The transport of vitamin C and effects of disease

BY C. J. SCHORAH

Department of Chemical Pathology and Immunology, University of Leeds, Leeds LS2 9JT

It has been known for a number of years that high concentrations of vitamin C (ascorbic acid + dehydroascorbic acid) occur in some cells, but it is only recently that we have begun to discover how high the levels are and also that the vitamin seems to be secreted onto epithelial surfaces (Table 1). If we assume that such concentrations and secretions are needed for the appropriate biological activity of the vitamin, it follows that transport of vitamin C will be required in order to sustain normal metabolic functions. Further, if transport is central to the metabolism of vitamin C, any defect in the process could lead to the development of disease. The study of transport mechanisms is, therefore, important in our understanding of vitamin C metabolism and this review will examine our present knowledge in this area and speculate on diseases that may impair these processes.

GENERAL CONSIDERATIONS AND PROBLEMS WITH THE STUDY OF VITAMIN C TRANSPORT

Any material which cannot be synthesized within the cell needs to be transported in two ways: across cell membranes and through biological fluids. Transport through biological

Table 1. *Approximate average concentrations of vitamin C* in normal cells and biological fluids*

	Vitamin C (mmol/l)	Source
Cell†		
Cervicovaginal	16·0‡	Basu *et al.* (1990)
Adrenal medulla§‖	10·5	Dhariwal *et al.* (1989)
Monocyte	8·0	Bergsten *et al.* (1990)
Neutrophil	1·3	Washko *et al.* (1990)
Brain	1·3‡	Mefford *et al.* (1981)
Gastric	2·0‡	C. J. Schorah (unpublished results)
Fluids		
Aqueous humour§	1·00	Socci & Delamere (1988)
Tears	0·77	Paterson & O'Rourke (1987)
Seminal fluid	0·65	Patriarca *et al.* (1991)
Gastric juice	0·25	Schorah *et al.* (1991)
Spinal fluid	0·08	Spector (1977)
Plasma	0·04	Basu & Schorah (1982)

* Ascorbic acid predominates, dehydroascorbic acid <5% of total, except gastric juice where dehydroascorbic acid averages 38% (see Table 5).

† Values expressed per litre cell water.

‡ Estimates of concentration in cell water from wet weights.

§ Animal studies, all other values are in man.

‖ Chromaffin granules.

fluids can involve simple diffusion, hydrodynamic flow and protein binding. As a general rule, the less water soluble, more toxic or larger the component the greater the importance of protein binding. Currently, it is believed that protein binding is of little consequence in the transport of vitamin C in biological fluids and its retention in cells (Rose, 1989; Washko *et al.* 1989). Vitamin C can be filtered by the kidney (Basu & Schorah, 1982) and ultrafiltered from plasma suggesting that protein binding in the blood is minimal. It is also rapidly lost from energy-depleted cells (Rose, 1988) indicating that binding within the cell is probably limited. On the other hand, Lovstad (1987) has shown that plasma protein can increase the stability of vitamin C. Whilst this could be explained by protein-binding of trace metals or free radicals, both of which tend to oxidize the vitamin, there is evidence of binding of ascorbic acid to serum albumin (Molloy & Wilson, 1980; Meucci *et al.* 1987). It is also energy sparing to maintain the very high cell concentration of vitamin C with the assistance of protein binding rather than allowing the concentration to be expressed fully within the cell water. At the moment we can say no more than that vitamin C probably forms some associations with protein, such as albumin, which may, therefore, assist in its transport. The full significance of such associations and the strength of the binding remains to be determined.

The polarity of ascorbic acid, which encourages its presence in aqueous fluids, also hinders its passage across the hydrophobic cell membrane (Rose, 1987). This, and the high concentration within the cell, makes it almost certain that the vitamin will need carrier mechanisms to enter the cell. Because researchers have long been aware of this, there has been considerable investigation of the membrane transport of vitamin C. Unfortunately, our understanding of the mechanism remains restricted. This is because of the limitations of the techniques available and difficulties with the interpretation of much of the data. Problems caused by the use of different species, some requiring vitamin C in the diet and others not, and the use of unphysiological concentrations of vitamin C are being resolved. However, some major technical problems are only just beginning to be addressed.

