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Na^+-independent dehydro-L-ascorbic acid uptake in renal brush-border membrane vesicles

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A membrane preparation enriched in the brush-border component of the plasma membrane was isolated from rat renal superficial cortex by a divalent cation precipitation procedure. Uptake of dehydro-L-ascorbic acid, the oxidized form of L-ascorbic acid, by the brush-border membrane vesicles was studied. The uptake mechanism was found to be sodium-independent and insensitive to the trans-membrane electrical potential difference. Uptake was saturable and subject to cis-inhibition. Concentrative uptake was demonstrated only under conditions of trans-stimulation by structural analogs. The results suggest a mechanism of facilitated diffusion for the uptake of dehydro-L-ascorbic acid in renal brush-border membranes.

Introduction

L-Ascorbic acid is required in the diets of man and guinea pig as both species lack an enzyme necessary for its biosynthesis [1]. In each of these mammals a specific small intestinal transporter has been postulated for L-ascorbic acid on the basis of kinetic data [2,3]. The use of membrane vesicles from the guinea pig ileum has allowed demonstration of an L-ascorbic acid specific transport system [4]. The use of membrane vesicles from renal cortical proximal tubules has demonstrated medium transport of L-ascorbic acid both in guinea pig and in rat [5], an animal that does have de novo L-ascorbic acid synthetic capability. The rat, however, lacks an intestinal transport system for L-ascorbic acid.

Dehydro-L-ascorbic acid is the first compound formed in the catabolism of L-ascorbic acid (Fig. 1). Dehydro-L-ascorbic acid differs chemically from L-ascorbic acid in that it lacks a dissociating proton at the carbon 3 position and is, therefore, an electrically neutral particle at physiological conditions. Dehydro-L-ascorbic acid is a chemically labile compound being hydrolyzed to 2,3-diketo-L-gulonic acid. The hydrolysis is pH and temperature dependent, having a half-time of 6.5 min at pH 7.0, 37°C [6]. Dehydro-L-ascorbic acid has the same antiscorbutic activity as L-ascorbic acid with the two being interconvertible in vivo [7], although

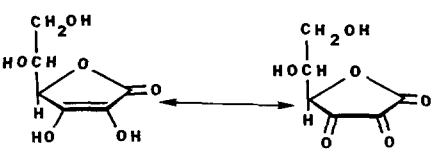

Fig. 1. Structures of the biologically interconvertible L-ascorbic acid and dehydro-L-ascorbic acid. Note that the oxidized molecule lacks the dissociable hydrogens that allow the reduced form to ionize.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

it is presently unknown where the bulk of metabolism occurs. Little is known of the transport properties of dehydro-L-ascorbic acid in the kidney. Dehydro-L-ascorbic acid is reported to be present in human plasma at a concentration of 0.6–2.8 μM [8]. On the basis of its physicochemical properties, it is likely to be filtered in the glomerulus. The present studies were done to ascertain whether or not the kidney has transport properties that participate in the conversion of dehydro-L-ascorbic acid.

Materials and Methods

(1) Preparation of kidney brush-border vesicles. Kidney brush-border vesicles were prepared by a procedure slightly modified from Malathi et al. [9]. For each experiment three rats were killed by a blow to the head. The kidneys were removed and placed in ice-cold physiological saline. The capsules were removed, and the superficial cortex was dissected from the medulla. The resulting cortical slices were placed in 30.0 ml of 100 mM mannitol/2 mM Tris-HCl, pH 7.1 (Buffer 1), on ice (all steps carried out at 2–4°C). The cortical tissue was then homogenized in a Waring-type blender at high speed for five minutes. An aliquot was removed for protein and enzyme determinations. Sufficient 1 M $CaCl_2$ was added to bring the final concentration to 10 mM $CaCl_2$. The mixture remained on ice for 15 min and was then diluted with 100 mM mannitol/2 mM Tris-HCl/10 mM $CaCl_2$/pH 7.1 (Buffer 1a) to yield a final volume 20-times that of cortical weight.

