Annu. Rev. Nutr. 2005. 25:105–25
doi: 10.1146/annurev.nutr.25.050304.092647

First published online as a Review in Advance on February 10, 2005

REGULATION OF VITAMIN C TRANSPORT

John X. Wilson
Department of Exercise and Nutrition Sciences, School of Public Health and Health Professions, University at Buffalo, Buffalo, New York 14214-3079;
email: jxwilson@buffalo.edu

Key Words ascorbic acid, dehydroascorbic acid, transporters, ion channels, bioavailability

■ **Abstract** Ascorbic acid and dehydroascorbic acid (DHAA, oxidized vitamin C) are dietary sources of vitamin C in humans. Both nutrients are absorbed from the lumen of the intestine and renal tubules by, respectively, enterocytes and renal epithelial cells. Subsequently vitamin C circulates in the blood and enters all of the other cells of the body. Concerning flux across the plasma membrane, simple diffusion of ascorbic acid plays only a small or negligible role. More important are specific mechanisms of transport and metabolism that concentrate vitamin C intracellularly to enhance its function as an enzyme cofactor and antioxidant. The known transport mechanisms are facilitated diffusion of DHAA through glucose-sensitive and -insensitive transporters, facilitated diffusion of ascorbate through channels, exocytosis of ascorbate in secretory vesicles, and secondary active transport of ascorbate through the sodium-dependent vitamin C transporters SVCT1 and SVCT2 proteins that are encoded by the genes Slc23a1 and Slc23a2, respectively. Evidence is reviewed indicating that these transport pathways are regulated under physiological conditions and altered by aging and disease.

CONTENTS

INTRODUCTION 105
SIMPLE DIFFUSION 106
FACILITATED DIFFUSION 106
Uptake of Dehydroascorbic Acid into Cells 107
Ascorbate Efflux from Cells 111
ACTIVE TRANSPORT 112
Sodium-Ascorbate Cotransport Mediates High-Affinity Absorption 112
Distinct Properties of the Cotransporters SVCT1 and SVCT2 115
PERSPECTIVES AND FUTURE DIRECTIONS 119

INTRODUCTION

Most mammalian species synthesize ascorbic acid de novo from glucose in the liver, through a biosynthetic pathway involving gulono-gamma-lactone oxidase for the terminal step. But primates and guinea pigs are absolutely dependent on

exogenously supplied dietary vitamin C due to inactivation of the gulono-gamma-lactone oxidase gene by mutation (33).

Ingestion of ascorbic acid or its reversibly oxidized metabolite, dehydroascorbic acid (DHAA), raises the plasma concentration of ascorbate (the predominant form of reduced vitamin C at physiological pH) in normal human subjects (40, 79). Transport systems associated with cells' plasma membranes determine the distribution of vitamin C between extracellular and intracellular fluids. Simple diffusion, facilitated diffusion, and active mechanisms may potentially contribute to the membrane transport of vitamin C.

SIMPLE DIFFUSION

Ascorbic acid is a lactone ($C_6H_8O_6$) and its hydroxyl groups at positions 2 and 3 ionize with pK values of 4.17 and 11.57. Therefore, reduced vitamin C exists predominantly as the ascorbate anion in most body fluids. Molecules that are comparably water soluble but smaller and nonionized, such as ethanol and glycerol, diffuse rapidly through nonspecific pathways in cell membranes, especially through the lipid bilayer. In contrast, ascorbate, because of its size and charge, does not readily permeate the lipid bilayer. Simple diffusion of DHAA into cells is negligible, too, because the oil:water distribution coefficient of DHAA is similar to that of an osmotic diuretic, mannitol, which is excluded from lipid bilayers (76).

Weak organic acids can enter cells by simple diffusion of their undissociated forms. Once in the cytoplasm, these acids dissociate into organic ions (e.g., propionic acid becomes propionate) and protons. To investigate if appreciable uptake of ascorbic acid might occur this way, bone-derived osteoblasts were incubated with 5 mM sodium ascorbate or sodium propionate at pH 7.3 and cytoplasmic pH was monitored to detect uptake of the organic acids (99). Sodium propionate caused rapid acidification of the cytoplasm but ascorbate had little effect, which indicates that osteoblasts do not take up appreciable amounts of undissociated ascorbic acid. Further studies showed that these cells increase their intracellular concentration of vitamin C through plasma membrane transport systems that translocate either the ascorbate anion (99) or DHAA (72). Therefore, simple diffusion across the plasma membrane at physiological pH comprises only a slow component of vitamin C accumulation and may be negligibly small in those cells that contain transport systems with high affinity for ascorbate or DHAA.

FACILITATED DIFFUSION

Specific proteins mediate the entry and exit of vitamin C in cells by facilitated diffusion or active transport. Facilitated diffusion achieves net movement only in the direction of a chemical or electrochemical gradient of the transported solute, whereas active transport can move solute against this gradient by using energy

derived from cellular metabolism. The facilitated diffusion of DHAA and ascorbate is discussed in this section.

Uptake of Dehydroascorbic Acid into Cells

When ascorbate acts as an antioxidant or enzyme cofactor, it becomes oxidized to DHAA. This oxidization product evidently plays physiologically important roles because it can be used by cells to regenerate ascorbate, and directly or indirectly, it can change the redox state of many other molecules. DHAA is a dietary source of vitamin C in humans because cellular mechanisms of transport and metabolism convert DHAA to ascorbate (40, 79). It is widely understood that dietary ascorbate and DHAA possess roughly equivalent bioavailability, which is the fraction of the ingested amount that has the potential to meet the functional requirements of tissues for vitamin C. Indeed, the vitamin C content of food is commonly reported as the sum of the ascorbate and DHAA contents.

Bioavailability is determined by the rates of absorption, distribution, and metabolism within the body, and by excretion. Absorption of vitamin C from food occurs across the epithelium of the small intestine, which is comprised of polarized enterocytes. Both ascorbate and DHAA are absorbed along the entire length of the human intestine, as has been shown by measuring transport activities in luminal (brush-border) membrane vesicles (59). For both the DHAA and ascorbate transport systems, initial rates of uptake saturate with increasing external substrate concentration, reflecting high-affinity interactions that can be described by Michaelis-Menten kinetics. A transport system's affinity is reflected in the substrate concentrations required for half-maximal flux, which is the Michaelis constant, K_m. In human intestinal brush-border membranes, DHAA is taken up with lower affinity than ascorbate because the apparent K_m values are 0.8 and 0.2 mM, respectively. However, the maximal rates of uptake are similar for DHAA and ascorbate when glucose is absent.

The mechanism of DHAA uptake by luminal membranes of human jejunum has pharmacological characteristics that clearly differ from those of ascorbate uptake. Sodium-independent carriers take up DHAA by facilitated diffusion, and these are distinct from the sodium-dependent transporters of ascorbate. Glucose inhibits ascorbate uptake but not DHAA uptake, which raises the possibility that glucose derived from food may increase the bioavailability of DHAA relative to ascorbate (59). Human enterocytes contain reductases that convert DHAA to ascorbate (12). This conversion maintains the intracellular level of DHAA low and the resulting concentration gradient favors uptake of oxidized vitamin C across the enterocytes' plasma membrane. Intestinal DHAA absorption may be especially important during gastritis, since chronic gastritis decreases the concentration of ascorbate in gastric juice and yet does not cause scurvy (80). Inflammation in the gastric mucosa may accelerate oxidation of ascorbate to DHAA, but intestinal absorption of the latter may prevent gastritis patients from becoming scorbutic.

Any DHAA that escapes reduction in enterocytes and enters the blood, or that is formed by oxidation of ascorbate in extracellular fluid, may be taken up and reduced to ascorbate by other cell types. Many cells have been shown capable of using extracellular DHAA to produce intracellular ascorbate, including adipocytes, astrocytes, endothelial cells, erythrocytes, granulosa cells, hepatocytes, neutrophils, osteoblasts and smooth muscle cells (17, 34, 36, 46, 63, 72, 93, 94, 98). DHAA uptake is obviously not mediated by sodium-ascorbate cotransporters because it is neither dependent on sodium nor blocked by an antagonist of sodium-ascorbate cotransport (4,4'-diisothiocyanatostilbene-2,2′-disulfonic acid) (17). Human neutrophils reportedly lack sodium-ascorbate cotransporters but incubation of these myeloid cells with DHAA in vitro increases their intracellular ascorbate concentration to levels equal to those found in mature neutrophils in vivo (94). Many other cell types possess simultaneously both sodium-ascorbate cotransporters and the ability to take up and reduce DHAA. For instance, astrocytes incubated with either ascorbate or DHAA raise intracellular ascorbate concentration to the same level, although that level is achieved more quickly when DHAA is the source (17, 84).

