ELSEVIER

Review

Absorption, transport, and disposition of ascorbic acid in humans

Steven C. Rumsey and Mark Levine

Molecular and Clinical Nutrition Section, National Institutes of Health, Bethesda, MD USA

L-ascorbic acid (ascorbate or vitamin C) is a required nutrient for humans. Absorption, transport, and disposition of ingested ascorbate involve the following: (1) bioavailability and absorption in the gastrointestinal tract; (2) presence in the circulation; (3) tissue distribution; (4) excretion; and, (5) metabolism. Fundamental to each of the above are ascorbate chemistry and mechanisms of transport of ascorbate across membranes. Ascorbate can be reversibly oxidized to dehydroascorbic acid, which can be irreversibly degraded. Both reduced and oxidized forms cross cell membranes. Differences in transport kinetics, tissue specificity, and Na^+ and energy dependence strongly support the existence of separate transport mechanisms. An important consideration in the analysis of ascorbate transport is that of substrate availability. Reduced ascorbate is by far the most predominant form found in plasma and tissues. Dehydroascorbic acid is rapidly reduced intracellularly to ascorbate by both enzymatic and chemical mechanisms. Despite constitutively low levels of dehydroascorbic acid, conditions that promote oxidation of ascorbate can profoundly alter both the nature and availability of substrate. Elucidation of mechanisms that modulate the delivery of ascorbate to tissues and its utilization under different metabolic conditions will be invaluable for making recommendations for ascorbate ingestion. (J. Nutr. Biochem. 9:116–130, 1998) *Published by Elsevier Science Inc. 1998*

Keywords: ascorbic acid; vitamin C; dehydroascorbic acid; transport; pharmacokinetics; ascorbate recycling

Introduction

L-ascorbic acid, also referred to as ascorbate or vitamin C, is a required nutrient for humans. Ascorbate is synthesized by plants and most mammals but not by human and nonhuman primates, guinea pigs, the Indian fruit bat, several birds, and some fish.[1,2] Primates lack the enzyme gulonolactone oxidase, which catalyzes the last enzymatic step in ascorbate synthesis.[3,4] Lack of dietary ascorbate results in the clinical syndrome scurvy, which is fatal if untreated. Human scurvy is characterized by symptoms of weakness and fatigue and signs of hyperkeratosis at hair follicles, perifollicular hemorrhages, ecchymoses, and bleeding gums.[5–9] The current recommended dietary allowance (RDA) for ascorbate (vitamin C) is 60 mg/d and is based on prevention of scurvy with an additional margin for protection. This rationale has been questioned,[10–14] however, and other criteria have been proposed as the basis for new recommendations for ascorbate intake.[15]

The biological basis of recommendations for humans should account for ascorbate absorption and disposition, which is governed by the following: (1) bioavailability and absorption in the gastrointestinal tract; (2) concentrations in circulation; (3) tissue distribution; (4) excretion; and (5) metabolism. Fundamental to all of these issues are principles of ascorbate chemistry and mechanisms of membrane transport and cellular accumulation.

Ascorbate chemistry

Ascorbate is reversibly oxidized with the loss of one electron to form the free radical, semidehydroascorbic acid, which is further oxidized to dehydroascorbic acid[16] (*Figure 1*). Dehydroascorbic acid can be reduced to ascorbate via the same intermediate radical, or the ring structure of dehydroascorbic acid can irreversibly hydrolyze to yield

Address correspondence and reprint requests to Mark Levine, Molecular and Clinical Nutrition Section, Building 10, Room 4D52, MSC 1372, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-1372 USA.
Received December 8, 1997; accepted January 6, 1998.

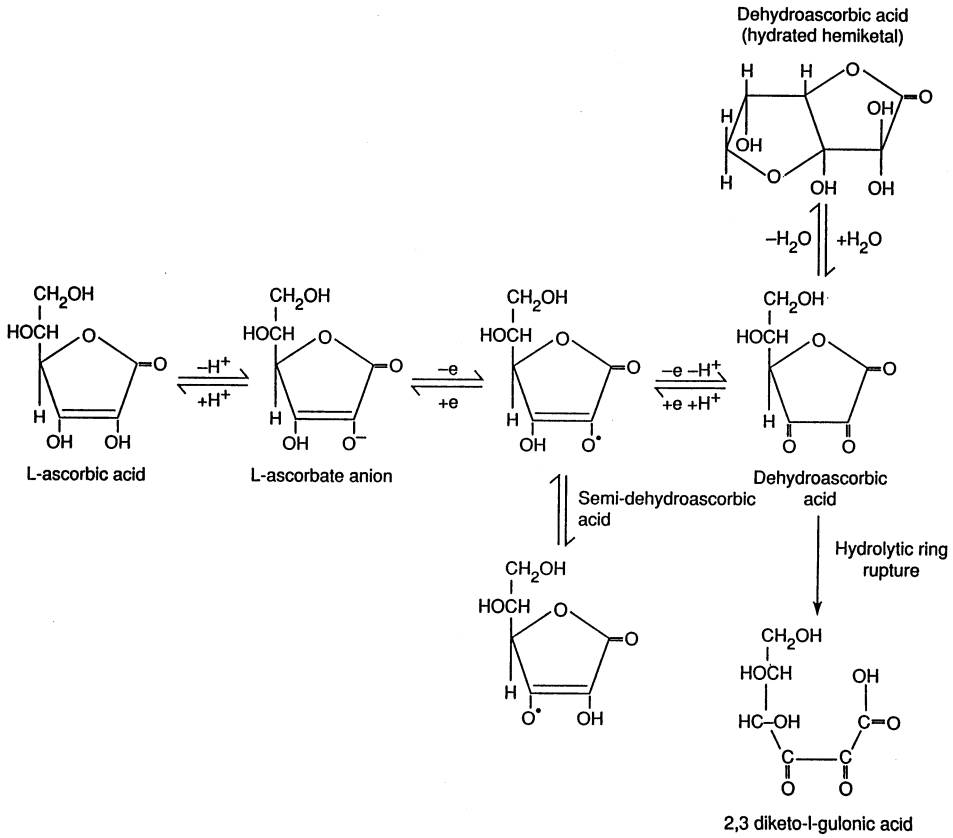

Figure 1 Ascorbate and its oxidation products. Dehydroascorbic acid might exist in multiple forms, although only two are shown here for simplicity. The hydrated hemiketal is proposed to be the favored form in aqueous solutions,[16] but it is unknown which form is found in biological systems. Semidehydroascorbic acid might also have other configurations that are omitted here.[17,209] Formation of 2,3,-diketogulonic acid by hydrolytic ring rupture is probably irreversible. (Figure from Washko, et al.[192])

diketogulonic acid. The latter can be metabolized further to form oxalate, threonate, xylose, xylonic acid, and lynxonic acid.[17] Dehydroascorbic acid is unstable in aqueous solution, with a half-life at 37°C of approximately 6–20 min as a function of concentration.[18–20]

Ascorbate is often called an outstanding antioxidant. In chemical terms this is simply a reflection of its redox properties as a reducing agent. In physiologic terms this means that ascorbate provides electrons for enzymes, for chemical compounds that are oxidants, or for other electron acceptors. In addition to its redox potential, other properties of ascorbate make it an excellent electron donor in biological systems. First, its intermediate free radical is relatively nonreactive, especially with oxygen.[21] Second, the ascorbate oxidation product dehydroascorbic acid is reduced by cells to ascorbate, which then becomes available for reuse.[22,23] Mechanisms of dehydroascorbic acid reduction are discussed below.

Transport mechanisms

Ascorbate and dehydroascorbic acid transport

Both ascorbate and dehydroascorbic acid are transported across cell membranes. Ascorbate transport exhibits saturable kinetics, as shown in normal human cells, immortalized human cells, a variety of animal cells, and in tissues from many species. In most cases, the transport of ascorbate is Na^+-dependent in tissues from animals[24–31] and humans.[22,32–37] Ascorbate transport has been specifically shown to require metabolic energy,[24,26,36–38] with a stoichiometry for Na^+ from 1:1[39–41] to 2:1.[42–44] Some tissues display sodium-independent ascorbate transport,[45,46] but these results are questionable because of lack of adequate control data, low transport rates, or nonspecific assays. The apparent transport affinity (K_m) of ascorbate has been determined in human fibroblasts, neutrophils, lymphocytes, and osteoblasts and ranges from 5–20 μM.[22,32–34,36,37,47] The protein responsible for ascorbate transport has not yet been isolated, as discussed below.

Transport of dehydroascorbic acid is primarily Na^+-independent in tissues from animals[27,42,48,49] and humans,[20,22,50] and does not require metabolic energy. The proposed mechanism is one of cellular trapping. Upon cell entry, dehydroascorbic acid is immediately reduced to ascorbate, which produces an effective gradient of dehydroascorbic acid across the membrane.[20,51] Although some reports describe Na^+-dependent uptake of dehydroascorbic acid, the data may not be relevant because of the extremely high concentrations of dehydroascorbic acid used,[35,52] which are toxic to cells and tissues.[22,53] The apparent K_m reported for dehydroascorbic acid transport is generally much higher than that of ascorbate and ranges from 0.75 to

3.7 mM in human neutrophils, fibroblasts, and HL60 cells.[54–56] A more recent study of human neutrophils demonstrated both low and high affinity dehydroascorbic acid transport. Only the high affinity component could be quantified, yielding an apparent K_m of 35 μM.[22]

Interactions between dehydroascorbic acid and glucose transport have been studied for many years.[49,50,54,57] Kinetic evidence suggests that in some tissues dehydroascorbic acid may be transported by the same transporter as glucose.[50,54,58,59] Seven glucose transporter isoforms (GLUT 1–5, SGLT1, and SGLT2) have been characterized,[60–62] which vary in substrate affinity and tissue distribution.

Experiments utilizing *Xenopus laevis* oocytes to express individual glucose transporter isoforms have demonstrated that GLUT1[63,64] and GLUT3[64] act as dehydroascorbic acid transport proteins with an affinity similar to or lower than that for glucose (1–2 mM).[64] Human GLUT4 also mediates the transport of dehydroascorbic acid, but with a 2–3-fold lower rate than for glucose.[64] Human GLUT2, GLUT5, and SGLT1 were unable to transport dehydroascorbic acid. None of the glucose transporters (GLUT1–5 and SGLT1) transported ascorbate.[64]

Studies in neutrophils showed two components of dehydroascorbic acid transport, only one of which could be calculated (transport K_M of 35.5 μM).[22] Rates of intracellular reduction in neutrophils were a limiting factor for dehydroascorbic acid uptake at high concentrations (>800 μM).[22] The reasons for kinetic differences between oocytes and cells could be due to multiple GLUT transporter activities and/or intracellular reduction pathways. Intracellular dehydroascorbic acid reduction mechanisms will be discussed below. It is not known whether other membrane proteins also mediate dehydroascorbic acid transport.

Interactions between glucose and ascorbate transport activity have also been demonstrated. In neutrophils, ascorbate transport had two apparent components.[34] It is not clear if one of these components was actually dehydroascorbic acid transport, because small amounts of dehydroascorbic acid could have been present but were not detectable. Nevertheless, both transport components were inhibited by glucose, although by different mechanisms. Glucose inhibited the high affinity component noncompetitively and the low affinity component competitively.[34] In fibroblasts, ascorbate transport was not inhibited by glucose.[36] The reasons that glucose inhibited ascorbate transport in some cells is not known but may be due to cell-specific differences in the putative ascorbate transporter.

Besides glucose, other compounds have been proposed to regulate ascorbate transport. Endotoxin inhibited ascorbate transport in 3T3 fibroblasts. The suggested mechanism involved activation of the complement system and production of a 9 kD inhibitory protein that has identity with or is very similar to fragment C3a of complement component C3.[65] The inhibitory protein had no effect on glucose transport; however, it remains possible that these findings were due to oxidation of ascorbate to dehydroascorbic acid and hydrolysis to diketogulonic acid. The latter is poorly transported by cells (S.C. Rumsey and M. Levine, unpublished results). In addition, no information is available on the effect of the inhibitory protein on dehydroascorbic acid transport.

TGF-β increased ascorbate transport in the osteosarcoma cell line UMR-106 by 20–30%.[33] Changes were attributed to increased transport V_{max}; however, the experiments did not adequately address whether increased ascorbate uptake was in the reduced or oxidized form. The increases were small and may have been due to increased cell volume or cell surface area induced by TGF-β. Other transport processes were not adequately examined to test the specificity of TGF-β for ascorbate transport.

