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Transport of Vitamin C in Animal and Human Cells

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The transport systems of animal and human tissues for vitamin C are reviewed with respect to their properties. It emerges that pure diffusion plays only a very minor role, while a variety of more or less specific transporters is found on cellular membranes. Although most tissues prefer the reduced ascorbate over the oxidized dehydroascorbic acid and have high-affinity transporters for it, there are several examples for the reversed situation. Special attention is given to similarity or identity with glucose transporters, especially the GLUT-1 and the sodium-dependent intestinal and renal transporters, and to the very widespread dependence of ascorbate transport on sodium ions. The significance of ascorbate transport for vitamin C-requiring and nonrequiring species as well as alterations in states of disease can be seen from ample experimental evidence.

KEY WORDS: Ascorbate transport; oxidized dehydroascorbic acid; high affinity transporters.

INTRODUCTION

In a thorough review article on the transport of water-soluble vitamins which appeared in 1988, R. C. Rose explained why transport systems for these substances are necessary for a sufficient supply of organisms and cells (Rose, 1988). Among the water-soluble vitamins, vitamin C has always been at center stage because it needs to be taken in relatively large (some say mega-) quantities by the human organism. Primates share this fate with guinea pigs and some other animal species, because their liver lacks the enzyme gulonolactone oxidase which generates ascorbic acid from gulonic acid in other animals (King, 1973; Halver *et al.*, 1975; Chatterjee *et al.*, 1975). These species differ from others in that they need a special uptake system for vitamin C in their intestines. As to all other organs and tissues of the body which require the vitamin for various biosyntheses, all animals need transport systems to accumulate sufficient quantities. The functions of vitamin C have been reviewed in textbooks and monographs frequently and will not be discussed in detail here.

In the mentioned review, the emphasis was on the transport systems of various organs and tissues for acquiring ascorbic acid, or rather its anion ascorbate (asc[2]), and its oxidized form, dehydroascorbic acid (DHA). In the meantime specific properties of such transport systems have been described in some detail. Recent data point to a possibility of understanding the molecular biology and the genetic background of vitamin C transport (Vera *et al.*, 1993).

This review will not scale up the earlier one to the present state of knowledge, but will concentrate on the known properties of vitamin C transport molecules, of which there seem to exist several different types. Pre-1988 literature will be cited again if pertinent to the topic of the chapter and if the mechanism proposed therein is still in discussion. The chapters of this article concentrate on different possible features of transport of oxidized and reduced vitamin C in several cell types, tissues, and organs with an attempt to draw conclusions on possible mechanisms of transport through biological membranes. Most of the evidence concentrates on the cell surface

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[2] Abbreviations: Asc, reduced ascorbate; DHA, dehydroascorbic acid (oxidized ascorbate).

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membrane, but there are also some data on intracellular ascorbate transport. Many cells and tissues can accumulate ascorbate against a concentration gradient with intracellular concentrations well into the mM region (Washko *et al.*, 1989). Compared to the normal concentration in blood plasma (around 50 μM, Lentner, 1981), this means up to 40-fold enrichment. In principle, two possibilities exist to establish these concentration gradients: active transport systems driven directly or indirectly by ATP hydrolysis (i.e., by a sodium gradient), or, since intracellular vitamin C is practically exclusively in the reduced asc state, preferential uptake of oxidized vitamin (DHA) in a concentration gradient, intracellular reduction, and impermeability of the membrane, sometimes together in one cell membrane, will be presented.

SIGNIFICANCE OF FREE DIFFUSION

Though free diffusion of vitamin C (in one of the two forms) does not account for all transport events, it cannot be definitely excluded as an additional mechanism. Neutral ascorbic acid (which exists only at pH below 5) as well as dehydroascorbic acid are relatively lipophilic compared to the ascorbate anion (Mann and Newton, 1975; Sapper *et al.*, 1985), but the degree of hydrophobicity is apparently insufficient to enable the uncharged species to diffuse freely through membranes (Rose, 1987). Neutral ascorbate could pass biological membranes along a pH gradient, if this is established by proton movement in the opposite direction (Sapper *et al.*, 1985).