The acute influence of glucose on DHAA uptake varies between cell types. Some are largely inhibited by physiological concentrations of glucose (adipocytes, erythrocytes, granulosa cells, neutrophils, osteoblasts, smooth muscle cells), whereas others are less sensitive (erythrocytes, astrocytes), and still other cells' DHAA uptake is not changed detectably by glucose (luminal membranes of intestinal enterocytes and renal tubular cells) (17, 34, 36, 46, 59, 60, 72, 77, 94).

DHAA competes with glucose for uptake through the mammalian facilitative glucose transporters GLUT1, GLUT3, and GLUT4 (47, 77, 78, 94). GLUT proteins do not transport ascorbic acid or ascorbate. The results obtained with mammalian transporters that have been expressed experimentally in *Xenopus* oocytes are generally consistent with the hypothesis that GLUT1 and GLUT3 transport DHAA with affinities and maximal capacities similar to their transport of glucose. The hypothesis is also supported by a case report that uptake of DHAA by erythrocytes of a patient with GLUT1 deficiency was impaired to the same degree as that of the glucose analog, 3-O-methylglucose (45). Furthermore, enhancement of the activity of endogenous facilitative glucose transporters in mammalian cells increases the initial rate of DHAA uptake: This dual phenomenon is induced by insulin and insulin-like growth factor I in osteoblasts (72), insulin-like growth factor I and follicle-stimulating hormone in granulosa cells (46), and colony-stimulating factors in neutrophils (94).

The separate transporters for DHAA and ascorbate can be regulated independently of each other. For instance, colony-stimulating factors enhance DHAA uptake in human neutrophils that lack sodium-ascorbate cotransporters (94). Bone-derived osteoblasts provide another intriguing example: Transforming growth factor-β increases the maximal rate of ascorbate uptake through sodium-ascorbate cotransporters, but does not stimulate DHAA uptake (100), whereas insulin increases the maximal rate of DHAA uptake through glucose transporters without

changing sodium-ascorbate cotransport activity (72). Thus, transforming growth factor-β and insulin both increase intracellular ascorbate concentration in osteoblasts but act through different vitamin C transport systems.

Oxidation and regeneration of extracellular ascorbate may be an important way for the osteoid-resorbing activity of osteoclasts to stimulate the osteoid-forming activity of osteoblasts in bone. Osteoclasts are sources of reactive oxygen species that oxidize extracellular vitamin C. As DHAA is produced in the extracellular fluid, it may be taken up by a sodium-independent, facilitated transport system in neighboring osteoblasts and reduced to ascorbate just in time and place to support the synthesis of extracellular matrix by those cells (72). Intracellular ascorbate stimulates the osteoblasts to produce collagenous osteoid that subsequently becomes calcified (29). Thus, DHAA uptake may couple the osteoid resorption and formation that are essential for bone remodeling and repair.

Plasma and leukocyte ascorbate concentrations are decreased in patients during inflammation, such as that associated with diabetes, surgery, trauma, and sepsis (8, 25, 53, 56, 81). Orthopedic surgery has been shown to increase the DHAA:ascorbate ratio in urine, which indicates that surgical stress causes net conversion of ascorbate to DHAA (50). This may be partially due to net conversion of ascorbate to DHAA at sites where inflammation occurs; for instance, the DHAA:ascorbate ratio rises in wounded skin (43). A possible clinical consequence is that the depressed ascorbate levels in surgical and critically ill patients can cause diffuse hemorrhage (6).

An excess of glucose, such as occurs during the hyperglycemia of uncontrolled diabetes or surgical stress or sepsis, may competitively block most DHAA uptake through facilitative glucose transporters and thus impair the clearance of DHAA by cells. In addition to direct competition between glucose and DHAA for binding sites on transporters, chronic changes in glucose supply can have noncompetitive effects on the amount of DHAA uptake mediated by glucose transporters. For example, glucose pretreatment slows DHAA uptake (measured during 30- to 90-second transport assays in glucose-free medium) by the L6 skeletal muscle cell line by downregulating facilitative glucose transporters in the plasma membrane, principally GLUT1 (47). Conversely, overnight incubation in low-glucose medium upregulates the facilitative glucose transporters and increases the cells' DHAA transport capacity (47).

Glucose-sensitive DHAA transport mechanisms are subject to hormonal regulation and this may account for local deficiencies within tissues. For example, insulin increases the maximal rate of DHAA uptake by facilitative glucose transporters in target cells such as osteoblasts, thereby raising intracellular ascorbate concentration (72). Insulin insufficiency may impair DHAA uptake through facilitative glucose transporters during type I diabetes. Insulin-like growth factor I can activate insulin receptors to stimulate cellular uptake of DHAA but has only one-tenth the potency of insulin (72). Indeed, the maximal rate of DHAA uptake is decreased in lymphoblasts from patients with type I diabetes and nephropathy (65). Slowing of DHAA uptake impairs regeneration of ascorbate. For instance,

ascorbate concentration is decreased and DHAA is increased in the plasma and sciatic nerve of rats made diabetic by streptozotocin (66, 67). Moreover, because intracellular ascorbate is required for collagen synthesis by osteoblasts (29), deficient recycling of ascorbate in this bone cell type may contribute to the development of osteopenia. It is consistent with this hypothesis that feeding ascorbate to diabetic pregnant rats has been found to improve skeletal development in their offspring (9).

DHAA uptake occurs through relatively glucose-insensitive mechanisms in rat astrocytes and human erythrocytes, enterocytes, and renal epithelial cells.

DHAA is filtered from the plasma at the renal glomerular capillaries and then reabsorbed across the luminal membranes of the epithelial cells that comprise the renal proximal tubules. These cells are the principal sites where vitamin C is removed from the lumen of the renal tubule and translocated toward the blood. Studies of brush-border membrane vesicles prepared from rat renal cortex, which are representative of the luminal membranes of proximal tubule cells, have shown that ascorbate uptake occurs through sodium-ascorbate cotransport, whereas DHAA uptake occurs by a sodium-independent process (60). Neither ascorbate nor DHAA uptake is inhibited by glucose; hence, the facilitative and sodium-dependent glucose transporters present in the luminal membranes cannot be the routes of vitamin C uptake. Perhaps a previously unidentified, specific DHAA transporter exists in epithelial cells.

Uptake of DHAA (5–200 uM) by brain-derived astrocytes is inhibited only partially by a supraphysiological concentration of glucose (10 mM) (17). The remaining glucose-insensitive accumulation of intracellular ascorbate from DHAA is blocked by phloretin and cytochalasin B, which are antagonists of facilitative glucose transporters, but it is also inhibited reversibly by sulfinpyrazone. Astrocytes are an abundant cell type in the brain. These nonneuronal, glial cells regulate the composition of extracellular fluid and thus influence the environment and activity of neurons. DHAA is lethal to neurons in the absence of astrocytes (87), whereas the latter cells are capable of regenerating ascorbate without ill effect (17, 84). Brain cells produce cyclic AMP in response to neurotransmitters and ischemia and this intracellular messenger stimulates astrocytic uptake of DHAA and accumulation of ascorbate (84). Thus, astrocytes clear neurotoxic DHAA from extracellular fluid through a transport system that is not very sensitive to inhibition by glucose and that is upregulated by cyclic AMP.

It has been suggested that DHAA can be injected as a prodrug to treat cerebral ischemia (1, 38). The aim is to increase cerebral ascorbate concentration and thereby counter the oxidative stress caused by ischemia and reperfusion. Radiotracer experiments have shown that blood-borne DHAA enters the brain and is converted to ascorbate (1). Intravenous injection of DHAA improves neurologic outcome in mice subjected to experimental stroke, although it is not known if elevation of the vitamin C concentration in brain parenchyma is the cause of this improvement (38). The acute effect of DHAA on normal brain parenchymal cells may be to inhibit behavioral activation (i.e., open-field locomotion, approach of novel objects, and social interactions with other rats), since infusion of ascorbate oxidase into rat brain converts extracellular ascorbate to DHAA and leads to a rapid

decline in behavioral activation; in fact, oxidation of 50% to 70% of extracellular ascorbate is associated with a near-total inhibition of all recorded behavior (75).

Within various cell types, the most important enzymes for DHAA reduction are thioredoxin, protein disulfide isomerase, and thioredoxin reductase. Additionally, 3-hydroxysteroid dehydrogenase may reduce DHAA in hepatocytes. Glucose-6-phosphate dehydrogenase (G6PD) activity provides nicotinamide adenine dinucleotide phosphate (NADPH) as a source of reducing equivalents for DHAA reduction. Using DHAA-stimulated reduction of cationic nitroxides, which is an indirect measure of the rate of DHAA reduction, Mehlhorn (62) found that DHAA reduction is slower in human erythrocytes deficient in G6PD than in normal erythrocytes. Because low-activity alleles of G6PD are common genetic polymorphisms in human populations and the resulting G6PD deficiency could impair DHAA reduction (62), it may be desirable to screen for G6PD status before administering high doses of DHAA parenterally. If the amount of DHAA administered exceeds the capacity for its reduction, then DHAA concentration may rise high enough to inhibit transporters and enzymes (27).