The mechanisms of transport of ascorbate across cell membranes have been controversial for many years. Some investigators contended that all ascorbate transport was due to conversion of ascorbate to dehydroascorbic acid with subsequent transport of only dehydroascorbic acid.[63,66,67] In these studies, ascorbate uptake was not detected. This was likely due to assay conditions utilizing very short time periods or insensitive or nonspecific assay conditions. Indeed, because of the ease of interconversion between ascorbate and dehydroascorbic acid under some redox conditions, the nature of the substrate present in vitro is not always straightforward, and care must be taken to ensure substrate purity. The rate of dehydroascorbic acid uptake appears to be much greater than that of ascorbate for most tissues studied. When both ascorbic and dehydroascorbic acid transport were measured in myeloid cell lines, 15–30-fold more dehydroascorbic acid was transported compared to ascorbate.[22] Ascorbate and dehydroascorbic acid transport are, nonetheless, different with respect to Na^+ dependence, energy dependence, rate constants, effect of reducing agents, and tissue specificity. Explanation of ascorbate transport by oxidation to dehydroascorbic acid alone does not account for these differences.

Several distinct experimental approaches indicate that ascorbate and dehydroascorbic acid transport are different. First, experiments performed in human neutrophils using ascorbate analogs and cross-competition with dehydroascorbic acid in the presence and absence of Na^+ conclusively demonstrated separate ascorbate and dehydroascorbic acid transport activities.[22] Second, expression of size-fractionated mRNA from rabbit kidney (1.8–3.1 kb) in *Xenopus laevis* oocytes resulted in ascorbate transport.[68] Induced transport activity was Na^+-dependent, saturable, and not inhibited by glucose. Despite these promising experiments, the ascorbate transport protein has not yet been isolated. Third, although dehydroascorbic acid transport was efficiently mediated by glucose transporter proteins GLUT1, GLUT3, and to some extent GLUT4 when expressed in oocytes, no glucose transporters transported ascorbate.[64] Taken together, recent experimental data in conjunction with the extensive literature on ascorbate transport strongly support the existence of two separate and distinct transport mechanisms for ascorbate and dehydroascorbic acid.[22,69]

The contribution of ascorbate and/or dehydroascorbic acid to cellular transport in vivo remains an open issue. Transport depends on both substrate affinity and substrate availability. Plasma concentrations of ascorbate (5–100 μM) are in the range reported for ascorbate transport affinity. By contrast, dehydroascorbic acid is either not detectable or found only at very low levels in the circulation

of healthy humans.[15,70] Because there is currently no adequate direct assay for dehydroascorbic acid, the lower limit of detection of dehydroascorbic acid using HPLC with electrochemical detection is 1–2% of the concentration of ascorbate present. Smaller amounts of dehydroascorbic acid cannot be distinguished from 0.[70,71] If dehydroascorbic acid is present at all in normal humans, the concentrations are likely to be less than 2 μM. Absence of dehydroascorbic acid in plasma is consistent with the proposed mechanisms of dehydroascorbic acid reduction and ascorbate recycling, which are discussed below. In addition, transport of both ascorbic and dehydroascorbic acids may be affected in vivo by glucose, whose concentration is 3–6 mM in normal people and as much as 30 mM in diabetics. The effect of alterations in glucose level on cellular accumulation of either ascorbate or dehydroascorbic acid in vivo is unknown.

Intracellular dehydroascorbic acid reduction

It has been known for many years that a variety of tissues can reduce dehydroascorbic acid to ascorbate.[72,73] Intracellular dehydroascorbic acid is immediately reduced to ascorbate.[20,22,23,51,66,74,75] Intracellular reduction is mediated by two major pathways: chemical reduction by glutathione[76] and enzymatic reduction.[23,77]

The role of these two processes in vivo is probably tissue specific and remains to be resolved.[76–78] In erythrocytes, pig heart tissue, and ocular tissues from rat and rabbit, reduction of dehydroascorbic acid has been completely accounted for by glutathione;[76,79–81] however, these findings can be questioned.[82] Tissue homogenization can result in a many-fold dilution of intracellular proteins and could have inactivated or decreased intrinsic protein activity. Protein activity could also be masked by assay conditions, pH changes, and concurrent nonenzymatic chemical reduction. Another consideration is that the tissues selected for study may not have been enriched in reducing protein. The contribution of glutathione-mediated chemical reduction and protein-mediated reduction may vary in different tissues and in the same tissues with different stimuli.

Dehydroascorbic acid reductase activity was recently isolated from human neutrophils by activity-based purification. The responsible activity was glutaredoxin, an 11,000 D glutathione-dependent protein. Glutaredoxin accounted for as much as 80% of total cellular dehydroascorbic acid reducing activity.[23]

Other isolated proteins that have been shown to have dehydroascorbic acid reductase activity include glutaredoxin from pig liver, bovine thymus and human placenta;[83] bovine protein disulfide isomerase;[77,83] 3-α-hydroxysteroid dehydrogenase;[84] and an as yet unidentified protein of MW 31,000 isolated from rat liver[85] and human red blood cells.[82] Although glutaredoxin is responsible for most of dehydroascorbic acid reduction in human neutrophils, the relative role of these other proteins in tissues is not known. Protein disulfide isomerase and 3-α-hydroxysteroid dehydrogenase[84] have a 5- to 10-fold lower affinity for dehydroascorbic acid (K_m of 2.8 and 4.6 mM, respectively) than do the 31,000 D protein[82,85] or glutaredoxin isolated from other mammalian tissues (K_m of 0.2–1.0 mM).[23,77] 3-α-hydroxysteroid dehydrogenase[84] and the 31,000 D protein have the lowest measured V_{max} (1.5 and 1.9 nmol/min/μg protein, respectively), with the remaining proteins ranging from 6 to 27 nmol/min/μg protein.[23,77,83–85] The tissue distribution of glutaredoxin and other dehydroascorbic acid–reducing protein activities and their regulation under normal and oxidative stress conditions in humans remain to be examined.[23,77,86]

As mentioned above, it is likely that dehydroascorbic acid reduction is the driving force for dehydroascorbic acid transport across cell membranes.[20,22,74] Intracellular reduction to ascorbate produces an outside to inside dehydroascorbic acid gradient, favoring entry. Because ascorbate exits cells at comparatively slow rates,[22,36,51,87] high intracellular concentrations of ascorbate can be maintained. Data in leukocytes,[67,74] neutrophils,[22] and oocytes[64] indicate that dehydroascorbic acid transport may be limited at high concentrations by the capacity of intracellular reduction to ascorbate; thus, in experiments of dehydroascorbic acid transport, apparent transport affinities can only be determined if intracellular reduction is confirmed to be complete.

Ascorbate recycling

The most comprehensive model of ascorbate accumulation consistent with available data is ascorbate recycling (*Figure 2*). In this model, transport of both dehydroascorbic acid and ascorbate occurs, but is dependent on substrate availability.[20,22,64,88] Under nonoxidizing conditions, ascorbate is predicted to account for the majority of ascorbate accumulation in the circulation and in the extracellular space because it is expected to be the only substrate available. In the presence of oxidants, ascorbate will oxidize, and dehydroascorbic acid may transiently form. In blood, dehydroascorbic acid would be expected to be rapidly transported and reduced within red blood cells, which have abundant GLUT1, glutathione, and glutaredoxin.[60,79,86] This is consistent with observations that dehydroascorbic acid is not detected in normal human plasma.[15,70,71] In the extracellular space under oxidizing conditions (i.e., infection), dehydroascorbic acid would form and be available to surrounding cells. Compared to ascorbate, dehydroascorbic acid would then be preferentially transported and immediately reduced within cells.

Experiments using human neutrophils provide strong evidence for ascorbate recycling.[20,22,23,88] In resting cells, ascorbate is transported as ascorbate.[32] It is likely that ascorbate transport activity is responsible for at least some part of the high internal concentration of ascorbate (mM levels) in resting cells. Upon neutrophil activation, reactive oxygen species are produced. Extracellular ascorbate is oxidized to dehydroascorbic acid, which is rapidly transported into cells and immediately reduced intracellularly to ascorbate (*Figure 2*). In this manner, as much as 10-fold increases in intracellular ascorbate concentration can be achieved.[20,22] In activated cells, ascorbate transport also continues independently but at a much slower rate as compared to dehydroascorbic acid transport. The results imply that dehydroascorbic acid formation and transport could occur during inflammation. Indeed, exposure of human neutrophils to bacteria in the presence of ascorbate is

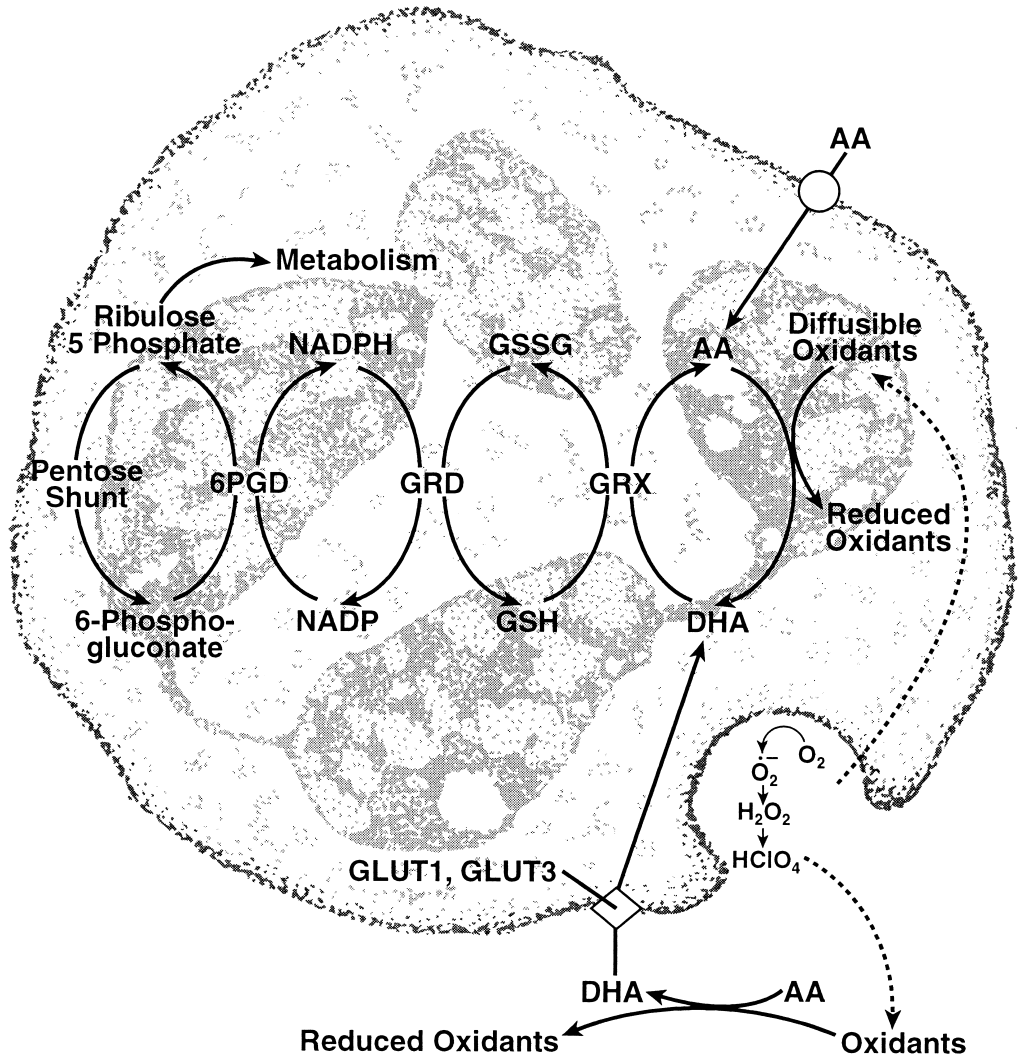

Figure 2 A model of dehydroascorbic acid and ascorbate transport and recycling in human neutrophils. Ascorbate and dehydroascorbic acid are transported differently.[20,22,64,68] The putative ascorbate transporter (open circle) transports ascorbate and probably maintains mM concentrations of ascorbate inside neutrophils.[20,22,32] The ascorbate transport protein has not been isolated. With activation, neutrophils secrete reactive oxygen species, which oxidize extracellular ascorbate to dehydroascorbic acid.[20] Dehydroascorbic acid is rapidly transported by glucose transporter isoforms GLUT1 and GLUT3 (open diamond).[64] Intracellular dehydroascorbic acid is immediately reduced to ascorbate.[20] In neutrophils, glutaredoxin is responsible for the majority of intracellular reduction.[23] As a result of dehydroascorbic acid transport and reduction, as much as 10-fold higher ascorbate internal concentrations are achieved compared to activity of the ascorbate transporter alone.[20,22] The proposed mechanism of reduction could require glutathione, NADPH, and the enzymes shown.[23] Although dehydroascorbic acid is transported by glucose transporter isoforms GLUT1 and GLUT3,[64] it is unknown whether one or both isoforms are responsible for dehydroascorbic acid entry in neutrophils. Abbreviations: AA, ascorbate; DHA, dehydroascorbic acid; GRX, glutaredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; 6-PGD, 6-phosphogluconate dehydrogenase; GRD, glutathione reductase.

an extremely potent inducer of ascorbate recycling, with as much as 30-fold increases in intracellular ascorbate compared to controls without bacteria.[88]

Transport of both ascorbate and dehydroascorbic acid could occur in vivo. Substrate availability probably plays a key role in which species is transported at a particular time. Under nonoxidizing conditions, ascorbate is the form likely to be transported by cells because of its ubiquitous presence in the intravascular and extracellular spaces and probable absence of dehydroascorbic acid. Ascorbate transport can be considered as constitutive-substrate transport. By contrast, dehydroascorbic acid transport can be considered as induced-substrate transport, because dehydroascorbic acid transport is dependent on substrate formation and is limited by substrate availability.