Ascorbate may also pass a lipid membrane as a sodium complex (Lohmann and Winzenburg, 1983), which may be the diffusible species where diffusion takes place. Uptake of ascorbate via diffusion could be expected in the intestine because there the resorbed vitamin is carried away by the blood. Diffusion contributes a significant part of the total uptake (Maffia *et al.*, 1993), but is not sufficient for an adequate supply (Rose, 1985). In eel intestine, the rates become comparable at concentrations around 50 μM. At higher concentrations, the carrier-mediated uptake prevails (Maffia *et al.*, 1993).

Diffusion may contribute to uptake of ascorbate in cerebral capillaries (Lam and Daniel, 1986), in placental syncytiotrophoblasts (Ingermann *et al.*, 1986; Choi and Rose, 1989), heart endothelial cells (Bassingthwaighte *et al.*, 1985), and in paracellular flux of ascorbate into the aqueous humor of the eye in rats (DiMattio, 1989a) and bullfrogs (DiMattio and Streitman, 1991). Preparation of biological samples must be handled with great care, because impurities like blood vessel endothelia, etc. give erroneous evidence for apparent diffusion (Zhou *et al.*, 1990).

Ascorbate has been used as a nondiffusible extracellular marker in heart (Bassingthwaighte *et al.*, 1985, Reil *et al.*, 1987). In osteoblasts, diffusion has been shown to be very unlikely because in contrast to other small organic acid molecules ascorbate uptake did not decrease the intracellular pH (Wilson and Dixon, 1989a). Recently, Welch *et al.* (1993) experimentally excluded diffusion of ascorbate as a significant mechanism of uptake in human fibroblasts.

In the case of intracellular transport and release of ascorbate or dehydroascorbate from cells, the situation is much less clear. Vitamin C taken up by the small intestine (Rose *et al.*, 1988) or reabsorbed by the kidneys (Rose, 1986a) is released into the blood plasma by transport through the basolateral membranes. This may be diffusion in a concentration gradient, whereby both forms of the vitamin are released, at least in intestine. In kidneys, a gradient of pH (alkaline outside) may contribute to drive diffusion (Sapper *et al.*, 1985).

Release of ascorbate by diffusion from some eye tissues like retinal pigment epithelium (Khatami *et al.*, 1986), ciliary body (Socci and Delamere, 1988), aqueous humor (Chu and Candia, 1988), and from placenta (Choi and Rose, 1989) seems possible, but not from nervous tissues (Thorn *et al.*, 1985; Knoth *et al.*, 1987). In any case, membranes must be quite selective in their permeability for ascorbate, because ascorbate does not bind to intracellular structures in cells with high intracellular concentration (Rose, 1989; Washko *et al.*, 1989).

Intracellular transport of ascorbate, though very important in nervous tissues containing chromaffin granules, has not been studied very thoroughly. Ascorbate uptake into the secretory granules of neurohypophyses could be a diffusion process, since it is nonsaturable and does not prefer either form of the vitamin (Thorn *et al.*, 1985, 1986). However, uptake of DHA into the neurosecretory endings is slower than into the secretory granules; thus, the existence of a carrier in the membranes of the latter is quite likely (Thorn *et al.*, 1986).

UPTAKE OF DEHYDROASCORBIC ACID AS THE PREFERRED SPECIES

The discussion on whether DHA is the preferred membrane transport form of vitamin C has several aspects. First, regeneration of ascorbate, which is an important antioxidant, from the oxidized forms has recently become the subject of intense investigation. DHA as well as ascorbate are highly unstable. Chemical or enzymatic reduction are often preceded by cellular uptake of DHA. Second, under physiological conditions (notably, pH) DHA is more lipophilic than ascorbate and may thus be a better membrane permeator (Mann and Newton, 1975). This notion has been challenged by thorough investigation concerning the physical properties of vitamin C species (Lohmann and Winzenburg; 1983, Sapper *et al.*, 1985; Rose, 1987).

Impermeability of microsomal (endoplasmic reticulum derived) membranes for DHA argues against its free passage through membranes (Peterkofsky *et al.*, 1987). Third, DHA may be transported by a glucose transporter (see below) and this transport may be much more efficient than that of the reduced vitamin. Care has to be taken in judging the physiological significance of these data because of the much lower concentration and the biological instability of DHA (Wang *et al.*, 1992).