Ascorbate Efflux from Cells

There has been relatively little research on how vitamin C is transported out of cells. Incubation with extracellular vitamin C stimulates efflux of preloaded [^{14}C]vitamin C, as has been shown for various cells of both epithelial and nonepithelial types (26, 42, 85). This effect has been interpreted as transstimulation of ascorbate efflux by extracellular ascorbate, which is supposed to enter cells through a homeoexchange transport system. However, the existence of ascorbate homeoexchange systems has not been demonstrated conclusively and the molecular identities of the membrane proteins responsible for homeoexchange have not been determined.

Intracellular reduction of DHAA to ascorbate can lead to ascorbate efflux, as reported for erythrocytes, endothelial cells, and hepatocyte-like HepG cells (63, 93). It is not known if a single transporter exchanges extracellular DHAA for intracellular ascorbate or if different transporters mediate uptake than efflux. Nevertheless, this recycling mechanism allows the reducing equivalents derived from cell metabolism to be transferred to DHAA and carried into the extracellular fluid as ascorbate, thus becoming available to neighboring cells.

Efflux of ascorbate from enterocytes and renal tubular cells, to the blood, is essential for intestinal absorption and renal conservation of vitamin C. Because enterocytes and renal tubular cells swell markedly during transepithelial transport of nutrients such as glucose or alanine (4, 10, 58), it may be hypothesized that ascorbate diffuses from the cytoplasm to the extracellular fluid through volume-sensitive anion channels in the epithelial cells' basolateral membrane and then enters the blood plasma through discontinuities in the capillary wall. Indeed, there is functional evidence that volume-sensitive anion channels are permeant to ascorbate, although the molecular identities of these channels have not been determined (31).

Volume-sensitive pathways may mediate ascorbate efflux from nonepithelial cells, too. Adrenal chromaffin cells release vitamin C from multiple intracellular

compartments to the extracellular fluid when incubated with high-potassium solutions that both depolarize and swell the cells (19), whereas depolarization of the plasma membrane by itself is not a sufficient stimulus to elicit ascorbate efflux (13). Cerebral astrocytes also rapidly release ascorbate when stimulated appropriately. Swelling of astrocytes transiently and reversibly permits large quantities of ascorbate to move from the cells to the extracellular fluid (83, 104). Based on its osmotic and pharmacological characteristics, it may be inferred that volume-sensitive anion channels in the plasma membrane mediate this efflux (83, 104). Thus, astrocytes condition the extracellular fluid for neurons by clearing DHAA and replacing ascorbate.

Glutamate uptake into brain cells triggers ascorbate efflux (74, 106). In vitro experiments have shown that astrocytes release ascorbate when exposed to glutamate (104). Astrocytes are in close contact with neuronal synapses and respond to the glutamate released there. Thus, a demand-driven component of ascorbate efflux from astrocytes may appear just in time and place to reach active neurons. A putative glutamate-ascorbate heteroexchanger has been proposed to mediate the counter movements of glutamate and ascorbate (74). However, it is more likely that glutamate uptake through sodium-glutamate cotransporters, located in astrocyte processes (end feet), causes swelling of the end feet and thereby activates a volume-sensitive efflux pathway (104). Exocytosis of ascorbate in secretory vesicles may also raise extracellular ascorbate (95). The physiological consequences may be enormous because changes in the brain's extracellular level of ascorbate play a critical role in behavioral activation. In rats that are awake, for example, ascorbate iontophoresis enhances the magnitude of glutamate-induced neuronal excitations (44). In contrast, intrastriatal infusion of ascorbate oxidase, to convert extracellular ascorbate to DHAA, causes a rapid and severe inhibition of behavior in rats (75).

Connexin proteins, which aggregate to form gap junctions and hemichannels in plasma membranes, may increase membrane permeability to ascorbate. This has been shown by reconstituting microsomes containing connexin proteins into liposomes (2). The liposomes were incubated with ascorbate for 2 minutes and ascorbate uptake was detected by measuring the reduction of cytochrome c that had been trapped in the lumen of the liposomes during their formation. Incorporation of connexin 26 into the liposomes greatly increased their apparent ascorbate uptake (2). Presumably, the connexin 26 formed gap-junction hemichannels that are bidirectional and can facilitate ascorbate efflux as well as uptake.

ACTIVE TRANSPORT

Sodium-Ascorbate Cotransport Mediates High-Affinity Absorption

Ascorbate is absorbed from the lumen of the human intestine by sodium-ascorbate cotransport in enterocytes, as has been shown by measuring transport activities

in luminal (brush-border) membrane vesicles (59). This is an example of secondary active transport because it couples ascorbate uptake to the concentration gradient of sodium ion across the plasma membrane that is maintained by sodium/potassium-ATPase. It is likely because of the limited capacity of enterocytes for sodium-ascorbate cotransport that large oral doses of ascorbate are absorbed less completely than are small doses. How ascorbate is administered affects how much reaches the bloodstream and may affect the results of studies of its potential effect on health and disease. For instance, peak plasma ascorbate concentrations are higher after administration of intravenous ascorbate doses than after administration of oral doses and the difference increases with increasing dose (70). Clinical consequences arise from the limited bioavailability of oral ascorbate. For example, when oral and intravenous routes of ascorbate administration (500 mg/day for 30 days) were compared in sedentary older men, only intravenous ascorbate restored endothelial function as indicated by flow-mediated dilatation in the brachial artery (24).

Absorption sites for ascorbate are found along the entire length of the small intestine (59). In vesicles prepared from the jejunum, intravesicular (cytoplasmic) glucose inhibits ascorbate uptake. This observation does not implicate sodium-glucose cotransporters (i.e., SGLT proteins) as carriers of ascorbate because only glucose on the cytoplasmic side of the plasma membrane slows sodium-dependent ascorbate uptake (59). If trans-inhibition by glucose occurs in vivo then it may permit ingested glucose and hyperglycemia to decrease ascorbate bioavailability by slowing intestinal absorption of the vitamin. Other ingredients in foods and drugs may have comparable effects. For instance, the inhibition by salicylate of sodium-ascorbate cotransport that has been demonstrated in vitro (20) may explain the decrease in ascorbate bioavailability caused by aspirin (39).

Flavonoids, such as the dietary component quercetin, reversibly inhibit vitamin C uptake in human colon cancer cells and rat nontransformed intestinal crypt cells, as well as in Chinese hamster ovary cells and Xenopus laevis oocytes transfected with human sodium-dependent vitamin C transporter SVCT1 (52, 86). However, it has not been determined if inhibition of SVCTs and glucose transporters by quercetin, or oxidation of ascorbate by quercetin metabolites (32), causes the decrease in ascorbate bioavailability after quercetin administration in vivo (86). The high content of quercetin and other flavonoids in red grape juice may account for the observation that drinking this juice decreases absorption into blood of [^{13}C]ascorbate administered orally to human subjects (3).

Besides intestinal absorption, another important determinant of bioavailability is secondary active transport of ascorbate in the kidney. Most vitamin C circulates in the blood in the form of the ascorbate anion. The ascorbate in the blood plasma is freely filtered at the renal glomerulus, but much of it is reabsorbed in the proximal tubule. Ascorbate uptake across the luminal membranes of renal proximal tubule cells occurs through sodium-ascorbate cotransport. The amount of ascorbate lost in the urine rises when the plasma ascorbate concentration exceeds the renal threshold. Above this threshold the tubular reabsorptive capacity

is overwhelmed. This phenomenon is exploited clinically when urinary acidification with ascorbate is used for treatment and prevention of urinary tract infection (14). The renal threshold for vitamin C is reported to be slightly higher in men than in women (plasma ascorbate concentrations of 86 and 71 uM, respectively), but the underlying mechanism and physiological importance of this difference are unknown (68). Unlike the sodium-dependent ascorbate transport system in the luminal membrane of human jejunum that is inhibited by cytoplasmic glucose (59), Malo & Wilson (60) observed that the initial rate of ascorbate uptake by the luminal membrane of rat proximal tubule is not retarded by glucose. A previous study found a small, low-potency inhibitory action of glucose (30% inhibition by 100 mM glucose) during relatively long uptake periods, which is attributable to sodium loading of the membrane vesicles by prolonged, maximal operation of the sodium-glucose cotransporter (91). The briefer uptake period employed by Malo & Wilson (60) did not allow glucose-coupled sodium entry to increase intracellular sodium concentration to levels that inhibit ascorbate uptake during the transport assay.