The roles of ascorbate and dehydroascorbic acid in accumulation somewhat resemble the interplay between thyroxin (T4) and triiodothyronine (T3) in thyroid hormone metabolism. Approximately 97% of total circulating thyroid hormone is T4, and <3% is T3. The majority of both forms are protein bound, however, and it is free hormone that provides metabolic feedback. There is approximately 4-fold more free T4 than T3.[89] At the molecular level, it is likely that only T3 is active in the nucleus and that all of the

eventual metabolic activity of T4 is due to its conversion to T3. Both hormones are, nevertheless, important for thyroid hormone action because of their circulating concentrations, plasma protein binding, tissue distribution, intracellular transport, and conversion of T4 to T3.[89]

There are several possible reasons that ascorbate recycling occurs. In neutrophils, ascorbate recycling provides rapid increases in intracellular ascorbate at the same time that oxidants are produced. Oxidants in neutrophils are produced in the phagosome and on the external surface of the cell membrane, and also leak into the cytosol and extracellular space.[20,90] Higher concentrations of ascorbate in neutrophils could provide better protection against intracellular reactive oxygen intermediates. In neutrophils, these intermediates are an integral component of microbicidal pathways and mediate apoptosis.[91–93] Increased intracellular ascorbate concentrations could be effective in quenching cytosolic and extracellular oxidants and possibly delay neutrophil apoptosis.[94,95] Prolongation of neutrophil survival could also translate into enhanced microbicidal activity.

It is also possible that increased intracellular ascorbate has an extracellular protective function. This could occur by active extrusion of ascorbate under certain conditions or by leakage of intracellular ascorbate as neutrophils die. Because ascorbate recycling causes such large increases in intracellular neutrophil ascorbate, these concentrations could affect extracellular concentrations as a function of neutrophil cell density and/or limitations of extracellular diffusion of ascorbate into an area of inflammation. Increased extracellular ascorbate would be protective against oxidant damage to collagen and surrounding cells. Experimental systems that will need to be developed to test these possibilities include new models of inflammation with bacteria and multiple cell types, new ascorbate and dehydroascorbic acid analogs to distinguish between the effects of extracellular and intracellular ascorbate, cell lines transfected with glutaredoxin or with glutaredoxin knocked out, and new methods to measure oxidant production and collagen degradation. These experiments could determine the functional consequences of ascorbate recycling and its regulation in vivo, with potential clinical application.

Ascorbate recycling could occur in other cell types as a function of oxidants produced. It is possible that extracellular ascorbate oxidation could be induced by low concentrations of superoxide or other oxidants arising from mitochondria or cellular metabolism; however, it remains to be determined for other cell types which oxidants are produced and whether their quantitites are sufficient for ascorbate oxidation.

Intestinal absorption and bioavailability

To provide ascorbate to tissues, humans must ingest the vitamin and absorb it in the gastrointestinal tract. Because both ascorbic and dehydroascorbic acids are transported in vivo, we discuss intestinal absorption of both forms. Ascorbate in the reduced form constitutes the majority (80–90%) of this vitamin in food.[96,97] Studies utilizing sections of human ileum[24,98] determined that ascorbate is absorbed in the human intestine by a Na^+-dependent active transport system. Absorption is most effective in the proximal intestine.[99] Absorption of ascorbate in proximal intestine was studied directly in human subjects using intestinal perfusion to measure uptake rates, and the calculated maximal transport rate was 50 mg/cm-hr.[100] The K_m was expressed as 5 mmols, indicating that the calculation may be incorrect because of an unclear effect of volume. Results of other uptake studies vary considerably,[99,101–103] perhaps as a consequence of assay imprecision or variations in subject repletion status and dose. These studies are also problematic because they used the indirect measure of urinary output as the criteria for ascorbate uptake.

It is not known whether there are individual differences in ascorbate absorption. In addition, the possibility exists that vitamin C intestinal transport is regulated. In guinea pigs, prior feeding of large doses of ascorbate decreased transport across isolated intestinal mucosa;[104] however, this issue has not been examined in humans.

Little information is available on the intestinal absorption of dehydroascorbic acid in humans. In isolated guinea pig intestinal mucosa, dehydroascorbic acid absorption is a Na^+-independent, saturating process.[105,106] The majority of dehydroascorbic acid is immediately reduced upon crossing the serosal membrane and is present as ascorbate.[75] Although dehydroascorbic acid is anti-scorbutic in humans,[107–109] some data show that absorption is less than that of ascorbate.[110] It is also not known whether the local environment in the gastrointestinal tract affects dehydroascorbic acid reduction to ascorbate or hydrolysis to diketogulonic acid.

It is unclear which forms physically cross intestinal membranes: ascorbate or dehydroascorbic acid or both. Consistent with ascorbate recycling, the data imply that both species are transported but by separate mechanisms, as in human neutrophils. Direct experiments to validate this concept remain to be performed.

Absorption is determined clinically using the pharmacokinetic principles of bioavailability. True bioavailability is defined as the increase in the amount of a substance in plasma after an oral dose compared with the increase in plasma after the same dose is given intravenously.[111,112] To achieve accurate baseline calculations, measurements must be performed at steady-state for the dose tested.

Bioavailability for ascorbate is most relevant since it is the dominant substrate in foods and supplements and the dominant (if not only) substrate in plasma. A comprehensive study of true bioavailability at different ascorbate doses was reported recently.[15,113] Patients were brought to steady-state plasma concentrations at each dose before measurements were made. Based on a five compartment phamacokinetic model, ascorbate bioavailability of a liquid solution given in the fasted state was 90% for $\leq$200 mg, 73% for 500 mg, and 49% for 1250 mg.[15,113] Bioavailability calculations assume that clearance is constant at different doses. This assumption was not met at ascorbate doses $<$200 mg but could be determined using the new model, which accounts for changing clearance.[113]

Other studies examined the relative bioavailability of ascorbate provided to subjects in different forms or at varying doses.[103,114–119] Relative bioavailability is calculated by comparing measurements that are assumed to represent bioavailability. Relative bioavailability is often

determined by comparing changes in urinary or plasma ascorbate after two different oral doses of ascorbate. This method can be potentially valuable as a tool to evaluate different acute dosage forms, but it cannot give information about true absorption of ascorbate. Inherent problems with this methodology include lack of control over subject repletion status and inability accurately to judge baseline concentrations.

The effect of different foods on ascorbate bioavailability is still largely unknown. True bioavailability of ascorbate has not been determined with different food preparations. Studies examining relative bioavailability have found little difference in absorption between pure ascorbate and ascorbate in foods,[120–125] with the exception of one study that showed that ascorbate relative bioavailability was increased by 35% when the vitamin was accompanied with a citrus extract.[126] One must keep in mind, however, that the majority of these studies were performed using imprecise assays, and further study into this area is warranted.

As mentioned above, dehydroascorbic acid given orally has been shown to be absorbed in humans.[108–110] These studies only examined relative bioavailability, however, and have similar problems to those described above for ascorbate. No information is available on the true bioavailability of dehydroascorbic acid.

Ascorbate in the circulation

Ascorbate is present in the blood at concentrations of 5–90 μM in normal individuals,[15] whereas dehydroascorbic acid is only present at very low concentrations (<2% of ascorbate) or not at all.[70,71,127] Some investigators measured concentrations of dehydroascorbic acid at as much as 20% of total ascorbate in plasma,[110,128–131] but such values may be a consequence of lack of specificity of the assays used or of inadvertent oxidation of ascorbate to dehydroascorbic acid during sample procurement, preparation, or analysis.[34] Ascorbate in plasma and serum is available to tissues and cell transporters directly without a protein-bound intermediate.[70,132]

Different cell types in blood transport both ascorbate and dehydroascorbic acid in vitro.[20,51,52,87,133,134] Although the rate of transport of dehydroascorbic acid is greater than that of ascorbate in all of these cells, as discussed above, the concentration of dehydroascorbic acid present in blood as substrate is negligible in most cases. Reported content of ascorbate in erythrocytes[134] is difficult to interpret because ascorbate is easily oxidized by iron and/or hemoglobin. The concentration of ascorbate in erythrocytes is probably less than that of plasma. Ascorbate is accumulated in mM concentration in neutrophils, lymphocytes, monocytes, and platelets.[15,20,51,52,87,133,134]

Until recently, data concerning the relationship between ascorbate intake and its concentration in plasma have been extremely limited. Earlier inpatient studies did not address this issue. Prisoner volunteer subjects were given ≤64 mg of ascorbate daily, and the diet was deficient in other vitamins and minerals.[6,135] In follow-up studies, prisoner volunteer subjects received either 6.5 or 66.5 mg of ascorbate daily, although one subject received 130.5 mg daily.[136,137] In two later volunteer studies, only three daily

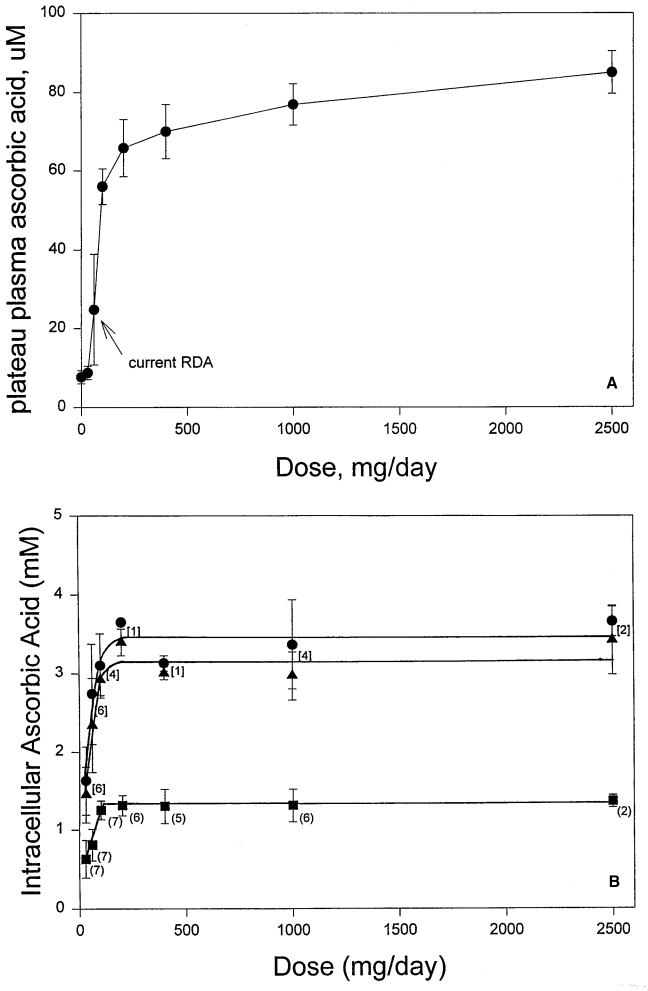

Figure 3 Relationships between ascorbate dose and plasma and intracellular ascorbate concentrations. Seven healthy volunteers were hospitalized for 4–6 months and consumed a diet containing <5 mg vitamin C daily.[15,145] Steady-state plasma and tissue concentrations were determined at seven vitamin C daily doses from 30 to 2500 mg.[15] **A.** Steady-state plateau ascorbate concentrations in plasma as a function of ingested dose. **B.** Intracellular ascorbate concentrations (mM) in circulating immune cells as a function of dose. Dose is expressed as mg of ascorbate administered daily. Neutrophils (■), monocytes (▲), and lymphocytes (●) were isolated as described[32,87] when plateau was achieved for each dose. Numbers in parentheses at each dose indicate the number of volunteers from whom neutrophils were obtained; numbers in brackets at each dose indicate the number of volunteers from whom lymphocytes and monocytes were obtained. (Figure from Levine, et al.[15])

doses of ascorbate were used,[138] or only one dose above 60 mg was used.[139] Outpatient studies examining dose-response information have also been limited because, by design, they cannot strictly control the amount of ascorbate in a subject's diet.[140–143] Use of dietary surveys to obtain vitamin ingestion information is often misleading, especially over narrow dose ranges.[144]

