the end of the experiment (30 days), 11 of 16 eyes (68.8%) in the control group had ulcerated or formed descemetoceles, and in the treated group, 15 of 20 eyes (75%) had ulcerated, formed descemetoceles, or perforated. In the second set of experiments, burned rabbit eyes received topical 10% Ascorbic Acid whereas control eyes were treated with the vehicle only. At the termination of the experiment (34 days), 16 of 20 eyes (80%) in the control group had ulcerated or perforated corneas, compared to 5 of 18 eyes (27.5%) in the topical ascorbate treated group. Immediate topical treatment of the burned eyes achieved greatly increased aqueous humor ascorbate concentrations (Pfister et al. 1980).

Nitrites induced the oxidation of corneal thiols and reduced glutathione (GSH) in the presence of irradiation with UV light (365 nm). Nitrites are considered potent phototoxicants. Studies were carried out to measure the impact of ascorbate as a possible compatible antioxidant. This study did not specify whether the ascorbate was L-Ascorbic Acid or Sodium Ascorbate. Aqueous corneal bovine epithelial cells were incubated in the absence or presence of UV light for 15 and 30 min, with a nitrite concentration of 0.2 mM, and ascorbate, when used, at 1, 2.5, or 5.0 mM.

**TABLE 13**
SH content of the bovine corneal epithelial cell proteins (Varma et al. 1997)

| | SH Content (nmole/ml) | |
|---|---|---|
| Incubation conditions | 15 Minutes | 30 Minutes |
| Dark | 19.91 ± 0.6 | 19.33 ± 0.58 |
| Dark + nitrite | 19.34 ± 0.48 | 18.65 ± 1.14 |
| Dark + ascorbate | 17.65 ± 0.66 | 19.32 ± 0.45 |
| Dark + nitrite + ascorbate | 15.56 ± 0.37 | 17.47 ± 0.74 |
| UV | 19.7 ± 0.36 | 18.17 ± 1.07 |
| UV + nitrite | 12.78 ± 1.64 | 10.09 ± 1.37 |
| UV + nitrite + ascorbate (1.0 mM) | 15.15 ± 1.7 | 14.96 ± 1.71 |
| UV + nitrite + ascorbate (5.0 mM) | 17.01 ± 1.24 | 16.62 ± 1.93 |
| UV + nitrite + ascorbate (2.5 mM) | 18.28 ± 1.55 | 18.83 ± 2.31 |

The -SH content of the protein was determined. The authors concluded that Ascorbic Acid was found to be effective in preventing thiol oxidation, suggesting the possibility of preventing nitrogen oxide-based smog irritation to the eye by this physiologically compatible antioxidant. The results are presented in Table 13 (Varma et al. 1997).

*Sodium Ascorbate*

Pfister et al. (1991) studied the combined effect of citrate/ascorbate treatment in alkali-injured rabbit eyes. Rabbit eyes were injured with 1 M NaOH for 35 s and then were rinsed. Sodium Ascorbate (11.250 g) was dissolved in a 10% solution of citrate and was administered in the lower cul-de-sac of each eye one hour after injury. Both eyes of the rabbits received the same medication. Group I (47 eyes) received two drops of citrate every hour and Adsorbotear without EDTA (saline vehicle for both Sodium Ascorbate and the citrate) on the half-hour for 14 h/day. Group II (48 eyes) received two drops of 10% citrate every hour and 10% Sodium Ascorbate every hour for 14 h/day.

The citrate/ascorbate group had significantly fewer ulcerations than did group I (2 of 48 versus 10 of 47). Both anterior ulcers in the citrate/ascorbate group and five ulcers in group I healed by the end of the experiment (0 of 48 versus 5 of 47). The average depth of ulceration was significantly less for the citrate/ascorbate group (Pfister et al. 1991).

## Wound Healing

Sakurai et al. (1997) conducted a study in which 12 guinea pigs were shaved over the anterior neck and from the midchest level caudally and underwent subxiphoid immersion in 100°C water for 3 s to produce a 70% burn surface area second degree burn. The animals were resuscitated with Ringer's lactate solution from 6 h post burn, after which the resuscitation fluid volume was reduced to 25%. The animals were divided into two groups.

Six of the animals received 14.2 mg/kg/h Vitamin C 6 h postburn. The control animals received no Vitamin C only Ringer's solution. Both groups received identical resuscitation volumes. Twenty-four hours after injury, the animals were killed and approximately 1 $cm^2$ of skin was excised. Heart rates, mean arterial blood pressure, cardiac output, hematocrit level, and water content of burned and unburned tissue were measured before injury and at intervals thereafter.

There were no significant differences in the heart rates and mean arterial blood pressure between the groups at any time during the 24-h study period. The hematocrit values of the control group were significantly higher than those of the treated group 8 h post-burn and thereafter. Cardiac outputs between the groups showed no significant differences until 6 h after injury. The control group had significantly lower blood CO values at 7, 8, and 24 h postburn. In the treated group at 24 h postburn, the burned skin had a significantly lower water content (73.1 ± 1.1) than the skin of the control group (76 ± 0.8). This indicated lesser burn wound edema in the treated group (Sakurai et al. 1997).

Personelle et al. (1998) conducted a study to determine the effect of Ascorbic Acid on wound healing. Eight white adult New Zealand rats (four male and four female) underwent a surgical procedure in which three flaps of skin were mobilized and each triangular flap presented 5 cm length × 1 cm at the base with a ratio of 5:1. The surgery was performed at the fascia level from the vertex to the base and then sutured to its normal position with 5-0 Nylon isolated stitches. From left to right the flaps were labeled A, B, C, respectively.

Twenty-four hours later, all three flaps in each animal, presented from vertex-based direction, had deep dermo-epidermic necrosis in 16% of their extension and superficial sloft toward the base. All skin flaps C underwent biopsy 24 h after mobilization, with the necrosis already intact. All skin flaps B remained untouched as a control group, and all flaps A were treated with ACE Pool (0.02% Vitamin A, 0.20% Ascorbic Acid, and 0.05% Vitamin E) injectable solution. The injections were administered daily for 7 days beginning 24 h after the flap mobilization and 2 h after the animals were fed. The intradermal injection was consistently given in the same place and distributed around the flap base and toward the vertex including the necrotic area. One day after the last injection, biopsies from the flaps A and B were performed for microscopic examination.

The histology of flap C had dermoepidermal necrosis with acute inflammatory reactions, dilated capillary networks, increased neutrophil number, and skin annex necrosis. Flap B had the epidermis with a thick stratum corneum, ectasia of the capillary network, fibroblast proliferation with histiocytic cells within a fibrin network, and the proliferation of the collagen fibers. The biopsy A had an epidermis with a thick stratum corneum, spots of dermoepidermal necrosis, concentrated neutrophilic exudate, and skin annex with spots of dermal regeneration.

During the next 7 days, flap A treated with ACE Pool healed more quickly compared with the nontreated flaps B. Total necrosis resolution occurred, and all cutaneous structures were

restored in 87.5% of the treated flaps A. Group C flaps had serofibrin over the necrotic area in 62.5% of the rats and 25% had a thick crust attached to the dermis (Personelle et al. 1998).

### Miscellaneous

Owen et al. (1970) added Ascorbic Acid, followed by heparin, to freshly drawn blood obtained from normal mongrel dogs. Preliminary tests showed that 10 mg of Ascorbic Acid overcame the anticoagulant activity of up to 5 units (~0.05 mg) of heparin. The same result was obtained when heparin was added first, the freshly drawn blood added, and then the solution of Ascorbic Acid.

Herbert and Jacob (1974) conducted a study in which two sets of four identical meals containing moderate or high concentrations of Vitamin $B_{12}$ were homogenized and then incubated for 30 min at room temperature to mimic the digestive actions of the mouth and stomach. Doses of 0, 0.1 g, 0.25 g, and 0.5 g Ascorbic Acid were added and following incubation, the Vitamin $B_{12}$ contents of the meals were determined by radioassay.

Ascorbic Acid at 0.1 g had a minimal effect on the Vitamin $B_{12}$ content of the high-$B_{12}$ meal but appeared to destroy 43% of the $B_{12}$ content in the meal of moderate content. Larger doses of Ascorbic Acid had proportionally greater effects, with 0.25 g of Ascorbic Acid destroying 81% of the vitamin $B_{12}$ content in the moderate $B_{12}$ meal and 25% of that in the high-$B_{12}$ meal; 0.5 g destroyed slightly less than half of the Vitamin $B_{12}$ in the high-content meal, but 95% in the moderate content meal (Herbert and Jacob 1974).

According to David (1976), high doses of tyrosine are lethal to mice and Ascorbic Acid pretreatment afforded a marked protection against tyrosine toxicity. Ascorbic Acid reportedly prevented the increase of tissue tyrosine levels by stimulating *p*-hydroxyphenylpyruvic acid oxidase, increasing the urinary excretion, and inhibiting the gastrointestinal absorption of tyrosine.

Fann et al. (1986) incubated human lung parenchymal slices (100 to 200 mg) for 60 min in oxygenated Tyrode's solution alone or with Sodium Ascorbate (0.001 to 1 M) and/or methacholine (1 to 100 M) and/or indomethacin (0.17 to 17 M). Aliquots of the incubation media were assayed by radioimmunoassay for prostagladins (PGs) $PGE_2$, $PGF_{2\alpha}$, thromboxane $B_2$, and 6-keto-$PGF_{1\alpha}$. Ascorbic Acid increased the accumulation of all four prostanoids in the incubation medium, especially thromboxane $B_2$ and 6-keto-$PGF_{1\alpha}$. The effect was concentration dependent and was inhibited by indomethacin.

Kameyama et al. (1996) studied the effect of Magnesium Ascorbyl Phosphate in a cream base (material designated VC-PMG) on melanogenesis in vitro and in B16F10 murine melanoma cells and KH-$^1/_4$ human melanoma cells. Melanogenic activity was measured by radiometric assays using [U-$^{14}$C]-tyrosine for total melanin production. Purified tyrosine showed melanin formation activity of 18 ± 2 pmol at 16 h.

VC-PMG suppressed melanin formation in a dose-dependent manner, and a concentration of 0.001% significantly suppressed melanin formation by tyrosine. VC-PMG 0.1% or 1% significantly suppressed melanin formation more than 90% in B16 cell extracts. KH-$^1/_4$ cells showed 40 pmol melanin formation/$10^5$ cells per hour without VC-PMG. When cultured with 1% VC-PMG for 3 days, melanin formation was inhibited by 48% ± 5% and cell growth was slightly suppressed (Kameyama et al. 1996).

Zhang et al. (1999) reported the feasibility of eletroporation-mediated topical delivery (EMTD) of Ascorbic Acid for potential cosmetic applications. A cream containing 20% Ascorbic Acid and a crystal suspension of 33% Ascorbic Acid were applied to human cadaver skin and fresh surgical skin (full thickness skin). Six exponential pulses at 60 or 100 V and pulse lengths of 2.7 to 30 ms were used. A control group received the Ascorbic Acid only. The authors conclude that EMTD increased Ascorbic Acid penetration, but no statistical analysis was performed to determine if the findings were significant.

## ANIMAL TOXICOLOGY

### Acute Oral

*Ascorbic Acid*

The following Ascorbic Acid $LD_{50}$s were estimated: mouse >5000 mg/kg bw, rat >5000 mg/kg bw, rabbit >2000 mg/kg bw, cat >1000 mg/kg bw, dog >5000 mg/kg bw, and guinea pig >5000 mg/kg bw (Demole 1934).

Adult albino CD-1 mice fasted 18 h prior to the dosage of 4, 4.5, 5, 5.5, and 7 g/kg bw Ascorbic Acid. The acute oral $LD_{50}$ for mice dosed with Ascorbic Acid was 5.2 ± 0.19 g/kg bw. With higher doses, stupor, prostration, and death were observed (Food and Drug Research Laboratories 1974).

*Sodium Ascorbate*

FDA (1999a) listed the following acute oral $LD_{50}$ values for Sodium Ascorbate administration: mice, >5000 mg/kg bw; rats >5000 mg/kg bw; and guinea pigs >5000 mg/kg bw.

### Short-Term Oral

*Ascorbic Acid*

Demole (1934) administered daily oral doses of Ascorbic Acid (500 to 1000 mg/kg bw) for seven days. Treated mice showed no difference in appetite, weight gain and general behavior from controls receiving the same amount of biologically inactive galacturonic acid. Histological examination of various organs (kidney, pancreas, liver, heart, and lungs) showed no changes.

Guinea pigs were given Ascorbic Acid orally in daily doses of 400 to 2500 mg/kg bw for 6 days. Again there were no significant differences between control animals given the same dose of galacturonic acid and treated animals. Guinea pigs, given

Ascorbic Acid orally in daily doses of 400 to 2500 mg/kg bw for 6 days, showed no difference in appetite, weight gain, or general behavior from controls receiving the same dose of biologically inactive galaturonic acid. Various organs (kidney, liver, pancreas, heart, and lungs) had no microscopic changes (Demole 1934).

Kieckebusch et al. (1963) observed no harmful effects in rats following the oral administration of 6.5 g/kg bw Ascorbic Acid daily for a period of 10 weeks. Daily doses that reached 27.3 g/kg bw were toxic; the mortality rose to 77% within 4 weeks. The maximum nontoxic dose was 10 g/kg bw in rats.

In a study by Imai et al. (1967), male guinea pigs received either a stock diet or a scorbutigenic diet and water ad libitum for 10 days. The guinea pigs fed the scorbutigenic diet were divided into groups after the 10 day feeding period and were given orally 0.5 to 5.0 mg/day Ascorbic Acid or 1.05 to 10.5 mg/day Magnesium Ascorbyl Phosphate. Animals fed 0.5 mg/animal/day Ascorbic Acid had positive weight gains. However, this dose did not prevent the development of scurvy as efficiently as 1 mg/animal/day of Ascorbic Acid. Magnesium Ascorbyl Phosphate had a considerable effect on the prevention of scorbutic syndrome at a dose of 2.1 mg/animal/day (equimolar to 1 mg/animal/day Ascorbic Acid).

De Albuquerque and Henriques (1970) reported a study in which four groups of young rats had Ascorbic Acid added to their diet at 0%, 1%, 5%, and 10% (estimated to be 1, 5, and 10 g/kg/day). Weight gain was slightly reduced in the group receiving 1% and increasingly reduced in the other two groups. A laxative effect was noted in the group receiving 10% Ascorbic Acid, and two of the six rats in this group died.

Frith et al. (1980) conducted a study in which BALB/c male mice (288) were allocated into four groups: group 1 (48 animals), control diet; group 2 (48 animals), control diet and 500 ppm 2-acetylaminofluorene (2-AAF); group 3 (96 animals), control diet and 250 mg/ml of Ascorbic Acid in water; group 4 (96 animals), control diet, 2-AAF, and Ascorbic Acid. Food and water consumptions were measured at weekly intervals. The animals were killed at 28 days and necropsied.

There were no detectable differences in relative food consumption due to the addition of Ascorbic Acid or to an interaction of Ascorbic Acid with 2-AAF. However the presence of Ascorbic Acid in the water was associated with a significant reduction in relative water consumption. The addition of 2-AAF caused a significant increase in relative water consumption and a significant interaction of Ascorbic Acid with 2-AAF was detected.

Major histological findings were restricted to the urinary bladder. Vacuolization of the transitional epithelium, simple and nodular urothelial hyperplasia, fibrosis, and chronic inflammation of the lamina propria were found in varying degrees in the urinary bladders of mice receiving 2-AAF alone and in combination with Ascorbic Acid. The most severe lesions were seen in the mice given the combination of 2-AAF and Ascorbic Acid. The urinary bladders of mice receiving the control diet and Ascorbic Acid alone were normal. The chronic inflammation and fibrosis were restricted primarily to the fundus of the urinary bladder. The lamina propria contained an increased amount of collagen, an increase in the vasculature and an infiltration of mononuclear inflammatory cells (Frith et al. 1980).

In a National Toxicology Program study (1983) male and female F344/N rats and $B6C3F_1$ mice (groups of five males and five females of each species) were fed diets containing 0, 6000, 12,500, 25,000, 50,000, or 100,000 ppm L-Ascorbic Acid for 14 days. Rats and mice were observed twice daily for mortality and were weighed on days 1 and 15. Necropsies were performed on all animals on day 15 or 16. All animals of both species survived to the end of the dosing period.

A decrease in mean body weight gain relative to controls was greater than 10% in all dosed groups of male rats except those fed diets containing 25,000 ppm Ascorbic Acid. Weight gains for dosed female rats were increased more than 17% relative to controls, except in the 6000 ppm group (+8%) and the 25,000 ppm group (−12%). Weight gain differences were considered to be unrelated to treatment. Mice of each sex receiving 100,000 ppm lost weight. Female mice receiving 12,500 ppm gained only 0 to 0.2 g. Decreases in mean body weight gains were not dose related in male or female mice that received dietary concentrations between 6000 and 50,000 ppm. No compound-related clinical signs or gross or microscopic pathological effects were observed in either species (National Toxicology Program 1983).

Takahashi (1995) fed male Jcl:SD rats a laboratory ration for 1 week and then an experimental diet containing 5% Ascorbic Acid. Control rats continued on the basal diet. All rats were weighed daily and food consumption was recorded. Dead rats and those killed were necropsied and hemorrhagic foci were counted. Diarrhea was observed in the treated rats throughout the experiment. Ascorbic Acid did not have a hemorrhagic effect. At necropsy, edema of the stomach, hypertrophy of the kidneys and enlargement of the cecum were found in 2/6, 1/6, and 6/6 rats, respectively.

FDA (1999b) listed the following oral $LD_{50}$ values for Ascorbic Acid administration: mice, 8021 mg/kg/day for 10 days; rats, >6500 mg/kg/day for 6 days; guinea pigs, >8900 mg/kg/day for >6 days; and dogs 100 mg/kg/day for 7 days.

*Magnesium Ascorbyl Phosphate*

Male guinea pigs were fed either a stock diet or a scorbutigenic diet and water ad libitum for 10 days. The guinea pigs fed the scorbutigenic diet were divided into groups after the 10-day feeding period and were orally dosed with 0.5 to 5.0 mg/day Ascorbic Acid or 1.05–10.5 mg/day Magnesium Ascorbyl Phosphate. Animals receiving 0.5 mg/animal/day Ascorbic Acid had positive weight gains. However, this dose did not prevent the development of scurvy as efficiently as 1 mg/animal/day of Ascorbic Acid. Magnesium Ascorbyl Phosphate had a considerable effect on the prevention of

scorbutic syndrome at the dosage of 2.1 mg/animal/day, equimolar to 1 mg/animal/day Ascorbic Acid (Imai et al. 1967).

## Subchronic Oral

*Ascorbic Acid*

Nandi et al. (1973) fed male guinea pigs a control basal diet and administered 0.5 mg to 250 mg Ascorbic Acid in 0.75 ml water orally for 20 weeks. Control animals were fed only the basal diet and the daily consumption of Ascorbic Acid from the basal diet was approximately 1.03 to 1.20 mg per animal.

Supplementation had no influence on food consumption, growth rate, physical appearance, or behavioral pattern of the guinea pigs. The urinary excretion of Ascorbic Acid of the treated animals was greater than it was in the control animals. The amount of Ascorbic Acid excreted in treated animals was 2.5% of the administered dose. The average total glucuronic acid content of the urine of guinea pigs fed 250 mg Ascorbic Acid per guinea pig per day was similar to that of the control animals. The average 24-h urinary excretion of oxalic acid by guinea pigs fed 250 mg Ascorbic Acid daily was $3.5 \pm 0.8$ mg, similar to that of the control animals.

Hemoglobin, blood glucose, serum iron, liver iron and liver glycogen of guinea pigs fed 250 mg Ascorbic Acid was also similar to control values. The authors concluded that large doses of Ascorbic Acid were neither beneficial nor toxic to guinea pigs (Nandi et al. 1973).

Odumosu and Wilson (1973) reported a study in which Duncan-Hartley guinea pigs were maintained on a normal diet of rabbit pellets containing 27 mg Ascorbic Acid/100 g. After 2 weeks of acclimation to the diet, the animals were transferred to a scorbutigenic diet with supplementary Ascorbic Acid (50 mg 100 $ml^{-1}$) added to the drinking water. Following this 4-week maintenance period, a control group of females continued treatment with the scorbutic diet and 15 mg of supplementary Ascorbic Acid given daily by intraperitoneal injection; a treated group, comprising equal numbers of males and females, received the scorbutic diet together with 100 mg Ascorbic Acid given daily by stomach intubation; and separate groups of males and females continued to receive the scorbutic diet.

The supplemented group gained weight rapidly and by day 24 was significantly heavier than the control group. There was a trend in both male and female scorbutic groups to gain more weight earlier and then lose the weight quickly. All scorbutic males died. The scorbutic females were divided into two groups at day 24; potential diers and potential survivors. From day 24 until the end of the experiment, the potential diers showed a rapid and steady decrease in weight. The potential survivor females had a gradual decline in weight and at day 36, their weight had returned to the initial experiment level where it remained steady. When potential survivor females died, they showed no severe scorbutic signs like those of the scorbutic males and potential dier females. At day 24 scorbutic male food intake was 57% of controls and at the end of the experiment potential surviving females food intake was 82% of controls.

Plasma and liver Ascorbic Acid concentrations were measured in scorbutic males and in the potential survivor and potential dier females. Both sexes had continually decreased concentrations of liver Ascorbic Acid, but the females tended to have a faster decrease. After day 24, the mean liver Ascorbic Acid concentration ceased to fall in the potential survivors and started to rise again. Plasma Ascorbic Acid levels were elevated in both sexes at the beginning and reached a minimum at day 30. The mean plasma concentration rose in potential female survivors. Plasma concentrations in the potential diers continued to decrease as the liver concentrations began to rise (Odumosu and Wilson 1973).

Ohno and Myoga (1981) fed 12 female guinea pigs a scorbutic diet supplemented with 200 mg Ascorbic Acid and water ad libitum for 12 days. After this preliminary period, the guinea pigs were divided into two groups; one group continued with the Ascorbic Acid supplementation for 112 days and the other was placed on the scorbutic diet only for 29 days and thereafter supplemented with 200 mg Ascorbic Acid. Guinea pigs fed Ascorbic Acid gained body weight gradually, whereas those fed only the scorbutic diet lost weight markedly.

The food intake remained almost constant in the animals supplemented with Ascorbic Acid but decreased in those fed only the scorbutic diet. Guinea pigs receiving Ascorbic Acid showed a gradual increase in total urinary excretion of Ascorbic Acid, whereas the scorbutic group showed a decrease. Two animals fed the scorbutic diet died of scurvy on the 25th day and 30th days of testing. The others did not have any clinical signs of scurvy. Among the guinea pigs fed Ascorbic Acid, one died on day 57, one on day 100, and two on day 109. These animals had varying degrees of abnormal macroscopic manifestations in the liver, indicated mainly by congestion (Ohno and Myoga 1981).

Studies were conducted by the National Toxicology Program (1983) to evaluate the toxicity of cumulative administration of L-Ascorbic Acid and to determine the concentrations to be used in a 2-year study. Male and female F344/N rats and B5C3$F_1$ mice (groups of 10 rats and 10 mice of both sex) were fed diets containing 0, 25,000, 50,000, or 100,000 ppm L-Ascorbic Acid. The animals were checked for mortality and signs of moribundity twice daily. Those animals that were judged moribund were killed and necropsied. Body weight and feed consumption data were collected weekly. At the end of the 91-day study, survivors were killed and necropsied.

The following examinations were made in the control and 100,000 ppm groups: tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary glands, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, brain, pituitary, and

spinal cord. Femoral bone marrow sections were examined from female rats in the controls, 25,000, 50,000, and 100,000 ppm groups.

No rats died. One male mouse receiving 50,000 ppm died on day 84. Mean body weight gains were unchanged for male rats and depressed 13% to 16% among female rats fed diets containing 25,000 ppm or more. Feed consumption by dosed rats of each sex was higher than that of controls. Mean body weight gain relative to controls was depressed 37% in male mice receiving 50,000 or 100,000 ppm. Weight gains of dosed and control female mice were not depressed more than 10% to 13%, and the depressions were not dose related. Feed consumption by dosed and control mice were comparable. Cystic endometrial glands were found in the uteri of 4/9 female mice receiving 100,000 ppm compared to none in the controls. No other compound-related effects in mice were observed. Doses selected for mice in the 2-year study were 25,000 and 50,000 ppm L-Ascorbic Acid.

Alterations of the femur bone marrow (reticulum-cell hyperplasia) were observed in 2/10 female rats receiving 25,000 ppm, 1/10 female rats receiving 50,000, and 4/10 female rats receiving 100,000 ppm; these changes were not seen in female controls or in any male rat groups. Myeloid depletion was observed in 2/10 female rats receiving 50,000 ppm and in 4/10 female rats receiving 100,000. The femoral bone marrow lesion was characterized by multiple foci of cells that appeared to be proliferating fibroblasts replacing the normal myeloid elements and fat cells of the marrow. A few, somewhat nodular, groups of lymphocytes were observed in association with these foci in the two most affected rats in the 100,000 ppm groups. Some residual myeloid elements in the fibroblast foci were observed in all the affected rats in the 50,000 or 100,000 ppm groups, whereas in two animals in the 25,000 ppm groups the myeloid elements appeared normal, but the lymphocytes were absent.

A second 13-week study was conducted to gather additional data on the myelofibrosis observed in female rats in the previous 13-week study. Groups of 20 female F344/N rats were fed diets containing 0, 25,000, or 50,000 ppm L-Ascorbic Acid for 91 days. Initial and final body weights were measured; samples for hematologic analysis were collected from the orbital sinuses of all animals on days 0, 7, 30, and 90. Bone marrow smears were taken from one femur and one rib per animal at necropsy. Both femurs and one rib were examined microscopically.

All animals survived to the end. Mean body weight gain was decreased by 13% among female rats fed diets containing 50,000 ppm L-Ascorbic Acid. Although some mean corpuscular hemoglobin concentration values were lower in dosed groups than in controls. No consistent statistical differences were observed, and the results of hematologic analyses were within the clinically normal range for all groups of animals. Mild reticulum cell hyperplasia was found in the bone marrow of 2/20 female rats receiving 25,000 ppm and in 2/20 females receiving 50,000 ppm. Foci of reticulum cells were found in 2/20 female rats receiving 50,000 ppm Ascorbic Acid. The femoral lesions noted in the female rats were not considered to be potentially life threatening. Doses selected for male and female rats for the 2-year study were 25,000 and 50,000 ppm (National Toxicology Program 1983).

*Sodium Ascorbate*

Takada et al. (1996) administered Sodium Ascorbate and/or sodium nitrite for 6 months to male and female Wistar rats (5 rats/group). The control group was fed a basal diet and water only. Treated groups were administered the following: 0.075%, 0.15%, or 0.3% sodium nitrite dissolved in water; 1%, 2%, or 4% Sodium Ascorbate; or a combination with both chemicals at low + low, middle + middle, and high + high doses.

Body weight gain was significantly decreased in the combined-high dose group. Significant decreases of serum total protein, increase of BUN (blood urea nitrogen) and relative kidney weight were also found in the combined-high dose group. Histopathological examination showed moderate or severe squamous cell hyperplasia of the forestomach in the combined-high dose group and slight hyperplasia in the combined-middle dose group. No differences were seen between the sexes. The minimum toxic dose was 0.15% sodium nitrite + 2% Sodium Ascorbate (Takada et al. 1996).