Evidently, ascorbate and DHAA uptakes are not mediated by glucose transporters in these rat kidney membranes, since they are not inhibited by glucose directly or by the SGLT blocker phlorizin (60). Nevertheless, patients with diabetic nephropathy have lower plasma ascorbate concentration and higher renal clearance of ascorbate than do control subjects (35). Since tubular atrophy and interstitial fibrosis are common in the diabetic kidney, it is likely that structural damage impairs renal tubular reabsorption of ascorbate in these patients and thus diminishes plasma ascorbate concentration (35).

Many nonepithelial cell types also are capable of high-affinity vitamin C uptake through sodium-ascorbate cotransporters, including bone osteoblasts, cerebral astrocytes, ovarian granulosa cells, and vascular smooth muscle and endothelial cells (37, 49, 100, 101, 107). The apparent K_m values for sodium-ascorbate cotransport range from 200 uM L-ascorbate in absorptive epithelia [luminal membranes of human jejunum (59) and rodent renal cortex (91)] to 20 uM L-ascorbate in nonepithelial cells [osteoblasts (21)]. Thus, the secondary active transport of ascorbate occurs with higher affinity than does facilitated transport of DHAA.

Sodium-ascorbate cotransporters are remarkably specific for L-ascorbate (20, 29, 54, 59, 99). Among the molecules that have been tested and found not to be substrates for these cotransporters are ascorbate-2-O-phosphate, DHAA, glucose, 2-deoxyglucose, xanthine, hypoxanthine, L-gulono-lactone, formate, lactate, pyruvate, gluconate, oxalate, malonate, succinate, and an assortment of nucleosides and nucleotides. The cotransporters' stereoselectivity has been demonstrated as a greater affinity for L-ascorbate over the epimer D-isoascorbate, which leads to higher intracellular ascorbate concentrations of L-ascorbate than D-isoascorbate at steady state (29). The cotransporters are absolutely dependent on sodium and translocate at least two sodium cations with each ascorbate anion, thus employing the electrochemical gradient of sodium ion across the plasma membrane to provide the energy required for concentrative uptake of ascorbate (29, 54, 59, 99, 102).

Distinct Properties of the Cotransporters SVCT1 and SVCT2

Two isoforms of sodium-ascorbate cotransporters have been cloned and named SVCT1 and SVCT2 (16, 54, 55, 73, 88, 92, 96, 97). They are encoded by the genes Slc23a1 and Slc23a2, respectively. The majority of single nucleotide polymorphisms (SNPs) in Slc23a1 are population-specific in either African Americans or Caucasians, including three of four nonsynonymous SNPs (23). Most SNPs in Slc23a2 are shared between African Americans and Caucasians, and there are no nonsynonymous SNPs in Slc23a2 (23). In humans, rats, and mice, SVCT1 and SVCT2 have extensive sequence identity with each other but do not share structural homology with other families of sodium cotransporters. Hydropathy plots of the amino acid sequences are consistent with 12 transmembrane domains. Both SVCT isoforms are glycoproteins containing *N*-linked oligosaccharides when expressed in heterologous systems such as *Xenopus* oocytes, HRPE cells, and COS-1 cells (55).

Heterologous expression of either SVCT1 or SVCT2 confers concentrative ascorbate uptake. SVCT1 has consistently been found to have a higher capacity for ascorbate than does the other isoform. It has not been determined if this greater capacity is an inherent property of the SVCT1 protein (i.e., a larger turnover number) or simply reflects a larger abundance of SVCT1 than SVCT2 in the plasma membrane of the transfected cells (55).

SVCT1 and SVCT2 are remarkably specific for L-ascorbate (54, 73, 96). Their stereoselectivity for L-ascorbate over the epimer D-isoascorbate (erythorbate) likely accounts for the low antiscorbutic activity of the latter. Most studies have found that SVCT2 has a higher affinity for L-ascorbate than does SVCT1, with SVCT2 being approximately half saturated at the low end of the normal range of plasma ascorbate concentrations (90).

Endogenous mRNA for one or both of the SVCT isoforms has been found in most organs (16, 51, 73, 92, 96, 97). Reverse transcription-polymerase chain reaction (RT-PCR) analysis showed that brain, skeletal muscle, and spleen express predominantly SVCT2, whereas liver and kidney express mainly SVCT1; indeed, quantitative RT-PCR showed that SVCT2 mRNA levels in liver and kidney are less than 5% of SVCT1 levels (51). SVCT1 is localized mostly in epithelial tissues, whereas SVCT2 has a wider distribution. On the one hand, the high capacity of SVCT1 is appropriate for epithelial cells that transport much more ascorbate than required for their own internal use. On the other hand, the affinities of both SVCT1 and SVCT2 are sufficiently high to enable cells to absorb ascorbate effectively from extracellular fluid where—in most tissues except the stomach, eye, and central nervous system—the concentration of ascorbate may approximate the 20–80 uM normally found in plasma.

Transfection of an antisense oligonucleotide of SVCT2 into cells that otherwise express this transporter endogenously suppresses their sodium-dependent uptake of ascorbate in vitro (82). Concerning manipulation of this transporter in vivo, heterozygous SVCT2 knockout (*Slc23a2+/−*) mice comprise a valuable model

because they survive to adulthood, unlike the homozygous *Slc23a2−/−* mice that die shortly after birth (88). The SVCT isoforms appear to function independently of each other because SVCT1 expression and ascorbate concentrations in SVCT1-predominant organs are not affected by SVCT2 deficiency (51). Ascorbate concentrations are lower for *Slc23a2+/−* than wild-type (*Slc23a2+/+*) mice in tissues where SVCT2 is the main isoform, such as brain, spleen, and skeletal muscle (51, 88). Therefore, SVCT2 is a major determinant of ascorbate accumulation in tissues lacking SVCT1.

Selective sorting to the apical plasma membrane has been demonstrated for SVCT1 in the human colon adenocarcinoma cell line CaCo-2 (61) and the human nasal epithelial cell line CF15 (28). The evidence for the latter is that, first, cells cultured from human airway epithelia express SVCT1 and SVCT2 mRNA and are capable of sodium-dependent ascorbate uptake, and second, recombinantly expressed SVCT2-enhanced green fluorescent fusion protein is targeted exclusively to the apical membrane pole of the CF15 nasal epithelial cell line (28). Confocal imaging of hSVCT1 truncation mutants expressed in CaCo-2 cells and Madin-Darby canine kidney cells demonstrated that hSVCT1 was expressed at the apical cell surface and also resided in a heterogeneous population of intracellular organelles (89). Progressive truncation of the cytoplasmic COOH-terminal tail of hSVCT1 showed that an embedded ten-amino-acid sequence PICPVFKGFS, in amino acids 563–572, was required for targeting of hSVCT1 (89).

Elderly human subjects require more vitamin C in their diet than do young subjects to reach a desired plasma ascorbate concentration (11). Animal studies have revealed a diminished expression of SVCT1 mRNA and a decline in the capacity of cells to absorb vitamin C during aging (64). The ascorbate concentration in the liver of male rats decreases with age even though the rate of de novo ascorbate synthesis does not (64). When incubated with ascorbate, isolated hepatocytes from old as compared with young rats show decreased maximal rate of ascorbate uptake and lower steady state intracellular ascorbate concentration. Sodium-free media significantly reduces ascorbate uptake, implicating sodium-ascorbate cotransporters. Hepatic SVCT1 mRNA levels decline 45% with age, with no significant changes in SVCT2 mRNA abundance. It thus appears likely that a fall in SVCT1 expression changes hepatic ascorbate concentration. This finding may indicate a dominant role for plasma membrane transporters in the regulation of ascorbate levels, even in cells capable of synthesizing the vitamin. Moreover, the deficit in intracellular ascorbate concentration can be overcome by increasing the external supply of the vitamin, which suggests that intervention to increase plasma ascorbate concentrations might be beneficial for hepatic function in elderly subjects (64). Another potential therapeutic approach is to elevate the activity of sodium-ascorbate cotransporters.

Much research has been directed to identifying mechanisms that control the ascorbate transport in various cell types. Transport activity may be altered by changes in the affinity of substrate binding, the translocation capacity (i.e., turnover

number) of each transporter protein, or the number of transporter proteins present in the plasma membrane.

Sodium-ascorbate cotransport may be regulated kinetically by changes in either the concentrations of the transported solutes or membrane potential. Ascorbate downregulates the maximal rate of sodium-ascorbate cotransport. This has been shown for absorptive epithelia by feeding excess ascorbate to guinea pigs and afterward measuring the rate of sodium-dependent uptake of vitamin C into the intestinal mucosal (41). Downregulation of SVCT1 by ascorbate may limit its usefulness for raising intracellular ascorbate concentration. Evidence for downregulation is that incubation of the human colon adenocarcinoma cell line CaCo-2 TC7 with ascorbate for 24 hours leads to decreases in SVCT1 mRNA level and ascorbate uptake rate (57). Thus, it appears that the activity of SVCT1 in enterocytes is regulated to adjust for the recent history of ascorbate absorption.