A recent inpatient study examined the relationship between ascorbate intake over a wide range (30–2500 mg daily) and concentrations of ascorbate in plasma and tissue.[15] Seven men 20–26 years old were hospitalized for 4–6 months and consumed a strictly controlled diet.[145]

Plasma concentrations as a function of dose displayed sigmoid saturating kinetics (*Figure 3A*). The first ascorbate dose beyond the steep portion of the curve was 200 mg daily. The most marked alterations in plasma ascorbate concentrations occurred between 30 and 100 mg/d; therefore, variations of intake within this range may markedly affect ascorbate availability to tissue. Doses above 400 mg/d resulted in little further increase in plasma ascorbate concentrations, and absorbed doses were almost entirely excreted in the urine,[15] as discussed later.

The approximate half-time of ascorbate in plasma can be inferred from several studies in which subjects ingested $<$10 mg daily.[15,125,138,139] Half-time in plasma was approximately 7–14 days. However, half-time is probably affected by initial starting plasma concentration, and individual subjects varied in half-time parameters. The reasons for individual variation are unknown but might be due to any of the processes involved in ascorbate recycling.

Tissue accumulation and distribution

Ascorbate content of human tissues varies over a wide range. Tissues with the greatest quantity of ascorbate/100 g tissue include adrenal and pituitary glands with 30–50 mg/100 g, followed by liver, spleen, eye lens, pancreas, kidney, and brain with between 10 and 30 mg/100 g.[69] By total weight and because of its size, liver has the greatest store. In humans it is difficult to study relationships between intake and tissue concentrations. Tissue samples are not easily obtained, and degradation occurs unless samples are processed quickly. Since guinea pigs do not synthesize ascorbate, they have been used as one model of tissue distribution. Tissue distribution of ascorbate appears to be similar in guinea pigs and humans. In one guinea pig study with graded intake (from 0.2–10 mg/100 g body weight), an intake-dependent increase to saturation was reported for various tissues including heart, brain, spleen, leukocytes, and adrenal gland.[146] Examination of the results of many studies in animals using different ascorbate intake amounts showed a similar trend, with higher doses resulting in higher tissue concentrations.[69]

The half-time of ascorbic acid in specific human cells or tissues under conditions of minimal ascorbate intake has not been well studied. Based on mathematical modeling of urinary excretion of radiolabelled ascorbate, estimations have been made of ascorbate loss from a theoretical total body pool amounting to 2.6 to 4.1% per day.[136,140] These estimates, however, do not give information about ascorbate content of specific tissues. One study reported that concentrations of ascorbic acid in circulating mononuclear leukocytes had a half-time of approximately 30 days.[139] Factors that likely influence intracellular ascorbate half-time include initial intracellular starting concentration and extracellular plasma concentration.

Dehydroascorbic acid is not found in appreciable amounts in tissue.[20,22,36,147] When radiolabelled ascorbate and dehydroascorbic acid were injected in guinea pigs, clear differences were seen in tissue distribution and rates of uptake.[148] Ascorbate was rapidly accumulated by adrenal and pituitary glands, lungs, liver, kidneys, bone, and skin. Dehydroascorbic acid remained primarily associated with blood and blood cells. Even with injection of dehydroascorbic acid, all radiolabel isolated from tissue was found in the reduced form. Because dehydroascorbic acid is rapidly transported into and reduced by blood cells, it is probably not available to most tissues via the circulation. This concept is supported by isolation of glutaredoxin from red blood cells[86] and neutrophils,[23] and by the high dehydroascorbic acid reductive capacity of red cells mediated by glutathione chemically.[79]

Unlike other cells, circulating blood cells can be obtained relatively easily. It has therefore been possible to examine the concentrations of ascorbate in these cells and the relationship between ascorbate intake and cellular concentration in humans. Similar to plasma data, until very recently there have been no well-controlled studies examining the relationship between circulating cell ascorbate content and dietary intake over a wide range. Some investigators suggested that erythrocytes and platelets contain the highest fraction of ascorbate among blood cells because of their abundance;[134] however, as noted above, determination of erythrocyte ascorbate is problematic because of the presence of very large quantities of highly reactive heme-iron, which can easily oxidize ascorbate during sample preparation or storage. To date, rigorous analysis of erythrocyte ascorbate content has not been performed. The recent NIH study examined the relationship between ascorbate dose and ascorbate content of lymphocytes, neutrophils, and monocytes.[15] Intracellular ascorbate in these cells saturated at lower doses than did plasma, by 100 mg daily (*Figure 3B*), and achieved intracellular ascorbate concentrations (1–4 mM) at least 14-fold higher than plasma. These findings are one explanation of the sigmoid relationship between dose and plasma concentration.

Renal excretion

In all species studied, ascorbate is filtered at the glomerulus and is reabsorbed at the proximal tubule by an active transport process.[149–152] In humans, the upper range of ascorbate concentration in blood is limited by renal reabsorption.[15,153] Maximal tubule reabsorption rates were determined in men and women of different ages and found to be relatively constant between groups at approximately 1.5 mg/100 ml glomerular filtrate.[149,154] As in intestine, ascorbate transport in the kidney tubule is Na^+-dependent.[150–152,155] Ascorbate transport in brush border vesicles from kidney cortex was saturable, potential-sensitive, and partially inhibited by glucose.[155] Tubule reabsorption presumably is related to the tubule concentration of ascorbate. It is unknown whether ascorbate reabsorption can be regulated by other mechanisms, and it is also unknown whether ascorbate is actively secreted into renal tubules.

The threshold ascorbate dose for urinary excretion was believed to represent a near saturating dose, and the current recommended dietary allowance for ascorbate is based in part on this concept.[156,157] One deficiency-repletion clinical study implied that ascorbate was excreted in the urine of volunteers who were at steady-state for a 60-mg daily dose.[135] These data were used to set the current recommended dietary allowance of ascorbate at 60 mg dai-

ly.[156,157] The original findings are questionable, however, because the assay for ascorbate was subject to false-positive interference, especially at low ascorbate concentrations,[34] and the data from these volunteers was referred to but not published in the scientific literature. More importantly, it remained unknown whether threshold urine excretion was a valid index of saturation. The threshold for ascorbate urinary excretion was examined in the recent NIH study.[15] Ascorbate excretion was measured at steady-state for 30–1250-mg doses using a sensitive electrochemical HPLC assay. The results showed that ascorbate appeared in urine only at doses ≥100 mg/d,[15,113] corresponding to an average plasma concentration of 55–60 μM (*Figure 3A*). At this dose, lymphocytes, neutrophils, and monocytes were fully saturated and plasma was approximately 70% saturated. The complete saturating ascorbate dose was 1000 mg daily.

In contrast to ascorbate, dehydroascorbic acid transport in isolated kidney tubules is Na^+-independent, insensitive to transmembrane electrical potential difference, and is not concentrated against a gradient.[45,158] Transported dehydroascorbic acid is rapidly reduced to ascorbate in isolated tubules from rat and guinea pig,[106] and transport of ascorbate and dehydroascorbic acid at the renal basolateral membrane appeared to be an Na^+-independent process of comparable rate.[106] No evidence is available on the renal excretion or reuptake of dehydroascorbic acid in vivo because no measurable amounts can be found in plasma. Measurement of dehydroascorbic acid in urine is fraught with similar methodological difficulties as with plasma. Most likely, dehydroascorbic acid is present in very low quantities or not at all in urine, and greater quantities are probably a result of experimental artifact.[159]

Ascorbate metabolism in health and disease

In humans, urine is the primary and perhaps the exclusive route of ascorbate and ascorbate metabolite excretion. Metabolites of known structure found in urine utilizing ^{14}C-labeled ascorbate include dehydroascorbic acid, diketogulonic acid, oxalate, ascorbate-2-sulfate, and methyl ascorbate.[69,160–166] Nonhuman primates and guinea pigs excrete up to 90% of ingested ascorbate in the form of CO_2. Humans, however, do not appear to utilize this metabolic route.[163,167] One study in humans found that as much as 30% of ascorbate given in doses >180 mg was excreted as CO_2, but the findings were attributed to presystemic bacterial or chemical degradation of ascorbate in the intestine.[166] Little quantitative information exists on the relationship between repletion status or intake and the formation and excretion of ascorbate metabolites in humans. Qualitative data suggest that there may be a constitutive amount of metabolite formation at all doses.[140] The relative proportion of metabolic products is not clear but is probably dependent on dose, repletion status, and other unknown factors. In one study, patients were given [^{14}C]ascorbate intravenously, and urinary metabolites were measured: 40% was oxalate, 20% was diketogulonic acid, 2% was dehydroascorbic acid, and the rest was of unknown form.[168] Assay imprecision and artifact due to ascorbate degradation during handling, however, cloud interpretation of these results. It remains unclear what factors may regulate production of ascorbate metabolites. Production of two major metabolites, oxalate and diketogulonic acid, has dehydroascorbic acid as a precursor,[165] even though dehydroascorbic acid itself is not detectable in urine.[159] Factors that increase oxidation of ascorbate to dehydroascorbic acid could theoretically increase irreversible production of metabolites and thereby lead to increased ascorbate utilization. When given orally or intravenously to volunteers at steady-state, ascorbate doses of 500 mg and 1250 mg were almost completely accounted for by urinary excretion of ascorbate;[15] nevertheless, oxalate excretion was highest at an intake of 1 g ascorbate daily. At this dose, the amount of oxalate excreted was <10% of ascorbate excreted, and oxalate measurements reflect its metabolism from all sources. We conclude that, for the steady-state condition at doses ≥500 mg, most ascorbate in healthy humans is probably excreted as such in urine. At doses ≤60 mg daily, ascorbate itself is not excreted, and only metabolites such as oxalate are expected in urine.

Abnormal kidney function may profoundly affect ascorbate metabolism. The entire plasma content of ascorbate is filtered and reabsorbed approximately once per hour in healthy people. It is unknown whether there are mechanisms that regulate these processes. In patients with renal disease an inverse correlation was seen between creatinine clearance and ascorbate excretion;[169] however, it is impossible to draw firm conclusions from these observations for a number of reasons. No distinctions were made between different types of renal diseases, and no data were obtained concerning ascorbate intake and ascorbate steady-state plasma values. It would be valuable to examine excretion of ascorbate in patients with different types of renal insufficiency under steady-state conditions, with intake rigorously controlled.

Ascorbate is oxidized by a variety of enzymatic and nonenzymatic reactions, as reviewed elsewhere.[170] In particular, ascorbate might be consumed in chemical reactions in which oxidants are reduced. Suggestive evidence supports the hypothesis that oxidative processes regulate ascorbate catabolism in humans, although direct proof is currently lacking. Other antioxidants are present in plasma, including uric acid, tocopherols, and ubiquinone. Ascorbate, however, might be oxidized preferentially and may have an important role as a first defense against oxidative stress.[171]

Smokers present one example of the relationship between oxidants and ascorbate since they expose themselves to oxidants via inhaled smoke. These gas phase oxidants have been demonstrated to induce lipid peroxidation in vitro, which is prevented by the presence of ascorbate.[172] The body turnover of ^{14}C-labeled ascorbate has been shown to be 40% greater in smokers compared with nonsmokers,[173] and estimation of ascorbate requirements of smokers based on intake and serum concentrations show that smokers may require as much as three times the dose of ascorbate than nonsmokers to avoid risk of deficiency.[174] Unfortunately, clinical studies in smokers have even more problems than those described for nonsmokers, including narrow dose ranges, imprecise assays, lack of strict intake control, and lack of information regarding steady-state endpoints.