Toxicity of oxidized vitamin C toward the integrity of membranes not only makes considerations of the physiological significance of uptake of DHA difficult, but may also experimentally impede a clean investigation (Bianchi and Rose, 1986a; Rose *et al.*, 1992).

Nevertheless, it seems unequivocally proven that blood cells and related tumor cell lines prefer DHA over reduced ascorbate (Bigley and Stankova, 1974; Orringer and Roer, 1979; Bigley *et al.*, 1983; Stahl *et al.*, 1985; Bianchi and Rose, 1986b; Rose, 1986b; McGown *et al.*, 1990; Schweinzer and Goldenberg, 1992; Washko *et al.*, 1993; Vera *et al.*, 1993).

In erythrocytes, uptake of DHA seems to operate in concert with release of reduced ascorbate and so to contribute to stabilization of ascorbate in plasma (Orringer and Roer, 1979; Schipfer *et al.*, 1985; McGown *et al.*, 1990; Iheanacho *et al.*, 1993). In cultured tumor cells, this mechanism of ascorbate stabilization could not be verified (Schweinzer *et al.*, 1993; Schweinzer and Goldenberg, 1993) and may be unique for the red blood cells.

In neutrophils, Washko *et al.* (1989, 1992, 1993) report transport of both ascorbate and DHA (the latter being preferred) and report affinities for the ascorbate transport (see below), while Vera *et al.* (1993) find transport of DHA only [similar to what is found in tumor cells by Schweinzer and Goldenberg (1992)], but with affinities similar to those reported for ascorbate by the other group. A K_M of 2 mM was reported for neutrophils (Bigley *et al.*, 1983), which would be characteristic for a low-affinity transporter. 2 mM is an unrealistically high concentration of DHA, such that this transporter would never be saturated. Thus nonsaturability is frequently reported (Schweinzer and Goldenberg, 1992) in the physiological concentration range.

Kidney and intestine both seem to be able to take up DHA from the luminal side, a logical property considering the important of (re)absorption of the vitamin. But wherever studied, DHA transport into the cells through the basolateral membrane was faster than through the luminal membrane and nonsaturable (Bianchi and Rose, 1985a,b; Bianchi *et al.*, 1986; Rose *et al.*, 1988; Rose, 1989; Rose and Choi, 1990). The model for intestinal and renal ascorbate transport (Rose *et al.*, 1988) can still be considered as valid. According to this model, DHA can be taken up both from the lumen and from the blood and is reduced inside the cell. Ascorbate can then either be released to the blood or can contribute to protection of the mucosal cells from oxidative stress.

Whether the transport systems for reduced and oxidized vitamin C are different or equal in kidney cell membranes is still a matter of debate (Bowers-Komro and McCormick, 1991). A clear difference for transport of ascorbate and DHA was found in pancreas (Zhou *et al.*, 1991), where ascorbate transport is saturable and DHA transport is not.

In the human placenta, the situation seems to be different. DHA uptake in syncytiotrophoblasts is preferred over that of ascorbate. Apart from supplying the fetus with vitamin C, preferential uptake of the oxidized vitamin and its subsequent reduction protects the placenta from the toxicity of DHA (Ingermann *et al.*, 1988; Choi and Rose, 1989; Rose *et al.*, 1992).

Ocular tissues belong to those best studied for ascorbate transport because they need vitamin C for protection against photoinduced oxidative damage (Clemetson, 1989). As to the preferences for the two vitamin forms, there are differences between various tissue types. While ascorbate is preferred in most cases, the reverse situation has been reported in

ocular lens (Kern and Zolot, 1987), in ciliary epithelium (Helbig *et al.*, 1989), in corneal epithelium (Bode *et al.*, 1991), and in lacrimal gland (Dreyer and Rose, 1993). Two different transport systems have been described for a virus-transformed retinal pigment epithelial cell line (Lam *et al.*, 1992), but considering the affinities (high for ascorbate, low for DHA) and the physiological concentrations, ascorbate can be considered to be the preferred species as generally reported for the tissues of this organ.