*Ascorbic Acid and Sodium Ascorbate*

A total of 180 male F344 rats were randomly allocated to two treatment and one control group. They were fed a basal diet containing 5% Ascorbic Acid, 5% Sodium Ascorbate, or no added chemical for 36 weeks. Urinalyses were performed on five rats in each group. Five rats in each group were injected intraperitoneally with 100 mg/kg bw of 5-bromo-2′-deoxyuridine (BrdU) at weeks 2, 4, 8, 16, and 36 and killed after administration. At 16 weeks, the urinary bladders from one rat in each group was removed in order to determine prostaglandin $E_2$ ($PGE_2$), cAMP, and Ascorbic Acid. No deaths occurred during the treatment period and clinical signs of toxicity were not observed in any of the treated animals. A statistically significant reduction in weight gain was noted in the group treated with Ascorbic Acid from weeks 4 to 32. Animals receiving Sodium Ascorbate consumed more water than controls. Urinary pH was decreased in rats given Ascorbic Acid, whereas it was consistently increased in animals receiving Sodium Ascorbate. High concentrations of Ascorbic Acid (150 mg/dl) in the urine were detected in groups treated with Ascorbic Acid or Sodium Ascorbate throughout the study. Ascorbic Acid did not elevate DNA synthesis, while Sodium Ascorbate induced a significant increase in DNA synthesis at weeks 2 to 16. Simple hyperplasia appeared at week 8 in Sodium Ascorbate treated rats. The hyperplasia was no longer evident at weeks 24 and 36. The urinary bladders of rats administered Sodium Ascorbate contained significantly increased concentrations of $PGE_2$, cAMP, and Ascorbic Acid (Shibata et al. 1989).

## Chronic Oral

*Ascorbic Acid*

In a review article, Lang (1965) reported that Ascorbic Acid only exerts toxic effects above a dosage of 25 mg/kg bw in chronic feeding studies using rats. The limiting dose tolerated without symptoms was at least 10 mg/kg bw. Trials with guinea pigs gave this same threshold. Guinea pigs pretreated with high doses of Ascorbic Acid survived a scorbutigenic diet and a protein-deficient diet better than control animals receiving only physiological amounts of Ascorbic Acid with feed.

Surber and Cerioli (1971) reported a study in which four groups of 26 male and 26 female rats received in their diet for two years daily doses of 0, 1000, 1500, or 2000 mg/kg bw of Ascorbic Acid.

Hematological examinations, urine analysis, blood enzyme activity, and liver and renal function tests were within the normal range of values observed in the control group. No gross or microscopic detectable toxic lesions were observed which could be attributed to the daily ingestion of large doses of Ascorbic Acid. Age-dependent degenerative processes in organs, increasing predisposition of aging animals to intercurrent diseases and the appearance of spontaneous tumors occurred at the same rate in control and treated animals (Surber and Cerioli 1971).

Sorensen et al. (1974) reported a study in which young male guinea pigs were fed diets containing either 2 g (control) or 86 g (experimental) of Ascorbic Acid/kg of diet for 275 days. The average weight gain of the control group was significantly greater than the experimental group after 150 days of consuming the treated diet. The food intake of both groups was similar. After 5 months on the diets the average daily intakes of Ascorbic Acid, computed over one week were 45.9 $\pm$ 2.1 g and 53.3 $\pm$ 1.5 g per animal, respectively.

## Acute Parenteral

*Ascorbic Acid*

Table 14 lists the acute parenteral LD50 values for Ascorbic Acid in various animal strains reported by Demole (1934).

Calgli et al. (1965) stated that Ascorbic Acid neutralized with sodium carbonate and administered intravenously, resulted in an $LD_{100}$ between 6.4 and 7.3 g/kg in rabbits. No toxic effects were apparent at 5.3 g/kg.

El-Bana et al. (1978) injected Ascorbic Acid intraperitoneally into thirty male and female albino rats at a dose of 100 mg/kg bw. Blood samples were collected from the tail of each animal at 15 min, at 30 min, and at 60 min post injection. There was a rapid and significant rise in blood glucose after the injection and the hyperglycemic effect peaked at 30 min and declined at 60 min. A significant decrease of plasma cholesterol from a marked lipolytic effect was suggested by the significant rise of plasma glycerol. There was no effect on the BUN at 15 and 30 min, but a significant lowering of BUN at 60 min.

**TABLE 14**
Acute parenteral $LD_{50}$ values (Demole 1934)

| | $LD_{50}$ values as a function of delivery route (mg/kg/day) | | |
|---|---|---|---|
| Species | Subcutaneous | Intravenous | Intraperitoneal |
| Mouse | 5000 | 1058 | 2000 |
| Rat | 5000 | 1000 | — |
| Guinea pig | 1000 | 500 | 2000 |
| Rabbit | 1000 | 1000 | 1000 |
| Cat | 1000 | 500 | 500 |
| Dog | 200 | 200 | — |

## Short-Term Parenteral

*Ascorbic Acid*

Demole (1934) reported that mice were given Ascorbic Acid subcutaneously or intravenously daily doses of 500 to 1000 mg/kg bw for 7 days. In both treatment groups, the treated mice showed no difference in appetite, weight gain, and general behavior from controls receiving the same amount of biologically inactive galacturonic acid. Histological examination (kidney, pancreas, liver, heart, and lungs) of various organs detected no changes.

Guinea pigs given Ascorbic Acid subcutaneously and intravenously in daily doses of 400 to 2500 mg/kg bw for 6 days showed no differences in appetite, weight gain, and general behavior from control animals receiving the same amounts of biologically inactive galacturonic acid. Histological examination of various organs (kidney, pancreas, liver, heart, and lungs) were negative (Demole 1934).

Mallick and Deb (1975) fed female rats a standard laboratory diet for 7 days, then divided the animals into several groups and supplied each group with different percentages of protein (2%, 18%, 28%). The number of rats in each group was not stated. The rats were also injected with Ascorbic Acid (10 mg, 40 mg/100 g bw) for several days (injection route and number of days not specified). The animals were killed at 21 days. Low doses of Ascorbic Acid stimulated and high doses inhibited thyroid activity of rats supplied with normal and high percentages of protein. Ascorbic Acid had no significant effect on the thyroid of low protein fed animals.

Marcusen and Heninger (1976) injected four groups of 7 to 10 rats daily with 1, 10, or 100 mg Ascorbic Acid/100 g bw. Control animals received no injections. After 21 days of treatment, the rats were killed and thyroid and pituitary glands were extracted for thyroxine ($T_4$), tri-iodothyroxine ($T_3$), and thyroid-stimulating hormone (TSH).

Body weights were less than control animals in the 10 and 100 mg groups. Thyroid and pituitary weights were not significantly different between any groups, but when expressed on a body weight basis, there was an increase in both thyroid and pituitary weights in rats fed the higher doses. Serum $T_4$

**TABLE 15**
Short-term parenteral $LD_{50}$ values for Ascorbic Acid (FDA 1999c)

| Animal | $LD_{50}$ as a function of delivery route (mg/kg/day) | | | Duration of treatment (days) |
|---|---|---|---|---|
| | Subcutaneous | Intravenous | Intraperitoneal | |
| Mouse | — | 1058 | — | 10 |
| Rat | >600 | — | — | 28 |
| Guinea pig | — | — | 100 | 7 |
| Rabbit | — | 500 | — | 7 |
| Rabbit | — | — | 100 | 16 |
| Cat | — | >500 | — | 9 |
| Dog | — | >2000 | — | 3 |

concentrations were decreased in the 10 and 100 mg groups; serum $T_3$ was unaltered. There was an increase in serum $T_3$ in animals fed the 1 mg dose of Ascorbic Acid. Thyroid concentrations of $T_4$ were increased in all treated groups; $T_3$ concentrations were increased in only the 10 and 100 mg groups. Serum and pituitary concentrations of TSH showed opposite patterns; serum TSH concentrations were decreased in the 10 and 100 mg groups, whereas pituitary concentrations were increased (Marcusen and Heninger 1976).

FDA (1999c) listed the $LD_{50}$ values after the administration of Ascorbic Acid shown in Table 15.

### Sodium Ascorbate

In a study by Osswald et al. (1987), the toxicity (lethal effects and body weight loss) of daily subcutaneously administered 500 mg/kg *N*-methylformamide (NMF) during a period of 8 days in female CD-mice was ameliorated when 100 mg/kg Sodium Ascorbate was administered simultaneously. In a second study, P 388 leukemic cells were implanted intraperitoneally into female $B_6D_2F_1$ mice at a dose of $10^6$ cells/mouse. Sodium Ascorbate with and without NMF was administered daily subcutaneously to the mice. The onset of treatment began 24 h after implantation and lasted for 16 days.

The simultaneous combination of 360 mg/kg NMF with 60 mg/kg Sodium Ascorbate caused a 133% increase in the life span of the mice. The combination of 720 mg/kg NMF with 120 mg/kg Sodium Ascorbate administered at intervals of 48 h had a 146% increase in life span. In a separate experiment, M 5076 sarcoma were implanted intramuscularly into $B_6D_2F_1$ female mice. Again Sodium Ascorbate with or without NMF was administered to the animals. In rats with advanced M5076 sarcoma the daily subcutaneous injection of 360 mg/kg NMF with 60 mg/kg Sodium Ascorbate had a 135% increase in life span compared to controls (Osswald et al. 1987).

### Dermal

*Ascorbic Acid and Magnesium Ascorbyl Phosphate*

Imai et al. (1967) administered either a stock diet or a scorbutigenic diet and water ad libitum for 10 days to male guinea pigs. Guinea pigs fed the scorbutigenic diet were divided into groups after the 10-day feeding period and were percutaneously given Ascorbic Acid at doses from 3.5 to 14.0 mg/day or Magnesium Ascorbyl Phosphate at doses from 7.5 to 30.0 mg/day, both in a cream. The cream was applied on the clipped skin of the back just posterior to the neck. The cream base consisted of cetyl alcohol 3%, hydrogenated lanolin 4%, vegetable oil such as olive oil 3%, isopropyl myristate 6%, polyethylene glycol 6%, nonionic surface-active agents such as polyoxyethylene stearate and glycerol monostearate 16%, and preservatives. One-third the daily dose of the cream was applied at 8 AM, 12 PM, and 4 PM. After application the animals were observed.

The L-Ascorbic Acid dose of 7 mg/animal/day (0.5 g cream/day) and 15 mg/animal/day of Magnesium Ascorbyl Phosphate (0.5 g cream/day) prevented the development of scurvy; the activity of Magnesium Ascorbyl Phosphate was somewhat weaker than that of L-Ascorbic Acid.

Sixteen hours after the last application of cream, the skin of the back (treated) and of the abdomen (non-treated) were examined microscopically. The applied skin of the backs in the treated groups indicated the existence of Ascorbic Acid in the intercellular space of the epithelium, contrasting with the absence of Ascorbic Acid in the control group and in the skin of the abdomen in all groups (Imai et al. 1967).

### Toxic Interactions

Many reports state that the interaction of Ascorbic Acid with metals influences metal toxicity. These studies are summarized in Table 16. In some cases there was reduced toxicity, in some there was negligible change, and in some cases the effect of the metal was increased.

Fowler et al. (1993) injected male F344 rats intraperitoneally with 0, 458, or 687 $\mu$mol/kg 4-aminophenol (PAP). PAP caused selective necrosis to the pars recta of the proximal tubule. Coadministration of Ascorbic Acid (457 and 687 $\mu$mol/kg) with PAP protected the rats against the nephrotoxicity, markedly reduced the effect on renal function, and limited the extent of renal tubular necrosis.

## REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

### In Vivo

*Ascorbic acid*

Frohberg et al. (1973) administered daily oral doses of 150, 250, 500, or 1000 mg/kg Ascorbic Acid to pregnant rats in a first trial from day 6 to day 15 of pregnancy and in a second trial from day 0, preconception, to day 21 postpartum. Also, mice received daily oral doses of 250, 500, or 1000 mg/kg Ascorbic Acid from

**TABLE 16**
Effect of Ascorbic Acid on metal toxicity

| Metal | Species | Effects of Ascorbic Acid | Reference |
|---|---|---|---|
| Copper | Pig | Reduced iron deficiency induced by copper | Gipp et al. (1974) |
| Rubidium | Rat | Ascorbic Acid supplementation afforded some protection against the alterations of certain liver enzymes as well as in regard to the histological changes of either liver and kidney effects caused by Rb | Chatterjee et al. (1979) |
| Lead (Pb) | Human | Ameliorated toxicity | Federation of American Societies for Experimental Biology (1979) |
| Pb | Guinea pig | Variable toxicity | |
| Pb | Human | No effect on toxicity | Evans et al. (1943) |
| Pb | Rat | 1% Ascorbic Acid prevented growth depression, reduction of food consumption, anemia, and decreased the accumulation of lead in tissues (long-term) | Suzuki and Yoshida (1979) |
| Selenium | Rat | Marginal benefit from toxicity | Levander and Morris (1970) |
| Vandium | Chick | Ameliorated toxicity | Berg and Lawrence (1971) |
| Chromate ($CrO_4$) pigments (Na, Ca, Zn, and basic Pb chromates) | Cell culture media | Increased production of reactive oxygen species (ROS) | Lefebvre and Pezerat (1994) |
| $CrO_4$ | Rat | $CrO_4$ and Ascorbic Acid administered concomitantly completely prevented proteinuria by enhancement of extracellular reduction of chromate IV to III | Appenroth et al. (1994) |
| Aluminum | Rat | Aluminum concentrations in the bone, kidney, liver, and spleen were significantly increased, as was the overall cumulative urinary excretion of Al due to the gastric intubation of Ascorbic Acid | Domingo et al. (1994) |

day 6 to day 15 of pregnancy. There were no indications of maternal toxicity, terata, or fetal toxicity. There was no effect on the embryonic and postpartum development of the young or on breeding behavior, pregnancy, parturition, or lactation capacity of the mother animals.

Nandi et al. (1973) fed Charles Foster albino rats a fortified wheat diet consisting of whole grain wheat flour, 63 g; sucrose, 10 g; caesin, 15 g; groundnut oil, 5 g; shark liver oil, 5 g; USP XVII salt mixture, 4 g; and AOAC (Association of Official Agricultural Chemists) vitamin mixture, 1 g. Ascorbic Acid (0.5 mg to 250 mg in 0.75 ml water) was administered orally to individual male and female rats (100 mg/100g bw $day^{-1}$) for 2 weeks before, and then during mating. Females were separated from males after becoming pregnant. Ascorbic Acid feeding was continued during the period of gestation and lactation. Control animals received no Ascorbic Acid supplementation.

The average body weight of male rats receiving 100 mg Ascorbic Acid was not significantly different from controls. The administration of large doses of Ascorbic Acid had no influence on pregnancy or growth of the litters. The average number of pups born per litter and body weight of the pups from Ascorbic Acid-fed parents were similar to those from the control litters (Nandi et al. 1973).

FDRL (1975a) reported that the administration of 520 mg/kg bw of Ascorbic Acid to pregnant mice for 10 consecutive days had no clear effect on nidation or on maternal or fetal survival. The number of abnormalities observed in either soft or skeletal tissues of the treated group did not differ from those observed in the negative control group.

The administration of 550 mg/kg bw of Ascorbic Acid to pregnant rats for 10 consecutive days had no clear effect on nidation or on maternal or fetal survival. The number of abnormalities

observed in either soft or skeletal tissues of the treated group did not differ from those observed in the negative-control group (FDRL 1975b).

Alleva et al. (1976) exposed guinea pigs, rats, and hamsters to large daily doses of Ascorbic Acid during pregnancy. Guinea pigs received twice daily subcutaneous injections of Sodium Ascorbate (400 mg/kg/day) after being housed 6 days with a male. The control group received only saline injections. After pregnancy was established, the treated guinea pigs received daily oral treatments of Ascorbic Acid. Control animals received water. Female Holtzman rats were exposed to males and those with vaginal sperm the following morning were given a single oral dose of 0, 50, 150, or 450 mg of Ascorbic Acid daily from days 1 to 19 of pregnancy. Lakeview hamsters with fresh vaginal sperm were given Ascorbic Acid in the same daily doses given to the rats, from day 1 to day 15 of pregnancy. No increases in abortion or mortality of offspring were observed in guinea pigs, rats, or hamsters given daily doses of Ascorbic Acid as described. A slight increase in pup weight was observed in treated guinea pigs and hamsters.

In a study described earlier in "Absorption, Distribution, Metabolism, and Excretion," Norkus and Rosso (1981) fed guinea pigs a control (0.04% Ascorbic Acid) diet from day 3 of gestation. At day 31 the animals were divided into control, 0.56% Ascorbic Acid, and 0.82% Ascorbic Acid diets. There were no differences found in litter size, mean birth weight, and body weight among offspring from all groups at either 5 or 10 days after birth.

In a study by Seidenberg et al. (1986), a group of 30 adult ICR/SIM mice received 3200 mg/kg/day of Ascorbic Acid in water by oral intubation on days 8 through 12 of gestation. The mice were allowed to deliver and the neonates were examined, counted, and weighed. Two of the pregnant mice died and their average weight gain was $6.6 \pm 2.6$ g. Twenty-five litters were born and there was no resorption. The survival rate of the neonates was 99%. The average neonatal weight on day 1 and day 3 was $1.7 \pm 0.09$ and $2.3 \pm 0.2$ g, respectively. Ascorbic Acid was considered non-teratogenic in this study.

Basu (1985) examined the influence of prolonged exposure of guinea pigs to excessive Ascorbic Acid on the outcome of pregnancy, as well as the adaptive effect of the vitamin either during preweanling life or following. Duncan-Hartley guinea pigs were maintained on a stock pellet diet containing 70 mg Ascorbic Acid/100 g, and water was given ad libitum containing either 1 mg or 0.1 mg Ascorbic Acid/ml. The total intake of Ascorbic Acid per animal was not recorded. Females in two groups (test and control) were mated. The test groups received extra dietary Ascorbic Acid in their drinking water each day for two weeks before mating. In treated groups, the pregnant females continued to receive extra Ascorbic Acid.

Following birth three pups were put with one lactating mother, and at 21 days of age, the offspring were separated from their mothers and the females were put back with males for further mating. These weanlings were divided into two groups: group A was maintained on 1 mg Ascorbic Acid/ml drinking water, while group B was reduced to 0.1 mg/ml during the first 31 days of postweanling life. At 31 days these animals were killed.

The control animals were also mated and the offspring were separated from their mothers at 21 days. For four weeks, the offspring were maintained on 1 mg/ml Ascorbic Acid in the drinking water. The animals were then divided into two groups: group 1 continued on 1 mg/ml Ascorbic Acid for the next 4 weeks, while group 2 received 0.1 mg/ml. At the end of 4 weeks, the animals were killed. All animals became pregnant; no significant difference was observed between the groups, neither in terms of the number of offspring per pregnancy, nor in their weights at birth.

Continuous dietary administration of 0.1 mg/ml Ascorbic Acid from intrauterine life resulted in a significantly higher body weight gain at all periods studied compared to normal intake. However, change in the Ascorbic Acid treatment from high to normal amounts resulted in a marked loss in body weight by 31 days. This reduction also led to the development of scurvy-like signs, which were characterized by deep elevations on the paws and legs, swollen knee joints, enlarged epiphyses of ribs, and impaired joint movement. The plasma, leucocyte, and adrenal concentrations of Ascorbic Acid were measured in all groups of animals at the end of 31 days. Concentrations were significantly higher in animals in the high-supplementation group compared to those who received 0.1 mg/ml Ascorbic Acid. However the offspring of guinea pigs given high supplementation throughout pregnancy and lactation, followed by normal Ascorbic Acid had significantly lower Ascorbic Acid concentrations in their plasma, leucocyte, and adrenals than did controls (Basu 1985).

In a study by Pillans et al. (1990) pregnant C3H mice were exposed to 3.43 or 6.68 g Ascorbic Acid/kg bw on the 11th day postcopulation, and to coadministration of a teratogenic dose of cyclophosphamide (CP; 15 mg/kg bw). Sixteen hours after drug administration, embryonal cephalic DNA strand breaks were assessed. The mice were killed on day 18 after copulation and the fetal weights, gross morphological abnormalities, and fetal deaths were recorded.

The administration of 3.43 g/kg Ascorbic Acid was not associated with demonstrable toxic effects, but with 6.68 g/kg Ascorbic Acid there was a 46% incidence of fetal deaths. When Ascorbic Acid (3.34 g/kg) was coadministered with CP the incidence of DNA strand breaks was unchanged. CP-treated mice had 59% cephalic double-stranded DNA and controls had 81%. All fetuses treated with Ascorbic Acid and CP were morphologically normal and there was no reduction in fetal weight. These findings demonstrate that the administration of 6.68 g/kg Ascorbic Acid was toxic to the mouse embryo, but the lower dose was not and had a protective effect against the toxic manifestations of CP (Pillans et al. 1990).

Colomina et al. (1994) dosed three groups of pregnant Swiss mice daily with aluminum hydroxide, Ascorbic Acid (85 mg/kg), or aluminum hydroxide (300 mg/kg) concurrent with Ascorbic

Acid (85 mg/kg) on gestational days 6 to 15 by gavage. A fourth group of pregnant females received distilled water and served as the control group. Aluminum levels were determined in fetuses and in maternal organs and tissues.

The authors concluded that the reproductive data did not suggest embryotoxic or fetotoxic effects in any group. No gross, internal, or skeletal malformations or variations related to the different treatments were found. There were no differences between control and treated groups on the aluminum levels in maternal liver and bone as well as in whole body fetuses, whereas aluminum concentrations were significantly higher in placenta and kidney of dams receiving aluminum hydroxide and aluminum hydroxide plus Ascorbic Acid. No signs of maternal or developmental toxicity were observed when aluminum hydroxide was given alone or concurrently with Ascorbic Acid (Colomina et al. 1994).

*Sodium Ascorbate*

Siman and Eriksson (1997) fed normal and streptozotocin diabetic rats either a standard diet or a diet enriched with 0.9%, 1.8%, or 4% Sodium Ascorbate throughout pregnancy. On gestational day 20, the litters of normal and diabetic rats without Sodium Ascorbate supplement contained 9% and 12% resorptions, 2% and 17% late resorptions, and 1% and 27% malformations, respectively. Sodium Ascorbate treatment reduced the rates of late resorptions and malformations in the diabetic groups in proportion to the dose administered. In the diabetic group with 4% ascorbate treatment, unchanged numbers of early resorptions, 7% late resorptions, and 8% malformations were observed. Maternal diabetes did not alter tissue levels of Ascorbic Acid in the fetuses at term but Sodium Ascorbate supplementation caused an accumulation of Ascorbic Acid in the placenta and maternal and fetal liver.

Cohen et al. (1998) evaluated the effects of Sodium Ascorbate in a two-generation bioassay that involved feeding male and female F344 rats 4 weeks before mating, feeding the dams during gestation and lactation, and then feeding the weaned (28 days old) male $F_1$ generation rats for the remainder of their lifetime (up to 128 weeks). Dietary levels of 1%, 5%, and 7% Sodium Ascorbate were tested. Ammonium Chloride ($NH_4Cl$) was administered in two groups at 2.04% and 2.78% with Sodium Ascorbate. Control animals received no added chemicals to their diet.

No abnormalities were noted in the $F_0$ generation during feeding of the respective diets before conception and during lactation, nor was there evidence of increased morbidity or mortality. $F_1$ males coadministered $NH_4Cl$ showed significantly lower weights than the other groups. Rats fed 7% Sodium Ascorbate at week 0 and 5% Sodium Ascorbate at week 24 weighed significantly less than controls.

Mortality in $F_1$ rats was increased only in the high Sodium Ascorbate/high $NH_4Cl$ group. The most common cause of death in all groups was leukemia, with a grossly enlarged spleen and usually diffuse infiltrates of leukemic cells in other tissues, especially in the lungs and liver. At 5% and 7% Sodium Ascorbate, there was an increase in urinary bladder urothelial papillary and nodular hyperplasia and the induction of a few papillomas and carcinomas.

There was a dose-response increase in renal pelvic calcification and hyperplasia and inhibition of the aging nephropathy of rats even at the level of 1% Sodium Ascorbate. The group fed 5% Sodium Ascorbate and 2.04% $NH_4Cl$ showed complete inhibition of the urothelial effects of Sodium Ascorbate and significant inhibition of its renal effects (Cohen et al. 1998).

## In Vitro

*Ascorbic Acid*

Mummery et al. (1984) screened Ascorbic Acid for induction of differentiation in mouse N1E-115 neuroblastoma cells. These investigators reported Ascorbic Acid as a non-teratogen. The toxic dose and no effect dose of Ascorbic Acid were reported as $1 \times 10^{-3}$ M and $1 \times 10^{-7}$ M, respectively.

According to Pratt and Willis (1985), Ascorbic Acid was screened for growth inhibition of human embryonic palatal mesenchymal cells. The $IC_{50}$ (inhibitory concentration of 50% of the culture) for Ascorbic Acid was 300 $\mu$g/ml. Ascorbic Acid was considered a non-teratogen that was not inhibitory in vitro.

Uphill et al. (1990) assessed Ascorbic Acid for its teratogenic potential in the in vitro micromass assay (single cell suspensions of midbrain [CNS] and limb-buds [LB] from 13-day rat embryos). Concentrations of Ascorbic Acid were assessed for effects on inhibition of cell differentiation and cell survival by 50% compared to control values ($IC_{50}$). The $IC_{50}$ values for the CNS cells based on differentiation and survival were 120 and 100 $\mu$g/ml, respectively; the $IC_{50}$ values for the LB cells based on differentiation and survival were 335 and 370 $\mu$g/ml, respectively. Ascorbic Acid was considered a non-teratogen.

DeYoung et al. (1991) evaluated the developmental toxicity of Ascorbic Acid with the frog embryo teratogenesis assay: *Xenopus* (FETAX). Small cell *Xenopus laevis* blastulae were exposed to Ascorbic Acid for 96 h. The most common malformations induced by Ascorbic Acid was failure of the gut to coil (10 mg/ml). At 13 mg/ml, facial, eye, and brain malformations were noted. Growth was stunted and severe malformations of the gut, musculoskeletal system, face, eye, and heart occurred at 19 mg/ml. According to the authors, the FETAX protocol compares TI values, embryo growth, and the type and severity of induced malformations, and, in general, TI values $<1.5$ indicate low teratogenic potential (Ascorbic Acid averaged 1.633 in three tests). Nonetheless, these authors stated that Ascorbic Acid tested negative in FETAX. The results are given in Table 17.

Anderson and Francis (1993) measured the malformations and growth reductions in whole rat embryo cultures after treatment with the oxygen radical generating system of xanthine/xanthine oxidase and/or L-Ascorbic Acid. Treatment with xanthine/xanthine oxidase caused a significant linear trend towards

**TABLE 17**
Developmental toxicity of Ascorbic Acid with in vitro FETAX assay (DeYoung et al. 1991)

| Test no. | $LC_{50}$[a] | $EC_{50}$[b] | TI[c] | MCIG[d] | MCIG[e] |
|---|---|---|---|---|---|
| 1 | 19.2 | 11.6 | 1.7 | 10.0 | 52 |
| 2 | 20.3 | 12.8 | 1.6 | 10.0 | 49 |
| 3 | 19.6 | 12.0 | 1.6 | 10.0 | 51 |

[a]Median lethal concentration (mg/ml).
[b]Concentration inducing malformations in 50% of the surviving embryos (mg/ml).
[c]Teratogenic index TI = $LC_{50}/EC_{50}$.
[d]Minimum concentration to inhibit growth (mg/L).
[e]Minimum to inhibit growth as a percent of $LC_{50}$.

increasingly severe abnormalities when compared to controls (xanthine only). Low concentrations of Ascorbic Acid (10 or 100 $\mu$m) added to cultures containing xanthine/xanthine oxidase did not abolish this trend. These cultures were not significantly different from cultures without Ascorbic Acid. However xanthine/xanthine oxidase plus 1000 $\mu$m Ascorbic Acid caused a significant linear trend toward decreasingly severe abnormalities when compared with xanthine/xanthine oxidase. Ascorbic Acid (10, 100, or 1000 $\mu$m) added to xanthine control cultures did not differ significantly from the control cultures. No biologically significant effects on growth parameters of the embryos treated with L-Ascorbic Acid were observed. Germ cell detachment was also measured in mixed cultures of Sertoli and germ cells treated with xanthine/xanthine oxidase and/or L-Ascorbic Acid. Treatment with xanthine/xanthine oxidase in studies significantly increased germ cell detachment when compared to controls. Detachment was also significant with Ascorbic Acid doses of 1 mM and 2 mM. With the highest dose of Ascorbic Acid with the xanthine/xanthine oxidase system there was a significant decrease in detachment. Ascorbic Acid treatment alone had no effect on the system.