When tissues slices or biopsies are used it is important to distinguish uptake into interstitial fluid from uptake into the cell (Raghoebar *et al.* 1987; Rose, 1989). Even studies using isolated cells can fail to distinguish between membrane binding and actual cell uptake (Raghoebar *et al.* 1987). This is particularly so where cells have been pulsed for short time intervals with isotopes of the vitamin (Mann & Newton, 1975). Short incubations clearly have a disadvantage here, but have a considerable advantage when it comes to the much greater problem of vitamin C stability.

There are two compounds with biological activity, ascorbic acid and dehydroascorbic acid. Both are unstable, and whilst ascorbic acid is less reactive in some biological fluids, such as plasma, its half-life is short in tissue culture media and buffers that have been used in transport and cell uptake studies. Table 2 gives some indication of the rate of loss of total vitamin C (ascorbic acid + dehydroascorbic acid) and ascorbic acid from various fluids. Clearly, incubation in tissue culture medium quickly results in the production of breakdown products. The stability of the vitamin will depend on the medium/buffer used, the type and presence of cells, the amount of oxygen in the gas phase and the initial concentration of ascorbic acid (Cullen *et al.* 1986; Padh & Aleo, 1987; Raghoebar *et al.* 1987; Choi & Rose, 1989; Bergsten *et al.* 1990). Reducing agents, such as glutathione, are able to stabilize vitamin C (Table 2), but the high concentration needed is unphysiological and may affect transport of the vitamin. In addition, some reducing

Table 2. *Stability of ascorbic acid (100 μmol/l) in different solutions*

(Mean values with their standard errors)

	Percentage remaining (3 h at 37°)		
	Mean	SE	Half-life (h)
Total (ascorbic + dehydroascorbic)			
Plasma	94	3·0	>24
Medium	52	3·8	4·4
Medium + glutathione (10 mM)	91	4·7	18·1
Ascorbic acid			
Plasma	74	3·8	—
Medium	49	3·3	3·7
Medium + glutathione (10 mM)	90	4·5	16·9

The medium was RPMI 1640 containing fetal calf serum (100 ml/l) and antibiotics.

agents (dithiothreitol) can be cell toxic. The similarity of the results for total vitamin C and ascorbic acid in Table 2 probably reflect the even greater instability of dehydroascorbic acid at neutral pH, with reported half-lives of less than 30 min (Penney & Zilva, 1943; Bode *et al.* 1990). It is, therefore, essential that for in vitro studies there is an attempt to measure which metabolite is concentrated into the cell. Unfortunately, many studies that have used [^{14}C]ascorbic acid have only assesssed cell uptake of total radioactivity and no attempt has been made to ascertain in what form that radioactivity is present.

The final problem concerns potential losses of the vitamin during cell isolation and separation. It has been shown recently that different isolation procedures produce different levels of the vitamin associated with the cell (Bowers-Komro & McCormick, 1991). The technique which results in the lowest values has been considered the poorer as it has been assumed that loss has occurred from within the cell. This, however, may not be the case as increased washing may remove non-specific membrane binding of the vitamin and, therefore, give a more accurate measure of the true intracellular level.

These problems make the interpretation of many studies of vitamin C membrane transport in isolated cells and tissues difficult. However, it is possible to draw some conclusions from studies where attempts have been made to measure the concentrations of ascorbic acid and its metabolites.

MECHANISMS OF VITAMIN C TRANSPORT

Because ascorbic acid and dehydroascorbic acid both have vitamin C-like activity it is necessary to consider the transport of both components. Tables 3 and 4 attempt to summarize the most reliable findings for the transport of ascorbic acid. Only the placental and leucocyte studies (neutrophil, mononuclear cells) have been undertaken in man. The concentration of the vitamin within the cells studied, its poor membrane permeability (Rose, 1987) and the need for energy make it almost certain that the form of transport of ascorbic acid is facilitated, i.e. requiring a membrane carrier, and active.

Table 3. *Ascorbic acid transport in cells primarily utilizing vitamin C*

Cell	Facilitated Active	Facilitated Passive	Sodium dependent	Inhibited by glucose	Specific
Neutrophil*	Yes	?	No	Yes	Yes
Mononuclear†	Yes	?	–	Yes	?
Adrenal medulla‡	Yes	–	Yes	–	–
Brain§	Yes	–	Yes	No	Yes
Fibroblast, osteoblast‖	Yes	–	Yes	–	Yes

? Uncertainty; –, not investigated adequately.
* Moser & Weber (1984), Raghoebar *et al.* (1987), Washko *et al.* (1989, 1990).
† Davis *et al.* (1983), Bergsten *et al.* (1990).
‡ Diliberto *et al.* (1983), Rose (1988).
§ Cullen *et al.* (1986), Mooradian (1987), Wilson & Dixon (1989*a*), Wilson *et al.* (1990).
‖ Padh & Aleo (1987), Wilson & Dixon (1989*b*).