The preparation was then centrifuged at $350 \times g$ for 12 min in a Beckman J-21 centrifuge at 2°C. The pellet was discarded. The supernatant was spun down at $20\,000 \times g$ for 12 min. The pellet was resuspended in 15 ml of Buffer 1 with ten strokes of a glass/Teflon homogenizer at high speed. The mixture was made 10 mM in $CaCl_2$, remained for 15 min on ice and was brought to 30.0 ml with Buffer 1a. The mixture was centrifuged for 12 min at $700 \times g$. After discarding the pellet, the supernatant was centrifuged at $35\,000 \times g$ for 12 min. The pellet was resuspended in 30.0 ml of 100 mM mannitol/20 mM Hepes, adjusted to pH 7.4 with Tris-base (Buffer 2). The mixture was then homogenized with ten strokes of a glass/Teflon homogenizer at high speed. After centrifuging the mixture at $40\,000 \times g$ for 30 min, the resultant pellet was resuspended in 4.0 ml of Buffer 2 by passing the mixture 20 times through a 25-G stainless steel needle. The suspension was centrifuged at $2000 \times g$ for 5 min, and the small pellet was discarded. After a final centrifugation at $48\,000 \times g$ for 30 min, the pellet of purified brush-border membrane vesicles was resuspended in an appropriate pre-incubation buffer at a 1 : 5 dilution (pellet volume/buffer volume). The vesicles equilibrated on ice for 1 h.

(2) Transport measurements. The composition of incubation buffers are indicated in figure legends. The pre-incubation medium was 300 mM mannitol, 10 mM Hepes-Tris (pH 7.0) with exceptions noted. All incubations were carried out at 20°C. To a tube containing 100 μl of transport buffer containing the radiolabelled substrate, a 20 μl aliquot of a membrane vesicle suspension was added and mixed by a vortex. At a chosen time, 20 μl of the mixture were removed, added to 2.0 ml of ice-cold stop solution (transport buffer without substrate or inhibitors), mixed, and rapidly filtered through a nitrocellulose membrane filter (Whatman, 0.45 μm pore size). The filter was washed with 2.0 ml of cold stop solution. After dissolving the membranes in a dioxane based liquid scintillator, the samples were assayed for radioactive content by liquid scintillation counting. After subtracting non-specific retention of radioactivity by the membrane filters, the counts per minute were expressed as moles of substrate.

(3) Chemicals. All chemicals used were reagent grade. Water was deionized and glass distilled. L-[1-^{14}C]Ascorbic acid (8.4 mCi/mmol) and D-[1-^{3}H]glucose were obtained from New England Nuclear, Boston, MA. Dehydro-L-ascorbic acid was freshly prepared for each study from L-ascorbic acid by oxidation with elemental Br_2 [10]. Excess Br_2 was removed by bubbling with humidified N_2. All solutions were filtered (0.22 μm, Millipore) prior to use.

Purity of dehydro-L-[1-^{14}C]ascorbic acid. The lability of the L-ascorbic acid/dehydro-L-ascorbic acid/2,3-diketo-L-gulonic acid system necessitates that careful analysis of the ^{14}C-label must be performed on a routine basis. We used HPLC to insure that complete conversion of L-ascorbic acid

to dehydro-L-ascorbic acid was achieved and to determine the extent of dehydro-L-[1-^{14}C]ascorbic acid breakdown during each protocol [11].

(4) Protein and enzyme assays. Protein determinations were by the method of Lowry et al. [11] with bovine serum albumin used as reference protein. Specific membrane marker enzymes were chosen for assay in the starting tissue homogenates and final pellets as means of estimating the purity of the preparation. The brush-border specific enzyme chosen was leucine aminopeptidase, the activity of which was determined by a commercially available procedure (bmc Single Vial$_{TM}$ LAP, Cat. No. 124869, Biodynamics, Indianapolis, IN 46250, U.S.A.). The basolateral membrane marker enzyme chosen was ($Na^+ + K^+$)-ATPase. The activity of this marker enzyme was determined by the method of Scharschmidt et al. [12]. Cytosolic enzymes were represented by D-glucose-6-phosphate dehydrogenase activity, assayed by Sigma No. 345-UV (Sigma Chemical Company, St. Louis, MO, U.S.A.). Lysosomal contamination was estimated by the level of total acid phosphatase activity (Sigma No. 104). The possible contamination of the preparation by fragments of the endoplasmic reticulum was monitored by assaying for the level of NAD oxidoreductase by the method of Sottocasa et al. [13]. Mitochondrial contamination was determined by using the method of Fleischer and Fleischer [14] to assay for succinate-cytochrome *c* reductase activity.