Substrate downregulation of SVCT2 activity has been induced by preincubating astrocytes and osteoblasts with ascorbate for 10 to 24 hours and subsequently determining the kinetic properties of the initial rate of [^{14}C]ascorbate uptake (22, 103). Changes in SVCT2 activity are rapid and large. The observation that SVCT2 activity varies inversely with intracellular ascorbate concentration is consistent with the hypothesis that this transporter regulates the intracellular concentration of its organic substrate. Upregulation of SVCT2 in cells depleted of ascorbate leads to more efficient absorption of extracellular ascorbate and tends to restore the intracellular concentration of the vitamin (22, 103). Therefore, in the absence of neurohormonal or paracrine signals, SVCT2 acts to maintain intracellular ascorbate concentration constant. Experiments with transgenic mice lacking Slc23a2 indicate that SVCT2 normally maintains the high ascorbate concentration found in brain (51, 88). In situ hybridization of mRNA indicated that the ependymal cells of the choroid plexus express SVCT2, where it may be involved in the transepithelial transport of ascorbate between the blood and the cerebrospinal fluid (92). Brain neurons and astrocytes also express SVCT2 (5, 15, 49, 92). However, because sodium-ascorbate cotransporters become downregulated when intracellular ascorbate concentration is high (103), they may not be suitable targets for therapeutic strategies that attempt to raise intracellular ascorbate to supraphysiological levels. Instead it has been suggested that DHAA be injected as a prodrug to increase tissue—particularly brain—ascorbate concentration (1, 38), as discussed in the "Uptake of Dehydroascorbic Acid into Cells" section of this review.

The limited capacities of SVCT isoforms and their susceptibility to downregulation by ascorbate may have influenced the many human clinical trials with oral vitamin C supplements that failed to confer antioxidant protection or clinical benefit. Optimization of the dosing regime may be critical to the success of future intervention studies using vitamin C (69). An important consequence of substrate regulation of SVCT1 and SVCT2 activities may be more efficient absorption by the intestine, conservation by the kidney, and uptake into target cells of intermittent doses than of continuously ingested doses of ascorbate. This phenomenon

may account for the finding in rainbow trout (which require vitamin C from food) that hepatic ascorbate concentration at the end of a three-month experiment was higher if ascorbate was fed at 10-day intervals (i.e., pulse and withdrawal) instead of continuously (7). Although ascorbate transport activity was not measured, the intermittent withdrawal of dietary ascorbate may have caused a compensatory up-regulation of sodium-ascorbate cotransporters and thereby improved vitamin C bioavailability.

Because SVCT transporters translocate at least two sodium cations with each ascorbate anion, changes in membrane potential affect ascorbate flux (59, 102). This can be understood from a thermodynamic viewpoint to result from changes in membrane potential altering the sodium electrochemical gradient and thus the free energy of the transport system. In particular, membrane depolarization slows ascorbate uptake into cells reliant on SVCT.

Ascorbate transport by membranes expressing SVCT1 or SVCT2 occurs with a pH optimum of approximately 7.5 (54, 59). Acidification of the aqueous compartments on either the extracellular or cytoplasmic side of the plasma membrane inhibits ascorbate transport. The potency of protons for this effect greatly exceeds that which can be explained by protonation of ascorbate to ascorbic acid. Thus, protons inhibit the ascorbate transport systems and may act directly on the SVCT proteins. It has been suggested that protonation of histidine residues in the transporters decreases ascorbate binding affinity (54).

Hormones, paracrine factors, and intracellular signaling molecules regulate the expression of sodium-ascorbate cotransporters. For example, agents that elevate cyclic AMP levels in cultures of rat brain astrocytes increase SVCT2 mRNA levels and subsequently elevate the maximal rate of high-affinity, sodium-dependent ascorbate uptake (49, 84). The stimulation of ascorbate transport capacity appears to require de novo protein synthesis because it is inhibited by cycloheximide. Another example comes from studies of cultured bone cells. Transforming growth factor-β (100) and glucocorticoids (30, 71) increase the maximal rate of ascorbate uptake through sodium-ascorbate cotransporters in osteoblasts. Fujita et al. (30) showed that a glucocorticoid, dexamethasone, increases expression of mRNA for SVCT2 in the MC3T3-E1 osteoblast-like cell line. Overexpression of SVCT2 in these cells increases ascorbate uptake rate and promotes mineralization, indicating that SVCT2 stimulates osteoblast differentiation and mineralization (105).

Posttranslational modification and redistribution of the SVCT proteins may be involved in the control of sodium-ascorbate cotransport. For instance, they may explain how drugs that activate protein kinase C (PKC) inhibit sodium-dependent ascorbate uptake by rabbit nonpigmented ciliary epithelial cells (54), monkey kidney COS-1 cells (55), and *Xenopus* oocytes heterologously expressing either hSVCT1 or hSVCT2 (16). The mechanisms of PKC regulation of the human isoforms hSVCT1 and hSVCT2 have been studied in monkey kidney COS-1 cells (55). Transient transfection of recombinant carboxyl-terminal V5 epitope-tagged forms of the transporters was used to permit their immunodetection.

The PKC activator phorbol 12-myristate 13-acetate (PMA) induced rapidly (5 to 80 minutes) a fall in the maximal velocity of ascorbate uptake through hSVCT1 and hSVCT2. Western blot and confocal microscopy analyses indicated that the total amounts of hSVCT1 or hSVCT2 proteins in the transfected COS-1 cells were not altered by PMA treatment. However, PMA caused a net redistribution of hSVCT1 protein from the cell surface to intracellular membranes. PMA did not induce a detectable change in the amount of hSVCT2 protein in the plasma membrane, so it appears that activation of PKC decreases the catalytic transport efficiency (i.e., translocation capacity, turnover number) of this isoform (55). A possible confound is that transfection in COS-1 cells achieves higher protein levels and transport activities for hSVCT1 than hSVCT2 prior to PMA exposure (55).

Little is known about sodium-ascorbate cotransport in disease. However, elevation of extracellular ascorbate concentration by infusion of the vitamin may not succeed in raising the intracellular ascorbate concentration in inflamed tissues (18). Concerning the underlying mechanism, the inflammatory cytokines tumor necrosis factor-alpha and interleukin-1beta have been shown to inhibit ascorbate uptake in human endothelial cells that spontaneously express SVCT2 (82). Moreover, septic insult (i.e., incubation with lipopolysaccharide and the inflammatory cytokine interferon-γ) in astrocytes inhibits DHAA reduction and SVCT2-mediated ascorbate uptake, thereby decreasing the rate of intracellular ascorbate accumulation from extracellular vitamin C (48).

PERSPECTIVES AND FUTURE DIRECTIONS

Cellular mechanisms of transport and metabolism concentrate vitamin C to enhance its function as an enzyme cofactor and antioxidant. Concerning flux across the plasma membrane, simple diffusion of ascorbic acid plays only a small or negligible role. More significant are the facilitated diffusion of DHAA through glucose-sensitive and -insensitive transporters, facilitated diffusion of ascorbate through channels, exocytosis of ascorbate in secretory vesicles, and secondary active transport of ascorbate through SVCT1 and SVCT2 proteins. It remains to be determined if the best way to increase ascorbate levels acutely in patients is by administering ascorbate or DHAA. Future research may also lead to gene engineering of vitamin C transport systems to create new treatments for symptoms of aging and disease.