There are several other examples of the relationship between ascorbate and oxidant quenching. Ascorbate is

very effective at preventing lipid peroxidation from aqueous peroxyl radicals and from oxidants released by polymorphonuclear cells.[171] Ascorbate prevents both initiation and propagation of metal ion–induced oxidation of human low-density lipoprotein (LDL).[175,176] LDL oxidation is thought to be a key process in the initiation of atherosclerosis.[177] In addition, ascorbate may decrease LDL-induced leukocyte adhesion,[178] and endothelial dysfunction may also be improved by ascorbate in both smokers and patients with coronary artery disease.[179,180] It is not known whether oxidation conditions in in vitro experiments are relevant to oxidation conditions in vivo, especially for LDL oxidation.

As an electron donor, ascorbate could be involved in several disease processes. Ascorbate might be consumed by preventing free radical–induced damage of DNA,[181] which is thought to be an initiating step in cancer formation.[182,183] Increased dietary ascorbate has been shown to decrease sperm DNA oxidative adduct formation[184] and DNA strand breaks as a result of ionizing radiation.[185] Ascorbate has also been suggested to quench oxidants leading to cataract formation[186] or endothelial cell reactivity in hypertensive patients,[187] but data are inconclusive.

Despite these and other examples, it still remains unclear whether increased oxidative stress leads to greater ascorbate catabolism in humans, whether higher ascorbate intake can prevent these losses, and whether there are direct clinical consequences related to these events.

Other metabolic alterations may affect ascorbate by modulating its cellular transport and tissue distribution. It has been long suggested, although never proven, that diabetics may have alterations in ascorbate metabolism.[188,189] This issue could be significant when considered with the evidence that dehydroascorbic acid is transported by glucose transporters.[54,56,64] There have been reports of higher concentrations of dehydroascorbic acid in plasma of diabetics compared to nondiabetics.[190,191] It is unclear whether these data are authentic in vivo values because of possible oxidation during sample collection and possible assay artifacts concerning dehydroascorbic acid determination.[127,192] A major limitation to future experiments is the lack of a dehydroascorbic acid assay that is direct, sensitive, and specific.[192]

It has often been suggested that the adrenal-pituitary axis is involved in the regulation of ascorbate concentrations.[193–196] Adrenocorticotropic hormone (ACTH) was shown to deplete rat adrenal gland ascorbate in vivo.[197] Hypophysectomized rats show marked decreases in ascorbate uptake in various tissues, including adrenal glands, ovaries, pancreas, cerebrum, and eyes.[196] One report described two patients who developed scurvy while receiving high doses of ACTH.[198] In vitro, glucocorticoids inhibit ascorbate uptake in rat adrenal cortex,[194] pancreas,[199] and bovine adrenal chromaffin cells,[200] but it is unclear whether glucocorticoid concentrations used in these studies represent those that cells encounter in vivo. No changes were seen in plasma ascorbate concentrations in experimental subjects during daily circadian cycles in which glucocorticoids vary,[15] nor in a subject who was injected with a supraphysiologic dose of 1–24 corticotrophin, the synthetic ACTH peptide for clinical use (M. Levine, unpublished data).

Immune modulators might also influence ascorbate and/or dehydroascorbic acid transport. As discussed above, neutrophil activation alters ascorbate uptake rates via conversion of ascorbate to dehydroascorbic acid, and activation of the complement system can inhibit ascorbate cellular uptake.[65] Whether these effects occur in vivo remain to be demonstrated. The relationship between immune cell function and ascorbate transport and cell content of ascorbate remains largely undefined and deserves to be pursued.

Conclusions

New information is available regarding ascorbate and dehydroascorbic acid transport, dehydroascorbic acid reduction, ascorbate dose-concentration pharmacokinetics for plasma and cells, ascorbate excretion, and ascorbate bioavailability. With these data, it is now possible to form a new basis of recommendations for vitamin C intake in humans. Ideally, intake recommendations should be based in part on how concentration regulates function in situ.[14] We first proposed this concept more than a decade ago,[10] even though others have inappropriately ignored this contribution.[201] Unfortunately, definitive functional data in relation to dose/concentration for ascorbate in humans are not yet available. An intake recommendation is nevertheless necessary and can still be made with available data. Ascorbate absorption and disposition data play a key role in such a recommendation by providing information about physiologically relevant concentrations and how they are achieved.[14,15,78,202,203]

Concentration data could have a key role in future recommendations regarding vitamin C and other water soluble vitamins. Current vitamin recommendations focus on vitamin intake. With knowledge about absorption and distribution, we learn about resulting vitamin concentration. Vitamin concentration, not intake, is most relevant for function. It is possible that identical vitamin intakes in different people result in different vitamin concentrations. Different concentrations from identical intakes could occur due to genetic or functional differences in transport proteins or recycling mechanisms, or in relation to sex, age, or clinical condition. For example, disease could result in decreased vitamin absorption, increased metabolism, altered distribution in body compartments, or accelerated renal excretion;[113,204–208] thus, future ideal vitamin recommendations might focus on achieving specified vitamin concentrations rather than on ingesting specified nutrient amounts. For such a paradigm shift to occur, a function or outcome would have to be associated with a vitamin concentration. These principles are similar to those used for cholesterol and triglyceride intake recommendations, which are based on measured concentrations rather than on intake alone.

We still have much to learn regarding ascorbate absorption and distribution. Our understanding would be substantially enhanced at the molecular level by characterization of the ascorbate transporter and its regulation in different tissues; continued determination of dehydroascorbic acid reduction mechanisms by specific proteins in vitro and in cells; and by characterization of molecular and genomic regulation of ascorbate transport, dehydroascorbic acid transport, and dehydroascorbic acid reduction. At the ana-

lytical level, a specific and sensitive assay for dehydroascorbic acid remains an elusive problem that we must continue to attempt to solve. At the clinical level, we have only a small data window on pharmacokinetics, that is, healthy male subjects. Given the sigmoid relationship between dose and plasma concentration, curve shifting may occur under different physiologic conditions, analogous to the shift that occurs with oxygen-hemoglobin dissociation as a function of pH. It is thus essential to characterize ascorbate dose-concentration relationships in women, in male and female smokers, in diabetics, and in those patients who may have increased ascorbate utilization. These data will allow us to predict and then measure functional outcomes in people in order to provide clear answers for ideal vitamin C recommendations.

References

1. Burns, J.J., Peyser, P., and Moltz, A. (1956). Missing step in guinea pigs required for the biosynthesis of L-ascorbic acid. *Science* **124,** 1148–1149
2. Chatterjee, M.L. and De, M.S. (1969). Adrenal ascorbic acid content of rats after different rauwolfia alkaloids. *Bull. Calcutta. Sch. Trop. Med.* **17,** 15–16
3. Chatterjee, I.B., Majumder, A.K., Nandi, B.K., and Subramanian, N. (1975). Synthesis and some major functions of vitamin C in animals. *Ann. N.Y. Acad. Sci.* **258,** 24–47
4. Nishikimi, M., Fukuyama, R., Minoshima, S., Shimizu, N., and Yagi, K. (1994). Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J. Biol. Chem.* **269,** 13685–13688
5. Lind, J. (1753). *A Treatise on the Scurvy*. A. Millar, London, UK
6. Hodges, J.R. and Sadow, J. (1969). Hypothalamo-pituitary-adrenal function in the rat after prolonged treatment with cortisol, *Br. J. Pharmacol.* **36,** 489–495
7. Irwin, M.I. and Hutchins, B.K. (1976). A conspectus of research on vitamin C requirements of man, *J. Nutr.* **106,** 821–879
8. Hodges, R.E. (1976). Scurvy. *World Health Organ. Monogr. Ser.* 120–125
9. Mussini, E., Hutton, J.J., Jr., and Udenfriend, S. (1967). Collagen proline hydroxylase in wound healing, granuloma formation, scurvy, and growth. *Science* **157,** 927–929
10. Levine, M. (1986). New concepts in the biology and biochemistry of ascorbic acid. *N. Engl. J. Med.* **314,** 892–902
11. Levine, M. and Hartzell, W. (1987). Ascorbic acid: The concept of optimum requirements. *Ann. N.Y. Acad. Sci.* **498,** 424–444
12. Levine, M., Dhariwal, K.R., Washko, P.W., Butler, J.D., Welch, R.W., Wang, Y.H., and Bergsten, P. (1991). Ascorbic acid and in situ kinetics: A new approach to vitamin requirements. *Am. J. Clin. Nutr.* **54,** 1157S-1162S
13. Levine, M., Dhariwal, K.R., Washko, P., Welch, R., Wang, Y.H., Cantilena, C.C., and Yu, R. (1992). Ascorbic acid and reaction kinetics in situ: A new approach to vitamin requirements, *J. Nutr. Sci. Vitaminol. (Tokyo) (***Spec Issue**) 169–172
14. Food and Nutrition Board (U.S.R.C.). (1994). *How should the recommended dietary allowances be revised?* National Academy Press, Washington, D.C.
15. Levine, M., Conry-Cantilena, C., Wang, Y., Welch, R.W., Washko, P.W., Dhariwal, K.R., Park, J.B., Lazarev, A., Graumlich, J., King, J., and Cantilena, L.R. (1996). Vitamin C pharmacokinetics in healthy volunteers: Evidence for a Recommended Dietary Allowance. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 3704–3709
16. Tolbert, B.M. and Ward, J.B. (1982). Dehydroascorbic acid. In: *Ascorbic Acid: Chemistry, Metabolism, and Uses*. (P.A. Seib and B.M. Tolbert, eds.), pp. 101–123, American Chemical Society, Washington, D.C.
17. Lewin, S. (1976). *Vitamin C: Its Molecular Biology and Medical Potential*. Academic Press, London
18. Penney, J.R. and Zilva, S.S. (1943). Chemical behavior of dehydro-l-ascorbic acid in vitro and in vivo. *Biochem. J.* **37,** 403–417
19. Lewin, S. (1976). Hydrolytic rupture of ascorbate by adenosine 3′:5′-cyclic monophosphate phosphodiesterase. *Biochem. Soc. Trans.* **4,** 71–73
20. Washko, P.W., Wang, Y., and Levine, M. (1993). Ascorbic acid recycling in human neutrophils. *J. Biol. Chem.* **268,** 15531–15535
21. Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **300,** 535–543
22. Welch, R.W., Wang, Y., Crossman, A., Jr., Park, J.B., Kirk, K.L., and Levine, M. (1995). Accumulation of vitamin C (ascorbate) and its oxidized metabolite dehydroascorbic acid occurs by separate mechanisms. *J. Biol. Chem.* **270,** 12584–12592
23. Park, J.B. and Levine, M. (1996). Purification, cloning, and expression of dehydroascorbic acid reduction activity from human neutrophils: Identification as glutaredoxin. *Biochem. J.* **315,** 931–938
24. Stevenson, N.R. (1974). Active transport of L-ascorbic acid in the human ileum. *Gastroenterology* **67,** 952–956
25. Thorn, N.A., Nielsen, F.S., and Jeppesen, C.K. (1991). Characteristics of ascorbic acid uptake by isolated ox neurohypophyseal nerve terminals and the influence of glucocorticoid and triiodothyronine on uptake. *Acta Physiol. Scand.* **141,** 97–106
26. Wright, J.R., Castranova, V., Colby, H.D., and Miles, P.R. (1981). Ascorbate uptake by isolated rat lung cells. *J. Appl. Physiol.* **51,** 1477–1483
27. Bianchi, J., Wilson, F.A., and Rose, R.C. (1986). Dehydroascorbic acid and ascorbic acid transport systems in the guinea pig ileum. *Am. J. Physiol.* **250,** G461-G468
28. Socci, R.R. and Delamere, N.A. (1988). Characteristics of ascorbate transport in the rabbit iris-ciliary body. *Exp. Eye Res.* **46,** 853–861
29. Chu, T.C. and Candia, O.A. (1988). Active transport of ascorbate across the isolated rabbit ciliary epithelium. *Invest. Ophthalmol. Vis. Sci.* **29,** 594–599
30. Delamere, N.A., Coca-Prados, M., and Aggarwal, S. (1993). Studies on regulation of the ascorbic acid transporter in a cell line derived from rabbit non-pigmented ciliary epithelium. *Biochim. Biophys. Acta* **1149,** 102–108
31. Franceschi, R.T., Wilson, J.X., and Dixon, S.J. (1995). Requirement for Na(+)-dependent ascorbic acid transport in osteoblast function. *Am. J. Physiol.* **268,** C1430-C1439
32. Washko, P.W., Rotrosen, D., and Levine, M. (1989). Ascorbic acid transport and accumulation in human neutrophils. *J. Biol. Chem.* **264,** 18996–19002
33. Dixon, S.J. and Wilson, J.X. (1992). Adaptive regulation of ascorbate transport in osteoblastic cells. *J. Bone Miner. Res.* **7,** 675–681
34. Washko, P. and Levine, M. (1992) Inhibition of ascorbic acid transport in human neutrophils by glucose. *J. Biol. Chem.* **267,** 23568–23574
35. Lam, K.W., Yu, H.S., Glickman, R.D., and Lin, T. (1993). Sodium-dependent ascorbic and dehydroascorbic acid uptake by SV-40-transformed retinal pigment epithelial cells. *Ophthalmic Res.* **25,** 100–107
36. Welch, R.W., Bergsten, P., Butler, J.D., and Levine, M. (1993). Ascorbic acid accumulation and transport in human fibroblasts. *Biochem. J.* **294,** 505–510
37. Bergsten, P., Yu, R., Kehrl, J., and Levine, M. (1995). Ascorbic acid transport and distribution in human B lymphocytes. *Arch. Biochem. Biophys.* **317,** 208–214
38. Castranova, V., Wright, J.R., Colby, H.D., and Miles, P.R. (1983). Ascorbate uptake by isolated rat alveolar macrophages and type II cells. *J. Appl. Physiol.* **54,** 208–214
39. Siliprandi, L., Vanni, P., Kessler, M., and Semenza, G. (1979). Na+-dependent, electroneutral L-ascorbate transport across brush border membrane vesicles from guinea pig small intestine. *Biochim. Biophys. Acta* **552,** 129–142
40. Diliberto, E.J., Jr., Heckman, G.D., and Daniels, A.J. (1983). Characterization of ascorbic acid transport by adrenomedullary chromaffin cells. Evidence for Na+-dependent co-transport. *J. Biol. Chem.* **258,** 12886–12894
41. Padh, H. and Aleo, J.J. (1987). Characterization of the ascorbic