Neurosecretory cells and fibroblasts, the main users of metabolic ascorbate, prefer the reduced form (Diliberto *et al.*, 1983; Levine and Pollard, 1983; Thorn *et al.*, 1986; Padh and Aleo, 1987; Thorn *et al.*, 1991; Welch *et al.*, 1993).

HIGH-AFFINITY AND LOW-AFFINITY TRANSPORTERS

Reduced ascorbate is transported into several tissues by a transport system with affinity in the physiological range. A narrow range of affinities is reported for various investigation models. With the exception of the high-affinity transporter found by Levine and his associates in fibroblasts and neutrophils with K_M values below 10 μM (Washko *et al.*, 1989; Welch *et al.*, 1993), the values are from around 17 μM for pancreatic islet cells (Zhou *et al.*, 1991) to around 130 μM for bovine retinal pigment epithelium (Khatami, 1987).

Some tissues show notable exceptions of rather low to extremely low affinity, again those with a mainly absorptive function, namely intestine (Rose and Choi, 1990, Maffia *et al.*, 1993) and placenta, or at least microvilli isolated from it (Iioka *et al.*, 1987). In the latter case (K_M = 1.33 mM), a diffusion artefact caused by contaminating material cannot be excluded (see above, Zhou *et al.*, 1990). Also in eel intestine (Maffia *et al.*, 1993), the low affinity allows diffusion to compete with specific transport at physiological concentrations (see above).

Neutrophils and fibroblasts also contain a low-affinity transporter with affinities around 5 mM (Washko *et al.*, 1989; Welch *et al.*, 1993). It is possible that other tissues aside from fibroblasts and neutrophils also contain a low-affinity transporter with affinities in the millimolar range, but this has not been described yet.

The affinities in the most commonly found range sometimes depend on the sodium content of the medium (Padh and Aleo, 1987; Wilson and Dixon, 1989a; Wilson, 1989) (see below).

Affinities for DHA are reported for neutrophils (Bigley *et al.*, 1983; Vera *et al.*, 1993), lymphocytes (Stahl *et al.*, 1985), erythrocytes (Bianchi and Rose, 1986b), rat kidney (Bowers-Komro and McCormick, 1991, but estimated as inhibitory constant), and virus-transformed retinal pigment epithelial cells (Lam *et al.*, 1993). With the notable exception of the value found by Vera *et al.* (1993), all are significantly higher than those for ascorbate if compared in the same tissue (between 400 μM and 13 mM). In many other cases, no affinity constant is found for DHA (Thorn *et al.*, 1986; Helbig *et al.*, 1989; Rose and Choi, 1990; Zhou *et al.*, 1991), while ascorbate transport almost always follows saturation kinetics and is saturated at physiological concentrations (Thorn *et al.*, 1985, 1986, 1991; Chu and Candia, 1988; Raghoebar *et al.*, 1987; Zhou *et al.*, 1990, 1991). Where two transporters with different affinities are found, at least the high-affinity transporter can be assumed to be always saturated *in vivo* (Washko *et al.*, 1989; Welch *et al.*, 1993). Low-affinity transporters also have higher capacity of transport, whether for the same (Welch *et al.*, 1993) or for the other (Lam *et al.*, 1993) vitamin C species. Vera *et al.* (1993) transfected the glucose transporter GLUT-1 into oocytes to demonstrate its inherent ability to allow facilitated diffusion of DHA. The affinities for DHA found in neutrophils correspond to those of the transfected transporter. The higher K value is in accordance with the affinity found for DHA transport in neutrophils by Bigley *et al.* (1983) and strengthens evidence that this well-known transporter is able to transfer DHA, but is no proof that this actually happens in vivo. Some characteristic values of affinity constants are listed in Table I. The conclusion from the last two chapters seems to be quite straightforward: apart from exceptional situations (e.g., activation of neutrophils, oxidative stress) ascorbate is the preferred transport from wherever its transport is possible, simply because of its much higher physiological concentration and affinity. This by no means rules out the significance of uptake of DHA to prolong its lifetime via intracellular reduction.