## GENOTOXICITY

Ascorbic Acid and Sodium Ascorbate have been widely tested in both bacterial and mammalian genotoxicity assays. One mammalian genotoxicity study was reported for Calcium Ascorbate. The results of these assays (summarized in Table 18) are almost all negative; i.e., no increase in genotoxicity over the control values was reported. These findings are consistent with the antioxidant properties of these ingredients.

Weitberg (1987) reported that Ascorbic Acid and Sodium Ascorbate, in combination with xanthine oxidase plus hypoxanthine (free radical generating system), can be genotoxic. These data are presented in Table 19. The author concluded that these results suggest that these ingredients may act as pro-oxidants in the presence of certain enzyme systems.

Likewise, genotoxicity studies in which metal ions in combination with ascorbates were tested demonstrate that the combination is capable of acting as a pro-oxidant. These data are shown in Table 20.

## CARCINOGENICITY

The Food and Agriculture Organization/World Health Organization (1974) reported no adverse effects on hematological examination or urine analytical measures or liver and renal function tests in rats given daily doses of 1000, 1500, or 2000 mg/kg bw of L-Ascorbic Acid for 2 years. Gross examination revealed no toxic lesions attributable to L-Ascorbic Acid.

The National Toxicology Program (1983) conducted a 2-year oral carcinogenesis bioassay of L-Ascorbic Acid in F344/N rats and $B6C3F_1$ mice. The 25,000 or 50,000 ppm L-Ascorbic Acid diets were fed to 50 untreated rats and 50 untreated mice of each sex. The animals were observed twice daily for signs of morbidity or mortality and clinical signs were recorded daily. Necropsies were performed on all animals not autolyzed or cannibalized. Gross and microscopic examinations were performed on major tissues or organs.

Mean body weights of dosed and control male rats were not significantly different. Dosed female rats had lower mean body weights than those of controls during the second year. The average daily feed consumption per rat for low and high doses was 101% and 105% that of the controls of males, respectively. The survival of high-dose male rats was slightly greater than that of the controls. For male rats, 66% of controls, 70% of the low-dose, and 82% of the high-dose group lived to termination of the study. For female rats, 72% of controls, 72% of the low-dose, and 74% of the high-dose group lived to termination of the study.

Pairwise comparisons of low-dose females and controls found a statistically significant increase of undifferentiated leukemias (equivalent to mononuclear cell leukemia) in the low-dose group (control, 6/50; low-dose, 17/50). These leukemias also occurred in increased proportions in high-dose female rats and in slightly decreased proportions in low- and high-dose males, but none of these differences were statistically significant. Significant negative trends were observed in the incidences of males with adenocarcinomas of the preputial gland and of females with adenocarcinomas of the clitoral gland. Interstitial-cell tumors occurred with a significant negative trend, but none of the pairwise comparisons were statistically significant. Pituitary adenomas showed a decreased trend in dosed female rats when compared to controls.

Survival of the high-dose group of male mice was significantly greater than that of controls. In male mice, 72% of the controls, 82% of the low-dose, and 94% of the high-dose group lived to the termination of the study. All groups of female mice survived equally (78%). Most observational differences were confined to the female rat. The incidence of undifferentiated (mononuclear-cell) leukemias in low-dose female rats was significantly higher than that in controls. These tumors were not considered to be related to the administration of L-Ascorbic Acid because they did not occur in the female high-dose group at

**TABLE 18**
Genotoxicity assays

| Assay type | Treatment/conditions | Result | Reference |
|---|---|---|---|
| | *Ascorbic Acid* | | |
| **Bacterial assays** | | | |
| *Salmonella typhimurium* TA1535, TA1537, TA1538 | 0.0013 and 0.0025% Ascorbic Acid using liver, lung, and testes of mice, rats, and primates (*Macaca mulatta*) with or without metabolic activation | Negative | Litton Bionetics 1975 |
| *Saccharomyces cerevisiae* strain D4 | 0.0013 and 0.0025% Ascorbic Acid using liver, lung, and testes of mice, rats, and primates (*Macaca mulatta*) with or without metabolic activation | Negative | Litton Bionetics 1975 |
| *Salmonella typhimurium* TA100 | Ascorbic Acid alone | Negative | Stich et al. 1976 |
| *Salmonella typhimurium* TA1530 requiring histidine ($his^+$) | Ascorbic Acid + *N*-methyl-*N*-nitrosoguanidine (MNNG) or dimethylanitrosamine (DMN) both are positive mutagens; when DMN was used a rat liver extract containing the mixed function oxidase (MFO) was used | Ascorbic Acid had inhibitory effects on both MNNG and DMN; the higher the concentration the greater the inhibitory effect; no significant signs of Ascorbic Acid on cell toxicity was noted | Guttenplan 1977 |
| *Salmonella typhimurium* TA1535 | Ascorbic Acid + *N*-methyl-*N*-nitrosoguanidine (MNNG) and dimethylnitrosamine (DMN) and *N*-methyl-*N*-nitrosourea with rat microsomes ± metabolic activation | Ascorbic Acid inhibited mutagenesis induced by MNNG and DMN; the rate of this reaction was enhanced by Cu(II) and Fe(III); mutagenesis by N-methyl-N-nitrosourea was not inhibited by ascorbate | Guttenplan 1978 |
| *Salmonella typhimurium* TA100 Ames mutation test | 5 to 50 mM Ascorbic Acid | Negative | Omura et al. 1978 |
| *Salmonella typhimurium* TA92, TA1535, TA94, TA98 with and without metabolic activation | 5 mg/plate Ascorbic Acid in phosphate buffer rat liver microsome (S9) | Negative | Ishidate et al. 1983 |
| *Salmonella typhimurium* $his^-$ TA100 | 3 mM Ascorbic Acid when deionized water was used in preparation of incubation medium | Negative | Norkus et al. 1983 |
| *Salmonella typhimurium* G46 | Aminopyrine 90 mg/kg + potassium nitrite with and without Ascorbic Acid 373 or 622 mg/kg taken orally | Negative—Ascorbic Acid abolished mutagenicity | Pienkowska et al. 1985 |
| Ames Salmonella assy | 250 $\mu$g/ml Ascorbic Acid | Negative | Ishidate et al. 1988 |
| *Salmonella typhimurium* TA100, TA1535, TA1532, TA97, TA98 | 0–10000 $\mu$g/plate | Negative<br>Weakly mutagenic in a non–activated TA97 assay at doses 3333 and 10000 $\mu$g/plate | Zeiger et al. 1988 |
| *Salmonella typhimurium* TA102 bacterial mutation assay | Bleomycin (BLM) (0.5 $\mu$g) + Ascorbic Acid (0.05, 0.1, and 1 mg/plate)<br>Ascorbic Acid (0.05, 0.1, and 1 mg/plate) | $His^+$ revertants = 589 (no reduction)<br>347 (no effect) | Anderson et al. 1995 |

**TABLE 18**
Genotoxicity assays *(Continued)*

| Assay type | Treatment/conditions | Result | Reference |
|---|---|---|---|
| | Control | 359 | |
| **Mammalian cells** | | | |
| V79 Chinese hamster cell | $10^{-6}$–$10^{-3}$ M Ascorbic Acid | Positive—dose-dependent increase in SCE frequency in relation to Ascorbic Acid; induction of SCEs by Ascorbic Acid was reduced by cysteine and glutathione | Speit et al. 1980 |
| CHO cells | 0.3–1 mg/ml Ascorbic Acid | Positive—24–42% aberrations—authors noted that the study demonstrated the chromosome damaging capacity of vitamin C in one in vitro test system and does not provide information to the possible mutagenic or clastogenic action of Ascorbic Acid in mammals, including man | Stich et al. 1980 |
| L5178Y TK+/− mouse lymphoma assay | Ascorbic Acid and Sodium Ascorbate | Negative—cytotoxicity was seen in concentrations greater than 1.5 mM (AA) and 0.5 mM (SA) | Amacher and Paillet 1981 |
| Binding of 7,12-dimethylbenz[$\alpha$]-anthracene to DNA of murine epidermal cells in culture | Ascorbic Acid (100 $\mu$g/ml) | Negative—inhibitory effect of binding max at 67% | Shoyab 1981 |
| In vitro chromosomal aberration test using Chinese hamster fibroblast cell line | 0.3 mg/ml Ascorbic Acid in saline | Negative | Ishidate et al. 1983 |
| Ability of rat hepatocytes to synthesize DNA measured by [$^3$H]thymidine uptake | 0.1 mM Ascorbic Acid 48-h incubations | Negative | Novicki et al. 1985 |
| Micronucleus test in bone marrow of mice | Aminopyrine 90 mg/kg + potassium nitrite with and without Ascorbic Acid 373 or 622 mg/kg taken orally | Negative—Ascorbic Acid abolished mutagenicity | Pienkowska et al. 1985 |
| Cyclophosphamide (CPA)- and mytomycin C–induced (MMC) sister chromatid exchanges in mouse bone marrow and spleen cells in vivo | Ascorbic Acid (1.67, 3.34, and 6.68 g/kg) Mytomycin C (2.5 mg/kg) cyclophosphamide (40 mg/kg) | Negative—increasing concentrations of Ascorbic Acid caused decreasing levels of CPA- and MMC-induced SCEs in both cell types in vivo; at 6.68 g/kg Ascorbic Acid, ~75 and 40% SCE inhibition occurred in both cell types (CPA and MMC, respectively) | Krishna et al. 1986 |
| Chromosome abberation and SCEs in Chinese hamster ovary cells | 0–3000 $\mu$g/ml with and without metabolic activation (S9) rat liver fractions: there was a noticeable decrease in pH at doses ≥500 $\mu$g/ml | No chromosome aberrations ± S-9; increase in SCEs without S9 at 300 $\mu$g/ml only; no increase in SCEs + S-9 | Gulati et al. 1989 |
| Micronucleus test in bone marrow | Vitamin C and $K_2Cr_2O_7$ were fed to rats in the diet or injected intraperitoneally | Antimutagenic effect against bichromate | Chorvatoxicova et al. 1991 |

*(Continued on next page)*

**TABLE 18**
Genotoxicity assays *(Continued)*

| Assay type | Treatment/conditions | Result | Reference |
|---|---|---|---|
| TK locus in L5178Y mouse lymphoma cell mutation assay | 0–2000 $\mu$g/ml L-AA with and without metabolic activation using rat livers | Cell toxicity was effected by pH | Myhr and Caspary 1991 |
| In vivo–in vitro replicative DNA synthesis, F344 rat hepatocytes | 0–2000 mg/kg L-Ascorbic Acid | Negative | Uno et al. 1994 |
| Human peripheral lymphocyte chromosome aberration assay | BLM (10 $\mu$g) | BLM caused highly statistically significant increases in chromosomal aberrations | Anderson et al. 1995 |
| | Ascorbic Acid (1, 2, and 10 mM) | Ascorbic Acid alone did not produce chromosomal aberrations | |
| | BLM ( 10 $\mu$g/ml) + Ascorbic Acid (1, 2, and 10 mM) | Ascorbic Acid reduced levels of the aberrations to that of controls when aberrations were caused by BLM | |
| Mouse micronucleus assay (peripheral blood and bone marrow cells) | BLM (50 mg/kg bw)<br>Ascorbic Acid (1 g/kg bw) | Ascorbic Acid reduced the % of micronucleated cells in peripheral blood but not in the bone marrow | Anderson et al. 1995 |
| Bleomycin-induced chromosomal breaks in cultured peripheral blood lymphocytes | Lymphocytes from 25 volunteers were exposed to 0.03 unit/ml and the plasma Ascorbic Acid levels were assessed | Significant inverse correlation between mutagen sensitivity and plasma levels of Ascorbic Acid | Kucuk et al. 1995 |
| Human B lymphoblastoid cell line DNA damage assay | 500 $\mu$M Ascorbic Acid and 50 $\mu$M Cd | Negative—Ascorbate alone was not damaging | Littlefield and Hass 1995 |
| Human B lymphoblastoid cell line DNA damage assay | 500 $\mu$M Ascorbic Acid and 200 $\mu$M Ni | Negative—increased the amount of double-stranded DNA | Littlefield and Hass 1995 |
| In vivo–in vitro replicative DNA synthesis, B6C3F1 mice hepatocytes | 0–2000 mg/kg L-Ascorbic Acid | Negative | Miyagawa et al. 1995 |
| CHO cell line AS52 | 50 $\mu$M Ascorbic Acid 24 h before the treatment of the cells with a radical generating system | Negative—statistically significant inhibition of the cytotoxicity and mutagenicity | Bijur et al. 1997 |
| **In vivo** | | | |
| Dominant lethal test in male Wistar rats | Male rats were fed Ascorbic Acid at 0%, 1%, or 5% levels in diet for 2 weeks | Negative | Chauhan et al. 1978 |
| In vivo mutagenicity assay of bone marrow in Chinese hamsters | 200–10000 mg/kg bw Ascorbic Acid was injected intraperitoneally and orally | Negative | Speit et al. 1980 |
| Intrahepatic host-mediated mutagenicity assay | 2–5000 mg/kg bw Ascorbic acid was fed per day for 4 days to guinea pigs and *Salmonella typhimurium* was injected iv | Negative | Norkus et al. 1983 |
| In vivo bone marrow micronucleus assay without metabolic activation with lung fibroblasts | 250 $\mu$g/ml Ascorbic Acid | Negative | Ishidate et al. 1988 |

**TABLE 18**
Genotoxicity assays *(Continued)*

| Assay type | Treatment/conditions | Result | Reference |
|---|---|---|---|
| Bleomycin induced chromosomal breaks in whole heparinized blood | 228 smokers were assigned to one of four groups: placebo, 1 g Ascorbic Acid, and 2 g Ascorbic Acid, or 4 g Ascorbic Acid daily for 16 weeks | Negative—no dose-dependent relationship between Ascorbic Acid intake and mutagen sensitivity and no association between serum AA levels and mutagen sensitivity | King et al. 1997 |
| **Drosophila** | | | |
| *Drosophila melanogaster* wing spot test | 0, 100, and 300 mM Ascorbic Acid | Negative | Tripathy et al. 1990 |
| | *Calcium Ascorbate* | | |
| Binding of 7,12-dimethylbenz[$\alpha$]anthracene to DNA of murine epidermal cells in culture | Calcium Ascorbate (100 $\mu$g/ml) | Negative | Shoyab 1981 |
| | *Sodium Ascorbate* | | |
| **Bacterial assays** | | | |
| *Saccharomyces cerevisiae* strain D4 | Sodium Ascorbate using liver, lung, and testes of mice, rats, and primates (*Macaca mulatta*) with or without metabolic activation (1.25%, 2.5%, and 5%) | Negative | Litton Bionetics 1976a |
| *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 | Sodium Ascorbate using liver, lung, and testes of mice, rats, and primates (*Macaca mulatta*) with or without metabolic activation (0.075%, 0.150%, and 0.1%) | Negative | Litton Bionetics 1976a |
| 2 strains of plasmid-mediated penicillin-resistant *Staphylococcus aureus* | 1 mM Sodium Ascorbate | 12–35% colony-forming units irreversibly lost their ability to produce $\beta$-lactamase | Cuevas 1988 |
| Phagocytic human leukocytes and *Salmonella typhimurium* strain TA 100 with and without metabolic activation | 2.5 mM and 12.5 mM Sodium Ascorbate | Positive—increased mutation frequency—authors noted with respect to this system, it is possible that the ability of ascorbate to act either as an oxidizing or reducing agent, to increase phagocyte oxidative metabolism and superoxide generation, and to inhibit catalase function may all play a role | Weitzman and Stossel 1982 |
| **Mammallian Cells** | | | |
| Nitrosation of methylguanidine in human skin fibroblasts | 0.6 and 0.3 M Sodium Ascorbate | Negative—Sodium Ascorbate prevented the general toxic effect but does not completely abolish DNA repair synthesis, no detectable effect on the clone-forming capacity at the concentration range in this experiment was noted | Galloway and Painter 1979 |

*(Continued on next page)*

**TABLE 18**
Genotoxicity assays *(Continued)*

| Assay type | Treatment/conditions | Result | Reference |
|---|---|---|---|
| In vitro activation in human skin fibroblasts of dimethylnitrosamine (DMN) with and without metabolic activation | 0.25 ml Sodium Ascorbate, 0.25 ml DMN, and 0.5 S-9 mixture | Negative—the addition of Sodium Ascorbate to the S-9 preparation and DMN inhibited the formation of the reactive metabolites or prevented their action | Galloway and Painter 1979 |
| Sister-chromatid exchanges (SCEs) in Chinese Hamster Cells (CHO) | $10^{-4}$–$10^{-2}$ M Sodium Ascorbate for 2–3 h | Weak mutagen | Macrae and Stich 1979 |
| CHO cells | $2 \times 10^{-4}$, $5 \times 10^{-4}$, and $10^{-3}$ M Sodium Ascorbate | Positive—produced somatic mutations at the hypoxanthineguanine phosphoribosyl transferase locus—the authors noted the concentration at which ascorbate is active in inducing the mutants was very narrow: the peak mutation induction occured with $5 \times 10^{-4}$ M ascorbate—concentrations of $10^{-4}$ ascorbate resulted in declining cell survival | Rosin et al. 1980 |
| L5178Y $TK^{+/-}$ mouse lymphoma assay | Ascorbic Acid and Sodium Ascorbate | Negative—cytotoxicity was seen at concentrations greater than 1.5 mM (AA) and 0.5 mM (SA) | Amacher and Paillet 1981 |
| Binding of 7,12-dimethylbenz[$\alpha$]-anthracene to DNA of murine epidermal cells (MEC) in culture | Sodium Ascorbate (1, 10, 25, 50, 100, and 150 $\mu$g/ml) | Negative—100 $\mu$g/ml was nontoxic to MEC cells and the inhibitory effect increased with increasing concentration of Sodium Ascorbate | Shoyab 1981 |
| Chinese hamster ovary cells (CHO) sister chromatid exchanges (SCEs) | <0.1 mM Sodium Ascorbate | Significant protective effect (less SCEs) | Weitberg and Weitzman 1985 |
| | Sodium Ascorbate plus xanthine oxidase plus hypoxanthine | Reduced numbers of SCEs | |

**TABLE 19**
Genotoxicity of Ascorbic Acid and Sodium Ascorbate in combination with enzyme systems

| Assay type | Treatments/conditions | Result | Reference |
|---|---|---|---|
| Chinese hamster ovary cells | Ascorbic Acid and xanthine oxidase plus hypoxanthine (oxygen radical-generating system) | Positive—Ascorbic Acid $\geq$0.1 mM significantly increased the number of sister chromatid exchanges (SCEs) induced by the oxygen radical-generating system; superoxide dismutase and catalase inhibited the effect of Ascorbic Acid on radical-induced SCEs | Weitberg 1987 |

**TABLE 20**
Genotoxicity of Ascorbic Acid and Sodium Ascorbate in combination with metal ions

| Metal ion | Test system | Treatment | Effects | Reference |
|---|---|---|---|---|
| Cu | Cultured human fibroblasts | Treated with a mixture of Ascorbic Acid and $Cu^{2+}$ | Ascorbic Acid caused DNA fragmentation, DNA repair synthesis, and chromosome aberrations | Stich et al. (1976) |
| Cu | Colicin-induction test with E2 coliciongenic derivative of strain *Salmonella typhmurium* TA1537 | Ascorbic Acid and Sodium Ascorbate ($0.2 \times 10^{-2}$–$7 \times 10^{-2}$ M) with and without $CuSO_4$ | Ascorbic Acid induced colicin alone, but there was a greater effect with $CuSO_4$ | Ben-Gurion (1981) |
| Fe (II) and (III) Cu (II) Mn (II) | CHO cell | Sodium Ascorbate at concentrations from $4 \times 10^{-3}$ M to $2 \times 10^{-2}$ M | Sodium Ascorbate inhibited mitosis at concentrations of 2 and $1 \times 10^{-2}$ M and induced chromosomal aberrations at 7 and $4 \times 10^{-3}$ M | Stich et al. (1979) |
| Fe | Iron-mediated oxidative DNA damage in isolate rat liver nuclei | 10 and 100 mM Ascorbic Acid + 0–100 $\mu$M Fe | Ascorbic Acid increased DNA damage with increased Fe concentration; damage seen in all Fe concentrations except 0 | Sahu and Washington (1999) |

significantly greater differences than those in the controls. No increases were observed in male rats.

The incidence of hemangiosarcomas in low-dose male mice was significantly increased compared to the controls and occurred in the liver, bone marrow, and spleen. A hemangioma of the pancreas occurred in one high-dose male mouse.

A statistically significant negative trend occurred in the incidence of lymphocytic leukemia in female mice; significant negative trends were observed in the incidences of malignant lymphocytic lymphoma, all malignant lymphomas, and combined lymphoma or leukemia in male mice. A statistically significant negative trend occurred in the incidence of male mice with hepatocellular carcinomas. Under the conditions of this bioassay, L-Ascorbic Acid was not carcinogenic for male and female F344/N rats or male and female $B6C3F_1$ mice (National Toxicology Program 1983).

### Cell Proliferation

Yoshida et al. (1994) examined the short-term effects of combined treatment with antioxidants and sodium nitrite ($NaNO_2$) on forestomach proliferation in male F344 rats. Animals were treated for 4 weeks with 0.8% catechol, 0.8% hydroquinone, 1% *tert*-butylhydroquinone, 2% gallic acid, or 2% pyrogallol alone or in combination with 0.3% $NaNO_2$ in the drinking water and/or 1% Sodium Ascorbate in the diet. The thickness of the forestomach mucosa in rats treated with antioxidants and $NaNO_2$ in combination was increased compared to those receiving the antioxidant alone. Additional Sodium Ascorbate treatment further enhanced the thickening of mucosa.

In a second experiment, male F344 rats were dosed with 150 mg/kg bw of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) by stomach tube after a 16-h starvation. One week later, animals were treated as follows: group 1, Sodium Ascorbate plus $NaNO_2$; group 2, Ascorbic Acid plus $NaNO_2$; group 3, $NaNO_2$; group 4, Ascorbic Acid; group 5, Sodium Ascorbate; group 6, basal diet. Another six groups of rats were treated the same but without the MNNG exposure. Sodium Ascorbate and Ascorbic Acid were mixed in the basal diet at 1% and $NaNO_2$ was dissolved in tap water at 3%. All animals were killed at 52 weeks. Five rats in each group received an intraperitoneal injection of 100 mg/kg bw in saline 1 h prior to killing.

Relative kidney weights were increased in all treated groups. A few small tumors were observed grossly in the forestomach epithelium in rats treated with MNNG followed by Sodium Ascorbate or Ascorbic Acid. In animals treated with MNNG followed by Sodium Ascorbate and $NaNO_2$, Ascorbic Acid and $NaNO_2$ or $NaNO_2$ alone, the forestomachs contained large masses. A diffuse thickening and multiple small nodules were observed in the epithelia of animals treated with Sodium Ascorbate and $NaNO_2$ and Ascorbic Acid and $NaNO_2$ without MNNG pretreatment.

Treatment with MNNG and Sodium Ascorbate or Ascorbic Acid increased the incidences of squamous cell carcinoma (SCC) to 79% and 85%, respectively. Sodium Ascorbate and Ascorbic Acid alone did not modify the incidences of papillomas or SCCs. The incidence of moderate hyperplasia was increased to 100% and 85% by the treatment of $NaNO_2$ plus Sodium Ascorbate or Ascorbic Acid, respectively, as compared with 0% in the $NaNO_2$ group alone with MNNG pretreatment. Several hyperplasia were found in 20% of the animals in each of these groups, and papillomas were observed in 53% and 20% of the animals treated with $NaNO_2$ plus Sodium Ascorbate and Ascorbic Acid, respectively (Yoshida et al. 1994).

Cohen et al. (1995) fed male F344 rats (10) a 6.84% Sodium Ascorbate diet for 10 weeks. Fresh urine was collected and the rats were killed at the end of 10 weeks. Control animals received a basal diet. The urinary bladder, stomach, and kidneys were removed for microscopic examination. Sodium Ascorbate retarded body weight gain throughout the study period. An increase in urine volume corresponding to an increased water intake was observed. Urinary pH was also increased.

An increased incidence of urothelial simple hyperplasia was detected by routine light microscopic histopathology. Three rats had papillary and nodular hyperplasia. Significant changes in the urinary bladder, classified as level 4 or 5, which represent the most severe of the early proliferative effects in carcinogenesis, were seen by scanning electron microscopy. Kidney weight was slightly increased and urothelial hyperplasia of the renal papilla particularly at the fornix was seen in the rats administered Sodium Ascorbate.

The authors concluded that sodium salts enhance bladder carcinogenesis and increased urothelial proliferation and there is a general consensus that these chemicals are nongenotoxic but act by increasing urothelial cell proliferation (Cohen et al. 1995).

### Tumor Promotion

Banic (1981) injected sixty guinea pigs subcutaneously in the thigh of the right hind-leg with 20 mg of methylcholanthrene in 1 ml olive oil. These animals were divided into two groups: group 1, the treatment group, was injected with 100 mg/kg bw Sodium Ascorbate daily for 4 months, and group 2, the controls, did not receive the Sodium Ascorbate injections. In group 1, the treatment of Sodium Ascorbate was initiated again immediately after the development of tumors and was continued until the death of the animals. After 4 months, the animals were checked for the development of tumors every 14 days. Observations were made up to 584 days after the injection of methyl cholanthrene.

One animal from each group died before the end of the experiment. Tumors appeared after 25 weeks: two tumors in the treated group and two tumors in the control group. In following weeks, tumors developed with a higher frequency in the Sodium Ascorbate treated group. With the injection of methycholanthrene there was an obvious difference in the numbers of tumors between groups (14 in the treated and 5 in the control). At the end of the observation period, the number of tumors between groups was not statistically significant. The most common tumors were fibrosarcoma and liposarcoma (Banic 1981).

Ito et al. (1984) reported that Sodium Ascorbate fed at a 5% level to rats has been shown to promote urinary bladder carcinogenesis initiated by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine or *N*-methylnitrosourea, forestomach carcinogenesis initiated by *N*-methylnitrosourea or *N*-methyl-*N'*-nitro-*N*-nitroguanidine, and colon cancer initiated by 1,2-dimethylhydrazine. These authors gave male Fischer 344 rats, in groups of 20 to 30 rats, 0.01% or 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in drinking water for 4 weeks and then administered 5% Sodium Ascorbate in the drinking water for 32 or 34 weeks. The animals were killed and the bladders were examined microscopically. The incidences of hyperplasia, papilloma, and cancer were significantly larger after administration of the higher dose of BBN followed by Sodium Ascorbate, than BBN alone (Ito et al. 1984).

Shirai et al. (1985) injected Male F344 rats with 1,2-dimethylhydrazine (DMH) at a dose of 20 mg/kg bw subcutaneously once a week for 4 weeks. One week after the last injection, these animals were fed a 5% Sodium Ascorbate diet for 36 weeks. The control groups received a basal diet only or Sodium Ascorbate without the DMH injection. The animals were killed at 40 weeks and necropsied for all intestinal neoplasms. Rats receiving only the Sodium Ascorbate had no intestinal neoplasms. The incidence of adenomas in animals given DMH followed by Sodium Ascorbate was significantly greater (30%) than that in animals given DMH alone (5%). Modification of neoplasm development by Sodium Ascorbate was apparent, mainly in the distal colon.