Table 4. *Ascorbic acid transport in cells primarily transferring vitamin C*

Cell	Facilitated Active	Facilitated Passive	Sodium dependent	Inhibited by glucose	Specific
Kidney and intestine*:					
Mucosal	Yes	No	Yes	No	Yes
Serosal	No	Yes	No	–	Co-DHAA
Ciliary epithelium†:					
Influx	Yes	–	Yes	Yes	Yes
Efflux	–	Yes	No	No	–
Placental‡	Yes	–	–	–	–

Co-DHAA, the same process transports dehydroascorbic acid.
–, Not investigated adequately.
* Rose (1988, 1989), Rose & Choi (1990), Bowers-Komro & McCormick (1991).
† Chu & Candia (1988), Socci & Delamere (1988), Helbig *et al.* (1989, 1990).
‡ Choi & Rose (1989).

In the majority of cells studied the process also seems to be dependent on extracellular sodium. The presence of Na seems to decrease the Michaelis constant (K_m) and, therefore, increase the affinity of the vitamin for its membrane carrier protein rather than changing the maximum velocity V_{max}; (Diliberto *et al.* 1983; Padh & Aleo, 1987; Wilson & Dixon, 1989*b*). There is also some evidence that divalent metal ions, such as calcium, may also stimulate ascorbate transport (Padh & Aleo, 1987; Garcia & Municio, 1990; Washko *et al.* 1990).

The specificity of the process is less well understood. There are only a limited number of studies which have looked at the ability of analogues of ascorbic acid to either inhibit uptake, when on the same side of the membrane, or stimulate transport, when on the opposite side (so called *cis*-inhibition–*trans*-stimulation). Published reports indicate that the process seems to be fairly specific to ascorbic acid with pre-incubation in the absence of the vitamin enhancing uptake (Wilson *et al.* 1990). There is still controversy as to whether glucose is able to compete with ascorbic acid transport and, therefore, inhibit its uptake. This will be considered further in relation to the impact of disease on the transport of vitamin C.

Most cells seem to be able to transport dehydroascorbic acid, the oxidized form of the vitamin (Stankova *et al.* 1975, 1984; Bigley *et al.* 1983; Cullen *et al.* 1986; Rose, 1987, 1989; Choi & Rose, 1989; Helbig *et al.* 1990; Rose & Choi, 1990). Usually the process is facilitated diffusion, i.e. with a carrier, but not against an electrochemical gradient or requiring energy. Glucose may well inhibit the process. Net uptake of dehydroascorbate is probably maintained by the ability of cells to rapidly reduce it to ascorbic acid and, thus, maintain a low intracellular concentration of the oxidized form of the vitamin. It is possible that co-transport of ascorbic acid by this system provides an additional mechanism for moving ascorbic acid across cell membranes.

Two special situations need to be considered. Kidney tubules, intestinal mucosa and possibly the ciliary epithelium of the eye transport vitamin C across the cell (Table 4). Uptake of ascorbic acid from tubular fluid, intestinal lumen and plasma respectively seems to be the process described previously, i.e. facilitated, active and requiring Na. Once in the cell, passage down the concentration gradient into the blood, or in the case of the eye, the aqueous humour, is facilitated diffusion and this mechanism may also transport dehydroascorbic acid, but usually in the opposite direction, into the cell. Finally, there is evidence that white cells, such as neutrophils and mononuclear cells, have a different transport mechanism for vitamin C (Table 3). The process is active, but does not seem to be dependent on Na and is susceptible to inhibition by glucose. There is also evidence that the transport of dehydroascorbic acid is preferred (Stankova *et al.* 1975; Bigley *et al.* 1983; Davis *et al.* 1983; Moser & Weber, 1984; Raghoebar *et al.* 1987; Washko *et al.* 1989, 1990). However, there is a hypothesis which could account for these apparent differences without the need to propose a separate mechanism. Ascorbic acid is at risk of rapid oxidation close to the membrane of the neutrophil or the macrophage because of the reactive species generated by these cells as part of their immune function (Rossi *et al.* 1985). Because of the rapid generation of dehydroascorbic acid on the cell membrane, uptake of vitamin C may be predominantly in the oxidized form by the system of facilitated diffusion present in other cells with rapid reduction of dehydroascorbic acid within the cell maintaining a net inward flow. These cells will still actively transport ascorbic acid and the apparent inhibition of this process by glucose, and the lack of a requirement for Na (not found in other cells), could be an artifact created by the incubation conditions leading rapidly to the generation of dehydroascorbic acid, the subsequent transport of which is affected by glucose and does not require Na.