ROLE OF GLUCOSE IN TRANSPORT OF VITAMIN C

The structural similarity between glucose and vitamin C is evident such that some cross affinity of

Table I. Selected Affinity Constants for Vitamin C Transport

Tissue	Transported form	K_M	Reference
Adrenal	Asc	30 μM	Diliberto *et al.*, 1983
Adrenal	Asc	100 μM	Levine and Pollard, 1983
Astrocytes	Asc	32 μM	Wilson, 1989
Embryonic chick brain	Asc	37 μM	Wilson, 1990
Neurohypophysis	Asc	97 μM	Thorn *et al.*, 1991
Cerebral capillaries	Asc	125 μM	Lam and Daniel, 1986
Pancreatic islets	Asc	17.6 μM	Zhou *et al.*, 1991
Neutrophils	DHA	2 mM	Bigley *et al.*, 1983
Neutrophils	Asc	2–5 μM 6–7 mM	Washko *et al.*, 1989
CLL-lymphocytes	DHA	3.5 mM	Stahl *et al.*, 1985
Erythrocytes	DHA	412 μM	Bianchi and Rose, 1986b
Retinal pigment epithelium (cat)	Asc	42 μM	Khatami *et al.*, 1986
Retinal pigment epithelium (bovine)	Asc	125 μM	Khatami, 1987
Retinal pigment epithelial cells (SV-40-transformed)	Asc	41 μM	Lam *et al.*, 1993
Ciliary epithelium (bovine)	Asc	76 μM	Helbig *et al.*, 1989
Ciliary epithelium (rabbit)	Asc	80 μM	Delamere *et al.*, 1993
Leydig cells	Asc	33 μM	Moger, 1987
Placental microvilli	Asc	1.33 mM	Iioka *et al.*, 1987
3T6 fibroblasts	Asc	112 μM	Padh and Aleo, 1987
Human fibroblasts	Asc	6 μM 5 mM	Welch *et al.*, 1993
Osteoblasts (ROS 17/28)	Asc	30 μM	Wilson and Dixon, 1989a
Kidney cells	Asc	36 μM	Bowers-Komro and McCormick, 1991
	DHA	13 mM (Ki)	
Intestinal brush border (trout)	Asc	220 μM	Rose and Choi, 1990
Intestinal brush border (eel)	Asc	750 μM	Maffia *et al.*, 1993
	DHA	5.67 mM	
GLUT-1 transporter	DHA	60 μM 3.5 mM	Vera *et al.*, 1993

the respective transporters can be expected. However, the kinship is apparently more than incidental.

Inhibition of transport of ascorbate (Socci and Delamere, 1988; Helbig *et al.*, 1989; Zhou *et al.*, 1990; Fay *et al.*, 1990; Thorn *et al.*, 1991; Delamere *et al.*, 1993; Washko and Levine, 1992) or of DHA (Bigley *et al.*, 1983; Ingermann *et al.*, 1986; Kern and Zolot, 1987) by typical inhibitors of glucose transport (cytochalasin B, phloretin, phloridzin) falls into this incidental category because the inhibitors are not absolutely specific for the glucose transporters. The same can be stated for inhibition by high concentrations of glucose itself (Padh *et al.*, 1985; Khatami *et al.*, 1986; Khatami, 1987; Mooradian, 1987; Thorn *et al.*, 1991; Zhou *et al.*, 1991; Washko and Levine, 1992) because ascorbate transporters may bind glucose with low affinity.

In other tissues, the participation of a glucose transporter-related membrane protein has actually been disproved (Moger, 1987; DiMattio, 1989a,b; Choi and Rose, 1989; DiMattio and Streitman, 1991; Fisher *et al.*, 1991).

There is also considerable evidence for transport of ascorbate and/or DHA via the same transporter as glucose, resulting from thorough kinetic analysis (Ingermann *et al.*, 1986, 1988; Kern and Zolot, 1987; Diliberto *et al.*, 1983; Washko and Levine, 1992) or at least a shared responsibility of the glucose transporter for DHA uptake (Bianchi and Rose, 1986b).

The conclusive experiment to demonstrate the ability of the GLUT-1 glucose transporter to mediate uptake of DHA has been carried out by Vera *et al.* (1993) by transfection of the gene and expression in frog oocytes. This is in accordance with findings of Mooradian (1987) for preferred uptake of DHA via a glucose transporter in brain but not in muscle and those of Bianchi and Rose (1986b) for uptake in erythrocytes. The fact that GLUT-1 effectively mediates transport of DHA, however, does not

imply that this is the preferred way of vitamin C uptake in any tissue.