Fukushima et al. (1986) studied the role of sodium or potassium ion concentration and pH in Ascorbic Acid promotion of bladder cancer in rats. Male F344 rats were dosed orally with 0.05% BBN as an initiator for 4 weeks, and then were treated with 5% Sodium Ascorbate; 5% Ascorbic Acid, 3% $NaHCO_3$; 1% $NH_4Cl$ plus 5% Sodium Ascorbate; 5% Ascorbic Acid plus 3% $NaHCO_3$; 5% Ascorbic Acid plus 3% $K_2CO_3$; 5% Ascorbic Acid plus 5% $CaCO_3$ or 5% $MgCO_3$ in a powdered diet for 32 weeks. The urinary bladders were taken for microscopic analysis. On the basis of the results, as shown in Table 21, the authors concluded that the sodium and potassium ion concentration and pH of urine are important factors in urinary bladder carcinogenesis.

Ito et al. (1986) gave male F344 rats a subcutaneous injection of DMH at a dose of 20 mg/kg bw once a week for 4 consecutive weeks. One week after the last DMH injection, 20 rats were placed on a diet containing 5% Sodium Ascorbate and another 20 rats received normal diet for 36 weeks. The animals were killed 40 weeks after the first injection of DMH. Sodium Ascorbate significantly increased the number of colon tumors per rat ($0.80 \pm 1.00$) compared to DMH alone ($0.35 \pm 0.49$).

In a similar two stage bladder carcinogenesis model, Mori et al. (1987) gave two strains of inbred mice (80 of each strain) 0.5% BBN in their drinking water for four weeks and then a basal

**TABLE 21**
Ascorbate promotion of urinary bladder carcinogenesis (Fukushima et al. 1986)

| Treatment | Effect on urine | Promotion of BBN bladder carcinogenesis |
|---|---|---|
| 5% Ascorbic Acid | No elevation of pH | None |
| 5% Sodium Ascorbate | Elevated pH and sodium ion concentration | Promotion |
| 3% $NaHCO_3$ | Elevated pH and sodium ion concentration | Promotion |
| 3% $NaHCO_3$ plus 5% Ascorbic Acid | Elevated pH and sodium ion concentration | Promotion greater than 3% $NaHCO_3$ alone |
| 5% Sodium Ascorbate plus 1% $NH_4Cl$ | Elevated sodium ion concentration | Promotion less than Sodium Ascorbate alone |
| 5% Ascorbic Acid plus 3% $K_2CO_3$ | Elevated potassium ion concentration | Promotion |
| 5% Ascorbic Acid plus 5% $CaCO_3$ | Elevated calcium ion concentration | None |
| 5% Ascorbic Acid plus 5% $MgCO_3$ | Elevated magnesium ion concentration | None |

diet with or without 5% Sodium Ascorbate for 32 weeks. All animals were killed at the end of the experiment. Urine samples were taken at weeks 12, 24, and 34. Pathological examination was carried out on all animals.

In the groups treated with the BBN/Sodium Ascorbate diet, several rats had hematuria in the later stages of the experiment. The urinary bladder weights of the BBN/Sodium Ascorbate treated rats were greater than in the controls. Food intakes were similar between control and treated groups. Sodium Ascorbate administration increased the volume of water intake. The administration of Sodium Ascorbate significantly increased the induction of neoplastic lesions of the urinary bladder in both strains of rats (Mori et al. 1987).

Fukushima et al. (1987) studied the effects of treatment in F344 rats with Calcium Ascorbate, L-ascorbic dipalmitate, L-ascorbic stearate, and erythorbic acid on two-stage urinary bladder carcinogenicity after intiation with BBN. BBN was administered at a dose of 0.05% in drinking water for 4 weeks and thereafter, the test chemicals were given as a 5% supplementation in the diet for the following 32 weeks. No increase in the induction of preneoplastic lesions, papillomas, or carcinomas was observed. None of these chemicals possessed promoting activity for urinary bladder carcinogenesis.

Inoue et al. (1988) failed to find promoting activity for Ascorbic Acid and Sodium Ascorbate. BBN or *N*-ethyl-*N*-(4-hydroxybutyl)nitrosamine (EHBN) were added to the drinking water at equimolar concentrations (0.025% BBN and 0.021% EHBN). Ascorbic Acid and Sodium Ascorbate were mixed in the powdered basal diet at concentrations of 4.45%, 5%, 4.85%, and 6.5%. Male F344 rats were divided into groups of 15 rats. Rats in the treatment groups were treated simultaneously with the chemical and the carcinogen for 20 weeks. The control group received only a control diet. At 10 and 14 weeks, fresh urine samples were obtained from three rats in each group and the pH was measured. After the 20 weeks the rats were killed and the liver, kidneys, and urinary bladder were taken for microscopic examination.

The pH of the urine was decreased by Ascorbic Acid and increased by Sodium Ascorbate. Histopathologically, the incidences and numbers of preneoplastic and neoplastic lesions in groups treated with each test chemical were not different from those in the control groups (Inoue et al. 1988).

Thamavit et al. (1989) treated male F344 rats with 0.05% BBN, 0.2% *N*-bis(2-hydroxybutylpropyl)-nitrosamine (DHPN), and 0.2% *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) in the drinking water for 1 week each. For 3 days between treatments no chemicals were added to the drinking water. Subsequently the animals were given 5% Sodium Ascorbate in a basal diet. Two control groups were treated with only the Sodium Ascorbate or the precarcinogens. All surviving animals were killed at the end of 36 weeks. Sodium Ascorbate was found to inhibit liver but promote renal pelvis and urinary bladder carcinogenesis.

Asamoto et al. (1990) treated male F344 rats intraperitoneally with dimethylnitrosamine (DEN) at a dose of 200 mg/kg bw. After 2 weeks on a basal diet, the animals were divided into groups of 12 to 21 rats. One group was fed a diet containing 0.02% 2-AAF plus 5% Sodium Ascorbate. Another group received 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) and 5% Sodium Ascorbate and were given a partial hepatectomy. One group received Sodium Ascorbate only, and the control group received basal diet. All animals were killed at the end of 8 weeks and the number and area of glutathione *S*-transferase placental form (GST-P) positive foci per $cm^2$ were measured. Sodium Ascorbate did not have any effects on the development of DEN-initiated GST-P–positive foci under the influence of 2-AAF or 3′-Me-DAB plus partial hepatectomy.

In an extension of earlier work on the role of sodium ions in bladder carcinogenesis, Fukushima et al. (1990) treated male F344 rats with 0.05% BBN in the drinking water for 4 weeks. The rats were then randomly divided into six groups of 15 or 16. For 32 weeks the rats were fed (group 1) a control basal diet, (group 2) 5% sodium saccharin, (group 3) 5% sodium saccharin and 5% Sodium Ascorbate, (group 4) 5% Sodium Ascorbate, (group 5) 5% Ascorbic Acid, or (group 6) 5% sodium saccharin and 5% Ascorbic Acid.

Treatment of rats with sodium sacharin or Sodium Ascorbate alone significantly increased the induction of neoplastic and preneoplastic lesions of the urinary bladder. Treatment with

sodium saccharin plus Sodium Ascorbate also had significantly increased lesions when compared with controls, and the number of lesions was greater than animals treated with sodium saccharin or Sodium Ascorbate alone. The incidence of carcinomas and papillomas in the group that received 5% sodium saccharin and 5% Ascorbic Acid was not significantly different from controls. Consistent with earlier results (Fukushima et al. 1986), there was an increase in both urinary pH and sodium ion concentration in urine with sodium saccharin and Sodium Ascorbate together and alone; and sodium saccharin and Ascorbic Acid did not cause an increased urine pH, but did have an increase urine sodium ion concentrations (Fukushima et al. 1990).

Hasegawa et al. (1990) gave male F344 rats 0.1% *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) in the drinking water for 2 weeks. After this treatment period, the animals were administered a diet of 5% Sodium Ascorbate for 30 weeks. Another group received DHPN alone and the last group was given 5% Sodium Ascorbate for 30 weeks without prior DHPN administration. Each group contained 20 rats. At the end of 32 weeks the rats were killed and the lungs and other thoracic tissues were taken for microscopic examination.

Lung weights in the DHPN-initiated plus Sodium Ascorbate group were greater than in the noninitiated group. The increase in lung weight correlated to macroscopic findings: localized dark or whitish nodular lesions that were microscopically hyperplastic and/or neoplastic foci. Liver weight was not affected by Sodium Ascorbate administration. No lung neoplasms or adenomas were induced in rats receiving Sodium Ascorbate alone. Quantitative analysis of adenomas and carcinomas (numbers and areas of lesions per unit area of lung section) revealed obvious inhibitory effects of Sodium Ascorbate. The incidence of thyroid adenoma was increased with Sodium Ascorbate plus DHPN pretreatment but not significantly (Hasegawa et al. 1990).

Masui et al. (1991) studied H-*ras* mutations in rat urinary bladder carcinomas induced by various agents in an attempt to better understand the mechanism of promotion. Male F344 rats were fed 0.2% *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (tumor initiator) for 6 weeks and then fed a diet containinig either 3% to 5% sodium saccharin, 5% Sodium Ascorbate, 3.12% calcium saccharin, 1.34% sodium chloride, 5.2% calcium saccharin plus 1.34% sodium chloride, or basal diet for 72 weeks. The rats were killed at week 78, necropsied, and evaluated for urinary bladder and neoplasms.

Tumors were found in all groups (87 transitional cell carcinomas, 1 papillima, and 1 sarcoma). Protein and DNA were extracted from urinary bladder neoplasms. H-*ras* mutations were found in carcinomas from 24.4% of the rats, but there was no correlation with any of the promoting agents, including Sodium Ascorbate. Tumors with H-*ras* mutations were smaller than those without mutated *ras* (Masui et al. 1991).

Turusov et al. (1991) injected groups of 20 to 40 CBA female mice weekly for 8 weeks with 8 mg/kg bw DMH. Estradiol-dipropionate (EP) (5 $\mu$g/mouse) weekly subcutaneous injections (30) were given after the cessation of the DMH treatment. Two groups received 0.3% Ascorbic Acid or Sodium Ascorbate in the drinking water concurrently with the EP injections until the animals died. Control groups received DMH, EP, Ascorbic Acid, or Sodium Ascorbate. EP treatment increased the incidence of uterine sarcomas in CBA mice from 32.5% (DMH alone) to 62.5%. Ascorbic Acid given simultaneously with EP decreased the tumor incidence to 35%. Sodium Ascorbate did not affect tumor incidence. Ascorbic Acid inhibited the increased uterine weight produced in mice by EP but did not influence the growth of mouse transplantable uterine sarcomas.

Murai et al. (1997) studied differences in the promoting effect of Sodium Ascorbate between WS/Shi (WS), ODS/Shiod/od (ODS), and LEW/Crj (LEW) rats using a two-stage urinary bladder carcinogenesis model. Sodium Ascorbate increased the induction of neoplasms in the urinary bladder, whereas WS and ODS animals proved unresponsive to its promoting effect.

Another study reported differences in the promoting effect of Sodium Ascorbate on two-stage urinary bladder carcinogenesis. F344 rats are highly susceptible to the promoter effect of Sodium Ascorbate, but WS/Shi (WS) rats were not (Kamoto et al. 1997).

### Tumor Growth

Silverman et al. (1983) transplanted metastatic mouse colon carcinoma CA-51 subcutaneously into Balb/c mice. Another transplant was made around 13 to 20 days after the first and a third transplant (nonnecrotic tumor fragment) was also made. Immediately after the transplants, the mice were given 1% Sodium Ascorbate in their drinking water. Control groups received no Sodium Ascorbate. Subcutaneous tumors were removed when the tumors reached 1.5 cm. Animals were killed 30 days post transplant.

There were no differences in survival, in the number of mice with metastases, or in the size of metastases between treated and untreated groups. A second murine tumor, lymphosarcoma 6C3HED, was subcutaneously implanted into male and female C3H mice. Sodium Ascorbate was administered as above. Again, no significant differences in the number of mice with metastases were observed between treated and untreated groups, with the exception of brain and regional lymph node metastases which were enhanced in males by Sodium Ascorbate (Silverman et al. 1983).

Turusov et al. (1991) studied the effect of Ascorbic Acid on the growth of transplantable mouse uterine sarcoma. A piece of tumor (1 g) was minced and suspended in 10 ml of saline. Each of 4 groups of female CBA mice (20 animals/group) were injected with 0.5 ml of the suspension. Three groups had received Ascorbic Acid (0.3%, 0.75%, or 1.5%) in sucrose in drinking water 2 weeks before transplantation and continued to receive it. One group received only sucrose. The animals were killed 15 days after transplantation. There was no statistically significant effect of Ascorbic Acid on the growth of mouse transplantable uterine sarcomas, although the authors found the

results suggestive of a dose-dependent increase (Turusov et al. 1991).

### Inhibition of Carcinogenesis

Schlegel et al. (1970) studied the effect of Vitamin C and cholesterol on bladder tumors. A total of 310 Swiss albino female rats were divided into 6 groups: groups 1 and 2 were implanted with pellets containing cholesterol; groups 3 and 4 were implanted with cholesterol pellets containing 3-hydroxy-5-carboxybenzoquinone-(2-hydroxy-6-carboxy-anil)-(1) imide-(4); and groups 5 and 6 were implanted with cholesterol pellets containing 3-hydroxyanthranilic acid (3-HOA). The mice in groups 2, 4, and 6 were given L-Ascorbate (250 mg/L) in their drinking water ad libitum. Mice receiving Vitamin C in their drinking water showed an average urinary concentration of ascorbate of 400 mg/L compared to approximately 16 mg/L in the groups not receiving Vitamin C. All surviving mice were killed at 40 weeks and their bladders were examined for the incidence of bladder tumor formation.

Mice implanted with 3-HOA with no ascorbate (group 5) had a significantly greater incidence of bladder tumor formation than any other group (nine malignant tumors). Mice treated with 3-HOA and Ascorbic Acid had only 3 malignant tumors. Animals given cholosterol with 3-hydroxy-5-carboxybenzoquinone-(2-hydroxy-6-carboxy-anil)-(1) imide-(4) showed no higher incidence of bladder tumors than those mice implanted with cholesterol only, with no effect of ascorbate in the drinking water (Schlegel et al. 1970).

Rustia (1975) studied the effect of Sodium Ascorbate on the carcinogenic effect of ethylnitrosourea precursors. Ethylurea (EU) and sodium nitrite at doses of 200 mg/kg and 100 mg/kg in distilled water, respectively, were administered to one group of pregnant Syrian hamsters by intragastric intubation. Another group received EU and sodium nitrite and Sodium Ascorbate (200 mg/kg) by intragastric intubation at day 15 of gestation. Another group received EU alone and yet another, sodium nitrite alone. A control group had no treatment with EU, sodium nitrite, or Sodium Ascorbate. The animals were allowed to die spontaneously or were killed when moribund. Necropsies were performed on all progeny and treated mothers. Progeny of EU and sodium nitrite treated mothers developed significant incidences of neurogenic tumors of the peripheral nervous system, with a predominance in females. The concurrent administration of Sodium Ascorbate with EU and sodium nitrite prevented carcinogenic effects in the progeny. No tumors were seen in controls or in groups treated with EU alone or sodium nitrite alone.

Liehr and Wheeler (1983) tested the ability of Vitamin C (did not specify exact form) to inhibit induction of renal carcinoma by estrogens in male Syrian hamsters in vivo. The first group received the Vitamin-C purified hamster diet. The second group received the same diet enriched with 1 g/kg Vitamin C, and the third group was fed the same diet enriched with 2.5 g/kg Vitamin C. Each group consisted of 15 animals each. After two days, all animals received two 40-mg pellets implanted subcutaneously consisting of 90% (***E***)-diethylstilbestrol (DES) and 10% cholesterol. Three months later, each animal received an additional implant. After 172 days, the animals were killed. Macroscopic and microscopic renal carcinoma foci were determined.

The implantation of DES pellets into the hamsters resulted in a high incidence of renal carcinoma after 5.5 months. A kidney tumor frequency of ~85% of the animal population after 172 days of exposure to the implants agreed with high tumor incidences in earlier published papers. A statistical evaluation of the tumor frequency data showed that coadministration of Vitamin C resulted in a significant decrease in DES-induced kidney tumors in male Syrian hamsters.

In a second experiment, Vitamin C was administered in the drinking water of hamsters that was available ad libitum. The 120 hamsters were fed a Vitamin C–free diet. The drinking water contained 1% Vitamin C. A control group of 10 animals received the supplemented water but no 17$\beta$-estradiol. The second group, also 10 animals, received neither 17$\beta$-estradiol nor Vitamin C. The positive controls (25 hamsters) were provided with one 31-mg implant consisting of 90% 17$\beta$-estradiol and 10% cholesterol. All implants were placed subcutaneously. The experimental group (35 hamsters) was treated with the same implant but with the Vitamin C supplemented water. Another experimental group (20 hamsters) treated with the implant was allowed to drink the supplemented water from day 1 to day 92 of the experiment. The last experimental group (20 hamsters) received the implants and received the supplemented water from day 93 to the end of the experiment. All estrogen-treated animals were implanted with 17$\beta$-estradiol 3 months later. After 196 days, all animals were killed and their kidneys were examined microscopically.

Hamsters continually given Vitamin C in their drinking water and given the 17$\beta$-estradiol implant, developed renal carcinoma with a lower incidence than those which did not receive the Vitamin C supplementation. Administration of Vitamin C to estradiol-treated hamsters for only the first 3 months of the experiment had no effect on tumor incidence, but Vitamin C in drinking water for the last 3 months lowered incidence. Vitamin C supplementation did not significantly alter the absorption of estrogen from the implant. It did not change the estrogenic effect on the animals, nor did it significantly influence estrogen-dependent H-301 tumor cell growth (Liehr and Wheeler 1983).

Gardiner and Duncan (1989) incubated BL6 (mouse melanoma) and LLCMK (monkey kidney) cells with 200 $\mu$g/ml Ascorbic Acid for 24, 48, 72, and 96 h. Ascorbate addition at 200 $\mu$g/ml was found to inhibit the in vitro growth of BL6 cells but not LLCMK cells.

These same authors fed C57 mice 50 and 500 mg/kg bw Ascorbic Acid in the diet and then gave a subcutaneous injection of $5 \times 10^4$ BL6 mouse melanoma cells. Control groups were fed on the same diets but were injected with physiological saline instead of the melanoma cells. After 3 weeks, the mice were killed and blood, liver, and tumor samples were removed for Ascorbic Acid analysis.

In vivo growth of BL6 melanoma cells in mice was significantly inhibited by supplementary dietary Ascorbate at the 500 mg/kg bw level when compared to the 50 mg/kg bw concentration. Liver Ascorbate levels were reduced in tumor-bearing mice fed either of the diets when compared to animals without tumors on the same diet. Supplementary Ascorbate at 500 mg/kg bw increased the liver, tumor, tissue and blood Ascorbate concentrations (Gardiner and Duncan 1989).

De Pauw-Gillet et al. (1990) examined the effect of Ascorbic Acid and copper ions on B16 melanoma cells in vitro. B16 melanoma cells were incubated in minimal Eagle's medium (MEM) with 10% fetal calf serum (FCS) as the control group. Other cultures were incubated in MEM without the FCS and, hence, without added copper (designated: MEM-N2). Ascorbic Acid (50 $\mu$g/ml) added to cultures grown in MEM-N2 reduced the cell numbers, DNA content, melanin content, and protein content compared to MEM-N2 alone. All parameters in MEM-N2 and MEM-N2 plus Ascorbic Acid grown cultures were reduced compared to cultures grown in MEM-FCS. Addition of 1.0, 10.0, or 50 $\mu$M $CuSO_4$ with MEM-N2 plus Ascorbic Acid increased melanin levels to over 200% of that seen with MEM-FCS. At 100 $\mu$M $CuSO_4$, melanin was only slightly increased compared to cultures grown in MEM-FCS. With 100 $\mu$M $CuSO_4$ alone (no Ascorbic Acid), melanin increased to 175% of the level seen with cultures grown in MEM-FCS. The authors suggested that the relationship between melanin production, copper ion concentration, and Ascorbic Acid was complicated.

Meadows et al. (1991) reported that Sodium Ascorbate supplementation in drinking water inhibited subcutaneous tumor growth, enhanced levodopa methyl ester chemotherapy, and increased survival of B16 melanoma-bearing mice.

Shimpo et al. (1996) fed male ODS rats (a strain unable to synthesize Ascorbic Acid) a basal diet or a diet containing 0.06% 3′-Me-DAB, and drinking water containing 0.1% Ascorbic Acid. Rats were divided into the following eight groups: group 1, no treatment; group 2, Calcium Ascorbate; group 3, 2-*O*-octadecylascorbic acid; group 4, ascorbyl palmitate; group 5, 3′-Me-DAB; group 6, 3′-Me-DAB + Calcium Ascorbate; group 7, 3′-Me-DAB + 2-*O*-octadecylascorbic acid; and group 8, 3′-Me-DAB + ascorbyl palmitate. Calcium Ascorbate (2 g/kg), 2-*O*-octadecylascorbic acid (0.2 g/kg), and ascorbyl palmitate (0.6 g/kg) were administered once daily by gavage. 3′-Me-DAB was given in the basal diet. 2-*O*-octadecylascorbic acid and ascorbyl palmitate are lipophilic derivatives of Calcium Ascorbate. The animals were killed at 17 weeks, and the liver was weighed and processed for histological examination.

The authors reported that 2-*O*-octadecylascorbic acid treatment had a marked inhibitory effect on the development of 3′-Me-DAB-induced hepatocellular carcinomas as measured by multiplicity. Calcium Ascorbate and ascorbyl palmitate also had an inhibitory effect, but not to the extent of 2-*O*-octadecylascorbic acid. Erythrocyte polyamine levels were inhibited by Calcium Ascorbate and its derivatives as well (Shimpo et al. 1996).

## CLINICAL ASSESSMENT OF SAFETY

### Clinical Pharmacokinetics

Lowry et al. (1946) measured intracellular (white blood cells) and serum Ascorbic Acid concentrations in subjects who received standardized diets for 8 months containing 8, 23, or 78 mg/day of Ascorbic Acid. With 8 and 23 mg Ascorbic Acid the intracellular Ascorbic Acid levels averaged about 12 mg/100 ml compared to 25 mg/100 ml of intracellular Ascorbic Acid for subjects receiving 78 mg/day of Ascorbic Acid. Serum concentrations did not reach 0, averaging 0.2 mg/100 ml for subjects receiving 8 and 23 mg/day. After 8 months on the diet containing 8 mg/day an average of about 1800 mg Ascorbic Acid was retained following the ingestion of large doses of Ascorbic Acid. The authors concluded that the normal adult contains nearly 4 g of Ascorbic Acid.

Lamden and Chrystowski (1954) reported a study in which forty males were placed into groups ingesting Ascorbic Acid ranging from 0.25 to 8 g (divided into three doses as in the first experiment) daily for one week. The 51 control males without the extra dietary Ascorbic Acid had a mean oxalic acid excretion of 38.3 $\pm$ 0.7 mg. The daily ingestion of 4 or more grams of Ascorbic Acid increased the urinary excretion of oxalic acid; while the daily ingestion of less than 4 g produced no significant effect on oxalate excretion.

Willis and Fishman (1955) measured Ascorbic Acid in arteries from the following three groups of cases: cases of sudden death, routine hospital autopsies, and cases treated in the hospital with Ascorbic Acid for various lengths of time prior to death. Carotid sinus arteries, internal carotid arteries and aortas (the proximal 4 to 5 cm of the descending aorta) were examined. The authors concluded that Ascorbic Acid in the aorta of individuals who died of sudden death were generally higher than levels seen in routine hospital autopsies. The authors stated that the level in the aorta of patients undergoing Ascorbic Acid treatment was higher than in patients who died of sudden death, although no statistical analysis was provided. Data were presented for the other arteries only for routine hospital autopsies, so no comparisons were possible with the other two groups.

Hellman and Burns (1958) administered radiolabeled L-Ascorbic Acid (23.9 to 37.2 $\mu$Ci) intravenously to three patients. During a 10-day period an average of 42% of the administered dose was found in the urine and about 1% in the feces. No $^{14}C$ was detected in the respiratory $CO_2$, i.e., less than 5% of the dose during a 10-day period. The value for the half-life ranged from 13 to 20 days, with an average of 16 days. About 20% of total urinary $^{14}C$ was present as L-Ascorbic Acid, about the same amount as diketo-L-gulonic acid and less than 2% as dehydro-L-ascorbic acid. An average of 44% of the total radiocarbon excreted in the urine was recovered as oxalate. The average body pool of Ascorbic Acid was 20 mg/kg with a turnover rate averaging 1 mg/kg/day.

Rivers et al. (1963) subjected four healthy men to an Ascorbic Acid depletion diet for 8 weeks. Following this, two of the

men were given supplemented diets; 50 mg Ascorbic Acid for 2 weeks and 100 mg Ascorbic Acid for the last 2 weeks. White cell, plasma, and urinary total Ascorbic Acid determinations were made. The total white cell and plasma total Ascorbic Acid concentrations decreased significantly in the 8-week depletion phase. The average excretion of total Ascorbic Acid for all subjects during depletion was 9.4 mg/24 h. Both 50 and 100 mg Ascorbic Acid increased the white cell Ascorbic Acid concentration significantly over time and reached saturation in the first week for the 100-mg group. The 50-mg supplementation caused only slight increases in the plasma Ascorbic Acid concentration and at the 100 mg dose after the depletion phase increased to the beginning value before the depletion. This increase was highly significant. The L-Ascorbic Acid supplementations caused a slight but inconsistent increase in urinary excretion of Ascorbic Acid in the partially depleted subjects.

Atkins et al. (1964) reported 35% to 50% of the urinary oxalate to be derived from ingested Ascorbic Acid. However urinary oxalate represents only 20 to 40% of the metabolic turnover of Ascorbic Acid. These results were based on the Ascorbic Acid metabolic pool after the oral administration of a single physiological dose of Ascorbic Acid-*l*-$^{13}$C.

Kim and Song (1965) gave a dose of 500 mg L-Ascorbic Acid orally to healthy adults twice a day for a few days until they were saturated with the vitamin. After 12 h from the last administration of the vitamin, a baseline Ascorbic Acid concentration was determined and test dose of Ascorbic Acid (344 mg) was injected intravenously. The Ascorbic Acid concentration in the blood and urine was determined. The blood level rose immediately after the injection and returned to the baseline concentration in 3 h.

The urinary excretion in 3 h averaged 221.3 mg. The vitamin absorbed by the tissue cells and metabolized in the body or excreted into the gastrointestinal tract were found to average 74.1 mg in 3 h. When 300 mg of L-Ascorbic Acid was given intravenously twice at intervals of 3 h, an average of 101 mg of the vitamin was absorbed by the tissue cells and metabolized by the body or excreted into the gastrointestinal tract in 6 h. When the Ascorbic Acid was given orally to the saturated adults, the absorption rate increased as the test dose increased. When large amounts (3 g) of Ascorbic Acid were given, the absorption rate reached a maximum. The maximum absorption in 6 h was estimated as 681 mg of Ascorbic Acid (Kim and Song 1965).