Results

(1) Evaluation of the final preparation

The final membrane pellet was examined for possible contamination by cellular organelles. There was no significant enrichment of any marker enzyme assayed except leucine aminopeptidase. The activity of this enzyme was increased 14.7-fold with respect to the starting homogenate. The brush-border membrane vesicles prepared for this study were intact and capable of transport as may be seen in the concentrative overshoot beyond the equilibrium value for the uptake of D-glucose in the presence of an Na^+ gradient (out-to-in) (Fig. 2). The replacement of NaCl by KCl (Fig. 2) prevented the occurrence of concentrative uptake; similar results were obtained with choline chloride or mannitol (data not shown).

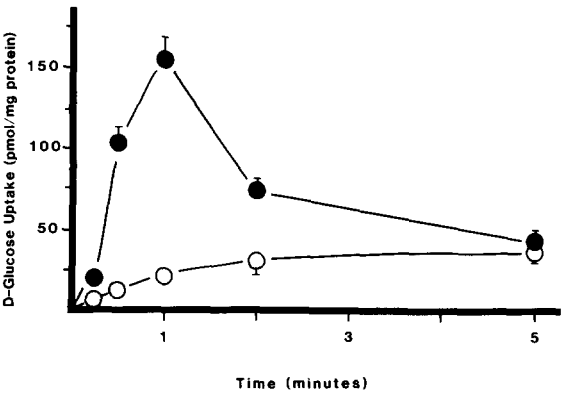

Fig. 2. D-Glucose uptake into microvillous membrane vesicles. Rat renal cortical microvillous membrane vesicles were prepared as described in the text. The vesicles, pre-equilibrated in 300 mM mannitol and 20 mM Hepes-Tris (pH 7.0), were incubated with 50 μM D-[^{3}H]glucose at 20°C in media containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.0), and either 100 mM NaCl (●) or 100 mM KCl (○); $n = 8$. The bars represent the standard error of the mean and are smaller than the symbol used when not shown in this and subsequent figures.

(2) Transport

As shown in Fig. 3, the uptake of dehydro-L-ascorbic acid into vesicles of the brush-border membrane of superficial rat renal cortical proximal tubules had no apparent cation dependency as the time-course of uptake ina 100 mM NaCl gradient (out-to-in) demonstrated no concentrative

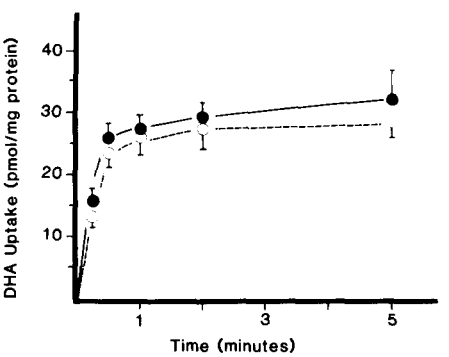

Fig. 3. The time-course of dehydro-L-ascorbic acid (DHA) uptake in brush-border membrane vesicles prepared from rat superficial renal cortex. The vesicles, pre-equilibrated in 300 mM mannitol, 20 mM Hepes-Tris (pH 7.0), were incubated at 20°C in the following medium: 100 mM NaCl (●) or 100 mM KCl (○), 100 mM mannitol, 20 mM Hepes-Tris (pH 7.0), 65 μM dehydro-L-[^{14}C]ascorbic acid ($n = 12$).