SIGNIFICANCE OF SODIUM IONS

It has been known for more than 10 years that uptake of ascorbate in adrenals is dependent on the presence of sodium, and that the main effect of sodium is the increase of the affinity, i.e., the lowering of the apparent dissociation constant of the transporter–ligand complex (Diliberto *et al.*, 1983). This specific property of the ascorbate transporter has been confirmed for some cell types, e.g., 3T6 fibroblasts (Padh and Aleo, 1987), osteoblastic cells (Wilson and Dixon, 1989a), astrocytes (Wilson, 1989), and embryonic chick brain (Wilson, 1990). This finding as well as the frequently found stoichiometry of 2 : 1 for the sodium/ascorbate ratio (Padh and Aleo, 1987; Helbig *et al.*, 1989; Wilson *et al.*, 1991; Maffia *et al.*, 1993) argue against significant involvement of the GLUT-type transporter in the investigated tissues. In most cases it is the uptake of reduced ascorbate which is found to depend on sodium, where sometimes this is definitely excluded for DHA (Bianchi and Rose, 1985b; Ingermann *et al.*, 1986; Helbig *et al.*, 1989; Zhou *et al.*, 1991). There are examples, however, for sodium dependence of DHA uptake (Kern and Zolot, 1987; Lam *et al.*, 1993) as well as independence of sodium for transport of ascorbate (Rose *et al.*, 1985; Bianchi and Rose, 1985a; Choi and Rose, 1989; Dreyer and Rose, 1993).

The majority of investigators consistently confirm the dependence on the presence of sodium (Thorn *et al.*, 1985, 1991; Khatami *et al.*, 1986; Khatami, 1987; Socci and Delamere, 1988; Chu and Candia, 1988; Zhou *et al.*, 1990; DiMattio and Streitman, 1991; Dixon and Wilson, 1992a,b) without reporting more specific characteristics.

A ratio of 2 : 1 is a rather definitive argument against diffusion of the Na-ascorbate complex through the lipid layer, as suggested by Lohmann and Winzenburg (1983), and raises the question of electrogenicity of the transport process. This has indeed been shown in some cases (Padh and Aleo, 1987; Wilson and Dixon, 1989b; Helbig *et al.*, 1989; Wilson *et al.*, 1991; Lam *et al.*, 1993), but not always (Moger, 1987). Intestinal and renal brush borders absorb ascorbate by sodium-gradient driven processes, which leaves open a possible identity with the sodium-dependent glucose transport in these membranes (Rose *et al.*, 1985; Rose, 1986a; Bianchi and Rose, 1985b, Rose and Choi, 1990; Bowers-Komro and McCormick, 1991; Maffia *et al.*, 1993).

STEREOSPECIFICITY OF ASCORBATE TRANSPORT

D-Araboascorbic acid (isoascorbic acid) is a largely but not completely ineffective isomer of vitamin C, i.e., it shows some biological potency (Stanek *et al.*, 1963). Lack of biological activity as a cofactor of vitamin C-dependent enzymatic syntheses might in part be due to inefficient transport into the cells, a hypothesis for which evidence has been given, at least in case of avian tendon cells (Kipp and Schwarz, 1990).

Isoascorbate can get into cells by diffusion as a potassium complex (Lohmann and Winzenburg, 1983), but this is not likely to be of physiological significance. High concentrations of isoascorbate (in the mM region) inhibit ascorbate uptake in many tissues (Khatami *et al.*, 1986; Khatami, 1987; Iioka *et al.*, 1987; Chu and Candia, 1988; Helbig *et al.*, 1989; Thorn *et al.*, 1991; Bowers-Komro and McCormick, 1991; Maffia *et al.*, 1993; Wilson and Dixon, 1989a). In the work cited last, the inhibitory constant for isoascorbate in osteoblastic cells is only 380 μM, one order of magnitude larger than the affinity constant for ascorbate (see Table I). This parallels the finding for tendon cells cited above. Connective tissue may be exceptional in this respect. However, isoascorbate has also been found to be a general inhibitor of vitamin C action (Arakawa *et al.*; 1986) and to be not transported at all in some cell types (Diliberto *et al.*, 1983; Moger, 1987). Definitive conclusions are difficult as long as the transport properties of the isomer are not studied *per se*. Yet the significance of studies like these appears questionable and more or less academic.