acid transport by 3T6 fibroblasts. *Biochim. Biophys. Acta* **901,** 283–290
42. Helbig, H., Korbmacher, C., Wohlfarth, J., Berweck, S., Kuhner, D., and Wiederholt, M. (1989). Electrogenic Na+-ascorbate co-transport in cultured bovine pigmented ciliary epithelial cells. *Am. J. Physiol.* **256,** C44–C49
43. Wilson, J.X., Jaworski, E.M., and Dixon, S.J. (1991). Evidence for electrogenic sodium-dependent ascorbate transport in rat astroglia. *Neurochem. Res.* **16,** 73–78
44. Maffia, M., Ahearn, G.A., Vilella, S., Zonno, V., and Storelli, C. (1993). Ascorbic acid transport by intestinal brush-border membrane vesicles of the teleost Anguilla anguilla. *Am. J. Physiol.* **264,** R1248–R1253
45. Bianchi, J. and Rose, R.C. (1985). Na+-independent dehydro-L-ascorbic acid uptake in renal brush-border membrane vesicles. *Biochim. Biophys. Acta* **819,** 75–82
46. Dreyer, R. and Rose, R.C. (1993). Lacrimal gland uptake and metabolism of ascorbic acid. *Proc. Soc. Exp. Biol. Med.* **202,** 212–216
47. Dixon, S.J. and Wilson, J.X. (1992). Transforming growth factor-beta stimulates ascorbate transport activity in osteoblastic cells. *Endocrinology* **130,** 484–489
48. Heath, H. and Fiddick, R. (1966). The active transport of ascorbic acid by the rat retina. *Exp. Eye Res.* **5,** 156–163
49. Kern, H.L. and Zolot, S.L. (1987). Transport of vitamin C in the lens. *Curr. Eye Res.* **6,** 885–896
50. Ingermann, R.L., Stankova, L., and Bigley, R.H. (1986). Role of monosaccharide transporter in vitamin C uptake by placental membrane vesicles. *Am. J. Physiol.* **250,** C637-C641
51. Hughes, R.E. and Maton, S.C. (1968). The passage of vitamin C across the erythrocyte membrane. *Br. J. Haematol.* **14,** 247–253
52. Hornig, D., Weber, F., and Wiss, O. (1971). Studies on the uptake of (1-^{14}C)ascorbic acid and (1-^{14}C)- dehydroascorbic acid by platelets of guinea pigs. *Clin. Chim. Acta* **33,** 187–196
53. Patterson, J.W. (1950). The diabetogenic effect of dehydroascorbic and dehydroisoascorbic acids. *J. Biol. Chem.* **183,** 81–88
54. Bigley, R., Wirth, M., Layman, D., Riddle, M., and Stankova, L. (1983). Interaction between glucose and dehydroascorbate transport in human neutrophils and fibroblasts. *Diabetes* **32,** 545–548
55. Stahl, R.L., Farber, C.M., Liebes, L.F., and Silber, R. (1985). Relationship of dehydroascorbic acid transport to cell lineage in lymphocytes from normal subjects and patients with chronic lymphocytic leukemia. *Cancer Res.* **45,** 6507–6512
56. Vera, J.C., Rivas, C.I., Zhang, R.H., Farber, C.M., and Golde, D.W. (1994). Human HL-60 myeloid leukemia cells transport dehydroascorbic acid via the glucose transporters and accumulate reduced ascorbic acid. *Blood* **84,** 1628–1634
57. Mann, G.V. and Newton, P. (1975). The membrane transport of ascorbic acid. *Ann. N.Y. Acad. Sci.* **258,** 243–252
58. Ingermann, R.L., Stankova, L., Bigley, R.H., and Bissonnette, J.M. (1988). Effect of monosaccharide on dehydroascorbic acid uptake by placental membrane vesicles. *J. Clin. Endocrinol. Metab.* **67,** 389–394
59. Mooradian, A.D. (1987). Effect of ascorbate and dehydroascorbate on tissue uptake of glucose. *Diabetes* **36,** 1001–1004
60. Keller, K. and Mueckler, M. (1990). Different mammalian facilitative glucose transporters expressed in Xenopus oocytes. *Biomed. Biochim. Acta* **49,** 1201–1203
61. Hediger, M.A., Coady, M.J., Ikeda, T.S., and Wright, E.M. (1987). Expression cloning and cDNA sequencing of the Na+/glucose co-transporter. *Nature* **330,** 379–381
62. Kanai, Y., Lee, W.S., You, G., Brown, D., and Hediger, M.A. (1994). The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. *J. Clin. Invest.* **93,** 397–404
63. Vera, J.C., Rivas, C.I., Fischbarg, J., and Golde, D.W. (1993). Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. *Nature* **364,** 79–82
64. Rumsey, S.C., Kwon, O., Xu, G.W., Burant, C.F., Simpson, I., and Levine, M. (1997). Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. *J. Biol. Chem.* **272,** 18982–18989
65. Padh, H. and Aleo, J.J. (1989). Ascorbic acid transport by 3T6 fibroblasts. Regulation by and purification of human serum complement factor. *J. Biol. Chem.* **264,** 6065–6069
66. Hendry, J.M., Easson, L.H., and Owen, J.A. (1964). The uptake and reduction of dehydroascorbic acid by human leukocytes. *Clin. Chim. Acta.* **9,** 498–499
67. Bigley, R.H. and Stankova, L. (1974). Uptake and reduction of oxidized and reduced ascorbate by human leukocytes. *J. Exp. Med.* **139,** 1084–1092
68. Dyer, D.L., Kanai, Y., Hediger, M.A., Rubin, S.A., and Said, H.M. (1994). Expression of a rabbit renal ascorbic acid transporter in Xenopus laevis oocytes. *Am. J. Physiol.* **267,** C301-C306
69. Hornig, D. (1975). Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. N.Y. Acad. Sci.* **258,** 103–118
70. Dhariwal, K.R., Hartzell, W.O., and Levine, M. (1991). Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. *Am. J. Clin. Nutr.* **54,** 712–716
71. Okamura, M. (1980). An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. *Clin. Chim. Acta* **103,** 259–268
72. Borsook, H., Davenport, H.W., Jeffreys, C.E.P., and Warner, R.C. (1936). The oxidation of ascorbic acid and its reduction in vitro and in vivo. *J. Biol. Chem.* **117,** 237–279
73. Schultze, M.O., Stotz, E., and King, C.G. (1937). Studies on the reduction of dehydroascorbic acid by guinea pig tissues. *J. Biol. Chem.* **122,** 395–406
74. Bigley, R., Stankova, L., Roos, D., and Loos, J. (1980). Glutathione-dependent dehydroascorbate reduction: A determinant of dehydroascorbate uptake by human polymorphonuclear leukocytes. *Enzyme* **25,** 200–204
75. Rose, R.C. (1988). Transport of ascorbic acid and other water-soluble vitamins. *Biochim. Biophys. Acta* **947,** 335–366
76. Winkler, B.S., Orselli, S.M., and Rex, T.S. (1994). The redox couple between glutathione and ascorbic acid: A chemical and physiological perspective. *Free Radic. Biol. Med.* **17,** 333–349
77. Wells, W.W. and Xu, D.P. (1994). Dehydroascorbate reduction. *J. Bioenerg. Biomembr.* **26,** 369–377
78. Levine, M., Rumsey, S.C., Wang, Y., Park, J.B., Xu, G.W., and Amano, N. (1996) Vitamin C. In: *Present Knowledge in Nutrition*. (L.J. Filer and E.E. Ziegler, eds.), pp. 146–159, International Life Sciences Institute, Washington, D.C.
79. Melhorn, R.J. (1991). Ascorbate- and dehydroascorbic acid-mediated reduction of free radicals in the human erythrocyte. *J. Biol. Chem.* **266,** 2724–2731
80. Coassin, M., Tomasi, A., Vannini, V., and Ursini, F. (1991). Enzymatic recycling of oxidized ascorbate in pig heart: One-electron vs two-electron pathway. *Arch. Biochem. Biophys.* **290,** 458–462
81. Winkler, B.S. (1992). Unequivocal evidence in support of the nonenzymatic redox coupling between glutathione/glutathione disulfide and ascorbic acid/dehydroascorbic acid. *Biochim. Biophys. Acta* **1117,** 287–290
82. Xu, D.P. and Wells, W.W. (1996). Alpha-Lipoic acid dependent regeneration of ascorbic acid from dehydroascorbic acid in rat liver mitochondria. *J. Bioenerg. Biomembr.* **28,** 77–85
83. Wells, W.W., Xu, D.P., Yang, Y., and Rocque, P.A. (1990). Mammalian thioltransferase (Glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *J. Biol. Chem.* **265,** 15361–15364
84. Del Bello, B., Maellaro, E., Sugherini, L., Santucci, A., Comporti, M., and Casini, A.F. (1994). Purification of NADPH-dependent dehydroascorbate reductase from rat liver and its identification with 3 alpha-hydroxysteroid dehydrogenase. *Biochem. J.* **304,** 385–390
85. Maellaro, E., Del Bello, B., Sugherini, L., Santucci, A., Comporti, M., and Casini, A.F. (1994). Purification and characterization of glutathione-dependent dehydroascorbate reductase from rat liver. *Biochem. J.* **301,** 471–476
86. Mieyal, J.J., Starke, D.W., Gravina, S.A., Dothey, C., and Chung, J.S. (1991). Thioltransferase in human red blood cells: Purification and properties. *Biochemistry* **30,** 6088–6097
87. Bergsten, P., Amitai, G., Kehrl, J., Dhariwal, K.R., Klein, H.G., and Levine, M. (1990). Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes. Depletion and reaccumulation. *J. Biol. Chem.* **265,** 2584–2587