IMPACT OF DISEASE ON TRANSPORT OF VITAMIN C

There are a number of conditions where low vitamin C concentrations have been reported in body fluids or in cell compartments and where these decreases cannot be explained entirely by a reduction in intake. Severe infection and other acute diseases have long been associated with low vitamin C levels in plasma and leucocytes (Basu & Schorah, 1982). Smoking is known to decrease vitamin C intake, but a number of studies have shown that levels in both plasma and leucocytes cannot be explained wholly by intake (Pelletier, 1975; Kallner *et al.* 1981; Schectman *et al.* 1989). Both situations will lead to some increase in reactive species generation and this will automatically lead to an increased turnover of ascorbic acid through oxidation (Cochrane *et al.* 1983; Anderson

Table 5. *Median gastric juice vitamin C levels in health and disease*

Gastric pathology	*n*	Gastric juice (μmol/l) Total vitamin C*	Gastric juice (μmol/l) Ascorbic acid	Plasma total vitamin C* (μmol/l)	Vitamin C intake (μmol/d)
Normal	23	249	154	39	410
Chronic gastritis	64	35	16	39	307
Reflux gastritis	14	118	111	72	474

* Ascorbic acid + dehydroascorbic acid.

et al. 1988; Frei *et al.* 1989). It is also possible that severe infection will, as part of the acute-phase response, lead to both a redistribution of vitamin C from the plasma compartment into interstitial fluid and a change in the proportion of the vitamin C-containing leucocytes (Schorah *et al.* 1986). It remains to be seen whether vitamin C transport is also impaired in these situations.

Reports of decreased levels in the eye following cataract formation (Bron & Brown, 1987) require more study to determine if transport problems are involved in the change.

The recent findings that vitamin C is found in high concentrations in gastric juice (Sobala *et al.* 1989; Schorah *et al.* 1991) and may well be secreted onto other epithelial surfaces (Paterson & O'Rourke, 1987; Patriarca *et al.* 1991), raises the possibility of it acting as a general antioxidant in these situations. Gastric juice is particularly interesting, because its normally high concentrations are considerably reduced by the presence of chronic gastritis, a cellular inflammatory response, but not by reflux chemical gastritis where there is little invasion of the gastric mucosa by leucocytes (Table 5). We have recently shown that, in patients with gastritis, oral vitamin C supplements were unable to restore gastric juice vitamin C, although plasma concentrations were increased significantly by this treatment (Table 6). We do not know why gastritis lowers gastric juice vitamin C. It may well represent a paralysis or poisoning of the transport system for ascorbic acid. An alternative explanation would be that ascorbic acid secretion continues normally but the leucocyte infiltration and associated free radical generation converts most of it to dehydroascorbic acid which is then rapidly re-absorbed. Destruction of the dehydroascorbic acid to products without vitamin C activity is unlikely as patients with gastritis do not develop clinical scurvy. More work is required because it is possible that vitamin C has important protective functions within gastric juice and its near absence in conditions such as chronic gastritis could contribute to the increased risk of gastric cancer seen in this group of patients (Sobala *et al.* 1991).

One of the most contentious situations where vitamin transport may be affected by disease is in diabetes. The hypothesis is developed in this way. Diabetes encourages low glucose levels in some cells and this reduces the activity of the pentose–phosphate pathway leading to decreased cell NADPH and reduced glutathione which in turn impairs the reduction of dehydroascorbic acid to ascorbic acid. Glucose and dehydroascorbic acid compete for transport across cell membranes and the increased blood glucose in diabetics, along with the impaired ability to reduce dehydroascorbic acid in the cell, could reduce cell uptake of dehydroascorbic acid. The result of this is to increase plasma dehydroascorbic acid levels and to decrease cell ascorbic acid concentrations.