ACKNOWLEDGMENTS

Research in the author's laboratory was supported by grants from the Canadian Institutes of Health Research, Heart and Stroke Foundation of Ontario, and Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. Agus DB, Gambhir SS, Pardridge WM, Spielholz C, Baselga J, et al. 1997. Vitamin C crosses the blood-brain barrier in the oxidized form through the glucose transporters. *J. Clin. Invest.* 100:2842–48
2. Ahmad S, Evans WH. 2002. Post-translational integration and oligomerization of connexin 26 in plasma membranes and evidence of formation of membrane pores: implications for the assembly of gap junctions. *Biochem. J.* 365:693–99
3. Bates CJ, Jones KS, Bluck LJ. 2004. Stable isotope-labelled vitamin C as a probe for vitamin C absorption by human subjects. *Br. J. Nutr*. 91:699–705
4. Beck JS, Potts DJ. 1990. Cell swelling, co-transport activation and potassium conductance in isolated perfused rabbit kidney proximal tubules. *J. Physiol*. 425:369–78
5. Berger UV, Lu XC, Liu W, Tang Z, Slusher BS, Hediger MA. 2003. Effect of middle cerebral artery occlusion on mRNA expression for the sodium-coupled vitamin C transporter SVCT2 in rat brain. *J. Neurochem.* 86:896–906
6. Blee TH, Cogbill TH, Lambert PJ. 2002. Hemorrhage associated with vitamin C deficiency in surgical patients. *Surgery* 131:408–12
7. Blom JH, Dabrowski K. 1998. Continuous or "pulse-and-withdraw" supply of ascorbic acid in the diet: a new approach to altering the bioavailability of ascorbic acid, using teleost fish as a scurvy-prone model. *Int. J. Vitam. Nutr. Res*. 68:88–93
8. Borrelli E, Roux-Lombard P, Grau GE, Girardin E, Ricou B. et al. 1996. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. *Crit. Care Med*. 24:392–97
9. Braddock R, Siman CM, Hamilton K, Garland HO, Sibley CP. 2002. Gamma-linoleic acid and ascorbate improves skeletal ossification in offspring of diabetic rats. *Pediatr. Res*. 51:647–52
10. Breton S, Marsolais M, Lapointe JY, Laprade R. 1996. Cell volume increases of physiologic amplitude activate basolateral K and CI conductances in the rabbit proximal convoluted tubule. *J. Am. Soc. Nephrol*. 7:2072–87
11. Brubacher D, Moser U, Jordan P. 2000. Vitamin C concentrations in plasma as a function of intake: a meta-analysis. *Int. J. Vitam. Nutr. Res*. 70:226–37
12. Buffinton GD, Doe WF. 1995. Altered ascorbic acid status in the mucosa from inflammatory bowel disease patients. *Free Radic. Res*. 22:131–43
13. Cahill PS, Wightman RM. 1995. Simultaneous amperometric measurement of ascorbate and catecholamine secretion from individual bovine adrenal medullary cells. *Anal. Chem*. 67:2599–605
14. Carlsson S, Wiklund NP, Engstrand L, Weitzberg E, Lundberg JON. 2001. Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* 5: 580–86
15. Castro M, Caprile T, Astuya A, Millan C, Reinicke K, et al. 2001. High-affinity sodium-vitamin C co-transporters (SVCT) expression in embryonic mouse neurons. *J. Neurochem*. 78:815–23
16. Daruwala R, Song J, Koh WS, Rumsey SC, Levine M. 1999. Cloning and functional characterization of the human sodium-dependent vitamin C transporters hSVCT1 and hSVCT2. *FEBS Lett*. 460:480–84
17. Daskalopoulos R, Korcok J, Tao L, Wilson JX. 2002. Accumulation of intracellular ascorbate from dehydroascorbic acid

by astrocytes is decreased after oxidative stress and restored by propofol. *Glia* 39:124–32

18. Demling R, Ikegami K, Picard L, Lalonde C. 1994. Administration of large doses of vitamin C does not decrease oxidant-induced lung lipid peroxidation caused by bacterial-independent acute peritonitis. *Inflammation* 18:499–510
19. Diliberto EJ, Menniti FS, Knoth J, Daniels AJ, Kizer JS, Viveros OH. 1987. Adrenomedullary chromaffin cells as a model to study the neurobiology of ascorbic acid: from monooxygenation to neuromodulation. *Ann. NY Acad. Sci*. 498:28–53
20. Dixon SJ, Kulaga A, Jaworski EM, Wilson JX. 1991. Ascorbate uptake by ROS 17/2.8 osteoblast-like cells: substrate specificity and sensitivity to transport inhibitors. *J. Bone Miner. Res*. 6:623–29
21. Dixon SJ, Wilson JX. 1992. Transforming growth factor-β stimulates ascorbate transport activity in osteoblastic cells. *Endocrinology* 130:484–89
22. Dixon SJ, Wilson JX. 1992. Adaptive regulation of ascorbate transport in osteoblastic cells. *J. Bone Miner. Res*. 7:675–81
23. Eck P, Erichsen HC, Taylor JG, Yeager M, Hughes AL, et al. 2004. Comparison of the genomic structure and variation in the two human sodium-dependent vitamin C transporters, SLC23A1 and SLC23A2. *Hum. Genet*. 115:285–94
24. Eskurza I, Monahan KD, Robinson JA, Seals DR. 2004. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J. Physiol*. 556:315–24
25. Fain O, Paries J, Jacquart B, Le Moel G, Kettaneh A, et al. 2003. Hypovitaminosis C in hospitalized patients. *Eur. J. Intern. Med.* 14:419–25
26. Finn FM, Johns PA. 1980. Ascorbic acid transport by isolated bovine adrenal cortical cells. *Endocrinology* 106:811–17
27. Fiorani M, De Sanctis R, Scarlatti F, Stocchi V. 1998. Substrates of hexokinase, glucose-6-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase prevent the inhibitory response induced by ascorbic acid/iron and dehydroascorbic acid in rabbit erythrocytes. *Arch. Biochem. Biophys*. 356:159–66
28. Fischer H, Schwarzer C, Illek B. 2004. Vitamin C controls the cystic fibrosis transmembrane conductance regulator chloride channel. *Proc. Natl. Acad. Sci. USA* 101:3691–96
29. Franceschi RT, Wilson JX, Dixon SJ. 1995. Requirement for Na^+-dependent ascorbic acid transport in osteoblast function. *Am. J. Physiol*. 268:C1430–39
30. Fujita I, Hirano J, Itoh N, Nakanishi T, Tanaka K. 2001. Dexamethasone induces sodium-dependant vitamin C transporter in a mouse osteoblastic cell line MC3T3-E1. *Br. J. Nutr*. 86:145–49
31. Furst J, Gschwentner M, Ritter M, Botta G, Jakab M, et al. 2002. Molecular and functional aspects of anionic channels activated during regulatory volume decrease in mammalian cells. *Pflugers Arch.* 444:1–25
32. Galati G, Sabzevari O, Wilson JX, O'Brien PJ. 2002. Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. *Toxicology* 177:91–104
33. Ha MN, Graham FL, D'Souza CK, Muller WJ, Igdoura SA, Schellhorn HE. 2004. Functional rescue of vitamin C synthesis deficiency in human cells using adenoviral-based expression of murine l-gulono-gamma-lactone oxidase. *Genomics* 83:482–92
34. Himmelreich U, Drew KN, Serianni AS, Kuchel PW. 1998. ^{13}C NMR studies of vitamin C transport and its redox cycling in human erythrocytes. *Biochemistry* 37:7578–88
35. Hirsch IB, Atchley DH, Tsai E, Labbe RF, Chait A. 1998. Ascorbic acid clearance in

diabetic nephropathy. *J. Diabetes Complications* 12:259–63
36. Holmes ME, Mwanjewe J, Samson SE, Haist JV, Wilson JX et al. 2002. Dehydroascorbic acid uptake by coronary artery smooth muscle: effect of intracellular acidification. *Biochem. J*. 362:507–12
37. Holmes ME, Samson SE, Wilson JX, Dixon SJ, Grover AK. 2000. Ascorbate transport in pig coronary artery smooth muscle: Na^+ removal and oxidative stress increase loss of accumulated cellular ascorbate. *J. Vasc. Res*. 37:390–98
38. Huang J, Agus DB, Winfree CJ, Kiss S, Mack WJ, et al. 2001. Dehydroascorbic acid, a blood-brain barrier transportable form of vitamin C, mediates potent cerebroprotection in experimental stroke. *Proc. Natl. Acad. Sci. USA* 98:11720–24
39. Ioannides C, Stone AN, Breacker PJ, Basu TK. 1982. Impairment of absorption of ascorbic acid following ingestion of aspirin in guinea pigs. *Biochem. Pharmacol*. 31:4035–38
40. Jacob RA, Spinozzi GM, Simon VA, Kelley DS, Prior RL, et al. 2003. Consumption of cherries lowers plasma urate in healthy women. *J. Nutr*. 133:1826–29
41. Karasov WH, Darken BW, Bottum MC. 1991. Dietary regulation of intestinal ascorbate uptake in guinea pigs. *Am. J. Physiol*. 260:G108–18
42. Khatami M, Stramm LE, Rockey JH. 1986. Ascorbate transport in cultured cat retinal pigment epithelial cells. *Exp. Eye Res*. 43:607–15
43. Kim M, Otsuka M, Yu R, Kurata T, Arakawa N. 1994. The distribution of ascorbic acid and dehydroascorbic acid during tissue regeneration in wounded dorsal skin of guinea pigs. *Int. J. Vitam. Nutr. Res*. 64:56–59
44. Kiyatkin EA, Rebec GV. 1998. Heterogeneity of ventral tegmental area neurons: single-unit recording and iontophoresis in awake unrestrained rats. *Neuroscience* 85:1285–309
45. Klepper J, Wang D, Fischbarg J, Vera JC, Jarjour IT, et al. 1999. Defective glucose transport across brain tissue barriers: a newly recognized neurological syndrome. *Neurochem. Res*. 24:587–94
46. Kodaman PH, Behrman HR. 1999. Hormone-regulated and glucose-sensitive transport of dehydroascorbic acid in immature rat granulosa cells. *Endocrinology* 140:3659–65
47. Korcok J, Dixon SJ, Lo TCY, Wilson JX. 2003. Differential effects of glucose on dehydroascorbic acid transport and intracellular ascorbate accumulation in astrocytes and skeletal myocytes. *Brain Res*. 993:201–7
48. Korcok J, Wu F, Tyml K, Hammond RR, Wilson JX. 2002. Sepsis inhibits reduction of dehydroascorbic acid and accumulation of ascorbate in astroglial cultures: Intracellular ascorbate depletion increases nitric oxide synthase induction and glutamate uptake inhibition. *J. Neurochem*. 81:185–93
49. Korcok J, Yan R, Siushansian R, Dixon SJ, Wilson JX. 2000. Sodium-ascorbate cotransport controls intracellular ascorbate concentration in primary astrocyte cultures expressing the SVCT2 transporter. *Brain Res*. 881:144–51
50. Kubin A, Kaudela K, Jindra R, Alth G, Grunberger W, et al. 2003. Dehydroascorbic acid in urine as a possible indicator of surgical stress. *Ann. Nutr. Metab*. 47:1–5
51. Kuo SM, MacLean ME, McCormick K, Wilson JX. 2004. Gender and sodium-ascorbate transporters determine ascorbate concentrations in mice. *J. Nutr*. 134:2216–21
52. Kuo SM, Morehouse HF Jr, Lin CP. 1997. Effect of antiproliferative flavonoids on ascorbic acid accumulation in human colon adenocarcinoma cells. *Cancer Lett*. 116:131–37
53. Lee W, Davis KA, Rettmer RL, Labbe RF. 1988. Ascorbic acid status: biochemical and clinical considerations. *Am. J. Clin. Nutr*. 48:286–90