88. Wang, Y., Russo, T.A., Kwon, O., Chanock, S., Rumsey, S.C., and Levine, M. (1997). Ascorbate recycling in human neutrophils: Induction by bacteria. *Proc. Natl. Acad. Sci. USA* **94,** 13816–13819
89. Larsen, P.R., and Ingbar, S.H. (1992). The thyroid gland. In: *Williams Textbook of Endocrinology*, (J.D. Wilson and D.W. Foster, eds.), pp. 365–371, Saunders, Philadelphia, PA, USA
90. DeChatelet, L.R. (1975). Oxidative bactericidal mechanisms of polymorphonuclear leukocytes. *J. Infect. Dis.* **131,** 295–303
91. Halliwell, B., Wasil, M., and Grootveld, M. (1987). Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid. Implications for antioxidant protection in the inflamed rheumatoid joint. *FEBS Lett.* **213,** 15–17
92. Liles, W.C., Kiener, P.A., Ledbetter, J.A., Aruffo, A., and Klebanoff, S.J. (1996). Differential expression of FAS (CD95) and Fas ligand on normal human phagocytes: Implications for the reulation of apoptosis in neutrophils. *J. Exp. Med.* **184,** 429–440
93. Segal, A., Gelsow, M., Garcia, R., Harper, A., and Miller, R. (1981). The respiratory burst of phagocytic cells is associated with a rise in vaculoar pH. *Nature* **290,** 406–408
94. Whyte, M.K.B., Meagher, L.C., MacDermot, J., and Haslet, C. (1993). Impairment of function in aging neutrophils is associated with apoptosis. *J. Immunol.* **150,** 5124–5134
95. Kasahara, Y., Iwai, K., Yachie, A., Ohta, K., Konno, S., Seki, H., Miyawaki, T., and Taniguchi, N. (1997). Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1) mediated apoptosis of neutrophils. *Blood* **89,** 1748–1753
96. Vanderslice, J.T. and Higgs, D.J. (1991). Vitamin C content of foods: sample variability. *Am. J. Clin. Nutr.* **54,** 1323S–1327S
97. Bilic, N. (1991). Assay for both ascorbic and dehydroascorbic acid in dairy foods by high-performance liquid chromatography using precolumn derivatization with methoxy- and ethoxy-1,2-phenylenediamine. *J. Chromatogr.* **543,** 367–374
98. Mellors, A.J., Nahrwold, D.L., and Rose, R.C. (1977). Ascorbic acid flux across mucosal border of guinea pig and human ileum. *Am. J. Physiol.* **233,** E374-E379
99. Stewart, J.S. and Booth, C.C. (1964). Ascorbic acid absorption in malabsorption. *Clin. Sci.* **27,** 15–22
100. Nelson, E.W., Lane, H., Fabri, P.J., and Scott, B. (1978). Demonstration of saturation kinetics in the intestinal absorption of vitamin C in man and the guinea pig. *J. Clin. Pharmacol.* **18,** 325–335
101. Kubler, W. and Gehler, J. (1970). [Kinetics of intestinal absorption of ascorbic acid. Calculation of non-dosage-dependent absorption processes]. *Int. A. Vitaminforsch.* **40,** 442–453
102. Mayersohn, M. (1972). Ascorbic acid absorption in man—pharmacokinetic implications. *Eur. J. Pharmacol.* **19,** 140–142
103. King, G., Beins, M., Larkin, J., Summers, B., and Ordman, A.B. (1994). Rate of excretion of vitamin C in human urine. *Age* **17,** 87–92
104. Karasov, W.H., Darken, B.W., and Bottum, M.C. (1991). Dietary regulation of intestinal ascorbate uptake in guinea pigs. *Am. J. Physiol.* **260,** G108-G118
105. Bianchi, J. and Rose, R.C. (1986). Dehydroascorbic acid and cell membranes: Possible disruptive effects. *Toxicology* **40,** 75–82
106. Rose, R.C., Choi, J.L., and Koch, M.J. (1988). Intestinal transport and metabolism of oxidized ascorbic acid (dehydroascorbic acid). *Am. J. Physiol.* **254,** G824–G828
107. Fox, F.W. and Levy, L.F. (1936). Experiments confirming the antiscorbutic activity of dehydroascorbic acid and a study of its storage and that of ascorbic acid by the guinea pig at different levels of intake. *Biochem. J.* **30,** 211–217
108. Todhunter, E.N., McMillan, T., and Ehmke, A. (1950). Utilization of dehydroascorbic acid by human subjects. *J. Nutr.* **42,** 297–308
109. Linkswiler, H. (1957). The effect of the ingestion of ascorbic acid and dehydroascorbic acid upon the blood levels of these two components in human subjects. *J. Nutr.* **63,** 43–54
110. Sabry, J.H., Fisher, K.H., and Dodds, M.L. (1958). Human Utilization of dehydroascorbic acid. *J. Nutr.* **68,** 457–466
111. Gibaldi, M., and Perrier, D. (1982). *Pharmacokinetics*. Marcel Dekker, New York, USA
112. Rowland, M., and Tozer, T.M. (1989). *Clinical Pharmacokinetics: Concepts and Applications*. Lea and Febiger, Philadelphia, USA
113. Graumlich, J.F., Ludden, T.M., Conry-Cantilena, C., Cantilena, L.R., Jr., Wang, Y., and Levine, M. (1997). Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. *Pharmaceutical Research* **14,** 1133–1139
114. Yung, S., Mayersohn, M., and Robinson, J.B. (1981). Ascorbic acid absorption in man: Influence of divided dose and food. *Life Sci.* **28,** 2505–2511
115. Piotrovskij, V.K., Kallay, Z., Gajdos, M., Gerykova, M., and Trnovec, T. (1993). The use of a nonlinear absorption model in the study of ascorbic acid bioavailability in man. *Biopharm. Drug Dispos.* **14,** 429–442
116. Zetler, G., Seidel, G., Siegers, C.P., and Ivens, H. (1976). Pharmacokinetics of ascorbic acid in man. *Eur. J. Clin. Pharmacol.* **10,** 273–282
117. Bhagavan, H.N. and Wolkoff, B.I. (1993). Correlation between the disintegration time and the bioavailability of vitamin C tablets. *Pharm. Res.* **10,** 239–242
118. Richards, T.W., Cheraskin, E., and Ringsdorf, W.M., Jr. (1969). Effect of sustained release versus regular multivitamin supplement upon vitamin C state. *Int. Z. Vitaminforsch.* **39,** 407–415
119. Yung, S., Mayersohn, M., and Robinson, J.B. (1982). Ascorbic acid absorption in humans: a comparison among several dosage forms. *J. Pharm. Sci.* **71,** 282–285
120. Clayton, M.M. and Folsom, M.T. (1940). A method for the study of the availability for human nutrition of the vitamin C in foods, with an application to the study of the potato. *J. Home Econ.* **32,** 390–395
121. Clayton, M.M. and Borden, R.A. (1943). The availability for human nutrition of the vitamin C in raw cabbage and home-canned tomato juice. *J. Nutr.* **25,** 349–369
122. Hawley, E.E., Stephens, D.J., and Anderson, G. (1936). The excretion of vitamin C in normal individuals following a comparable quantitative administration in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously. *J. Nutr.* **11,** 135–145
123. Todhunter, E.N. and Fatzer, A.S. (1940). A comparison of the utilization by college women of equivalent amounts of ascorbic acid (vitamin C) in red raspberries and in crystalline form. *J. Nutr.* **19,** 121–130
124. Hartzler, E.R. (1945). The availability of ascorbic acid in papayas and guavas. *J. Nutr.* **30,** 355–365
125. Mangels, A.R., Block, G., Frey, C.M., Patterson, B.H., Taylor, P.R., Norkus, E.P., and Levander, O.A. (1993). The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J. Nutr.* **123,** 1054–1061
126. Vinson, J.A. and Bose, P. (1988). Comparative bioavailability to humans of ascorbic acid alone or in a citrus extract. *Am. J. Clin. Nutr.* **48,** 601–604
127. Vanderslice, J.T., Higgs, D.J., Beecher, G.R., Higgs, H.E., and Bouma, J. (1992). On the presence of dehydroascorbic acid in human plasma. *Int. J. Vitam. Nutr. Res.* **62,** 101–102
128. Stewart, C.P., Horn, D.B., and Robson, J.S. (1953). The effect of cortisone and adrenocorticotropic hormone on the dehydroascorbic acid of human plasma. *Biochem. J.* **53,** 254–261
129. Cox, B.D. and Butterfield, W.J. (1975). Vitamin C supplements and diabetic cutaneous capillary fragility. *Br. Med. J.* **3,** 205
130. Margolis, S.A., Ziegler, R.G., and Helzlsouer, K.J. (1991). Ascorbic and dehydroascorbic acid measurement in human serum and plasma. *Am. J. Clin. Nutr.* **54,** 1315S-1318S
131. Koshiishi, I. and Imanari, T. (1997). Measurement of ascorbate and dehydroascorbate contents in biological fluids. *Anal. Chem.* **69,** 216–220
132. Sullivan, J.F. and Eisenstein, A.B. (1970). Ascorbic acid depletion in patients undergoing chronic hemodialysis. *Am. J. Clin. Nutr.* **23,** 1339–1346
133. Stankova, L., Rigas, D.A., and Bigley, R.H. (1975). Dehydroascorbate uptake and reduction by human blood neutrophils, erythrocytes, and lymphocytes. *Ann. N.Y. Acad. Sci.* **258,** 238–242
134. Evans, R.M., Currie, L., and Campbell, A. (1982). The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. *Br. J. Nutr.* **47,** 473–482
135. Baker, E.M., Hodges, R.E., Hood, J., Sauberlich, H.E., and March, S.C. (1969). Metabolism of ascorbic-1–14C acid in experimental human scurvy. *Am. J. Clin. Nutr.* **22,** 549–558