TRANSPORT OF VITAMIN C UNDER HEALTHY AND PATHOLOGICAL CONDITIONS

The literature on the pathology of relative vitamin C deficiency (apart from the more historical significance of scurvy) is widespread and reaches far into anecdotal medicine. Some aspects with clear

biochemical background emerge, however, and some of these can be connected with transport activities.

Only few species require ascorbate as a vitamin and therefore need intestinal uptake systems. Differences between species requiring and not requiring vitamin C have been confirmed (Rose, 1988).

A further, more interesting difference exists between the ascorbate requirements of eye tissues in diurnal and nocturnal animals, like guinea pigs (diurnal, high requirement of ascorbate in aqueous humour) and rats (low requirement) (DiMattio, 1989b) as well as different strains of mice (Koskela *et al.*, 1989).

The ability of several tissues to take up ascorbate is influenced by environmental conditions. One is the ascorbate supply: Low ascorbate increases the transport capacity, but not the affinity of astrocytes and osteoblasts (Wilson *et al.*, 1990; Dixon and Wilson, 1992). On the other hand, intestinal uptake in guinea pigs is repressed by hypervitaminosis (Karasov *et al.*, 1991).

Differentiation or transformation of cells also has some influence on ascorbate transport according to changes in metabolic activities (Stahl *et al.*, 1985; Wilson and Dixon, 1989b; Wilson, 1989), as well as oxygen supply (Wilson and Jaworski, 1992). Two types of pathological situations are prominent in the study of ascorbate transport activation of the immune system and diabetes mellitus.

The latter has naturally given rise to many studies concerning parallel properties of ascorbate/DHA and glucose transport. Yet no links with the insulin-sensitive GLUT-4 transporter (Gould and Holman, 1993) have ever been reported. Nevertheless, hyperglycemia can apparently inhibit uptake of ascorbate or DHA by other glucose transporters or the specific ascorbate transporter(s) (Mann and Newton, 1975; Bigley *et al.*, 1983; Padh *et al.*, 1985; Pecoraro and Chen, 1987; Ingermann *et al.*, 1988; DiMattio, 1992a,b; Vera *et al.*, 1993). Some reports contradict this assumption, however (Rose, 1986b). Increase of DHA as a consequence of impaired transport and toxic effects of the oxidized vitamin may contribute to diabetic complications.

Neutrophils are important users of ascorbate to protect themselves from unwanted consequences of the respiratory burst, and their capacity to accumulate this protecting molecule is greatly enhanced on activation (Raghoebar *et al.*, 1987; Washko *et al.*, 1993). Also, the cytokine TGF-β can induce ascorbate transport (Dixon and Wilson, 1992b).

Changes explainable by control of transporter expression have also been found in cataract (Ringvold *et al.*, 1985), adrenalectomy (Kipp and Rivers, 1987), and renal failure (Pahl *et al.*, 1989). The mechanism of induction may involve changes in protein kinase C activity, which inhibits ascorbate transport (Delamere *et al.*, 1993), or cAMP, which stimulates (Wilson and Dixon, 1989b; Wilson, 1989).

On the other hand, a component of the complement system, C3a, inhibits ascorbate uptake in fibroblasts; thus, the changes of ascorbate transport in the immune response are quite varied (Aleo and Padh, 1985; Padh and Aleo, 1987, 1989). Possibly in this stage ascorbate is directed from connective tissue to activated neutrophils and macrophages where it is badly needed.

Finally, malarial parasites seem to insert a transport system for ascorbate into the erythrocyte membrane as they do with other proteins (Stocker *et al.*, 1986). Malaria-infected erythrocytes thus differ from their normal counterparts in their preference for reduced ascorbate (Orringer and Roer, 1979; Bianchi and Rose, 1986b; McGown *et al.*, 1990). Interestingly, the release is also enhanced (Iheanacho *et al.*, 1993), a process which might contribute to enhanced stability of ascorbate in malaria-infected blood and enhance the immune response.

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