Table 6. *Gastric juice and plasma response following oral vitamin C (650 mg/d) in ten patients with gastritis*

(Vitamin C supplements were taken for 7 d; the change represents the mean difference in concentrations between the presupplement sample and that taken 24 h after the last dose)

Average change							
Gastric juice				Plasma			
Total vitamin C†		Ascorbic acid		Total vitamin C†		Ascorbic acid	
μmol/l	%	μmol/l	%	μmol/l	%	μmol/l	%
+16	17	+16	18	+40	90***	+34	118***

Values were significantly different from presupplement value (paired *t* test): ***$P<0.001$.
† Ascorbic acid + dehydroascorbic acid.

This could encourage diabetic complications by allowing oxidation of cell membranes and impairment of connective tissue production (Mann & Newton, 1975). Not all the evidence, however, supports the hypothesis. Uptake of glucose and dehydroascorbic acid into some cells seems to be impaired in the diabetic and this may also extend to uptake of ascorbic acid (Chen *et al.* 1983; Davis *et al.* 1983; Kapeghian & Verlangieri, 1984; Stankova *et al.* 1984; McLennan *et al.* 1988). Reports published some years ago also suggested that there was indeed accumulation of dehydroascorbic acid in plasma (Chatterjee *et al.* 1975; Chatterjee & Banerjee, 1979; Som *et al.* 1981; Banerjee, 1982). Unfortunately, there is reason to believe that technical problems have made these earlier studies unsound and more recent work has been unable to confirm these increases in dehydroascorbic acid (Newill *et al.* 1984; Stankova *et al.* 1984; Sinclair *et al.* 1991). Some publications have suggested a decrease in plasma vitamin C concentrations in diabetes but this has not been confirmed by other groups. The most recent studies, and those that have attempted to match for intake, suggest little change in the plasma levels of vitamin C. (Bryszawska & Kostrzewa, 1987; Schorah *et al.* 1988; Cunningham *et al.* 1991). Studies that have investigated changes in cellular vitamin C are more consistent in finding a decrease (Chen *et al.* 1983; Bryszawska & Kostrzewa, 1987; Cunningham *et al.* 1991) with only one finding little change (Schorah *et al.* 1988).

What can we make of all this? It seems clear that dehydroascorbic acid uptake can be impaired by increased glucose levels and that this may lead to some decrease in the vitamin C concentration in some cells (especially leucocytes). In contrast, there appears to be little change in plasma ascorbic acid or dehydroascorbic acid concentrations. Whether the decrease in the cell levels is sufficient to produce an effect on cell metabolism and encourage some diabetic complications remains to be investigated. The situation does appear to represent one of the most likely examples of a direct effect of disease on vitamin C transport.

CONCLUSIONS

Because of the technical problems found in studies of vitamin C transport it is difficult to draw firm conclusions. It is clear that vitamin C is concentrated into a number of cell types, sometimes to very high levels, and that this must require expenditure of cell energy. The limited cell membrane permeability of vitamin C means that transport must also be facilitated. The process in most cells seems to require Na and prefer ascorbic acid. There is some evidence that dehydroascorbic acid is transported by a separate mechanism which can also co-transport ascorbic acid and which uses facilitated diffusion. It is also possible that leucocytes have a different mechanism from other cells for the transport of ascorbic acid, but the findings can be explained by the tendency of these cells to produce reactive species which could rapidly oxidize ascorbic acid to dehydroascorbic acid encouraging this to be the form usually transported.

There are a number of diseases which may affect vitamin C transport. The most likely candidates are diabetes, as a result of disturbed cell or plasma glucose distribution, and damage, by a cellular inflammatory response or poisons, to mucosal surfaces which secrete vitamin C. The area is ripe for further investigation, but researchers must be careful to determine which component or metabolite of the vitamin is being transported.

REFERENCES

Anderson, R., Theron, A. J. & Ras, G. J. (1988). Ascorbic acid neutralizes reactive oxidants released by hyperactive phagocytes from cigarette smokers. *Lung* **166,** 149–159.

Banerjee, A. (1982). Blood dehydroascorbic acid and diabetes mellitus in human beings. *Annals of Clinical Biochemistry* **19,** 65–70.

Basu, J., Mikhail, M. S., Payraudeau, P. H., Palan, P. R. & Romney, S. L. (1990). Smoking and the antioxidant ascorbic acid: plasma, leukocyte and cervicovaginal cell concentrations in normal healthy women. *American Journal of Obstetrics and Gynecology* **163,** 1948–1952.