136. Baker, E.M., Hodges, R.E., Hood, J., Sauberlich, H.E., March, S.C., and Canham, J.E. (1971). Metabolism of 14C- and 3H-labeled L-ascorbic acid in human scurvy. *Am. J. Clin. Nutr.* **24,** 444–454
137. Hodges, J.R. and Hotston, R.T. (1971). Suppression of adrenocorticotrophic activity in the ascorbic acid deficient guinea-pig. *Br. J. Pharmacol.* **42,** 595–602
138. Jacob, R.A., Omaye, S.T., Skala, J.H., Leggott, P.J., Rothman, D.L., and Murray, P.A. (1987). Experimental vitamin C depletion and supplementation in young men. Nutrient interactions and dental health effects. *Ann. N. Y. Acad. Sci.* **498,** 333–346
139. Jacob, R.A., Pianalto, F.S., and Agee, R.E. (1992). Cellular ascorbate depletion in healthy men. *J. Nutr.* **122,** 1111–1118
140. Kallner, A., Hartmann, D., and Hornig, D. (1979). Steady-state turnover and body pool of ascorbic acid in man. *Am. J. Clin. Nutr.* **32,** 530–539
141. Garry, P.J., Goodwin, J.S., Hunt, W.C., and Gilbert, B.A. (1982). Nutritional status in a healthy elderly population: Vitamin C. *Am. J. Clin. Nutr.* **36,** 332–339
142. VanderJagt, D.J., Garry, P.J., and Bhagavan, H.N. (1987). Ascorbic acid intake and plasma levels in healthy elderly people. *Am. J. Clin. Nutr.* **46,** 290–294
143. Garry, P.J., VanderJagt, D.J., and Hunt, W.C. (1987). Ascorbic acid intakes and plasma levels in healthy elderly. *Ann. N.Y. Acad. Sci.* **498,** 90–99
144. Hegsted, D.M. (1992). Defining a nutritious diet: Need for new dietary standards. *J. Am. Coll. Nutr.* **11,** 241–245
145. King, J., Wang, Y., Welch, R.W., Dhariwal, K.R., Conry-Cantilena, C., and Levine, M. (1997). Use of a new vitamin C-deficient diet in a depletion/repletion clinical trial. *Am. J. Clin. Nutr.* **65,** 1434–1440
146. Keith, M.O. and Pelletier, O. (1974). Ascorbic acid concentrations in leukocytes and selected organs of guinea pigs in response to increasing ascorbic acid intake. *Am. J. Clin. Nutr.* **27,** 368–372
147. Damron, C.M., Monier, M.M., and Roe, J.H. (1952). Metabolism of L-ascorbic acid, dehydro L-ascorbic acid and diketo-L-gulonic acid in the guinea pig. *J. Biol. Chem.* **195,** 599–606
148. Hornig, D., Weber, F., and Wiss, O. (1972). Autoradiographic distribution of (1-^{14}C) ascorbic acid and (1-^{14}C) dehydroascorbic acid in male guinea pigs after intravenous injection. *Int. J. Vitam. Nutr. Res.* **42,** 223–241
149. Ralli, E.P., Friedman, G.J., and Rubin, S.H. (1938). The mechanism of the excretion of vitamin C by the human kidney. *J. Clin. Invest.* **17,** 765–770
150. Rose, R.C. (1986). Ascorbic acid transport in mammalian kidney. *Am. J. Physiol.* **250,** F627–F632
151. Martin, M., Ferrier, B., and Roch-Ramel, F. (1983). Renal excretion of ascorbic acid in the rat: A micropuncture study. *Am. J. Physiol.* **244,** F335–F341
152. Bowers-Komro, D.M. and McCormick, D.B. (1991). Characterization of ascorbic acid uptake by isolated rat kidney cells. *J. Nutr.* **121,** 57–64
153. Friedman, G.J., Sherry, S., and Ralli, E. (1940). The mechanism of excretion of vitamin C by the human kidney at low and normal plasma levels of ascorbic acid. *J. Clin. Invest.* **19,** 685–689
154. Oreopoulos, D.G., Lindeman, R.D., VanderJagt, D.J., Tzamaloukas, A.H., Bhagavan, H.N., and Garry, P.J. (1993). Renal excretion of ascorbic acid: Effect of age and sex. *J. Am. Coll. Nutr.* **12,** 537–542
155. Toggenburger, G., Hausermann, M., Mutsch, B., Genoni, G., Kessler, M., Weber, F., Hornig, D., O'Neill, B., and Semenza, G. (1981). Na+-dependent, potential-sensitive L-ascorbate transport across brush border membrane vesicles from kidney cortex. *Biochim. Biophys. Acta* **646,** 433–443
156. Food and Nutrition Board (U.S.R.C.). (1980). *Recommended Dietary Allowances.* National Academy Press, Washington, D.C.
157. Food and Nutrition Board (U.S.R.C.). (1985). *Recommended Dietary Allowances.* National Academy Press, Washington,D.C.
158. Bianchi, J. and Rose, R.C. (1985). Transport of L-ascorbic acid and dehydro-L-ascorbic acid across renal cortical basolateral membrane vesicles. *Biochim. Biophys. Acta* **820,** 265–273
159. Wang, Y.H., Dhariwal, K.R., and Levine, M. (1992). Ascorbic acid bioavailability in humans. Ascorbic acid in plasma, serum, and urine. *Ann. N.Y. Acad. Sci.* **669,** 383–386
160. Baker, E.M., Saari, J.C., and Tolbert, B.M. (1966). Ascorbic acid metabolism in man. *Am. J. Clin. Nutr.* **19,** 371–378
161. Tolbert, B.M., Chen, A.W., Bell, E.M., and Baker, E.M. (1967). Metabolism of 1-ascorbic-4–3H acid in man. *Am. J. Clin. Nutr.* **20,** 250–252
162. Tolbert, B.M., Downing, M., Carlson, R.W., Knight, M.K., and Baker, E.M. (1975). Chemistry and metabolism of ascorbic acid and ascorbate sulfate. *Ann. N.Y. Acad. Sci.* **258:**48–69, 48–69
163. Baker, E.M., Halver, J.E., Johnsen, D.O., Joyce, B.E., Knight, M.K., and Tolbert, B.M. (1975). Metabolism of ascorbic acid and ascorbic-2-sulfate in man and the subhuman primate. *Ann. N.Y. Acad. Sci.* **258,** 72–80
164. Tolbert, B.M., Harkrader, R.J., Johnson, D.O., and Joyce, B.A. (1976). C-6 oxidation of ascorbic acid: A major metabolic process in animals. *Biochem. Biophys. Res. Commun.* **71,** 1004–1009
165. Omaye, S.T., Tillotson, J.A., and Sauberlich, H.E. (1982). Metabolism of L-ascorbic acid in the monkey. In: *Ascorbic Acid: Chemistry, Metabolism and Uses*, (P.A. Seib and B.M. Tolbert, eds.), pp. 317–334, American Chemical Society, Washington, D.C.
166. Kallner, A., Hornig, D., and Pellikka, R. (1985). Formation of carbon dioxide from ascorbate in man. *Am. J. Clin. Nutr.* **41,** 609–613
167. Chatterjee, I.B. (1978). Ascorbic acid metabolism *World Rev. Nutr. Diet.* **30,** 69–87
168. Hellman, L. and Burns, J.J. (1957). Metabolism of L-Ascorbic acid-1-C^{14} in man. *J. Biol. Chem.* **230,** 923–930
169. Mydlik, M., Derzsiova, K., Pribylincova, V., Zvara, V., and Takac, M. (1986). Urinary excretion of vitamin C in chronic renal failure and after renal transplantation. *Int. Urol. Nephrol.* **18,** 457–462
170. Levine, M., Dhariwal, K.R., Welch, R.W., Wang, Y., and Park, J.B. (1995). Determination of optimal vitamin C requirements in humans. *Am. J. Clin. Nutr.* **62**(suppl), 1347S–1356S
171. Frei, B., Stocker, R., England, L., and Ames, B.N. (1990). Ascorbate: the most effective antioxidant in human blood plasma. *Adv. Exp. Med. Biol.* **264,** 155–163
172. Frei, B., Forte, T.M., Ames, B.N., and Cross, C.E. (1991). Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. *Biochem. J.* **277,** 133–138
173. Kallner, A.B., Hartmann, D., and Hornig, D.H. (1981). On the requirements of ascorbic acid in man: Steady-state turnover and body pool in smokers. *Am. J. Clin. Nutr.* **34,** 1347–1355
174. Schectman, G. (1993). Estimating ascorbic acid requirements for cigarette smokers. *Ann. N.Y. Acad. Sci.* **686,** 335–345
175. Jialal, I., Vega, G.L., and Grundy, S.M. (1990). Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoprotein. *Atherosclerosis* **82,** 185–191
176. Retsky, K.L. and Frei, B. (1995). Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. *Biochim. Biophys. Acta* **1257,** 279–287
177. Steinberg, D. (1995). Role of oxidized LDL and antioxidants in atherosclerosis. *Adv. Exp. Med. Biol.* **369,** 39–48
178. Lehr, H.A., Frei, B., Olofsson, A.M., Carew, T.E., and Arfors, K.E. (1995). Protection from oxidized LDL-induced leukocyte adhesion to microvascular and macrovascular endothelium in vivo by vitamin C but not by vitamin E. *Circulation* **91,** 1525–1532
179. Heitzer, T., Just, H., and Munzel, T. (1996). Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* **94,** 6–9
180. Levine, G.N., Frei, B., Koulouris, S.N., Gerhard, M.D., Keaney, J.F., and Vita, J.A. (1996). Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* **93,** 1107–1113
181. Feig, D.I. and Loeb, L.A. (1994). Oxygen radical induced mutagenesis is DNA polymerase specific. *J. Mol. Biol.* **235,** 33–41
182. Borek, C. (1993). Molecular mechanism in cancer induction and prevention. *Environ. Health Perspect.* **101**(Suppl 3), 237–245
183. Cerutti, P., Ghosh, R., Oya, Y., and Amstad, P. (1994). The role of the cellular antioxidant defense in oxidant carcinogenesis. *Environ. Health Perspect.* **102**(Suppl 10), 123–129
184. Fraga, C.G., Motchnik, P.A., Shigenaga, M.K., Helbock, H.J., Jacob, R.A., and Ames, B.N. (1991). Ascorbic acid protects against

endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. U.S.A.* **88,** 11003–11006
185. Green, M.H., Lowe, J.E., Waugh, A.P., Aldridge, K.E., Cole, J., and Arlett, C.F. (1994). Effect of diet and vitamin C on DNA strand breakage in freshly-isolated human white blood cells. *Mutat. Res.* **316,** 91–102
186. Mares-Perlman, J.A. (1997). Contribution of epidemiology to understanding relations of diet to age-related cataract. *Amer. J. Clin. Nutr.* **66,** 739–740
187. Solzbach, U., Hornig, B., Jeseich, M., and Just, H. (1997). Vitamin C improves endothelial dysfunction of epicardial coronary arteries in hypertensive patients. *Circulation* **96,** 1513–1519
188. Mann, G.V. (1974). Hypothesis: The role of vitamin C in diabetic angiopathy. *Perspect. Biol. Med.* **17,** 210–217
189. Cunningham, J.J. (1988). Altered vitamin C transport in diabetes mellitus. *Med. Hypotheses.* **26,** 263–265
190. Banerjee, A. (1982). Blood dehydroascorbic acid and diabetes mellitus in human beings. *Ann. Clin. Biochem.* **19,** 65–70
191. Cox, B.D. and Whichelow, M.J. (1975). The measurement of dehydroascorbic acid and diketogulonic acid in normal and diabetic plasma. *Biochem. Med.* **12,** 183–193
192. Washko, P.W., Welch, R.W., Dhariwal, K.R., Wang, Y., and Levine, M. (1992). Ascorbic acid and dehydroascorbic acid analyses in biological samples. *Anal. Biochem.* **204,** 1–14
193. Pirani, C.L. (1952). Relation of vitamin C to adrenocortical function and stress phenomena. *Metabolism* **1,** 197–222
194. Sharma, S.K., Johnstone, R.M., and Quastel, J.H. (1964). Corticosteroids and ascorbic acid transport in adrenal cortex in vitro. *Biochem. J.* **92,** 564–573
195. De Nicola, A.F., Clayman, M., and Johnstone, R.M. (1968). Hormonal control of ascorbic acid transport in rat adrenal glands. *Endocrinology* **82,** 436–446
196. Horning, D., Gallo-Torres, H.E., and Weiser, H. (1972). Tissue distribution of labelled ascorbic acid in normal and hypophysectomized rats. *Int. J. Vitam. Nutr. Res.* **42,** 487–496
197. Sayers, G., Sayers, M.A., Liang, T.Y., and Long, C.N.H. (1945). The cholesterol and ascorbic acid content of the adrenal, liver, brain, and plasma following hemorrhage. *Endocrinology* **37,** 96–110
198. Stefanini, M. and Rosenthal, M.C. (1950). Hemorrhagic diathesis with ascorbic acid deficiency during administration of anterior pituitary corticotropic hormone (ACTH). *Proc. Soc. Exptl. Biol. Med.* **75,** 806–808
199. Zhou, A. and Thorn, N.A. (1991). High ascorbic acid content in the rat endocrine pancreas. *Diabetologia* **34,** 839–842
200. Levine, R.L. (1983). Oxidative modification of glutamine synthetase. II. Characterization of the ascorbate model system, *J. Biol. Chem.* **258,** 11828–11833
201. Young, V.R. (1996). Evidence for a recommended dietary allowance for vitamin C from pharmacokinetics: A comment and analysis. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 14344–14348
202. Levine, M., Rumsey, S., and Wang, Y. (1997). Principles involved in formulating recommendations for vitamin C intake: A paradigm for water-soluble vitamins. *Methods Enzymol.* **279,** 43–54
203. Levine, M., Rumsey, S.C., Wang, Y., Park, J., Kwon, O., and Amano, N. (1997). In situ kinetics: An approach to recommended intake of vitamin C. *Methods Enzymol.* **281,** 425–437.
204. Vallance, B.D., Hume, R., and Weyers, E. (1978). Reassessment of changes in leucocyte and serum ascorbic acid after acute myocardial infarction. *Br. Heart J.* **40,** 64–68
205. Irvin, T.T. (1982). Vitamin C requirements in postoperative patients. *Int. J. Vitam. Nutr. Res. Suppl.* **23,** 277–286
206. Marcus, S.L., Petrylak, D.P., Dutcher, J.P., Paietta, E., Ciobanu, N., Strauman, J., Wiernik, P.H., Hutner, S.H., Frank, O., and Baker, H. (1991). Hypovitaminosis C in patients treated with high-dose interleukin 2 and lymphokine-activated killer cells. *Am. J. Clin. Nutr.* **54,** 1292S–1297S
207. Grant, J.P. (1994). Nutritional support in critically ill patients. *Ann. Surg.* **220,** 610–616
208. Schorah, C.J., Downing, C., Piripitsi, A., Gallivan, L., Al-Hazaa, A.H., Sanderson, M.J., and Bodenham, A. (1996). Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. *Am. J. Clin. Nutr.* **63,** 760–765
209. Bielski, G.H.J. (1982). Chemistry of ascorbic acid radicals. In: Ascorbic Acid: Chemistry, Metabolism, and Uses (P.A. Seib and B.M. Tolbert, eds.), pp. 81–100, American Chemical Society, Washington, D.C.