Takenouchi et al. (1966) gave 3 g Ascorbic Acid orally to three human subjects. Metabolites in the urine were measured before and after administration. The total amount of Ascorbic Acid metabolites found in the urine before administration (average of all three subjects) was 13 mg, of which 57% was Ascorbic Acid, 43% dehydroascorbic acid, 0% 2,3-diketogulonic acid, and 19 mg oxalic acid. The values at 24 and 28 h after administration of Ascorbic Acid were 446 and 430 mg of total Ascorbic Acid metabolites, of which 79% and 67% were Ascorbic Acid, 17% and 27% were dehydroascorbic acid, 4% and 5% were 2,3-diketogulonic acid, and 29 and 25 mg were oxalic acid, respectively.

These authors also administered 3 g Ascorbic Acid orally to three subjects daily for 2 consecutive weeks. The metabolites were estimated in 24-h urine collections on the last day of administration and the following values were obtained (the average): total Ascorbic Acid, 476 mg, of which 91% was Ascorbic Acid, 6% dehydroascorbic acid, 3% 2,3-diketogulonic acid, and 30.97 mg of oxalic acid. The amount of oxalic acid found in the 24-h urine sample following the oral daily administration of 9 g Ascorbic Acid (three installments) for 3 consecutive days was 43.2 mg. The major portion of administered Ascorbic Acid was excreted in the urine unchanged when administered both orally and intravenously for 2 consecutive weeks. The urinary 2,3-diketogulonic acid was increased with the rise of the amount of Ascorbic Acid administered; oxalic acid did not increase markedly until 9 g Ascorbic Acid were given orally for 3 consecutive days.

These same authors gave 3 g Ascorbic Acid intravenously to three human subjects. The metabolites in the urine were measured before and after administration. The total Ascorbic Acid eliminated in the urine before administration (average of all subjects) was 63 mg, of which 82.5% was Ascorbic Acid, 15% dehydroascorbic acid, 3% 2,3-diketogulonic acid, and 34.4 mg of oxalic acid. Following the administration of Ascorbic Acid, the corresponding figures were 457 mg for total Ascorbic Acid, of which 72% was Ascorbic Acid, 18% dehydroascorbic acid, 10% 2,3-diketogulonic acid, and 26.6 mg of oxalic acid after 24 h; and 79 mg total Ascorbic Acid, of which 84% was Ascorbic Acid, 14% dehydroascorbic acid, 21% 2,3-diketogulonic acid, and 35.6 mg of oxalic acid after 48 h (Takenouchi et al. 1966).

Takiguchi et al. (1966) divided 30 healthy subjects ranging in age from 23 to 45 years into three groups of 10 people each. The first group ingested 1 g of Ascorbic Acid daily for 90 days. The second group ingested 1 g Ascorbic Acid daily at breakfast and supper for 180 days (2 g total) and the third group ingested 2 g/day Ascorbic Acid plus an experimental diet on the last 3 days of the 90-day treatment period. No significant increase in urinary oxalic acid was observed following the ingestion of large doses of (1 to 2 g) Ascorbic Acid for 90 to 180 days. Ascorbic Acid was eliminated in the urine mainly in the reduced form and little was determined as diketogulonic acid, the precursor of oxalic acid.

Tobert et al. (1967) gave a male subject radiolabeled ($^{3}$H, 105 $\mu$Ci) Ascorbic Acid (10 mg). His ascorbate intake was $\sim$250 mg/day. The radiolabel did not enter the body water pool, but instead was excreted as organic-bound tritium. The excretion products were Ascorbic Acid and its immediate oxidation products, and unknown organic products. Kinetic analysis of the data shows a half-life of 2 days and 46 days for turnover of the labeled Ascorbic Acid and the unknown compound, respectively. There was an indication that the unknown metabolite may have been derivatives of L-threose and L-threonic acid.

Briggs et al. (1973) observed that with daily intakes of 4 g of Ascorbic Acid, the urinary oxalate level increased 10-fold, which may lead to kidney stones.

Nelson et al. (1978) conducted a study in which seven human subjects (five male and two female) served as volunteers in intestinal perfusion studies to characterize the kinetics of L-Ascorbic Acid uptake in the small intestine. Four solutions containing respectively 0.85, 2.84, 5.68, or 11.36 mM of L-Ascorbic Acid were perfused in a tandem manner in random order in each individual. In addition each solution contained 1000 mg/100 ml polyethylene glycol (PEG). All collected intestinal aspirates were measured for Ascorbic Acid by 2,4-dinitrophenylhydrazine reaction and PEG. The calculated $K_M$ and $V_{max}$ were 5.44 mM and 0.28 mM/cm-h, respectively. There was a nonlinear increase in Ascorbic Acid transport with increasing Ascorbic Acid concentrations (0.852 to 5.68 mM); the most efficient absorption occurred from the solution of lowest concentration. Net secretion of water was observed with the highest concentration of Ascorbic Acid (11.36 mM).

Kallner et al. (1979) followed the time course of radioactivity in plasma and urine after oral administration of a single dose of (L-$^{14}$C) Ascorbic Acid in healthy, nonsmoking male volunteers. The investigation was carried out under steady state conditions with regard to Ascorbic Acid intake (30 to 180 mg/day). The half-life of Ascorbic Acid in the normal adult male decreases with increasing total turnover, approaching about 10 days at a total turnover of 70 mg/day. The pool could be increased to about 20 mg/kg bw by increasing the dosage. The estimated average absorption of Ascorbic Acid in humans was 84%. Even at low dosages (30 mg/day) some Ascorbic Acid is excreted unchanged in the urine. The investigators concluded that on a daily intake of ~100 mg Ascorbic Acid this pool size would be reached in 95% of the population.

Kallner et al. (1981a) followed the time course of radioactivity in plasma and urine after oral administration of a single dose of (L-$^{14}$C) in healthy male volunteers smoking more than 20 cigarettes per day. The investigation was carried out under steady state conditions with regard to Ascorbic Acid plasma levels at intakes of about 30 to 180 mg/day. Smokers had a higher metabolic turnover of Ascorbic Acid than that found in nonsmokers. The investigators concluded that a daily intake of at least 140 mg was required for smokers to reach steady state concentrations as compared to 100 mg in nonsmokers as previously reported.

Kallner et al. (1981b) fed 42 healthy, male, nonsmoking volunteers a diet which limited their Ascorbic Acid intake for 3 weeks. The volunteers were supplemented with various doses of Ascorbic Acid (30 to 4000 mg/day) and on the experimental day, they received 10 $\mu$Ci of L-$^{14}$C Ascorbic Acid orally. All subjects fasted for 12 h before air collection of expired gas. The carbon dioxide content of the expired air ranged between 2.5 and 3.7 vol %. The expired volume from the subjects ranged between about 80 and 220 L per minute. Labeled carbon dioxide was recovered from the breath of participants having had daily intakes of more than 180 mg Ascorbic Acid and was identified between 1 and 8 h after administration of the isotope. The peak of excretion occurred between 3.5 and more than 8 h. Four subjects were given Ascorbic Acid intravenously (1000 mg) 3 h after the label administration. The recovery of radioactivity in the urine of these participants for 3 days was 50% to 60%.

Mitch et al. (1981) measured the effect of Ascorbic Acid on uric acid excretion in four men and two women ranging in age from 22 to 42 years. On the first day the subjects took no Ascorbic Acid; on the second, each subject took 1 g doses of Ascorbic Acid by mouth every 6 h. One week later, the study was repeated while four of the subjects (nine men and one woman) took 3 g of Ascorbic Acid orally every 6 h. In a separate study the time course of any changes in uric acid excretion after oral Ascorbic Acid was assessed in five subjects. They were given 500 ml of water followed by a single dose of 4 g Ascorbic Acid. The results indicated that 4 or 12 g of Ascorbic Acid taken in divided doses had no effect on serum uric acid concentration or uric acid excretion and clearance by the kidney.

Schmidt et al. (1981) measured the urinary oxalate excretion in five healthy male volunteers. One volunteer ingested 5 × 1 g doses of Ascorbic Acid per day (7, 9, and 11 AM and 1 and 3 PM) for 4 days and daily urine samples (7 AM, noon, and 6 and 11 PM) were collected. After 2 days without Ascorbic Acid intake, the remaining four volunteers ingested 5 × 2 g doses of Ascorbic Acid per day for 5 days and daily urine samples were taken. With intakes of 10 g Ascorbic Acid daily, the mean urinary oxalate excretion was enhanced from 50 mg to 87 mg per day. At least 25% of the Ascorbic Acid was absorbed and excreted in the urine. Discontinuation of this Ascorbic Acid administration caused oxalate excretion to return to baseline values within 24 h.

A time-course study with five additional volunteers ingesting 5 × 2 g doses of Ascorbic Acid per day was also conducted. Urine production was stimulated with 200 ml of a tea beverage and urine samples were taken every 2 hours. The time-course of oxalate excretion revealed that following the 3rd dose of 2 g Ascorbic Acid a plateau in urinary oxalate excretion was reached and was not exceeded despite additional 2 g doses of Ascorbic Acid. High-dose Ascorbic Acid ingestion had no effect on the daily urinary excretion of creatinine, uric acid, and inorganic phosphate. Calcium excretion was slightly reduced (Schmidt et al. 1981).

Sitren (1987) reported that L-Ascorbic Acid is absorbed by carrier-mediated transport from the upper small intestine and absorption is highly efficient with normal vitamin intake. Absorption does decrease at high doses. The bioavailability of Ascorbic Acid from foods ranges from 80% to 90%. L-Ascorbic Acid is distributed throughout the water-soluble compartments in the body. Typical body pool size is 1500 mg, of which 3% to 4% is utilized daily. The highest tissue concentrations are found in the adrenal and pituitary glands, with lower concentrations in the brain, pancreas, spleen and liver. Urine is the main excretory pathway and urinary metabolites include dehydroascorbic acid, diketogulonic acid, ascorbate-2-sulfate, oxalate, and methyl ascorbate. The renal threshold for Ascorbic Acid is approximately 1.5 mg/dl of plasma. At lower plasma

concentrations, Ascorbic Acid is reabsorbed by the renal tubules. Average 24-h excretion in normal adults is 8 to 27 mg. Depleted individuals excrete little or no Ascorbic Acid in the urine.

Blanchard (1991) compared the pharmacokinetics of Vitamin C in young and elderly male (15 in each) and female (14 in each) subjects. All subjects were nonsmokers. Vitamin C supplementation of 500 mg orally was given to each subject in two states of Vitamin C nutriture: a depleted state achieved by 4–5 weeks on a diet containing $<10$ mg Vitamin C/day and a supplemented state which was achieved following 500 mg/day of Vitamin C for 3 weeks. Males had a larger body surface area than females. However, young females had significantly greater percent body fat than the young males. Both young and elderly males had a larger fat-free mass than their female counterparts.

None of the pharmacokinetic parameters differed with respect to gender in depleted subjects. In the supplemented group, female subjects showed a significantly greater peak Vitamin C concentration ($C_{max}$), a longer absorption lag time ($t_{lag}$), apparent volume of distribution (AVd) in L/kg, clearance (CL) in ml/h/kg, and renal clearance ($CL_r$) in ml/h/kg. When AVd, CL, and $CL_r$ were expressed in absolute terms (L or ml/min), no gender-related differences were observed. A stepwise linear regression indicated that $C_{max}$ was inversely related to body weight, $t_{lag}$ was directly related to body surface area and dose, and AVd was inversely linearly related to body surface area (Blanchard 1991).

Wandzilak et al. (1994) investigated the effect of high dose ascorbate on urinary oxalate levels in healthy adults using a modified ion chromatography method. Subjects ingested 1, 5, or 10 g/day supplemental ascorbate for 5 days, separated by five days of no supplementation. Of the 15 subjects 13 tolerated all doses of Vitamin C with no evidence of gastrointestinal disturbances. Two people experienced diarrhea while taking the 10 g dose and were unable to continue at this level.

Urine ascorbate concentrations demonstrated variable increases with ascorbate supplementation. Addition of ascorbate directly to urine in vitro resulted in a statistically significant but modest increase in measured oxalate. Addition of 5.68 mM/L ascorbate increased measured urinary oxalate by 36 $\mu$M/L. Measurement of 24 h urinary oxalate levels with 5 and 10 g/day ascorbate showed similar, modest increases, which could be accounted entirely for by oxalate production during analytical procedures (Wandzilak et al. 1994).

## Tissue Distribution

Hornig (1975b) presented a review of studies on the distribution of Ascorbic Acid, its metabolites and analogues. Results were approximate values expressed in units of mg/100 g of wet tissue and are shown in Table 22.

Rose and Bode (1993) summarized data from more recent studies as shown in Table 23. They presented their data in units of $\mu$mol/l, calculated as though all tissue weight is water. The values in Table 23 may be compared to the range found in plasma, 0.04–0.06 $\mu$mol/l.

**TABLE 22**
Ascorbic Acid content in human tissues (Hornig 1975b)

| Tissue | Ascorbic Acid (mg/100 g wet tissue) |
|---|---|
| Adrenal glands | 30–40 |
| Pituitary gland | 40–50 |
| Liver | 10–16 |
| Spleen | 10–15 |
| Lungs | 7 |
| Kidneys | 5–15 |
| Testes | 3 |
| Thyroid | 2 |
| Heart muscle | 5–15 |
| Skeletal muscle | 3–4 |
| Brain | 13–15 |
| Pancreas | 10–15 |
| Eye lens | 25–31 |
| Plasma | 0.4–1 |
| Saliva | 0.07–0.09 |

The data presented in Tables 22 and 23 are consistent in that the highest levels of Ascorbic Acid are found in adrenal, pituitary, and ocular tissue. Hornig (1975b) did not report blood levels, but Rose and Bode (1993) did include blood data, concluding that Ascorbic Acid is highly concentrated in leukocytes.

## Clinical Toxicity

Widenbauer (1936) administered doses of Ascorbic Acid up to 6000 mg to 29 infants, 93 children of preschool and school age, and 20 adults for more than 1400 days. Toxic effects at

**TABLE 23**
Ascorbic Acid content in human tissues (Rose and Bode 1993)

| Tissue | Tissue concentration ($\mu$mol/l) |
|---|---|
| Blood | |
| Erythrocytes | 0.034 |
| Platelets | 1.9 |
| Granulocytes | 1.2 |
| Leukocytes | 3.8 |
| Eye | |
| Aqueous humor | 0.9 |
| Lens | 1.1 |
| Vitreous | 2 |
| Adrenal Gland | 1.9 |
| Brain | 0.7 |
| Kidney | 0.6 |
| Liver | 0.7 |
| Lungs | 3 |
| Skeletal Muscle | 0.1 |
| Pituitary Gland | 2.5 |
| Thyroid | 0.1 |

the higher doses were observed in five adults and four infants and included nausea, vomiting, diarrhea, flushing of the face, headache, fatigue, and disturbed sleep. Skin rashes were also seen in the infants.

Baker et al. (1969) conducted a study in which six volunteers were fed, initially, a synthetic liquid diet deficient in Vitamin C but adequate in all other nutrients. Isotopic labeling was accomplished through the administration of L-Ascorbic Acid-*l*-$^{14}$C on the 23rd day of deficiency. Repletion, which commenced on the 100th day, was accomplished through the daily administration of controlled amounts of the labeled vitamin.

The total Ascorbic Acid intake during the repletion phase ranged from 6.5 to 66.5 mg. Labeling the body Ascorbic Acid pool during the depletion phase resulted in no detectable urinary excretion of $^{14}$C-labeled reduced Ascorbic Acid or dehydroascorbic acid. Urinary excretion of $^{14}$C by all subjects occurred as a first order process during the depletion phase.

The first clinical signs of mild scurvy appeared in the subjects when their body Ascorbic Acid pool had been reduced to approximately 300 mg. Once the body pool of Ascorbic Acid was repleted to a level of 1.5 g, urinary loss of reduced Ascorbic Acid occurred. The rate of repletion of Ascorbic Acid was found to be a zero-order process and proportional to the daily intake of Ascorbic Acid. The daily intake of 6.5 mg/kg of Ascorbic Acid was sufficient to alleviate the clinical signs in one of the subjects (Baker et al. 1969).

In a study by Anderson et al. (1972), each of 407 test subjects ingested 1 g/day of a mixed Ascorbic Acid and Sodium Ascorbate preparation for at least 2 months, in addition to Ascorbic Acid in their regular diets. When the subjects first detected signs or symptoms of a cold, they increased their intake of the Ascorbic Acid and Sodium Ascorbate to 4 g/day. No toxic effects were noted in any of the subjects.

Barness (1974) reported oxaluria, renal stones, acidosis, glycosuria, renal tubular disease, gastrointestinal disturbances, and fatigue as toxic effects from intakes of 250 mg to 15 g Ascorbic Acid per day in humans.

Crawford et al. (1975) administered 1 g/day Ascorbic Acid for 3 months to 12 men and 12 women. Six of each sex served as controls. Blood samples were drawn before administration and one month and three months after administration. Ascorbic Acid did not significantly influence the levels of serum concentrations of cholesterol, plasminogen activator activity, plasminogen, fibrinogen, FR-antigen, partial thromboplastin time, platelet adhesiveness, $\alpha_1$-antitrypsin, or $\alpha_2$-macroglobulin.

Hines (1975) observed a Vitamin $B_{12}$ deficiency in patients who stated they ingested 1 g Ascorbic Acid with each meal (3 g/day total) for 3 years or more. These subjects had below normal serum Vitamin $B_{12}$ concentrations, hypersegmented neutrophils and occasional ovalomacrocytes, although none were anemic.

Shilotri and Bhat (1977) studied the effect of ingesting Ascorbic Acid on leukocyte function in five normal male volunteers. During the first 15 days the subjects received daily supplements of 200 mg of Ascorbic Acid, and during the next 2 weeks they were given 2 g of Ascorbic Acid per day. Supplementation of 200 mg of Ascorbic Acid per day did not affect bacterial killing by leukocytes. However, daily intake of 2 g Ascorbic Acid for 2 weeks significantly impaired bactericidal activity. Four weeks after withdrawal of vitamin supplementation, bactericidal activity returned to normal.

Gray (1994) listed diarrhea, abdominal cramps, nausea, heartburn, headache, flushing, dry ear/nose/throat, nose bleeds, sleep disturbances, kidney stones, tooth enamel destruction, and increased dental caries with excessive use of chewable tablets as symptoms of hypervitaminosis/acute toxicity of Ascorbic Acid.

Postaire et al. (1997) conducted a randomized, double-blind study in 20 healthy subjects to evaluate and compare the efficacy of two mixtures of dietary antioxidants. Twenty healthy Caucasians, 11 women and 9 men, with skin phototype II were used in the study. The formulations per capsule were 13 mg carotene, 2 mg of lycopene, 5 mg Vitamin E, and 30 mg Vitamin C (B13/L2) or 3 mg carotene, 3 mg lycopene, 5 mg Vitamin E, and 30 mg Vitamin C (B3/L3). The volunteers took two capsules of B13/L2 or B3/L3 daily for 8 weeks. A chromameter was used to measure redness, yellow components, and lightness of the skin. Multiple reflection spectrometry directly determined melanin and a reflection spectrometer determined carotene concentration.

For the B3/L3 dosage there was no significant influence on skin color (no cartenodermia) as far as redness, lightness of skin, and yellow components. The 8-week B12/L2 supplementation did lead to detectable cartenodermia. Significant increases in carotene concentrations in the skin were found after the 8 weeks with the group supplemented with the B13/L2 mixture. Significant increases of melanin concentration in the skin was found after 4, 5, 6, and 8 weeks of intake of both groups (Postaire et al. 1997).

CTFA (1998) reported a study in which the irritancy potential of a skin product containing 10% Ascorbic Acid ("Vitamin C") was evaluated via a 4-day Mini-Cum patch assay. A skin product without Ascorbic Acid was used as a negative control. Both materials were tested full strength under occlusive patch conditions. The primary irritation index (PII) of the test material was 0.40 compared to the negative control PII of 0.38. The test laboratory stated that the product with Ascorbic Acid had acceptable irritancy results.

KGL, Inc. (1998) tested a facial treatment containing 10% Ascorbic Acid on human skin in a maximization assay. Twenty-six of 29 male and female panelists completed the study. Approximately 0.1 ml of aqueous sodium lauryl sulfate (SLS) (0.25%) was applied to the volar forearm or the back of each subject and a patch was fastened to the skin with occlusive tape for 24 h. After 24 h, the SLS patch was removed and 0.1 ml of the test material was applied to the same site before the site was again covered with occlusive tape (induction patch). Since the test material contained volatile ingredients, it was allowed to air dry for ~30 min prior to application to the test site and was covered.

The induction patch was left in place for 48 h (or 72 h when placed over a weekend) following which it was removed and the site again examined for irritation.

If no irritation was present, a 0.25% aqueous SLS patch was again reapplied to the same site for 24 h, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence, 24-h SLS pretreatment followed by 48 h of test material application, was continued for a total of 5 induction exposures. If irritation developed at any time-point during the induction phase as previously outlined, the 24-h SLS pretreatment patch was eliminated and only the test material was reapplied to the same site after a 24-h rest period during which no patch was applied.

After a 10-day rest period that followed the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm, or side of back to determine if sensitization had developed. Pretreatment with SLS was performed prior to challenge. Approximately 0.1 ml of a 5% aqueous solution was applied to a fresh skin site under a 15-mm disc of cotton and covered with occlusive tape. The SLS patch was left in place for 1 hour. The challenge patch was then covered by occlusive tape and left in place for 48 h. After that period, the patch was removed and the site was graded one hour later and again 24 h later for any reaction. Under the conditions of this test, the test sample with 10% Ascorbic Acid did not possess a detectable contact-sensitizing potential and hence is not likely to cause contact sensitivity under normal use conditions (KGL, Inc. 1998).

Harrison Research Laboratories, Inc. (2000) tested an opaque cream containing 5% Ascorbic Acid for dermal sensitization in a repeat-insult patch test (RIPT). The odd-numbered subjects of these 200 subjects were patched with the test material (115 subjects were inducted); 103 subjects completed the test. The 12 subjects eliminated from the study dropped due to personal reasons, not due to the test material reaction. The 5% Ascorbic Acid cream did not induce dermal sensitization in human subjects.

## Clinical Treatment

*Ascorbic Acid*

Greenwood (1964) placed patients with early disc lesions, with either simple lumbosacral strain or definite sciatic nerve involvement, on Vitamin C, 750 to 1000 mg daily. Patients with severe disc involvement were given at least a short trial of Vitamin C therapy in spite of the obvious need for surgery. A small percentage of these recovered without surgery. Some recurred later.

All patients with chronic disease, particularly those with generalized disc involvement were placed on Vitamin C. All postoperative patients were placed on 500 mg/day indefinitely, increasing the dose to 750 to 1000 mg when discomfort occurred. The author stated that a significant percentage of patients with early disc lesions were able to avoid surgery by the use of large doses of Vitamin C. Many of these patients stopped their Vitamin C supplementation and the symptoms recurred. When they were placed back on the supplementation, the symptoms abated. Some of these patients turned to surgery (Greenwood 1964).

Halperin et al. (1993) conducted a study of 84 patients (43 males and 41 females) to ascertain the value of topical Ascorbic Acid in the prevention of radiation dermatitis. Patients with a primary brain tumor requiring external beam irradiation and patients with brain metastases requiring palliative external beam irradiation were eligible for inclusion in this study. Beginning at the initiation of radiotherapy patients were asked to apply a topical solution to the left and right sides of the irradiated head. The solutions were to be applied twice per day prior to and throughout the course of radiotherapy. In half of the bottles of solution, the coded bottle contained 10% Ascorbic Acid ($w/v$) solution in 20% propylene glycol ($v/v$) and the other half contained the vehicle only. The patients were randomized between those who were applying ASC solution to the left side of the head and the vehicle to the right side and the reverse. Each week the radiotherapist scored the skin reaction on both the left and right halves of the radiation field. A final scoring was done 1 week following the conclusion of irradiation, if possible.

Nineteen of the 84 patients dropped out of the study. In 10 patients there was a preference for the Ascorbic Acid solution (15%), in 20 patients there was a preference for the placebo (31%), and there was a preference for neither in 35 patients (54%). There was no discernible benefit of the Ascorbic Acid lotion for the prevention of radiation dermatitis (Halperin et al. 1993).

Alster and West (1998) conducted a study in order to determine the effectiveness of two formulations of topical Ascorbic Acid in reducing the degree and duration of post-$CO_2$ laser resurfacing erythema. Twenty-one patients (1 male and 20 female) had undergone a full-face $CO_2$ laser resurfacing. The study was limited to patients with skin types I, II, and III and the same high-energy, pulsed $CO_2$ laser was used by one operator. Skin type I is extremely fair skin—always burns, never tans. Skin type II is fair skin—always burns, sometimes tans. Skin type III is medium skin—sometimes burns, always tans. Each subject received two to three laser treatments.

Postoperative wound care included continuous topical application of ice and Catrix-10 ointment. At postoperative days 5 to 7, all patients were started on a petrolatum-based emollient cream. Thirteen to 42 days postoperatively, one-half of the patients were randomly selected to receive topical Vitamin C (10% Ascorbic Acid, 2% zinc sulfate, and 0.5% tyrosine) prepared in either an aqueous or cream-based formulation once a day. The other half of the face continued to be treated with a petrolatum-based emollient cream. Cutaneous erythema measurements were recorded with a hand-held reflectance spectrometer.

One patient who had applied the aqueous Ascorbic Acid formulation developed skin irritation and discontinued the study. Irritation was experienced by a second patient 1 week after beginning the aqueous application but was switched to the cream,

which was tolerated without further difficulty. Application of topical Ascorbic Acid in an aqueous formulation resulted in a significant decrease in post $CO_2$ laser resurfacing erythema by the 8th postoperative week when compared with laser-irradiated skin that had not received topical Vitamin C. The cream application of Ascorbic Acid did not result in a significant reduction in post $CO_2$ laser resurfacing erythema (Alster and West 1998).

Personelle et al. (1998) treated 21 patients with ACE Pool (0.02% Vitamin A, 0.05% Ascorbic Acid, and 0.05% Vitamin E) by dermal injection in specific areas after routine plastic surgeries; 4 rhytidectomy, 10 breast reduction, and 7 abdominoplasties. Each necrotic area received 1 to 3 cc of the injection daily for 15 days. The injection site was 5 mm behind the necrotic area at the intradermia superficial level and pointed toward the necrotic zone.

Treated areas healed faster than nontreated areas. The clinical evidence and the high levels of necrotic remission in the rat previously studied led the authors to suggest that Ascorbic Acid determines collagen tissue stabilization induced by the prolihydroxilation and that the association of these vitamins also determines vitamin E potentialization, which inhibits cellular lipid peroxidation (Personelle et al. 1998).

Traikovich (1999) determined the efficacy of topical Ascorbic Acid application in treating mild to moderate photodamage of facial skin in a 3-month, double-blind, vehicle controlled study. Nineteen evaluable volunteers between 36 and 72 years who were in good physical and mental health with mild to moderate photodamaged facial skin were considered for analysis (number in each sex not given). Coded, unmarked medications were randomly assigned to the left and right sides of each subject's face, one containing the active agent, topical Ascorbic Acid and the other, the vehicle serum. The primary composition of the active and vehicle serums contained Ascorbic Acid, tyrosine, zinc (active), bioflavinoid, hyaluronic acid, and water (vehicle). No concentrations were given. Three drops (0.5 ml) of each formulation were applied daily to the randomly assigned hemifaces over the 3-month study period. Treatment assignments were not disclosed to subjects or personnel.

Specific clinical parameters (fine wrinkling, tactile roughness, coarse rhytides, skin laxity/tone, sallowness/yellowing, and overall features) were evaluated and graded on a 0 to 9 point scale (0, none; 1 to 3, mild; 4 to 6, moderate; and 7 to 9, severe). Overall investigator scores were compared with baseline and graded as excellent (much improved), good (improved), fair (slightly improved), no change, or worse. Patient self-appraisal questionnaires were graded in the same manner, excellent to worse, and reported adverse effects (burning, stinging, redness, peeling, dryness, discoloration, itching, and rash).