Basu, T. K. & Schorah, C. J. (1982). *Vitamin C in Health and Disease*. London: Croom Helm Ltd.

Bergsten, P., Amitai, G., Kehrl, J., Dhariwal, K. R., Klein, H. G. & Levine, M. (1990). Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes. Depletion and reaccumulation. *Journal of Biological Chemistry* **265,** 2584–2587.

Bigley, R., Wirth, M., Layman, D., Riddle, M. & Stankova, L. (1983). Interaction between glucose and dehydroascorbate transport in human neutrophils and fibroblasts. *Diabetes* **32,** 545–548.

Bode, A. M., Cunningham, L. & Rose, R. C. (1990). Spontaneous decay of oxidised ascorbic acid (dehydro-L-ascorbic acid) evaluated by high-pressure liquid chromatography. *Clinical Chemistry* **36,** 1807–1809.

Bowers-Komro, D. M. & McCormick, D. B. (1991). Characterisation of ascorbic acid uptake by isolated rat kidney cells. *Journal of Nutrition* **121,** 57–64.

Bron, A. J. & Brown, N. A. P. (1987). Perinuclear lens retrodots – a role for ascorbate in cataractogenesis. *British Journal of Ophthalmology* **71,** 86–95.

Bryszawska, M. & Kostrzewa, E. (1987). Ascorbic acid content in plasma and erythrocytes of insulin dependent diabetic patients. *Medical Science Research* **15,** 1277–1278.

Chatterjee, I. B. & Banerjee, A. (1979). Estimation of dehydroascorbic acid in the blood of diabetic patients. *Analytical Biochemistry* **98,** 368–374.

Chatterjee, I. B., Majumder, A. K., Nandi, B. K. & Subramanian, N. (1975). Synthesis and some functions of vitamin C in animals. *Annals of the New York Academy of Sciences* **258,** 24–47.

Chen, M. S., Hutchinson, M. L., Pecoraro, R. E., Lee, W. Y. L. & Labbe, R. F. (1983). Hyperglycaemia induced intracellular depletion of ascorbic acid in human mononuclear leukocytes. *Diabetes* **32,** 1078–1081.

Choi, J. L. & Rose, R. C. (1989). Transport and metabolism of ascorbic acid in human placenta. *American Journal of Physiology* **257,** C110–C113.

Chu, T. C. & Candia, O. A. (1988). Active transport of ascorbate across the isolated rabbit ciliary epithelium. *Investigative Ophthalmology and Visual Science* **29,** 594–599.

Cochrane, C. G., Spragg, R. G. & Revak, S. D. (1983). Pathogenesis of the adult respiratory distress syndrome. Evidence of oxidant activity in bronchoalveolar lavage fluid. *Journal of Clinical Investigation* **71,** 754–761.

Cullen, E. I., May, V. & Eipper, B. A. (1986). Transport and stability of ascorbic acid in pituitary cultures. *Molecular and Cellular Endocrinology* **48,** 239–250.

Cunningham, J. J., Ellis, S. L., McVeigh, K. L., Levine, R. E. & Calles-Escandon, J. (1991). Reduced mononuclear leukocyte ascorbic acid content in adults with insulin-dependent diabetes mellitus consuming adequate vitamin C. *Metabolism: Clinical and Experimental* **40,** 146–149.

Davis, K. A., Lee, W. Y. L. & Labbe, R. F. (1983). Energy dependent transport of ascorbic acid into lymphocytes. *Federation Proceedings* **42,** 2011.

Dhariwal, K. R., Washko, P., Hartzell, W. O. & Levine, M. (1989). Ascorbic acid within chromaffin granules. *Journal of Biological Chemistry* **264,** 15404–15409.

Diliberto, E. J., Heckman, G. D. & Daniels, A. J. (1983). Characterization of ascorbic acid transport by adrenomedullary chromaffin cells. *Journal of Biological Chemistry* **258,** 12886–12894.

Frei, B., England, L. & Ames, B. N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences* **86,** 6377–6381.

Garcia, R. & Municio, A. M. (1990). Effect of *Escherichia coli* endotoxin on ascorbic acid transport in isolated adrenocortical cells. *Proceedings of the Society for Experimental Biology and Medicine* **193,** 280–284.