54. Liang WJ, Johnson D, Jarvis SM. 2001. Vitamin C transport systems of mammalian cells. *Mol. Membr. Biol*. 18:87–95
55. Liang WJ, Johnson D, Ma LS, Jarvis SM, Wei-Jun L. 2002. Regulation of the human vitamin C transporters expressed in COS-1 cells by protein kinase C. *Am. J. Physiol*. 283:C1696–704
56. Louw JA, Werbeck A, Louw ME, Kotze TJ, Cooper R, Labadarios D.1992. Blood vitamin concentrations during the acute-phase response. *Crit. Care Med*. 20:934–41
57. MacDonald L, Thumser AE, Sharp P. 2002. Decreased expression of the vitamin C transporter SVCT1 by ascorbic acid in a human intestinal epithelial cell line. *Br. J. Nutr*. 87:97–100
58. MacLeod RJ, Lembessis P, Hamilton JR. 1992. Differences in Ca^{2+}-mediation of hypotonic and Na^{+}-nutrient regulatory volume decrease in suspensions of jejunal enterocytes. *J. Membr. Biol*. 130:23–31
59. Malo C, Wilson JX. 2000. Glucose modulates vitamin C transport in adult human small intestinal brush border membrane vesicles. *J. Nutr*. 130:63–69
60. Malo C, Wilson JX. 2001. Transport of ascorbic acid and dehydroascorbic acid in rat kidney cortex. *FASEB J*. 15:A838
61. Maulen NP, Henriquez EA, Kempe S, Carcamo JG, Smid-Kotsas A, et al. 2003. Upregulation and polarized expression of the sodium-ascorbic acid transporter SVCT1 in post-confluent differentiated CaCo-2 cells. *J. Biol. Chem*. 278:9035–41
62. Mehlhorn RJ. 1991. Ascorbate- and dehydroascorbic acid–mediated reduction of free radicals in the human erythrocyte. *J. Biol. Chem*. 266:2724–31
63. Mendiratta S, Qu ZC, May JM. 1998. Erythrocyte ascorbate recycling: antioxidant effects in blood. *Free Radic. Biol. Med*. 24:789–97
64. Michels AJ, Joisher N, Hagen TM. 2003. Age-related decline of sodium-dependent ascorbic acid transport in isolated rat hepatocytes. *Arch. Biochem. Biophys*. 410: 112–20
65. Ng LL, Ngkeekwong FC, Quinn PA, Davies JE. 1998. Uptake mechanisms for ascorbate and dehydroascorbate in lymphoblasts from diabetic nephropathy and hypertensive patients. *Diabetologia* 41:435–42
66. Obrosova IG, Fathallah L, Liu E, Nourooz-Zadeh J. 2003. Early oxidative stress in the diabetic kidney: effect of DL-alpha-lipoic acid. *Free Radic. Biol. Med*. 34:186–95
67. Obrosova IG, Fathallah L, Stevens MJ. 2001. Taurine counteracts oxidative stress and nerve growth factor deficit in early experimental diabetic neuropathy. *Exp. Neurol*. 172:211–19
68. Oreopoulos DG, Lindeman RD, Vander-Jagt DJ, Tzamaloukas AH, Bhagavan HN, Garry PJ. 1993. Renal excretion of ascorbic acid: effect of age and sex. *J. Am. Coll. Nutr*. 12:537–42
69. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, et al. 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J. Am. Coll. Nutr*. 22:18–35
70. Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, et al. 2004. Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann. Intern. Med*. 140:533–37
71. Pandipati S, Driscoll JE, Franceschi RT. 1998. Glucocorticoid stimulation of Na^{+}-dependent ascorbic acid transport in osteoblast-like cells. *J. Cell. Physiol*. 176: 85–91
72. Qutob S, Dixon SJ, Wilson JX. 1998. Insulin stimulates vitamin C recycling and ascorbate accumulation in osteoblastic cells. *Endocrinology* 139:51–56
73. Rajan DP, Huang W, Dutta B, Devoe LD, Leibach FH, et al. 1999. Human placental sodium-dependent vitamin C transporter (SVCT2): molecular cloning and transport function. *Biochem. Biophys. Res. Commun*. 262:762–68

74. Rebec GV, Pierce RC. 1994. A vitamin as neuromodulator: Ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. *Prog. Neurobiol*. 43:537–65
75. Rebec GV, Wang Z. 2001. Behavioral activation in rats requires endogenous ascorbate release in striatum. *J. Neurosci*. 21:668–75
76. Rose RC. 1987. Solubility properties of reduced and oxidized ascorbate as determinants of membrane permeation. *Biochim. Biophys. Acta* 924:254–56
77. Rumsey SC, Daruwala R, Al-Hasani H, Zarnowski MJ, Simpson IA, Levine M. 2000. Dehydroascorbic acid transport by GLUT4 in *Xenopus* oocytes and isolated rat adipocytes. *J. Biol. Chem*. 275:28246–53
78. Rumsey SC, Kwon O, Xu GW, Burant CF, Simpson I, Levine M. 1997. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. *J. Biol. Chem*. 272:18982–89
79. Sabry JH, Fisher KH, Dodds ML.1958. Human utilization of dehydroascorbic acid. *J. Nutr*. 64:457–64
80. Schorah CJ. 1992. The transport of vitamin C and effects of disease. *Proc. Nutr. Soc*. 51:189–98
81. Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH, et al. 1996. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. *Am. J. Clin. Nutr*. 63:760–65
82. Seno T, Inoue N, Matsui K, Ejiri J, Hirata KI, et al. 2004. Functional expression of sodium-dependent vitamin C transporter 2 in human endothelial cells. *J. Vasc. Res*. 41:345–51
83. Siushansian R, Dixon SJ, Wilson JX. 1996. Osmotic swelling stimulates ascorbate efflux from cerebral astrocytes. *J. Neurochem*. 66:1227–33
84. Siushansian R, Tao L, Dixon SJ, Wilson JX. 1997. Cerebral astrocytes transport ascorbic acid and dehydroascorbic acid through distinct mechanisms regulated by cyclic AMP. *J. Neurochem*. 68:2378–85
85. Socci RR, Delamere NA. 1988. Characteristics of ascorbate transport in the rabbit iris-ciliary body. *Exp. Eye Res*. 46:853–61
86. Song J, Kwon O, Chen S, Daruwala R, Eck P, et al. 2002. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. *J. Biol. Chem.* 277:2252–60
87. Song JH, Shin SH, Ross GM. 2001. Oxidative stress induced by ascorbate causes neuronal damage in an in vitro system. *Brain Res*. 895:66–72
88. Sotiriou S, Gispert S, Cheng J, Wang Y, Chen A, et al. 2002. Ascorbic-acid transporter Slc23a1 is essential for vitamin C transport into the brain and for perinatal survival. *Nat. Med*. 8:514–17
89. Subramanian VS, Marchant JS, Boulware MJ, Said HM. 2004. A carboxy-terminal region dictates the apical plasma membrane targeting of the human sodium-dependent vitamin C transporter-1 in polarized epithelia. *J. Biol. Chem*. 279: 27719–28
90. Takanaga H, Mackenzie B, Hediger MA. 2004. Sodium-dependent ascorbic acid transporter family SLC23. *Pflugers Arch*. 447:677–82
91. Toggenburger G, Hausermann M, Mutsch B, Genoni G, Kessler M, et al. 1981. Na^+-dependent, potential-sensitive L-ascorbate transport across brush border membrane vesicles from kidney cortex. *Biochim. Biophys. Acta* 646:433–43
92. Tsukaguchi H, Tokui T, Mackenzie B, Berger UV, Chen XZ, et al. 1999. A family of mammalian Na^+-dependent L-ascorbic acid transporters. *Nature* 399:70–75
93. Upston JM, Karjalainen A, Bygrave FL, Stocker R. 1999. Efflux of hepatic

ascorbate: a potential contributor to the maintenance of plasma vitamin C. *Biochem. J*. 342:49–56