Optical profilometry analysis was performed, the resulting image was digitally analyzed, and numeric values were assigned to reflect surface features. The parameter obtained included $R_z$, $R_a$, and shadows ("z" is analysis that divides the scan into sections and looks at the minimum-maximum within each segment, and "a" generates an average line through the center of the profile and determines the area above and below this line). These values provided subjective data that document pretreatment and posttreatment texture changes proportional to the degree of wrinkling, roughness, and other surface irregularities.

Optical profilometry image analysis demonstrated a statistically significant (73.7%) improvement in the $R_a$ and shadows north-south facial axis values with active treatment greater than the vehicle control, as well as a trend for improvement in the $R_z$ north-south facial axis parameter, showing a 68.4% greater improvement with active treatment versus control. Clinical assessment demonstrated a greater significant improvement with active treatment greater than the controls for fine wrinkling, tactile roughness, coarse rhytides, skin laxity/tone, sallowness/yellowing, and overall features. Patient questionnaire results demonstrated statistically significant improvement overall, with active treatment 82.4% greater than control. Photographic assessment demonstrated significant improvement, with active treatment 57.9% greater than control (Traikovich 1999).

*Magnesium Ascorbyl Phosphate*

Magnesium Ascorbyl Phosphate (VC-PMG) cream (10%) was applied twice a day to the skin of 34 patients with ephelides, chloasma, senile freckles, nevus of Ota, or healthy skin. The effectiveness of the lightening of the pigmentation was judged by a color-difference meter. The VC-PMG cream was effective or fairly effective in 19 of 34 patients. VC-PMG cream applied to the healthy skin of 25 patients resulted in 1 effective, 2 fairly effective, 8 slightly effective, 12 not effective, and 2 possible darkenings (Kameyama et al. 1996).

## Photoprotection

Eberlein-Konig et al. (1998) conducted a double-blind study in which each of 10 subjects took daily either 2 g Ascorbic Acid with 1000 IU of d-$\alpha$-tocopherol or a placebo. Six men and four women (skin type II: $n = 9$; skin type III: $n = 1$) were assigned to the treated group and four men and six women (skin type II: $n = 7$; skin type III: $n = 3$) received a placebo. The sunburn reaction was assessed before and 8 days after treatment by the determination of the threshold UV dose for eliciting a MED and by measuring the cutaneous blood flow of skin irradiated with incremental UV doses against that of non-irradiated skin. Twelve areas measuring 1.8 $cm^2$ each on the right or left lower back were exposed to increasing UVB doses (20, 28, 40, 57, 80, 113, 124, 160, 175, 200, 210, and 226 $mJ/cm^2$). The second test after 8 days was done on the other side of the lower back.

MEDs were not significantly different between the two groups before the trial started. The median MED of the treatment group increased from 80 to 96.5 $mJ/cm^2$, whereas it declined from 80 to 68.5 $mJ/cm^2$ in the placebo group. Cutaneous blood flow measured at test sites before exposure did not significantly differ between groups. Cutaneous blood flow changed at most irradiated sites, with significant increases in the placebo group and

significant decreases in the treatment group. The photoprotection by the vitamins was particularly evident at UV doses well above the MED (Eberlein-Konig et al. 1998).

Fuchs and Kern (1998) studied whether oral supplementation with D-alpha-tocopherol ($\alpha$-Toc), L-Ascorbic Acid, or combined $\alpha$-Toc and Ascorbic Acid influenced the solar-simulated radiation (SSR) induced skin inflammation in 40 healthy volunteers. The human subjects were divided into four groups: group 1, 10 subjects receiving orally 2 g $\alpha$-Toc/day for 50 days; group 2, 10 subjects taking Ascorbic Acid 3 g/day for 50 days; group 3, 10 subjects taking $\alpha$-Toc 2 g and Ascorbic Acid 3 g/day for 50 days; and group 4, 10 subjects taking a placebo. Before and after 50 days of supplementation, $\alpha$-Toc and Ascorbic Acid concentrations were analyzed in buccal mucosal keratinocytes. The dose response curve of UV erythema was determined by reflectance spectrophotometry and the MED by visual grading before and after supplementation. Ten small areas (2 $cm^2$) of the untanned buttock skin were exposed to increasing doses (25, 50, 75, 100, 125, 150, 200, 250, 300, and 400 $mJ/cm^2$) of SSR for the determination of the dose response curve. 50 days after supplementation $\alpha$-Toc keratinocytes levels were increased in groups 1 and 3, Ascorbic Acid concentrations were elevated in groups 2 and 3.

The dose response curve of UVR induced erythema showed a significant flattening and the MED increased from $103 \pm 29$ $mJ/cm^2$ (before supplementation) to $183 \pm 35$ $mJ/cm^2$ (after supplementation) in group 3, whereas there were no significant changes in groups 1 and 2 after supplementation. The authors concluded that $\alpha$-Toc and Ascorbic Acid synergistically suppress sunburn reactions (Fuchs and Kern 1998).

Dreher er al. (1999) conducted a randomized, double-blinded, placebo controlled human (six subject, three males and three females) to study the short-term photoprotective effects of antioxidants when applied after UVR exposure. Melatonin, alpha-tocopherol, and Ascorbic Acid were topically administered alone or in combination as shown in Table 24.

Three controls were used. At predetermined time intervals after completing the irradiation with one minimal erythemal dose (determined on the mid-back) 5.85 $\mu$l of the respective formulation were pipetted on to the $1.5 \times 1.5$ cm premarked area on the lower back of the volunteer (resulting in 2.6 $\mu$l formulation per $cm^2$, corresponding to 2 $mg/cm^2$). The following measurements to evaluate the UV-induced skin response were performed just before irradiation (0 h) and 6, 24, and 48 h after irradiation: visual inspection of erythema, chromametry assessment of erythema, and dermal blood flow.

No significant protective effect of the antioxidants when applied alone or in combination were obtained when applied after UVR exposure. No improved photoprotective effect was obtained when multiple applications were done. The authors concluded that UVR-induced skin damage is a rapid event, and antioxidants possibly prevent such damage only when present at a relevant concentration at the site of action beginning and during oxidative stress (Dreher er al. 1999).

**TABLE 24**
Formulation composition % (Dreher et al. 1999)

| Formulation | Melatonin | $\alpha$-Tocopherol | Ascorbic Acid |
|---|---|---|---|
| K1 (unexposed and untreated skin) | 0 | 0 | 0 |
| K2 (UVR, untreated skin) | 0 | 0 | 0 |
| K3 (placebo-treated skin) | 0 | 0 | 0 |
| A1 | 1 | 0 | 0 |
| A2 | 2.5 | 0 | 0 |
| B1 | 0 | 2 | 0 |
| B2 | 1 | 2 | 0 |
| B3 | 2.5 | 2 | 0 |
| C1 | 0 | 0 | 5 |
| C2 | 0 | 2 | 5 |
| C3 | 1 | 2 | 5 |
| C4 | 2.5 | 2 | 5 |

## Case Studies

Klasson (1951) reported five cases in which Ascorbic Acid was used in treatment of burns. In case 1, a 22-year-old white male experienced first and second degree flash burns involving approximately 30% of his upper body surface. This included both arms, anterior chest wall, neck, and head. The patient was administered morphine and seen by doctors an hour later. A 1% solution of Ascorbic Acid in normal saline was applied to the wounds and there was an immediate relief of pain. The relief continued and no more morphine had to be given. During his hospital stay, his daily urinary output of Ascorbic Acid remained normal.

In case 2, a white male age 55 and a black male age 21 suffered flash burns at the same time involving the neck, face, scalp, both wrists, and both hands. These burns were classified as second degree burns. The head and neck regions were treated in the first 72 h with a 1% solution of Ascorbic Acid in normal saline, followed by dressings with a 2% Ascorbic Acid ointment. The hands and wrists were treated with sterile gauze and compression dressings, followed by dressings with Furacin ointment. Each patient received 200 mg orally of Ascorbic Acid four times daily throughout the treatment period. The daily urinary output of Ascorbic Acid was normal and at the end of 3 weeks, the head and neck of each was healed. The hands and wrists required approximately 30 more days of treatment before complete healing occurred.

In case 3, a white male, age 2.5 years, suffered second and third degree burns of his anterior chest wall and abdomen involving approximately 15% of his body surface. He was admitted to the hospital 1 week following the burns. The wounds were infected and he was in poor nutritional state. The child was placed on a high caloric diet and was given 900 mg Ascorbic

Acid orally. The infection was cleared by penicillin and later by daily local applications of a solution containing penicillin and saline followed by dressings containing 2% Ascorbic Acid ointment. The wound filled by gradual epithelization and required no grafting. Complete epithelization occurred in approximately 5 months without undue scarring.

In case 4, a 56-year-old male was admitted to a hospital with second degree burns of the face, neck and shoulder girdle, arm and hand, and third degree burns of the thumb, index, and middle finger. The total area burned was 25%. The burned areas were treated during the first 48 h with a 1% solution of Ascorbic Acid, followed by daily dressings with a 2% Ascorbic Acid ointment. During the first few days he was given 500 mg of Ascorbic Acid intramuscularly three times daily. This was followed by 300 mg Ascorbic Acid four times daily orally. His daily urinary output was normal and all wounds healed without undue scarring by the 69th hospital day.

In case 5, a 39-year-old white male received second degree burns involving his face, both hands, and wrists (12% body surface). The burned areas were treated during the first 48 h with a 1% Ascorbic Acid solution followed by daily dressings with a 2% Ascorbic Acid ointment. He received 300 mg of Ascorbic Acid four times daily orally throughout the treatment period. He was discharged on the 15th hospital day healed (Klasson 1951).

Lowry et al. (1952) administered 1000 mg/day of Ascorbic Acid to one woman and three men for 3 months. Ascorbic Acid concentration in the serum, white blood cells, and urine was measured. No progressive change of Ascorbic Acid concentration was observed in the serum, white cells, or urine. No harmful effects were observed during the 3 months.

Briggs et al. (1973) reported on a healthy man, 21 years old, who received two courses of Vitamin C supplementation at 4 g daily in divided doses. The volunteer's normal diet was free of oxalate-rich foods. His 24-h urinary oxalate level before supplementation was 58 mg, but rose to 622 mg and 478 mg, respectively (7 and 4 days). The usual 24-h oxalate increase after 4 g Ascorbic Acid daily for several days was about 12 mg.

## SUMMARY

This report reviews the safety of Ascorbic Acid the L-form, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate as used in cosmetic formulations. These ingredients function primarily as antioxidants in cosmetics. Related ingredients (Ascorbyl Palmitate, Ascorbyl Dipalmitate, Ascorbyl Stearate, Erythorbic Acid, and Sodium Erythorbate) have been previously reviewed by the CIR Expert Panel and found "to be safe for use as cosmetic ingredients in the present practices of good use."

Ascorbic Acid functions as an antioxidant and pH adjuster in cosmetic formulations. Of the 431 formulations reported by FDA, 310 were used in hair dyes and colors at concentrations between 0.3% and 0.6%. The reported concentrations for other product categories were either very low (<0.01%) or in the 5% to 10% range. One supplier reported preservative, skin-protectant, and sunscreen agent/UV filter functions for Ascorbic Acid in cosmetics.

Calcium Ascorbate and Magnesium Ascorbate function as antioxidants and as skin-conditioning agents—miscellaneous in cosmetics, but are not currently used. Sodium Ascorbyl Phosphate functions as an antioxidant in cosmetic products and is used at concentrations ranging from 0.01 to 3%. Magnesium Ascorbyl Phosphate functions as an antioxidant in cosmetics and was reported being used in 37 formulations over a wide concentration range (0.001% to 3%). Sodium Ascorbate also functions as an antioxidant in cosmetics and was reported being used in 6 formulations over a wide concentration range (0.0003% to 0.3%).

Ascorbic Acid is a GRAS substance for use as a chemical preservative in foods and as a nutrient and/or dietary supplement. Calcium Ascorbate and Sodium Ascorbate are listed as GRAS substances for use as chemical preservatives.

Estimates of median dietary intakes of Vitamin C for adults are 102 mg/day in the United States. The Tolerable Upper Intake Level for adults is set at 2 g/day according to the National Academy of Sciences. The adverse effects upon which the Upper Intake Level is based are osmotic diarrhea and gastrointestinal disturbances.

L-Ascorbic Acid is readily and reversibly oxidized to L-dehydroascorbic acid and both forms exist in equilibrium in the body. In alkaline solution, L-dehydroascorbic acid is hydrolyzed to L-diketogulonic acid and this reaction is not reversible within the body.

The Ascorbic Acid body pool of rats average ~10.7 mg/100 g/bw and was synthesized at an average of 2.6 mg/100 g/bw. Approximately 15% of Ascorbic Acid synthesized each day in rats was excreted in the urine. The remaining Ascorbic Acid was in part degraded to $CO_2$ or oxalic acid.

In guinea pigs, which require a dietary source of Ascorbic Acid, ~48% to 63% of ingested Ascorbic Acid was eliminated in the urine, 0.2% to 0.43% in the feces, and 5.5% in expired air. The incorporation of Ascorbic Acid was markedly greater in the adrenals, lungs, and bones of guinea pigs. In rats and guinea pigs devoid of Ascorbic Acid in the diet, an increased catabolism of Ascorbic Acid was evident. The degradation of Ascorbic Acid followed first order kinetics and the $K_M$ for the guinea pig small intestine was ~0.3 mM. Ascorbic Acid has an important relationship with the oxidation of transition metals such iron or copper at enzyme active sites and in food.

Ascorbic Acid and Sodium Ascorbate acted as a nitrosation inhibitor in several food and cosmetic product studies.

The octanol/water and stratum corneum/viable skin partition coefficients of Ascorbic Acid are $0.02 \pm 0.002$ and $0.25 \pm 0.13$, respectively. Permeation rates through whole and stripped mouse skin were $3.43 \pm 0.74$ $\mu g/cm^2/h$ and $33.2 \pm 5.2$ $\mu g/cm^2/h$.

The following acute oral $LD_{50}$s of Ascorbic Acid were reported: mouse >5000 mg/kg bw, rat >5000 mg/kg bw, rabbit

>2000 mg/kg bw, cat >1000 mg/kg bw, dog >5000 mg/kg bw, and guinea pig >5000 mg/kg bw. The following oral Sodium Ascorbate LD50 values were estimated; mice >5000 mg/kg bw, rats >5000 mg/kg bw, and guinea pigs >5000 mg/kg bw.

The following acute parenteral $LD_{50}$s of Ascorbic Acid were reported: mouse, 1058 to 5000 mg/kg/day; rat, 1000 to 5000 mg/kg/day; guinea pig, 500 to 2000 mg/kg/day; rabbit, 1000 mg/kg/day; cat, 500 to 1000 mg/kg/day; and dog, 200 mg/kg/day.

Ascorbic Acid stimulated collagen production in human skin fibroblasts, pig vascular smooth muscle cells, and Tenon's fibroblasts and enhanced mRNA transcription levels of type I and III collagen genes.

Mice (500 to 1000 mg/kg bw) and guinea pigs (400 to 2500 mg/kg bw) receiving Ascorbic Acid orally daily for 7 days had no difference in appetite, weight gain, and general behavior compared to controls receiving no Ascorbic Acid; histological examination of the kidney, pancreas, liver, heart, and lungs showed no change. The maximum toxic dose of Ascorbic Acid in rats over a period of 10 weeks was 10 g/kg bw. Male and female F344/N rats and $B6C3F_1$ mice were fed diets containing 0, 6000, 12,500, 25,000, 50,000, or 100,000 ppm Ascorbic Acid for 14 days. No compound-related clinical signs or gross or microscopic pathological effects were observed in either species.

The following short-term parenteral Ascorbic Acid LD50s were reported: mouse, 1058 mg/kg/day (10 days); rat, >600 mg/kg/day (28 days); guinea pig, 100 mg/kg/day (7 days); rabbit, 500 mg/kg/day (7 days); rabbit, 100 mg/kg/day (16 days); cat, >500 mg/kg/day (9 days); and dog, >2000 mg/kg/day (3 days).

Male guinea pigs fed a control basal diet and given 0.5 mg to 250 mg Ascorbic Acid orally for 20 weeks had similar hemoglobin, blood glucose, serum iron, liver iron, and liver glycogen levels compared to control values. These doses of Ascorbic Acid were neither beneficial nor toxic to the guinea pigs. Male and female F344/N rats and $B6C3F_1$ mice were fed diets containing 0, 6000, 12,500, 25,000, 50,000, or 100,000 ppm Ascorbic Acid for 91 days. Mean body weights were somewhat depressed in male mice and female rats receiving the greater doses of Ascorbic Acid. Cystic endometrial glands were found in the uteri of 4/9 female rats receiving 100,000 ppm compared to none of the controls. Alterations of the femoral bone marrow (reticulum-cell hyperplasia) were observed in 7/30 female rats receiving 25,000 ppm Ascorbic Acid or more. These changes were not seen in the female controls or in any male rat groups. Femoral bone marrow lesions were characterized by multiple foci of cells that appeared to be proliferating fibroblasts replacing the normal myeloid elements and fat cells of the marrow. Myeloid depletion was observed in 6/20 rats receiving 50,000 ppm or more Ascorbic Acid. Femoral lesions in the female rats were not considered to be potentially life-threatening. Minimum toxic doses of Sodium Ascorbate and Sodium Nitrite given to male and female Wistar rats concurrently for 6 months were 2% and 0.15%, respectively.

Chronic Ascorbic Acid feeding studies showed toxic effects at dosages above 25 mg/kg bw in rats and guinea pigs. Groups of male and female rats given daily doses of 0, 1000, 1500, or 2000 mg/kg bw Ascorbic Acid for two years had no macro- or microscopically detectable toxic lesions.

Mice given Ascorbic Acid subcutaneous and intravenous daily doses of Ascorbic Acid (500 to 1000 mg/kg bw) for 7 days had no changes in appetite, weight gain, and general behavior; histological examination of various organs showed no changes.

Ascorbic Acid administration elevated retinal ascorbate and reduced the loss of rhodopsin and photoreceptor nuclei resulting from intense light, and was a photoprotectant when applied to mice and pig skin before exposure to both UVA and UVB. The inhibition of UVR-induced suppression of contact hypersensitivity was also noted. Magnesium Ascorbyl Phosphate administration immediately after exposure in hairless mice significantly delayed skin tumor formation and hyperplasia induced by chronic exposure to 2 $kJ/m^2$ of UVB.

Rabbit eyes subjected to severe alkali burns and 10% topical Ascorbic Acid had significantly lower percentage of ulceration or perforation when compared to controls receiving no Ascorbic Acid; alkali-injured rabbit eyes receiving a 10% Sodium Ascorbate solution had significantly lower ulcerations than in nontreated eyes, consistent with antioxidant properties.

Pregnant mice and rats were given daily oral doses of 150, 250, 500, and 1000 mg/kg bw from days 6 to 15 of pregnancy. There were no indications of adult-toxic, teratogenic, or fetotoxic effects. There was no apparent effect on the embryonic and postpartum development of the young or on breeding behavior, pregnancy, parturition, and lactation capacity of the mother animals.

The administration of 520 mg/kg bw of Ascorbic Acid to pregnant mice for 10 consecutive days had no clear effect on nidation or on maternal or fetal survival. The number of abnormalities observed in either soft or skeletal tissues of the treated group did not differ from those observed in the negative-control group.

No increase in abortion or mortality was observed in offspring of guinea pigs, rats, and hamsters exposed to large daily doses of Ascorbic Acid during pregnancy. Ascorbic Acid was a nonteratogen in the in vitro micromass assay (single cell suspensions of midbrain and limb-buds from 13-day rat embryos).

Ascorbic Acid and Sodium Ascorbate were not genotoxic in several bacterial and mammalian test systems, consistent with the antioxidant properties of these chemicals. In the presence of certain enzyme systems or metal ions, positive results were seen, consistent with these chemicals acting as pro-oxidants in these test conditions.

In rats given daily doses of 1000, 1500, or 2000 mg/kg bw of L-Ascorbic Acid for 2 years, no adverse effects were observed in hematological examinations, urinalysis, liver, or renal function tests. Gross examination revealed no toxic lesions attributable to Ascorbic Acid. The National Toxicology Program conducted

a 2-year carcinogenesis bioassay of Ascorbic Acid (25,000 and 50,000 ppm) in F344/N rats and B6C3F$_1$ mice. Ascorbic Acid was not carcinogenic in either sex of both rats and mice.

Inhibition of carcinogenesis and tumor growth related to Ascorbic Acid's antioxidant properties has also been reported.

Sodium Ascorbate has been shown to promote the development of urinary carcinomas in two-stage carcinogenesis studies. This effect appears related to sodium ion concentration and pH in urine and can be produced by many chemicals.

Ascorbic Acid was found to effectively inhibit nitrosamine yield in several test systems.

Healthy adult males had increased oxalate excretion with the daily ingestion of 9 g of Ascorbic Acid. Excretion of Ascorbic Acid takes place by glomerular filtration and active tubular resorption. Ascorbic Acid was found in the following human tissues; adrenal glands, pituitary gland, liver, spleen, lungs, kidneys, testes, thyroid, heart muscle, skeletal muscle, brain, pancreas, eye lens, plasma, and saliva. The calculated $K_M$ and $V_{max}$ for Ascorbic Acid uptake in the human small intestine were 5.44 mM and 0.28 mM/cm/h, respectively. Absorption of Ascorbic Acid does decline at high doses. The typical body pool size is 1500 mg, of which 3% to 4% is utilized daily. The greatest human tissue concentrations are found in the adrenal and pituitary glands, with a lesser amount in the brain, pancreas, spleen, and liver. The renal threshold for Ascorbic Acid is reached at approximately 1.5 mg/dl of plasma. Average 24-h excretion in normal adults is 8 to 27 mg.

Doses of Ascorbic Acid up to 6000 mg given to adults and children for more than 1400 days had toxic effects in five adults and four infants and included nausea, vomiting, diarrhea, flushing of the face, headache, fatigue, and disturbed sleep. No harmful effects were observed in one woman and three men given 1000 mg/day Ascorbic Acid administration for 3 months. Oxaluria, renal stones, acidosis, glycosuria, renal tubular disease, gastrointestinal disturbances, and fatigue were reported toxic effects in humans taking 250 mg to 15 g Ascorbic Acid per day.

Dermal application of Ascorbic Acid to patients with radiation dermatitis and burn victims produced no adverse effects. Ascorbic Acid was a photoprotectant in clinical human UV studies at doses well above the MED.

An opaque cream containing 5% Ascorbic Acid did not induce dermal sensitization in 103 human subjects. A product containing 10% Ascorbic Acid was a nonirritant in a 4-day minicumulative patch assay on human skin and a facial treatment containing 10% Ascorbic Acid was not a contact sensitizer in a maximization assay on 26 humans.

## DISCUSSION

The CIR Expert Panel determined the data provided in this report to be sufficient to assess the safety of L-Ascorbic Acid, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate. Because of the structural and functional similarities of these ingredients, the Panel believes that the data on one ingredient can be extrapolated to all of them.

These ingredients exhibited little acute or short-term toxicity in animal studies and the toxicity seen in some clinical studies occurred only at extremely high ingestion levels which are not relevant to the use of these ingredients in cosmetics. Reproductive and developmental studies were negative.

The Expert Panel was concerned that Ascorbic Acid was genotoxic in a few assay systems. In most of the other assay systems, Ascorbic Acid was not genotoxic. The Panel attributed the finding that Ascorbic Acid was genotoxic in these few assay systems due to the presence of other chemicals, e.g., metals, or certain enzyme systems, which effectively convert Ascorbic Acid's antioxidant action to that of a pro-oxidant. When Ascorbic Acid acts as an antioxidant, the Panel concluded that Ascorbic Acid is not genotoxic. Supporting this view were the carcinogenicity studies conducted by the NTP, which demonstrated no evidence of carcinogenicity. The Panel did review studies in which Sodium Ascorbate acted as a tumor promoter in animals. These results were considered to be related to the concentration of sodium ions and the pH of urine in the test animals. Similar effects were seen with sodium bicarbonate. Because of the concern that certain metal ions may combine with these ingredients to produce pro-oxidant activity, the Panel cautioned formulators to be certain that these ingredients are acting as antioxidants in cosmetic formulations.

The Panel considered that the clinical experience in which Ascorbic Acid was used on damaged skin with no adverse effects and the RIPT using 5% Ascorbic Acid with negative results supports the finding that this group of ingredients do not present a risk of skin sensitization. This data coupled with an absence of reports in the clinical literature of Ascorbic Acid sensitization, strongly supports the safety of these ingredients.

## CONCLUSION

Based on the available data contained in this report, the CIR Expert Panel concludes that L-Ascorbic Acid, Calcium Ascorbate, Magnesium Ascorbate, Magnesium Ascorbyl Phosphate, Sodium Ascorbate, and Sodium Ascorbyl Phosphate are safe as used in cosmetic products.

## REFERENCES

Adams, J. B. 1997. Food additive-additive interactions involving sulphur dioxide and ascorbic and nitrous acids: A review. *Food Chem.* 59:401–406.

Alleva, F. R., J. J. Alleva, and T. Balazs. 1976. Effect of large daily doses of ascorbic acid on pregnancy in guinea pigs, rats, and hamsters. *Toxicol. Appl. Pharmacol.* 35:393–395.

Alster, T. S., and T. B. West. 1998. Effect of topical vitamin C on postoperative carbon dioxide laser resurfacing erythema. *Dermatol. Surg.* 24:331–334.

Amacher, D. E., and S. C. Paillet. 1981. Ascorbate is not detectably mutagenic in the L5178Y TK$^{+/-}$ cell mutation assay. *Cancer Lett.* 14:151–158.

Anderson, D., N. Basaran, S. D. Blowers, and A. J. Edwards. 1995. The effect of antioxidants on bleomycin treatment in in vitro and in vivo genotoxicity assays. *Mutat. Res.* 329:37–47.

Anderson, D., and A. J. Francis. 1993. The modulating effect of antioxidants in rat embryos and Sertoli cells in culture. *Basic Life Sci.* 61:189–200.

Anderson, T. W., D. B. W. Reid, and G. H. Beaton. 1972. Vitamin C and the common cold: A double blind trial. *Can. Med. Assoc. J.* 107:503–508.

Andersson, M., and K. Grankvist. 1995. Ascorbate-induced free radical toxicity to isolated islet cells. *Int. J. Biochem. Cell Biol.* 27:493–498.

Appenroth, D., K. Winnefeld, H. Schroter, and M. Rost. 1994. The ambiguous effect of ascorbic acid on chromate induced proteinuria in rats. *Arch. Toxicol.* 68:138–141.

Asamoto, M., Y. Shichino, H. Tsuda, and N. Ito. 1990. Inhibition of rat hepatic glutathione S-transferase placental form positive foci development by concomitant administration of antioxidants to carcinogen-treated rats. *Cancer Lett.* 55:25–29.

Atkins, G. L., B. M. Dean, W. J. Griffin, and R. W. E. Watts. 1964. Quantitative aspects of ascorbic acid metabolism in man. *J. Biol. Chem.* 239:211–216.

Baker, E. M., R. E. Hodges, J. Hood, H. E. Sauberlich, and S. C. March. 1969. Metabolism of ascorbic-l-$^{14}$C acid in experimental human scurvy. *Am. J. Clin. Nutr.* 22:549–558.

Banerjee, S., and H. D. Singh. 1958. Cholesterol metabolism in scorbutic guinea pigs. *J. Biol. Chem.* 233:336–339.

Banic, S. 1981. Vitamin C acts as a cocarcinogen to methylcholanthrene in guinea pigs. *Cancer Lett.* 11:239–242.

Barness, L. A. 1974. Safety considerations with high ascorbic acid dosage. *Trans. N. Y. Acad. Sci.* 36:818.