Helbig, H., Korbmacher, C. & Weiderholt, M. (1990). Mechanism of ascorbic acid transport in the aqueous humor. *Fortschritte der Ophthalmologie* **87,** 421–424.

Helbig, H., Korbmacher, C., Wohlfarth, J., Berweck, S., Kühner, D. & Wiederholt, M. (1989). Electrogenic Na^+-ascorbate co-transport in cultured bovine pigmented ciliary epithelial cells. *American Journal of Physiology* **256,** C44–C49.

Kallner, A. B., Hartmann, D. & Hornig, D. H. (1981). On the requirements of ascorbic acid in man: steady state turnover and body pool in smokers. *American Journal of Clinical Nutrition* **34,** 1347–1355.

Kapeghian, J. C. & Verlangieri, A. J. (1984). Effects of glucose on ascorbic acid uptake in heart endothelial cells. Possible pathogenesis of diabetic angiopathies. *Life Sciences* **34,** 577–584.

Lovstad, R. A. (1987). Copper catalysed oxidation of ascorbate (vitamin C). Inhibitory effect of catalase, superoxide, serum proteins and amino acids. *International Journal of Biochemistry* **19,** 309–313.

McLennan, S., Yue, D. K., Fisher, E., Capogreco, C., Heffernan, S., Ross, G. R. & Turtle, J. R. (1988). Deficiency of ascorbic acid in experimental diabetes. Relationship with collagen and polyol pathway abnormalities. *Diabetes* **37,** 359–361.

Mann, G. V. & Newton, P. (1975). The membrane transport of ascorbic acid. *Annals of the New York Academy of Sciences* **258,** 243–252.

Mefford, I. N., Oke, A. F. & Adams, R. N. (1981). Regional distribution of ascorbate in human brain. *Brain Research* **212,** 223–226.

Meucci, E., Mortorana, G. E., Ursitti, A., Miggiano, G. A. D., Mordente, A. & Castelli, A. (1987). Vitamin C bovine serum albumin binding behaviour. *Italian Journal of Biochemistry* **36,** 75–81.

Molloy, T. P. & Wilson, C. W. M. (1980). Protein binding of ascorbic acid. 1. Binding to bovine serum albumin. *International Journal for Vitamin and Nutrition Research* **50,** 380–386.

Mooradian, A. D. (1987). Effect of ascorbate and dehydroascorbate on tissue uptake of glucose. *Diabetes* **36,** 1001–1004.

Moser, U. & Weber, F. (1984). Uptake of ascorbic acid by human granulocytes. *International Journal for Vitamin and Nutrition Research* **54,** 47–53.

Newill, A., Habibzadeh, N., Bishop, N. & Schorah, C. J. (1984). Plasma levels of vitamin C components in normal and diabetic subjects. *Annals of Clinical Biochemistry* **21,** 488–490.

Padh, H. & Aleo, J. J. (1987). Characterisation of the ascorbic acid transport by 3T6 fibroblasts. *Biochimica et Biophysica Acta* **901,** 283–290.

Paterson, C. A. & O'Rourke, M. C. (1987). Vitamin C levels in human tears. *Archives of Ophthalmology* **105,** 376–377.

Patriarca, M., Menditto, A. & Morisi, G. (1991). Determination of ascorbic acid in blood plasma or serum or seminal plasma using a simplified sample preparation and high performance liquid chromatography coupled with UV detection. *Journal of Liquid Chromatography* **14,** 297–312.

Pelletier, O. (1975). Vitamin C and cigarette smokers. *Annals of the New York Academy of Sciences* **258,** 156–168.

Penney, J. R. & Zilva, S. S. (1943). The chemical behaviour of dehydro-L-ascorbic acid in vitro and in vivo. *Biochemical Journal* **37,** 403–417.

Raghoebar, M., Huisman, J. A. M., Van den Berg, W. B. & Van Ginneken, C. A. M. (1987). Characteristics of the transport of ascorbic acid into leucocytes. *Life Sciences* **40,** 499–510.

Rose, R. C. (1987). Solubility properties of reduced and oxidised ascorbate as determinants of membrane permeation. *Biochimica et Biophysica Acta* **924,** 254–256.

Rose, R. C. (1988). Transport of ascorbic acid and other water soluble vitamins. *Biochimica et Biophysica Acta* **947,** 335–366.

Rose, R. C. (1989). Renal metabolism of the oxidised form of ascorbic acid (dehydro-L-ascorbic acid). *American Journal of Physiology* **256,** F52–F56.