94. Vera JC, Rivas CI, Zhang RH, Golde DW. 1998. Colony-stimulating factors signal for increased transport of vitamin C in human host defense cells. *Blood* 91:2536–46
95. von Zastrow M, Tritton TR, Castle JD. 1986. Exocrine secretion granules contain peptide amidation activity. *Proc. Natl. Acad. Sci. USA* 83:3297–301
96. Wang H, Dutta B, Huang W, Devoe LD, Leibach FH, et al. 1999. Human Na^+-dependent vitamin C transporter 1 (hSVCT1): primary structure, functional characteristics and evidence for a nonfunctional splice variant. *Biochim. Biophys. Acta* 1461:1–9
97. Wang Y, Mackenzie B, Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. 2000. Human vitamin C (L-ascorbic acid) transporter SVCT1. *Biochem. Biophys. Res. Commun*. 267:488–94
98. Wilson JX. 1989. Ascorbic acid uptake by a high-affinity sodium-dependent mechanism in cultured rat astrocytes. *J. Neurochem*. 53:1064–71
99. Wilson JX, Dixon SJ. 1989. High-affinity sodium-dependent uptake of ascorbic acid by rat osteoblasts. *J. Membr. Biol*. 111:83–91
100. Wilson JX, Dixon SJ. 1995. Ascorbate concentration in osteoblastic cells is elevated by transforming growth factor-β. *Am. J. Physiol*. 268:E565–71
101. Wilson JX, Dixon SJ, Yu J, Nees S, Tyml K. 1996. Ascorbate uptake by microvascular endothelial cells of rat skeletal muscle. *Microcirculation* 3:211–21
102. Wilson JX, Jaworski EM, Dixon SJ. 1991. Evidence for electrogenic sodium-dependent ascorbate transport in rat astroglia. *Neurochem. Res*. 16:73–78
103. Wilson JX, Jaworski EM, Kulaga A, Dixon SJ. 1990. Substrate regulation of ascorbate transport activity in astrocytes. *Neurochem. Res*. 15:1037–43
104. Wilson JX, Peters C, Sitar SM, Daoust P, Gelb AW. 2000. Glutamate stimulates ascorbate transport by astrocytes. *Brain Res*. 858:61–66
105. Wu X, Itoh N, Taniguchi T, Hirano J, Nakanishi T, Tanaka K. 2004. Stimulation of differentiation in sodium-dependent vitamin C transporter 2 overexpressing MC3T3-E1 osteoblasts. *Biochem. Biophys. Res. Commun*. 317:1159–64
106. Yusa T. 2001. Increased extracellular ascorbate release reflects glutamate reuptake during the early stage of reperfusion after forebrain ischemia in rats. *Brain Res.* 897:104–13
107. Zreik TG, Kodaman PH, Jones EE, Olive DL, Behrman H. 1999. Identification and characterization of an ascorbic acid transporter in human granulosa-lutein cells. *Mol. Hum. Reprod*. 5:299–302

Annual Review of Nutrition
Volume 25, 2005

CONTENTS

DIETARY FIBER: HOW DID WE GET WHERE WE ARE?, *Martin Eastwood and David Kritchevsky* 1

DEFECTIVE GLUCOSE HOMEOSTASIS DURING INFECTION, *Owen P. McGuinness* 9

HUMAN MILK GLYCANS PROTECT INFANTS AGAINST ENTERIC PATHOGENS, *David S. Newburg, Guillermo M. Ruiz-Palacios, and Ardythe L. Morrow* 37

NUTRITIONAL CONTROL OF GENE EXPRESSION: HOW MAMMALIAN CELLS RESPOND TO AMINO ACID LIMITATION, *M.S. Kilberg, Y.-X. Pan, H. Chen, and V. Leung-Pineda* 59

MECHANISMS OF DIGESTION AND ABSORPTION OF DIETARY VITAMIN A, *Earl H. Harrison* 87

REGULATION OF VITAMIN C TRANSPORT, *John X. Wilson* 105

THE VITAMIN K-DEPENDENT CARBOXYLASE, *Kathleen L. Berkner* 127

VITAMIN E, OXIDATIVE STRESS, AND INFLAMMATION, *U. Singh, S. Devaraj, and Ishwarlal Jialal* 151

UPTAKE, LOCALIZATION, AND NONCARBOXYLASE ROLES OF BIOTIN, *Janos Zempleni* 175

REGULATION OF PHOSPHORUS HOMEOSTASIS BY THE TYPE IIa Na/PHOSPHATE COTRANSPORTER, *Harriet S. Tenenhouse* 197

SELENOPROTEIN P: AN EXTRACELLULAR PROTEIN WITH UNIQUE PHYSICAL CHARACTERISTICS AND A ROLE IN SELENIUM HOMEOSTASIS, *Raymond F. Burk and Kristina E. Hill* 215

ENERGY INTAKE, MEAL FREQUENCY, AND HEALTH: A NEUROBIOLOGICAL PERSPECTIVE, *Mark P. Mattson* 237

REDOX REGULATION BY INTRINSIC SPECIES AND EXTRINSIC NUTRIENTS IN NORMAL AND CANCER CELLS, *Archana Jaiswal McEligot, Sun Yang, and Frank L. Meyskens, Jr.* 261

REGULATION OF GENE TRANSCRIPTION BY BOTANICALS: NOVEL REGULATORY MECHANISMS, *Neil F. Shay and William J. Banz* 297

POLYUNSATURATED FATTY ACID REGULATION OF GENES OF LIPID METABOLISM, *Harini Sampath and James M. Ntambi* 317

SINGLE NUCLEOTIDE POLYMORPHISMS THAT INFLUENCE LIPID METABOLISM: INTERACTION WITH DIETARY FACTORS, *Dolores Corella and Jose M. Ordovas* 341

THE INSULIN RESISTANCE SYNDROME: DEFINITION AND DIETARY APPROACHES TO TREATMENT, *Gerald M. Reaven* 391

DEVELOPMENTAL DETERMINANTS OF BLOOD PRESSURE IN ADULTS, *Linda Adair and Darren Dahly* 407

PEDIATRIC OBESITY AND INSULIN RESISTANCE: CHRONIC DISEASE RISK AND IMPLICATIONS FOR TREATMENT AND PREVENTION BEYOND BODY WEIGHT MODIFICATION, *M.L. Cruz, G.Q. Shaibi, M.J. Weigensberg, D. Spruijt-Metz, G.D.C. Ball, and M.I. Goran* 435

ANNUAL LIPID CYCLES IN HIBERNATORS: INTEGRATION OF PHYSIOLOGY AND BEHAVIOR, *John Dark* 469

DROSOPHILA NUTRIGENOMICS CAN PROVIDE CLUES TO HUMAN GENE–NUTRIENT INTERACTIONS, *Douglas M. Ruden, Maria De Luca, Mark D. Garfinkel, Kerry L. Bynum, and Xiangyi Lu* 499

THE COW AS A MODEL TO STUDY FOOD INTAKE REGULATION, *Michael S. Allen, Barry J. Bradford, and Kevin J. Harvatine* 523

THE ROLE OF ESSENTIAL FATTY ACIDS IN DEVELOPMENT, *William C. Heird and Alexandre Lapillonne* 549

INDEXES

Subject Index 573

Cumulative Index of Contributing Authors, Volumes 21–25 605

Cumulative Index of Chapter Titles, Volumes 21–25 608

ERRATA

An online log of corrections to *Annual Review of Nutrition* chapters may be found at http://nutr.annualreviews.org/