Basu, T. K. 1985. The conditioning effect of large doses of ascorbic acid in guinea pigs. *Can. J. Physiol. Pharmacol.* 63:427–430.

Bates, C. J. 1997. Bioavailability of vitamin C. *Eur. J. Clin. Nutr.* 51:28–33.

Becker, R. R, H. B. Burch, L. L. Salomon, T. A. Venkitasurbramanian, and C. G. King. 1953. Ascorbic Acid deficiency and cholesterol synthesis. *J. Am. Chem. Soc.* 75:2020.

Ben-Gurion, R. 1981. Mutagenic and colicin-inducing activity of some antioxidants. *Prog. Mutat. Res.* 2:11–17.

Berg, L. R., and W. W. Lawrence. 1971. Cottonseed oil, dehydrated grass and ascorbic acid as dietary factors preventing toxicity of vandium for the chick. *Poult. Sci.* 50:1399–1404.

Bijur, G. N., M. E. Ariza, C. L. Hitchcock, and M. V. Williams. 1997. Antimutagenic and promutagenic activity of ascorbic acid during oxidative stress. *Environ. Mol. Mutagen.* 30:339–345.

Bissett, D. L., R. Chatterjee, and D. P. Hannon. 1992. Protective effect of a topically applied anti-oxidant plus an anti-inflammatory agent against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *J. Soc. Cosmet. Chem.* 43:85–92.

Black, H. S., and J. T. Chan. 1975. Supression of ultraviolet light-induced tumor formation by dietary antioxidants. *J Invest. Dermatol.* 65:412–414.

Blanchard, J. 1991. Effects of gender on vitamin C pharmacokinetics in man. *J. Am Coll. Nutr.* 10:453–459.

Briggs, M. H., P. Garcia-Webb, and P. Davies. 1973. Urinary oxalate and vitamin C supplements. *Lancet.* 2:201.

Budavari, S., ed. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed. Rahway, NJ: Merck & Co., Inc.

Burns, J., E. Mosbach, and S. Schulenburg. 1954. Ascorbic acid synthesis in normal and drug-treated rat studies with L-ascorbic-l-C$^{14}$ acid. *J. Biol. Chem.* 207:679–687.

Cagli, V., G. P. Cantore, J. Giraldi Pecori, and M. Virno. 1965. Azione diuretica dell'ascorbato di sodio: Osservazioni sugli animali e sull'uomo. *Policlinico Sez. Prat.* 72:836–842.

Cardesa, A., S. S. Mirivish, G. T. Haven, and P. Shubik. 1974. Inhibitory effect of ascorbic acid on the acute toxicity of dimethylamine plus nitrite in the rat. *Proc. Soc. Exp. Biol. Med.* 145:124–128.

Castranova, V., H. R. Wright, H. D. Colby, and P. R. Miles. 1983. Ascorbate uptake by isolated rat alveolar macrophages and type II cells. *J. Appl. Physiol.* 54:208–214.

Chatterjee, G. C., S. Chatterjee, K. Chatterjee, and A. Sahu. 1979. Studies on the protective effects of ascorbic acid in rubidium toxicity. *Toxicol. Appl. Pharmacol.* 51:47–58.

Chauhan, P. S., M. Aravindakshan, and K. Sundaram. 1978. Evaluation of ascorbic acid for mutagenicity by dominant lethal test in male Wistar rats. *Mutat. Res.* 53:166.

ChemID. 2000. Chemical synonyms. *ChemID database.* Bethesda, MD: National Library of Medicine.

Chorvatovicova, D., E. Ginter, A. Kosinova, and Z. Zloch. 1991. Effects of vitamins C and E on toxicity and mutagenicity of hexavalent chromium in rat and guinea pig. *Mutat. Res.* 262:41–46.

Cohen, S. M., M. Cano, E. M. Garland, M. S. John, and L. L. Arnold. 1995. Urinary and urothelial effects of sodium salts in male rats. *Carcinogenesis.* 16:343–348.

Cohen, S. M., T. A. Anderson, L. M. de Oliveira, and L. L. Arnold. 1998. Tumorigenicity of sodium ascorbate in male rats. *Cancer Res.* 58:2557–2561.

Colomina, M. T., M. Gomez, J. L. Domingo, and J. Corbella. 1994. Lack of maternal and developmental toxicity in mice given high doses of aluminum hydroxide and ascorbic acid during gestation. *Pharm . Toxicol.* 74:236–239.

Colvin, R. M., and S. R. Pinnell. 1996. Topical vitamin C in aging. *Clin. Dermatol.* 14:227–234.

Conney, A., G. Bray, C. Evans, and J. Burns. 1961. Metabolic interactions between L-ascorbic acid and drugs. *Ann. N. Y. Acad. Sci*. 92:115–127.

Cosmetic Ingredient Review (CIR). 1997. Final report on the safety assessment of ascorbyl palmitate, ascorbyl dipalmitate, ascorbyl stearate, erythorbic acid, and sodium erythrobate. Washington, DC:CIR

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2000. Concentration of use data for the ascorbic acid ingredients. Unpublished data submitted by CTFA. 3 pages.[2]

CTFA. 1998. 4-Day mini-cum irritancy assay of Anew Vitamin C Serum. Unpublished data submitted by CTFA. 1 page.[2]

Crawford, G. P. M., C. P. Warlow, B. Bennett, et al. 1975. The effect of vitamin C supplements on serum cholesterol, coagulation, fibrinolysis, and platelet adhesiveness. *Atherosclerosis.* 21:541–544.

Cuevas Amabile CH. 1988. Loss of penicillinase plasmids of *Staphylococcus aureus* after treatment with L-ascorbic acid. *Mutat. Res.* 207:107–109.

Curtain, C. H., and C. G. King. 1955. The metabolism of ascorbic acid-l-C$^{14}$ and oxalic acid in the rat. *J. Biol. Chem.* 216:539–549.

Darr, D., S. Combs, S. Dunston, T. Manning, and S. Pinnell. 1992. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br. J. Dermatol.* 127:247–253.

Darr, D., S. Dunston, H. Faust, and S. Pinnel. 1996. Effectiveness of antioxidants (vitamin C and vitamin E) with and without sunscreens as topical photoprotectansts. *Acta Derm. Venereol.* 76:264–268.

Das, M., K. Garg, G. B. Singh, and S. K. Khanna. 1994. Attenuation of benzathrone toxicity by ascorbic acid in guinea pigs. *Fundam. Appl. Toxicol.* 22:447–456.

Das, N., and S. Nebioglu. 1992. Vitamin C interactions in laboratory animals. *J. Clin. Pharm. Ther.* 17:343–346.

David, J. C. 1976. Lethality of tyrosine in mice. *Naunyn Schmiedebergs Arch. Pharmacol.* 293:15–23.

Davidson, J. M., P. A. LuValle, O. Zoia, D. Quaglino, Jr., and M. Giro. 1996. Ascorbate differentially regulates elastin and collagen biosynthesis in vascular smooth muscle cells and skin fibroblasts by pretranslational mechanisms. *Dermatology* 193:88–93.

De Albuquerque, A., and M. A. Henriques. 1970. Ensaios sobre a toxicidade do acido ascorbico. *Rev. Port. Farm.* 20:41–46.

De Pauw-Gillet, M. C., B. Siwek, G. Pozzi, E. Sabbioni, and R. J. Bassleer. 1990. Control of B16 melanoma cells differentiation and proliferation by $CuSO_4$ and vitamin C. *Anticancer Res.* 10:391–395.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036-4702, USA.

Demole, V. 1934. On the physiological action of ascorbic acid and some related compounds. *Biochem. J.* 28:770–773.

DeYoung, D. J., J. D. Bantle, and D. J. Fort. 1991. Assessment of the developmental toxicity of ascorbic acid, sodium selenate, coumarin, serotonin, and 13-cis retinoic acid using FETAX. *Drug Chem. Toxicol.* 14:127–141.

Domingo, J. L., M. Gomez, J. M. Llobet, D. del Castillo, and J. Corbella. 1994. Influence of citric, ascorbic and lactic acids on the gastrointestinal absorption of aluminum in uremic rats. *Nephron* 66:108–109.

Douglass, M. L., B. L. Kabacoff, G. A. Anderson, and M. C. Cheng. 1978. The chemistry of nitrosamine formation, inhibition, and destruction. *J. Soc. Cosmet. Chem.* 29:581–606.

Dreher, F., N. Denig, B. Gabard, D. A. Schwindt, and H. I. Maibach. 1999. Effect of topical antioxidants on UV-induced erythema formation when administered after application. *Dermatology.* 198:52–55.

Dreyer, R., and R. C. Rose. 1993. Lacrimal gland uptake and metabolism of ascorbic acid. *Proc. Soc. Exp. Biol. Med.* 202:212–216.

Dumas, M., C. Chaudagne, F. Bonte, and A. Meybeck. 1996. Age-related response of human dermal fibroblasts to L-ascorbic acid: Study of type I and III collagen synthesis. *C. R. Acad. Sci. Paris* 319:1127–1132.

Dunnett, P. C., and G. M. Telling. 1983. Study of the fate of bronopol and the effects of antioxidants on *N*-nitrosamine formation in shampoos and skin creams. Unpublished data submitted by CTFA. 6 pages.[2]

Eberlein-Konig, B., M. Placzek, and B. Przybilla. 1998. Protective effect against sunburn of combined systemic ascorbic acid and d-alpha-tocopherol. *J. Am. Acad. Dermtol.* 38:45–48.

El-Bana, M., M. Hani-Ayobe, A. Malak, A. M. Saleh, and N. A. El-Damarawy. 1978. Studies on the metabolic effects of L-ascorbic acid. *Ain Shams Med. J.* 29:57–60.

European Economic Community (EEC). 1999. EEC Cosmetics Directive 76/768/EEC, as amended Annexes I–VII. Brussels: EEC.

Evans, R. M., L. Currie, and A. Campbell. 1982. The distribution of ascorbic acid between cellular components of blood, and its relation to the plasma concentration. *Br. J. Nature* 47:473–482.

Fann, Y. D., K. G. Rothberg, P. G. Tremel, J. S. Douglas, and A. B. DuBois. 1986. Ascorbic acid promoted prostanoid release in human lung parenchyma. *Prostaglandins* 31:361–368.

Federation of American Societies for Experimental Biology. 1979. Evaluation of the health aspects of ascorbic acid, sodium ascorbate, calcium ascorbate, erythorbic acid, sodium erythorbate, and ascorbyl palmitate as food ingredients. Report No. 223-75-2004. Submitted by FDA in response to an FOI request in 1999. 46 pages.[2]

Fellion, Y., J. De Smedt, and N. Brundney. 2000. An HPLC-UV method for the direct evaluatin of *N*-nitrosodiethanolamine in some cosmetic products and raw materials. Unpublished data submitted by CTFA. 7 pages.[2]

Fiddler, W., J. W. Pensabene, E. G. Piotrowski, R. C. Doerr, and A. E. Wasserman. 1973. Use of sodium ascorbate or erythorbate to inhibit formation of *N*-nitrosodimethylamine in frankfurters. *J. Food Sci.* 38:1084.

Food and Agriculture Organization/World Health Organization. 1974. WHO Food Additive Ser. No. 5. Geneva: WHO.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. Washington, DC: FDA.

FDA. 1992. Modification in Voluntary Filing of Cosmetic Product Ingredient and Cosmetic Raw Composition Statements. Final Rule. *Federal Register* 57:3128–3130.

FDA. 1994. OTC Drug Review Ingredient Status Report. September 1, 1994. Washington, DC: FDA.

FDA. 1999a. Acute toxicity (LD50) of Sodium Ascorbate. Submitted by FDA in response to an FOI request in 1999. 1 page.[2]

FDA. 1999b. Short-term toxicity (LD50—oral) of Ascorbic Acid. Submitted by FDA in response to an FOI request in 1999. 1 page.[2]

FDA. 1999c. Short-term toxicity (LD50—parenteral) of ascorbic acid. Submitted by FDA in response to an FOI request in 1999. 1 page.[2]

FDA. 2001. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Food and Drug Research Laboratories, Inc. (FDRL). 1974. The acute oral toxicity for mice dosed with FDA 71-65 (ascorbic acid). Report No. 223-74-2176. Submitted by FDA in response to an FOI request in 1999. 2 pages.[2]

FDRL. 1975a. Teratologic evaluation of FDA 71-65 in mice. Report No. 223-74-2176. Submitted by FDA in response to an FOI request in 1999. 8 pages.[2]

FDRL. 1975b. Teratologic evaluation of FDA 71-65 in rats. Report No. 223-74-2176. Submitted by FDA in response to an FOI request in 1999. 8 pages.[2]

Food and Nutrition Board. 1980. *Recommended dietary allowances*, 9th ed., 72–82. Washington, DC: National Academy Press.

Fowler, L. M., J. R. Foster, and E. A. Lock. 1993. Effect of ascorbic acid, acivicin and probenecid on the nephrotoxicity of 4-aminophenol in the Fischer 344 rat. *Arch. Toxicol.* 67:613–621.

Frith, C. H., J. Rule, and R. L. Kodell. 1980. The effects of ascorbic acid on the induction of urothelial lesions in mice by 2-acetylaminofluorene. *Toxicol. Lett.* 6:309–318.

Frohberg, H., J. Gleich, and H. Kieser. 1973. Reproduktionstoxikologische studien mit ascorbinsaure an mausen and ratten. Translated from the German. *Arzneim. Forsch*. 23:1080–1082.

Fuchs, J., and H. Dern. 1998 Modulation of UV-light-induced skin inflammation by D-alpha-tocopherol and L-ascorbic acid: A clinical study using solar simulated radiation. *Free Radic. Biol. Med.* 25:1006–1012.

Fukushima, S., O. Tadashi, Y. Kurata, M. Shibata, and T. Kakizoe. 1987. Absence of promotion potential for calcium-L-acorbate, L-ascorbic dipalmitate, and erythorbic acid on rat urinary bladder carcinogenesis. *Cancer Lett.* 35:17–25.

Fukushima, S., S. Tomoyuki, M. Hirose, and N. Ito. 1986. Significance of L-ascorbic acid and urinary electrolytes in promotion of rat bladder carcinogenesis. *Princess Takamatsu Symp.* 16:159–168.

Fukushima, S., S. Uwagawa, T. Shirai, R. Hasegawa, and K. Ogawa. 1990. Synergism of sodium L-ascorbate but inhibition by L-ascorbic acid for sodium saccharin promotion of rat two-stage bladder carcinogenesis. *Cancer Res.* 50:4195–4198.

Galloway, S. M., and R. B. Painter. 1979. The use of short-term tests to measure the preventive action of reducing agents on formation and activation of carcinogenic nitroso compounds. *Mutat. Res.* 57:57–67.

Garcia Roche, M. O., A. Castillo, T. Gonzalez, M. Grillo, J. Rios, and N. Rodriguez. 1987. Effect of ascorbic acid on the hepatotoxicity due to the daily intake of nitrate, nitrite, and dimethylamine. *Die Nahrung.* 31:99–104.

Gardiner, N. S., and J. R. Duncan. 1989. Inhibition of murine melanoma growth by sodium ascorbate. *J. Nutr.* 119:586–590.

Ginter, E. 1970. Effect of dietary cholesterol on vitamin C metabolism in laboratory animals. *Acta Med. Sci. Hung.* 27:23–29.

Ginter, E. 1975. Ascorbic acid in cholesterol and bile acid metabolism. *Ann. N. Y. Acad. Sci.* 258:410–421.

Ginter, E., I. Kajaba, and O. Nizner. 1970. The effect of ascorbic acid on cholesterolemia in healthy subjects with seasonal deficit of vitamin C. *Nutr. Metab.* 12:76–86.

Ginter, E., R. Nemec, J. Cerven, and L. Mikus. 1973. Quantification of lowered cholesterol oxidation in guinea pigs with latent vitamin C deficeincy. *Lipids* 8:135–141.

Ginter, E., R. Ondreicka, P. Bobek, and V. Simko. 1969. The influence of chronic vitamin C deficiency on fatty acid composition of blood serum, liver triglycerides and cholesterol esters in guinea pigs. *J. Nutr.* 99:261–266.

Ginter, E, and Z. Zloch. 1972. Raised ascorbic acid consumption in cholesterol-fed guinea pigs. *Int. J. Vit. Nutr. Res.* 42:72–79.

Gipp, W. F., W. G. Pond, F. A. Kallfelz, et al. 1974. Effect of dietary copper, iron, and ascorbic acid levels on hematology, blood, tissue copper, iron, and zinc concentrations and 64Cu and 59Fe metabolism in young pigs. *J. Nutr.* 104:532–541.

Goode, H. F., M. J. Davies, and N. R. Webster. 1996. The effect of ascorbate loading on ascorbyl formation in patients with sepsis and healthy subjects. *Proc. Nutr. Soc.* 55:89a.

Gray, M. A. 1994. Vitamins: Nutrients, food additives, or medication. Vitamin C. *Orthop. Nurs.* 13:179–181.

Greenwood, J., Jr. 1964. Optimum vitamin C intake as a factor preservation of disc integrity. *Med. Ann. D.C.* 33:274–276.

Gulati, D. K., K. Witt, B. Anderson, E. Zeiger, and M. Shelby. 1989. Chromosome abberation and sister chromatid exchange test in Chinese hamster ovary cells in vitro III: Results of 27 chemicals. *Environ. Mol. Mutagen.* 13:133–193.

Guttenplan, J. B. 1977. Inhibition by L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds. *Nature* 268:368.

Guttenplan, J. B. 1978. Mechanisms of inhibition by ascorbate of microbial mutagenesis induced by *N*-nitroso coumpounds. *Cancer Res.* 38:2018–2022.

Halperin, E. C., L. Gaspar, S. George, D. Darr, and S. Pinnell. 1993. A double-blind, randomized, prospective trial to evaluate topical vitamin C solution for the prevention of radiation dermatitis. *Int. J. Radiat. Oncol. Biol. Phys.* 26:413–416.

Harrison Research Laboratories, Inc. 2000. RIPT containing 5% Ascorbic Acid. Unpublished data submitted by CTFA. 1 page.[2]

Hasegawa, R., F. Furukawa, K. Yoyoda, M. Takahashi, Y. Hayashi, et al. (1990) Inhibitory effects of antioxidants on *N*-Bis(2-hydroxypropyl)nitrosamine-induced lung carcinogenesis in rats. *Jpn. J. Cancer Res.* 81:871–877.

Hellman, L., and J. J. Burns. 1958. Metabolism of L-ascorbic acid-l-$C^{14}$ in man. *J. Biol. Chem.* 230:923–930.

Herbert, V., and E. Jacob. 1974. Destruction of vitamin $B_{12}$ by ascorbic acid. *JAMA.* 230:241–242.

Hines, J. D. 1975. Ascorbic acid and $B_{12}$ deficiency. *JAMA* 234:24.

Hoffman-La Roche Inc. 1998. Ascorbic acid impurity data. Unpublished data submitted by CTFA. 2 pages.[2]

Hornig, D. 1975a. Metabolism of ascorbic acid. *World Rev. Nutr. Diet.* 23:225–258.

Hornig, D. 1975b. Distribution of ascorbic acid, metabolites, and analogues in man and animals. *Ann. N. Y. Acad. Sci.* 258:103–118.

Husain, K., K. Sugendran, S. C. Pant, V. P. Sharma, and R. Vijayaghavan. 1992. Biochemical and pathological changes in response to hyperoxia and protection by antioxidants in rats. *Indian J. Physiol. Pharmacol*. 36:97–100.

Imai, Y., T. Usui, T. Matsuzaki, H. Yokotani, H. Mima, and Y. Aramaki. 1967. The antiscorbutic activity of L-ascorbic acid phosphate given orally and percutanously in guinea pigs. *Jap. J. Pharmacol.* 17:317–332.

Inoue, T., K. Imaida, E. Suzuke, M. Okada, and S. Fukushima. 1988. Combined effects of L-ascorbic acid, citric acid or their sodium salts on tumor induction by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine or *N*-ethyl-*N*-(4-hydroxybutyl)nitrosamine in the rat urinary bladder. *Cancer Lett.* 30:265–273.

Ishidate, M., M. C. Harnois, and T. Soduni. 1988. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell culture. *Mutat. Res.* 195:151–213.

Ishidate, M. Jr, T. Sofuni, K. Yoshikawa, et al. 1983. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol.* 22:623–626.

Ito, N., S. Fukushima, T. Shirai, A. Hagiwara, and K. Imaida. 1984. Drugs, food additives, and natural products as promoters in rat urinary bladder carcinogenesis. *IARC Sci. Publ.* 56:399–407.

Ito, N., M. Hirose, S. Fukushima, H. Tsuda, T. Shirai, and M. Tatematsu. 1986. Modifying effects of antioxidants on chemical carcinogenesis. *Toxicol. Pathol.* 14:315–323.

Ivanov, N., E. Popova, and E. Iskrenova. 1995. Determination of some bioactive substances in cosmetic products and half-finished products. *Folia Med. (Plovdiv).* 37:24.

Iwamoto, K., N. Ozawa, Y. Hayashi, T. Tsukamoto, and J. Watanabe. 1976. Gastrointestinal absorption of ascorbic acid in guinea pigs. *Chem. Pharm. Bull.* 24:2021–2025.

Kabacoff, B. L., M. L. Douglass, I. E. Rosenberg, et al. 2000. Formation of nitrosamines in non-ionic and anionic emulsions in the presence and absence of inhibitors. Unpublished data submitted by CTFA. 5 pages.[2]

Kabacoff, B. L., R. J. Lechnir, S. F. Vielhuber, and M. L. Douglass. 1981. Formation and inhibition of *N*-nitrosodiethanolamine in an anionic oil-water emulsion. Unpublished data submitted by CTFA. 8 pages.[2]

Kallner, A. B., D. Hartman, and D. H. Hornig. 1979. Steady-state turnover and body pool of ascorbic acid in man. *Am. J. Clin. Nutr.* 32:530–539.

Kallner, A. B., D. Hartman, and D. H. Hornig. 1981a. On the requirements of ascorbic acid in man: Steady-state turnover and body pool in smokers. *Am. J. Clin. Nutr.* 34:1347–1355.

Kallner, A. B., D. Hornig, and R. Pellikka. 1981b. Formation of carbon dioxide from ascorbate in man. *Am. J. Clin. Nutr.* 41:609–613.

Kameyama, K., C. Sakai, S. Kondoh, et al. 1996. Inhibitory effect of magnesium L-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. *J. Am. Acad. Dermatol.* 34:29–33.

Kamoto, T., S. Mori, T. Murai, Y. Yamada, S. Makino, O. Yoshida, and H. Hiai. 1997. Quantitative trait loci associated with promoting effects of sodium L-ascorbate on two-stage bladder carcinogenesis in rats. *Japn. J. Cancer Res.* 88:633–638.

KGL, Inc. 1998. An evaluation of the contact-sensitization potential of a topical coded product in human skin by means of the maximization assay. Unpublished data submitted by CTFA. 11 pages.[2]

Kiechebusch, W., W. Griem, and K. Lang. 1963. Untersuchungen uber die chronische toxizitat der ascorbinsaure bei der ratte. *Z. Ernahr*. 4:5–14.

Kim, J. H., and C. S. Song. 1965. The L-ascorbic acid absorption. *Yonsel Med. J.* 6:7–10.

King, T. M., Z. Trizna, X. Wu, et al. 1997. A clinical trial to evaluate the effect of vitamin C supplementation on in vitro mutagen sensitivity. *Cancer Epidemiol. Biomarkers Prev.* 6:537–542.

Klasson, D. H. 1951. Ascorbic acid in the treatment of burns. *N. Y. State J. Med.* 51:2388–2392.

Kobayashi, S., M. Takahana, M. Kanke, S. Itoh, and E. Ogata. 1998. Postadministration protective effect of magnesium-L-ascorbyl-phosphate on the development of UVB-induced cutaneous damage in mice. *Photochem. Photobiol.* 67:669–675.

Krishna, G., J. Nath, and T. Ong. 1986. Inhibition of cyclophosphamide and mitocycin C-induced sister chromatid exchanges in mice. *Cancer Res.* 46:2670–2674.

Kubo, H. 1966. Studies on intestinal absorption of vitamin c: I. Active transport of ascorbic acid in the intestine of guinea pig. *Shikoko Acta Med.* 22:34–41.

Kucuk, O., A. Pung, A. A. Franke, et al. 1995. Correlations between mutagen sensitivity and plasma nutrient levels of healthy individuals. *Cancer Epidemiol. Biomarkers Prev.* 4:217–221.

Lamden, M. P., and G. A. Chrystowski. 1954. Urinary oxalate excretion by man following ascorbic acid ingestion. *Proc. Soc. Exp. Biol. Med.* 85:190–192.

Lang, K. 1965. Wirkungen sehr hoher ascorbinsaure-dosen. *Wiss Veroff Dtsch ges Ernahr.* 14:149–154.

Lee, A. R., and K. Tojo. 1996. Evaluation for intrinsic skin permeation of unstable compounds. *Chem. Pharm. Bull.* 44:1621–1623.

Lee, A. R., and K. Tojo. 1998. Characterization of skin permeation of vitamin C: Theoretical analysis of penetration profiles and differential scanning calorimetry study. *Chem. Pharm. Bull.* 46:174–177.

Lefebvre, Y., and H. Pezerat. 1994. Reactive oxygen species produced from chromate pigments and ascorbate. *Environ. Health Perspect.* 102:243–245.

Levander, O. A., and V. C. Morris. 1970. Interactions of methionine, vitamin E, and antioxidants in selenium toxicity in the rat. *J. Nutr.* 100:1111–1117.

Levinson, R. A., C. A. Paterson, and R. R. Pfister. 1976. Ascorbic acid prevents corneal ulceration and perforation following experimental alkali burns. *Invest. Opthalmol.* 15:986–993.

Lewis, R. J., Sr. 1993. *Hazardous chemicals desk reference*, 3rd ed. New York, NY: Van Nostrand Reinhold.

Lide, D. R., ed. 1993. *CRC Handbook of Chemistry and Physics*, 74th ed. Boca Raton, FL: CRC Press.

Liehr, J. G., and W. J. Wheeler. 1983. Inhibition of estrogen-induced renal carcinoma in Syrian hamsters by vitamin C. *Cancer Res.* 10:4638–4642.

Littlefield, N. A., and B. S. Hass. 1995. Damage to DNA by cadmium or nickel in the presence of ascorbate. *Ann. Clin. Lab Sci.* 25:485–492.

Litton Bionetics. 1975. Mutagenicity evaluation of compound FDA 71–65 ascorbic acid. Report No. 223-74-2104. Submitted by FDA in response to an FOI request in 1999. 46 pages.[2]

Litton Bionetics. 1976a. Mutagenicity evaluation of sodium ascorbate USP, FCC, FDA 75-64 final report. Report No. 223-76-2101. Submitted by FDA in response to an FOI request in 1999. 32 pages.[2]

Lo, L. W., and H. F. Stich. 1978. The use of short-term tests to measure the preventive action of reducing agents on formation and activation of carciongenic nitroso compounds. *Mutat. Res.* 57:57–67.

Lowry, O. H., O. A. Bessey, M. J. Brock, and J. A. Lopez. 1946. The interrelationship of dietary, serum, white blood cell, and total body ascorbic acid. *J. Biol. Chem.* 166:111–119.

Lowry, O. H., O. A. Bessey, and H. B. Burch. 1952. Effects of prolonged high dosage with ascorbic acid. *Proc. Soc. Exp. Biol.* 80:361–362.

Macrae, W. D., and H. F. Stich. 1979. Induction of sister chromatid exchanges in Chinese hamster cells by the reducing agents bisulfite and ascobic acid. *Toxicology* 13:167–174.