Rose, R. C. & Choi, J. L. (1990). Intestinal absorption and metabolism of ascorbic acid in rainbow trout. *American Journal of Physiology* **258,** R1238–R1241.

Rossi, F., Bellavite, P., Berton, G., Grzeskowiak, M. & Papini, E. (1985). Mechanism of production of toxic oxygen radicals by granulocytes and macrophages and their function in the inflammatory process. *Pathology Research and Practice* **180,** 136–142.

Schectman, G., Byrd, J. C. & Gruchow, H. W. (1989). The influence of smoking on vitamin C status in adults. *American Journal of Public Health* **79,** 158–162.

Schorah, C. J., Bishop, N., Wales, J. K., Hansbro, P. M. & Habibzadeh, N. (1988). Blood vitamin C concentrations in patients with diabetes mellitus. *International Journal for Vitamin and Nutrition Research* **58,** 312–318.

Schorah, C. J., Habibzadeh, N., Hancock, M. & King, R. F. G. J. (1986). Changes in plasma and buffy layer vitamin C concentrations following major surgery: what do they reflect? *Annals of Clinical Biochemistry* **23,** 566–570.

Schorah, C. J., Sobala, G. M., Sanderson, M., Collis, N. & Primrose, J. N. (1991). Gastric juice ascorbic acid: effects of disease and implications for gastric carcinogenesis. *American Journal of Clinical Nutrition* **53,** Suppl., 287S–293S.

Sinclair, A. J., Girling, A. J., Gray, L., Le-Guen, C., Lunec, J. & Barnett, A. H. (1991). Disturbed handling of ascorbic acid in diabetic patients with and without microangiopathy during high dose ascorbate supplement. *Diabetologia* **34,** 171–175.

Sobala, G. M., Pignatelli, B., Schorah, C. J., Bartsch, H., Sanderson, M., Dixon, M. F., Shires, S., King, R. F. G. & Axon, A. T. R. (1991). Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach. *Carcinogenesis* **12,** 193–198.

Sobala, G. M., Schorah, C. J., Sanderson, M., Dixon, M. F., Tompkins, D. S., Godwin, P. & Axon, A. T. R. (1989). Ascorbic acid in the human stomach. *Gastroenterology* **97,** 357–363.

Socci, R. R. & Delamere, N. A. (1988). Characteristics of ascorbate transport in rabbit iris ciliary body. *Experimental Eye Research* **46,** 853–861.

Som, S., Basu, S., Mukherjee, D., Deb, S., Choudhury, P. R., Mukherjee, S., Chatterjee, S. N. & Chatterjee, I. B. (1981). Ascorbic acid in diabetes mellitus. *Metabolism* **30,** 572–577.

Spector, R. (1977). Vitamin homeostasis in the central nervous system. *New England Journal of Medicine* **296,** 1393–1398.

Stankova, L., Riddle, M., Larned, J., Burry, K., Menashe, D., Hart, J. & Bigley, R. (1984). Plasma ascorbate concentrations and blood dehydroascorbate transport in patients with diabetes mellitus. *Metabolism* **33,** 347–353.

Stankova, L., Rigas, D. A. & Bigley, R. H. (1975). Dehydroascorbate uptake and reduction by human blood neutrophils, erythrocytes and lymphocytes. *Annals of the New York Academy of Sciences* **258,** 238–242.

Washko, P., Rotrosen, D. & Levine, M. (1989). Ascorbic acid transport and accumulation in human neutrophils. *Journal of Biological Chemistry* **264,** 18996–19002.

Washko, P., Rotrosen, D. & Levine, M. (1990). Ascorbic acid accumulation in plated human neutrophils. *FEBS Letters* **260,** 101–104.

Wilson, J. X. & Dixon, S. J. (1989*a*). Ascorbic acid transport in mouse and rat astrocytes is reversibly inhibited by furosemide, SITS and DIDS. *Neurochemical Research* **14,** 1169–1175.

Wilson, J. X. & Dixon, S. J. (1989*b*). High-affinity sodium-dependent uptake of ascorbic acid by rat osteoblasts. *Journal of Membrane Biology* **111,** 83–81.

Wilson, J. X., Jaworski, E. M., Kulaga, A. & Dixon, S. J. (1990). Substrate regulation of ascorbate transport activity in astrocytes. *Neurochemical Research* **15,** 1037–1043.

Printed in Great Britain