Mallick, N., and C. Deb. 1975. Effect of different doses of ascorbic acid on thyroid activity in rats at different levels of dietary protein intake. *Endokrinologie* 66:333–339.

Marcusen, D. C., and R. W. Heninger. 1976. Affect of ascorbic acid on the pituitary-thyroid system in the rat. *J. Endocr.* 70:313–314.

Masui, T., A. M. Mann, T. L. Macatee, T. Okamura, E. M. Garland, H. Fuji, J. C. Pelling, and S. M. Cohen. 1991. H-ras mutations in rat urinary bladder carcinomas induced by *N*-[4-(5-nitro-2-furyl)-2-thiazolyformamide and sodium saccharin, sodium ascorbate, or related salts. *Cancer Res.* 51:3471–3475.

Meadows, G. G., H. F. Pierson, and R. M. Abdallah. 1991. Ascorbate in the treatment of experimental transplanted melanoma. *Am. J. Clin. Nutr.* 54:1284S–1291S.

Ministry of Health, Labor, and Welfare (MHLW). 2000. Pharmaceutical and Medical Safety Bureau Notification No. 990. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Divison, 2-2, 1-chrome, Kasumigaseki, Chiyoda-ku, Tokyo 100–8045, Japan.

Mirivish, S. S., A. Cardesa, L. Wallcave, and P. Shubik. 1975. Dietary and other factors affecting nitrosomethylurea (NMU) formation in the rat stomach. *J. Natl. Cancer Inst.* 3:633–636.

Mitch, W. E., M. W. Johnson, J. M. Kirshenbaum, and R. E. Lopez. 1981. Effect of large oral doses of ascorbic acid on uric acid excretion by normal subjects. *Clin. Pharmacol. Ther.* 29:318–321.

Miyagawa, M., H. Takasawa, A. Sugiyama, Y. Inoue, T. Murata, Y. Uno, and K. Yoshikawa. 1995. The in vitro-in vivo replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F1 mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocytes. *Mutat. Res.* 343:157–183.

Morcos, S. R., F. A. El-Shobaki, Z. El-Hawary, and N. Saleh. 1976. The effect of vitamin C and carotene on the adsorption of calcium from the intestine. *Z. Ernahrungswiss.* 15:387–390.

Mori, S., Y. Kurata, Y. Takeuchi, M. Toyama, S. Makino, and S. Fukushima. 1987. Influences of strain and diet on the promoting effects of sodium L-ascorbate in two-stage urinary bladder carcinogenesis in rats. *Cancer Res.* 47:3492–3495.

Mottram, D. S., and R. L. S. Patterson. 1977. The effect of ascorbate reductants on N-nitrosamine formation in a model system resembling bacon fat. *J. Sci. Food Agric.* 28:352–354.

Mummery, C. L., C. E. Van Den Brink, P. T. Van Der Saag, and S. W. De Laat. 1984. A short-term screening test for teratogens using differentiating neuroblastoma cells in vitro. *Teratology* 29:271–279.

Murad, S., D. Grove, K. A. Lindberg, G. Reynolds, A. Sivarajah, and S. R. Pinnell. 1981. Regulation of collagen synthesis by ascorbic acid. *Proc. Natl. Acad. Sci. U. S. A.* 78:2879–2882.

Murai, T., S. Mori, M. Hosono, et al. 1997. Strain differences in sensitivity to the promoting effect of sodium L-ascorbate in a two-stage rat urinary bladder carcinogenesis model. *Jpn. J. Cancer Res.* 88:245–253.

Myhr, B. C., and W. J. Caspary. 1991. Chemical mutagenesis at the TK locus in L5178Y, mouse lymphoma cells. I. Results for 31 coded compounds in the National Toxicology Program. *Environ. Mol. Mutagen.* 18:51–83.

Nakamura, T., S. R. Pinnell, D. Darr, I. Kurimoto, S. Itami, K. Yoshikawa, and J. W. Streilein. 1997. Vitamin C abrogates the deleterious effects of UVB radiation on cutaneous immunity by a mechanism that does not depend on TNF-$\alpha$. *J. Invest. Dermatol.* 109:20–24.

Nandi, B. K., A. K. Majumder, N. Subramanian, and I. B. Chatterjee. 1973. Effects of large doses of vitamin C in guinea pigs and rats. *J. Nutr.* 103:1688–1695.

National Academy of Sciences (NAS). 1996. *Food chemicals codex*, 4th ed. Washington, DC: National Academy Press.

NAS. 2000. *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academy Press.

National Toxicology Program. 1983. Carcinogenesis bioassay of L-ascorbic acid (vitamin C) (CAS no. 50-81-7) in F344/N rats and B6C3$F_1$ mice (feed study). NTIS no. PB83201194.

Nelson, E. W., H. Lane, P. J. Fabri, and B. Scott. 1978. Demonstration of saturation kinetics in the intestinal absorption of vitamin C in man and the guinea pig. *J. Clin. Pharmacol.* 18:325–335.

Nishihata, T., J. H. Rytting, K. Takahashi, and K. Sakai. 1988. Effects of dithiothreitol and ascorbate on the penetration of diclofenac across excised rat dorsal skin. *Pharm. Res.* 5:738–740.

Norkus, E. P., and P. Rosso. 1975. Changes in ascorbic acid metabolism of the offspring following high maternal intake of this vitamin in the pregnant guinea pig. *Ann. N. Y. Acad. Sci.* 258:401–409.

Norkus, E. P., and P. Rosso. 1981. Effects of maternal intake of ascorbic acid on the postnatal metabolism of this vitamin on the guinea pig. *J. Nutr.* 111:624–630.

Norkus, E. P., W. Kuenzig, and A. H. Conney. 1983. Studies on the mutagenic activity of ascorbic acid in vitro and in vivo. *Mutat. Res.* 8:183–191.

Noto, V., H. S. Taper, Y. H. Jiang, J. Janssens, J. Bonte, and W. De Loecker. 1989. Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4-naphthoquinone (vitamin $K_3$) treatment on human tumor cell growth in vitro. *Cancer* 63:901–906.

Novicki, D. L., M. R. Rosenberg, and G. Michalopoulos. 1985. Inhibition of DNA synthesis by chemical carcinogens in cultures of intiated and normal proliferating rat hepatocytes. *Cancer Res.* 45:337–344.

Odumosu, A., and C. W. M. Wilson. 1973. Metabolic availability of vitamin C in the guinea-pig. *Nature* 242:519–521.

Ohno, T., and K. Myoga. 1981. The possible toxicity of vitamin C in the guinea pigs. *Nutr. Rep. Int.* 24:291–294.

Omaye, S. T., E. E. Schaus, M. A. Kutnink, and W. C. Hawkes. 1987. Measurement of vitamin C in blood components by high pressure liquid chromatography. *Anal. N. Y. Acad. Sci.* 498:389–401.

Omura, H., K. Shinohara, H. Maeda, M. Noaka, and H. Murakami. 1978. Mutagenic action of triose reductone and ascorbic acid on *Salmonella typhimurium* TA 100 strain. *J. Nutr. Sci. Vitaminol.* 24:185–194.

Organisciak, D. T., H. M. Wang, Z. Y. Li, and M. O. Tso. 1985. The protective effect of ascorbate in the retinal light damage of rats. *Invest. Opthalmol. Vis. Sci.* 26:1580–1588.

Osswald, H., R. Herrmann, and M. Youssef. 1987. The influence of sodium ascorbate, menadione sodium bisulfite, or pyridoxal hydrochloride on the toxic and antineoplastic action of *N*-methylformamide in P 388 leukemia or M 5076 sarcoma in mice. *Toxicology* 43:183–191.

Owen, C. A., Jr, G. M. Tyce, E. V. Flock, and J. T. McCall. 1970. Heparin-ascorbic acid antagonism. *Mayo Clin. Proc.* 45:140–145.

Patterson, L. T., D. L. Nahrwold, and R. C. Rose. 1984. Ascorbic acid uptake in guinea pig intestinal mucosa. *Life Sci.* 31:2783–2791.

Pensabene, J. W., W. Fiddler, J. Feinberg, and A. E. Wasserman. 1976. Evaluation of ascorbyl monoesters for the inhibition of nitrosopyrrolidine formation in a model system. *J. Food Sci.* 41:199–200.

Personelle, J., E. Eolivar de Souza Pinto, and R. O. Ruiz. 1998. Injection of vitamin A, vitamin E, and vitamin C for treatment of tissue necrosis. *Aesth. Plast. Surg.* 22:58–64.

Peterkofsky, B., and W. Prather. 1977. Cytotoxicity of ascorbate and other reducing agents towards cultured fibroblasts as a result of hydrogen peroxide formation. *J. Cell Physiol.* 90:61–70.

Peterson, V. E., P. A. Crapo, I. Weiniger, H. Ginsberg, and J. Olefsky. 1975. Quantification of plasma cholesterol and triglyceride levels in hypercholesterolemic subjects receiving ascorbic acid supplements. *Am. J. Clin. Nutr.* 28:584–587.

Pfister, R. R., S. A. Paterson, J. W. Spiers, and S. A. Hayes. 1980. The efficacy of ascorbate treatment after severe experimental alkali burns depends upon the route of administration. *Invest. Opthalmol. Vis. Sci.* 19:1526–1529.

Pfister, R. R., J. L. Haddox, and D. Yuille-Barr. 1991. The combined effect of citrate/ascorbate treatment in alkali-injured rabbit eyes. *Cornea* 10:100–104.

Pienkowsha, K., H. Gajcy, and J. Koziorowska. 1985. Protective effect of ascorbic acid against mutagenicity of aminopyrine plus nitrite. *Pol. J. Pharmacol. Pharm.* 37:601–607.

Pillans, P. I., S. F. Ponzi, and M. J. Parker. 1990. Effects of ascorbic acid on the mouse embryo and on cyclophosphamide-induced cephalic DNA strand breaks in vivo. *Arch. Toxicol.* 64:423–425.

Ponec, M., A. Weerheim, J. A. Kempenaar, A. Mulder, G. S. Gooris, J. Bouswstra, and A. M. Mommaas. 1997. The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. *J. Invest. Dermatol.* 109:348–355.

Postaire, E., H. Jungmann, M. Bejot, U. Heinrich, and H. Tronnier. 1997. Evidence for antioxidant nutrients-induced pigmentation in skin: Results of a clinical trial. *Biochem. Mol. Biol. Int.* 42:1023–1033.

Pratt, R. M., and W. D. Willis. 1985. In vitro screening assay for teratogens using growth inhibition of human embryonic cells. *Proc. Natl. Acad. Sci. U. S. A.* 82:5791–5794.

Quevedo, W. C., Jr., T. J. Holstein, J. Dyckman, J. McDonald, C. J. McDonald, and E. L. Isaacson. 2000. Inhibition of UVR-induced tanning and immunosuppression by topical applications of vitamins C and E to the skin of hairless (*hr/hr*) mice. *Pigment Cell Res.* 13:89–98.

Ramos, K., and D. Acosta. 1983. Prevention by *L*(-)ascorbic acid of isoproterenol-induced cardiotoxicity in primary cultures of rat myocytes. *Toxicology* 26:81–90.

Reid, M. E. 1969. Gastrointestinal tract of guinea pig and elimination of ascorbic acid given intraperitoneally. *Proc. Soc. Exp. Biol. NY.* 68:403–406.

Rivers, J. M., E. D. Huang, and M. L. Dodds. 1963. Human metabolism of L-ascorbic acid and erythorbic acid. *J. Nutr.* 81:163–168.

Roller, P. P., L. K. Keefer, and B. W. Slavin. 1980. Inhibitory agents and chemical mechanisms in the dihalomethane-mediated nitrosation of amines with solid nitrite. *IARC Sci. Publ.* 31:119–128.

Rose, R. C. 1986. Ascorbic acid transport in mammalian kidney. *Am. J. Physiol.* 250:627–632.

Rose, R. C., and A. M. Bode. 1993. Biology of free radical scavengers: An evaluation of ascorbate. *FASEB J.* 7:1135–1142.

Rose, R. C., and D. L. Nahrwold. 1978. Intestinal ascorbic acid transport following high doses of high or low ascorbic acid content. *Int. J. Vit. Nutr. Res.* 48:382–386.

Rosin, M. P., R. H. C. San, and H. F. Stich. 1980. Mutagenic activity of ascorbate in mammalian cell cultures. *Cancer Lett.* 8:299–305.

Rustia, M. 1975. Inhibitory effect of sodium ascorbate on ethyl urea and sodium nitrite carcinogenesis and negative findings in the progeny after intestinal inoculation of precursors into pregnant hamsters. *J. Natl. Cancer Inst.* 55:1389–1394.

Sahagian, B. M., I. Harding-Barlow, and H. M. Perry. 1967. Transmural movements of zinc, manganese, cadmium, and mercury by rat small intestine. *J. Nutr.* 93:291–300.

Sahu, S. C., and M. C. Washington. 1999. Iron-mediated oxidative DNA damage detected by fluorometric analysis of DNA unwinding in isolated rat liver nuclei. Report No. 003530. Submitted by FDA in response to an FOI request in 1999. 25 pages.[2]

Saika, S. 1993. Ascorbic Acid and proliferation of cultured rabbit keratinocytes. *Cornea.* 13:191–198.

Sakagami, H., K. Satoh, H. Ohata, et al. 1996. Relationship between ascorbyl radical intensity and apoptosis-inducing activity. *Anticancer Res.* 16:2635–2644.

Sakurai, M., H. Tanka, T. Matsuda, T. Goya, S. Shimazaki, and H. Matsuda. 1997. Reduced resuscitation fluid volume for second-degree experimental burns with delayed initiation of vitamin C therapy. *J. Surg. Res*. 73:24–27.

Samborskaya, E. P. 1964. The effect of high ascorbic acid doses on the course of pregnancy and on the progeny in guinea pigs. *Byull. Eksp. Biol. Med.* 57:105–108.

Schlegel, J. U., G. E. Pipkin, R. Nishimura, and G. N. Shultz. 1970. The role of ascorbic acid in the prevention of bladder tumor formation. *J. Urol.* 103:155–159.

Schmidt, K. H., V. Hagmaier, D. H. Hornig, et al. 1981. Urinary excretion after large intakes of ascorbic acid in man. *Am. J. Clin. Nutr.* 34:305–311.

Schmidt, K., H. Oberritter, G. Brubhelt, V. Hagmaier, and D. Horning. 1983. Studies on the metabolic conversion of ascorbate. *Int. J. Vit. Nutr. Rev.* 53:77–85.

Schimdt, R. J., L. Y. Chung, A. M. Andrews, and T. D. Turner. 1993. Toxicity of *l*-ascorbic acid to L929 fibroblast cultures: Relevance to biocompatibilty testing of materials for use in wound management. *J. Biomed. Mater. Res.* 27:521–530.

Seidenberg, J. M., D. G. Anderson, and R. A. Becker. 1986. Validation of an in vivo developmental toxicity screen in the mouse. *Teratog. Carcinog. Mutagen.* 6:361–374.

Serrill, S., D. Jefferson, J. Quick, and C. E. Mengel. 1971. Effect of acetylsalicylic acid and ascorbic acid on oxygen toxicity. *Aerospace Med.* 42:436–438.

Shamberger, R. J. 1984. Genetic toxicology of ascorbic acid. *Mutat. Res.* 133:135–159.

Shibata, M. A., M. Yamada, E. Asakawa, A. Hagiwara, and S. Fukushima. 1989. Responses of rat urine and urothelium to bladder tumor promoters: Possible roles of prostaglandin $E_2$ and ascorbic acid in bladder carcinogenesis. *Carcinogenesis* 10:1651–1656.

Shilotri, P. G., and K. S. Bhat. 1977. Effect of mega doses of vitamin C on bactericidal activity of leukocytes. *Am. J. Clin. Nutr*. 30:1077–1081.

Shimpo, K., H. Takahashi, H. Tsuda, et al. 1996. Inhibition of hepatocellular carcinoma development and erythrocyte polyamine levels in ODS rats fed on 3′-methyl-4-dimethylaminoazobenzene by hemicalcium ascorbate, 2-*O*-Octadecylascorbic acid, and ascorbyl palmitate. *Cancer Detect. Prev.* 20:137–145.

Shirai, T., E. Ikawa, M. Hirose, W. Thamavit, and N. Ito. 1985. Modification by five antioxidants of 1,2-dimethylhydrazine-initiated colon carcinogenesis in F344 rats. *Carcinogenesis* 6:637–639.

Shoyab, M. 1981. Inhibition of the binding of 7,12-dimethylbenz[$\alpha$]anthracene to DNA of murine epidermal cells in culture by vitamin A and C. *Oncology* 38:187–192.

Siliprandi, L., M. Vanni, M. Dessler, and G. Semenza. 1979. $Na^+$-dependent, electroneutral L-ascorbate transport across brush border membrane vesicles from guinea pig small intestine. *Biochem. Biophys. Acta* 552:129–142.

Silverman, J., A. Rivenson, and B. Reddy. 1983. Effect of sodium ascorbate on transplantable murine tumors. *Nutr. Cancer* 4:192–197.

Siman, C. M., and U. J. Eriksson. 1997. Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. *Diabetologia* 40:1416–1424.

Sitren, H. S. 1987. Vitamin chart no. 13. *Nutr. Support Serv.* 7:45.

Solomons, N. W., R. A. Jacobs, O. Pineda, and F. E. Viteri. 1979. Studies on the bioavailability of zinc in man III. effects of ascorbic acid on zinc absorption. *Am. J. Clin. Nutr.* 32:2495–2499.

Soni, G. L., G. K. Nirwan, J. K. Mand, and R. Singh. 1984. Effect of megadoses of ascorbic acid on lipid metabolism and lipid peroxidation in albino rats. *J. Res. Punjab Agric. Univ.* 21:409–417.

Sorensen, D. I., M. M. Devine, and J. M. Rivers. 1974. Catabolism and tissue levels of ascorbic acid following long-term massive doses in the guinea pigs. *J. Nutr.* 104:1041–1048.

Speit, G., M. Wolf, and W. Vogel. 1980. The sce-inducing capacity of vitamin C: Investigations in vitro and in vivo. *Mutat. Res.* 78:273.

Steenvoorden, D. P., and G. Beijersbergen van Henegouwen. 1999. Protection against UV-induced systemic immunosuppression in mice by a single topical application of the antioxidant vitamins C and E. *Int. J. Radiat. Biol.* 75:747–755.

Stich, H. F., J. Karim, J. Koropatnick, and L. Lo. 1976. Mutagenic action of ascorbic acid. *Nature* 260:722.

Stich, H. F., L. Wei, and R. F. Whitting. 1979. Enhancement of the chromosome-damaging action of ascorbate by transition metals. *Cancer Res.* 39:4145–4151.

Stich, H. G., L. Wei, and R. F. Whitting. 1980. Chromosome aberrations in mammalian cells exposed to vitamin C and multiple vitamin pills. *Food Cosmet. Toxicol.* 18:497–501.

Strickland, F. M., R. P. Pelley, and M. L. Kripke. 1994. Prevention of ultraviolet-induced suppression of contact and delayed hypersensitivity by Aloe barbadensis gel extract. *J. Invest. Dermatol*. 102:197–204.

Surber, W., and A. Cerioli. 1971. Summary of two-year toxicity study with L-ascorbic acid on rats by Battelle Laboratories. Submitted by FDA in response to an FOI request in 1999. 1 page.[2]

Suzuki, T., and A. Yoshida. 1979. Effectiveness of dietary iron and ascorbic acid in the prevention and cure of moderately long-term toxicity in rats. *J. Nutr.* 109:1974–1979.

Switzer, B. R., and G. K. Summer. 1972. Collagen synthesis in human skin fibroblasts: Effects of ascorbate, $\alpha$-ketoglutarate and ferrous ion on proline hydroxylation. *J. Nutr.* 102:721–728.

Tajima, S., and S. R. Pinell. 1996. Ascorbic acid preferentially enhances type I and III collagen gene transcription in human skin fibroblasts. *J. Dermatol. Sci.* 11:250–253.

Takada, K., K. Naito, O. Uchida, et al. 1996. A combination toxicity study of sodium ascorbate and sodium nitrite in rats. *J. Toxicolog. Sci.* 21:377.

Takahashi, O. 1995. Haemorrhagic toxicity of a large dose of $\alpha$-, $\beta$-, $\gamma$- and $\delta$-tocopherols, ubiquinone, $\beta$-carotene, retinol acetate and L-ascorbic acid in the rat. *Food Chem. Toxicol.* 33:121–128.

Takenouchi, K., D. Aso, K. Kawase, H. Ichikawa, and T. Shiomi. 1966. On the metabolites of ascorbic acid, especially oxalic acid, eliminated in urine, following the administration of large amounts of ascorbic acid. *J. Vitaminol.* 12:49–58.

Takiguchi, H., S. Furuyama, and N. Shimazono. 1966. Urinary oxalic acid excretion by man following ingestion of large amounts of ascorbic acid. *J. Vitaminol.* 12:307–312.

Tamano, S., H. Tanaka, M. Kawabe, E. Asakawa, M. Sano, S. Shioya, and T. Shirai. 1993. No enhancing effects of calcium/magnesium salts of L-glutamate and L-ascorbate on tumor development in a rat medium-term multiorgan carcinogenesis bioassay. *J. Toxicol. Environ. Health* 39:43–58.

Thamavit, W., S. Fukushima, Y. Kurata, M. Asamoto, and N. Ito. 1989. Modification by sodium L-ascorbate, butylated hydroxytoulene, phenobarbital, and pepleomycin of lesion development in a wide-spectrum initiation rat model. *Cancer Lett.* 45:93–101.

Tojo, K., and A. C. Lee. 1989. Skin penetration of vitamins C and E. *J. Soc. Cosmet. Chem.* 40:119–125.

Tolbert, B. M., A. W. Chen, E. M. Bell, and E. M. Baker. 1967. Metabolism of L-ascorbic-4-$^3$H acid in man. *Am. J. Clin. Nutr.* 20:250–252.

Traikovich, S. S. 1999. Use of topical ascorbic acid and its effect on photodamaged skin topography. *Arch. Otolaryngol. Head Neck Surg.* 125:1091–1098.

Tripathy, N. K., F. E. W'urgler, and H. Frei. 1990. Genetic toxicity of six carcinogens and six non-carcinogens in the Drosophila wing spot test. *Mutat. Res.* 242:169–180.

Tsao, C. S., and P. Y. Leung. 1988. Urinary ascorbic acid levels following the withdrawal of large doses of ascorbic acid in guinea pigs. *J. Nutr.* 118:895–900.

Tsao, C. S., P. Y. Leung, and M. Young. 1990. Levels of minerals in serum and urine of guinea pigs following intraperitoneal administration of ascorbate. *Int. J. Vitam. Nutr. Res.* 60:121–125.

Turusov, V. S., L. S. Trukhanove, and Y. U. D. Psrfenov. 1991. Modifying effect of ascorbic acid and sodium ascorbate on the promoting stage of uterine sarcomogenesis induced in CBA mice by 1,2-dimethylhydrazine and estradiol-dipropionate. *Cancer Lett.* 56:29–35.

US Pharmacopeial Convention, Inc. 2000. *The United States Pharmacopeia*, Vol 24, and *The National Formulary*, Vol 19. Taunton, MA: Rand McNally.

Uno, Y., T. Hironao, M. Miyagawa, Y. Inoue, T. Murata, and Y. Kunie. 1994. An in vivo–in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early predicition assay for nongenotoxic hepatocarcinogens screening of 22 unknown positives and 25 noncarcinogens. *Mutat. Res.* 320:189–205.

Uphill, P. F., S. R. Wilkins, and J. A. Allen. 1990. In vitro micromass teratogen test: Results from a blind trial of 25 compounds. *Toxicol. In Vitro.* 4:623–626.

Van den Berg, G. J., S. Yu, A. G. Lemmens, and A. C. Beynen. 1994a. Dietary ascobic acid lowers the concentration of soluble copper in the small intestinal lumen of rats. *Br. J. Nutr.* 71:701–707.

Van Den Berg, G. J., S. Yu, A. G. Lemmens, and A. C. Beynen. 1994b. Ascorbic Acid feeding of rats reduces copper absorption, causing impaired copper status and depressed biliary copper excretion. *Biol. Trace Elem. Res.* 41:47–58.

Varma, S. D. 1987. Ascorbic acid and the eye with special reference to the lens. *Ann. N. Y. Acad. Sci.* 498:280–306.

Varma, S. D., A. H. Ali, P. S. Devamanoharan, and S. M. Morris. 1997. Nitrite-induced photo-oxidation of thiol and its implications in smog toxicity to the eye: Prevention by ascorbate. *J. Ocul. Pharmacol. Ther.* 13:179–187.

Wandzilak, T. R., S. D. D'Andre, P. A. Davis, and H. E. Williams. 1994. Effect of high dose vitamin C on urinary oxalate levels. *J. Urol.* 151:834–837.

Weight, M. J., P. Kotze, W. A. de Klerk, and N. Weight. 1974. The effect of dietary ascorbic acid on cholesterol biosynthesis in vitro. *Int. J. Biochem.* 5:287–290.

Weinberger, M. A., and E. J. Hayes. 1999. The effects of feeding ascorbic acid to miniature swine on stock or high cholesterol-fat diets. Report No. P-81. Submitted by FDA in response to an FOI request in 1999. 94 pages.[2]

Weitberg, A. B. 1987. Antioxidants inhibit the effect of vitamin C on oxygen radical-induced sister chromatid exchange. *Mutat. Res.* 191:53–56.

Weitberg, A. B., and S. A. Weitzman. 1985. The effect of vitamin C on oxygen radical-induced sister-chromatid exchanges. *Mutat. Res.* 144:23–26.

Weitzman, S. A., and T. P. Stossel. 1982. Effects of oxygen radical scavengers and antioxidants on phagocyte-induced mutagenesis. *J. Immunol.* 128:2770–2772.

Wendt, M. D., C. N. Soparkar, K. Louie, S. F. Basinger, and R. L. Gross. 1997. Ascorbate stimulates type I and type III collagen in human Tenon's fibroblasts. *J. Glaucoma* 6:402–407.

Wenninger, J. A., R. C. Canterbery, and G. N. McEwen, Jr., eds. 2000. *International Cosmetic Ingredient Dictionary and Handbook*, 8th ed., Vols. 1–2. Washington, DC: CTFA.

Westco Chemicals Inc. 1998. Cosmetic Ingredient Chemical Description Form on Ascorbic Acid. Unpublished data submitted by CTFA. 10 pages.[2]

Widenbauer, F. 1936. Toxische nebenwirkungen von ascorbinasure—C-hypervitaminose. *Kin. Wochschr.* 15:1158–1159.

Willis, G. C., and S. Fishman. 1955. Ascorbic acid content of human arterial tissue. *Can. Med. Assoc. J*. 72:500–503.

Wu, J., K. Karlsson, and A. Danielsson. 1997. Effects of vitamins E, C and catalase on bromobenzene and hydrogen peroxide-induced intracellular oxidation and DNA single-strand breakage in Hep $G_2$ cells. *J. Hepatol.* 26:669–677.

Yakuji Nippo, Ltd. 1998. *The Comprehensive Licensing Standards of Cosmetics by Category 1998*. Japan: Komiyama Printing Co. Ltd.

Yoshida, Y., M. Hirose, K. Takaba, J. Kimura, and N. Ito. 1994. Induction and promotion of forestomach tumors by sodium nitrite in combination with ascorbic acid or sodium ascorbate in rats with or without *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine pre-treatment. *Int. J. Cancer.* 56:124–128.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1988. *Salmonella* mutagenicity tests. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* 11:1–158.

Zhang, L., S. Lerner, W. V. Rustum, and G. A. Hofmann. 1999. Electroportion-mediated topical delivery of vitamin C for cosmetic applications. *Bioelectrochem. Bioenerg.* 48:453–461.