'overshoot'. When K^+ replaced Na^+ in the external medium no significant difference was noted in the time-course of dehydro-L-ascorbic acid uptake. Sodium chloride replacement by choline chloride or mannitol gave similar results as for K^+-medium (data not shown). Similar results were seen in guinea pig and rabbit. The vesicles prepared by our method are sealed and capable of transport in that Na^+-dependent concentrative uptake of D-[^{3}H]glucose was observed in control studies.

(3) Binding vs. transport

Total uptake of a solute by an isolated membrane preparation may be due to transport into an intravesicular space and/or binding to a membrane surface. One way of distinguishing between these two possibilities is to analyze the osmotic sensitivity of solute uptake by an isolated membrane preparation. The amount of solute transported into an internal space should be in direct proportion to the intravesicular volume at equilibrium conditions. The intravesicular volume may be modified by the addition of impermeant solutes to the external medium [14]. The results of such a study of the sensitivity of dehydro-L-ascorbic acid uptake to osmotic effects are illustrated in Fig. 4. The abscissa intercept indicates that there is no significant contribution of membrane binding to the total uptake of dehydro-L-ascorbic acid.

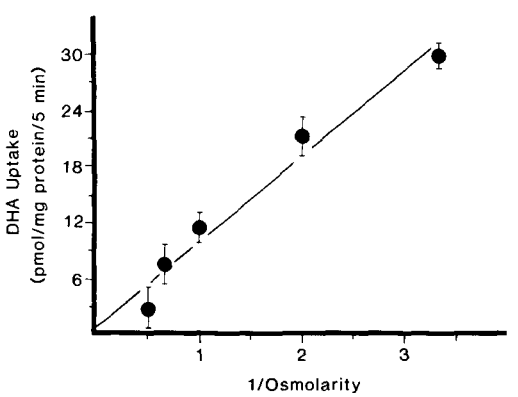

Fig. 4. Dehydro-L-ascorbic acid (DHA) uptake as a function of incubation medium osmolarity. The vesicles were pre-equilibrated as in Fig. 2 and incubated at 20°C for 5 min in 100 mM NaCl, 100 mM mannitol, 20 mM Hepes-Tris (pH 7.0), 65 μM dehydro-L-[^{14}C]ascorbic acid, and sufficient sucrose to give the indicated external osmolarity ($n = 8$; $r = 0.97$).

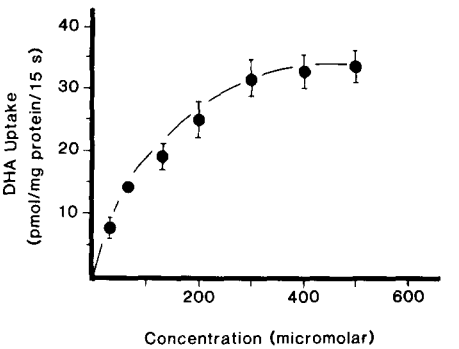

Fig. 5. The uptake of dehydro-L-ascorbic acid (DHA) in renal brush-border membranes at various substrate concentrations. The membranes were pre-equilibrated as in Fig. 2 and incubated at 20°C for 15 s in a medium containing 100 mM NaCl, 100 mM mannitol, 20 mM Hepes-Tris (pH 7.0), and dehydro-L-[^{14}C]ascorbic acid from 32.5 to 500 μM ($n = 12$).

(4) Kinetics of uptake

The transport of dehydro-L-ascorbic acid across the brush-border membrane of superficial proximal tubules from rat renal cortex is saturable as demonstrated in Fig. 5. The apparent kinetic constants were determined by double reciprocal plots from 15-s uptake values measured at 20°C in the presence of an initial 100 mM NaCl gradient (out-to-in). At shorter incubation times the amount of radioactivity taken up was too small to yield reliable results. It appears from Fig. 3 that values obtained at 15 s may yield a reasonable approximation of initial uptake rates. In the rat, $K_m = 167 \pm 20$ μM dehydro-L-ascorbic acid and $V = 11.6 \pm 1.3$ pmol · (mg protein)$^{-1}$ · (15 s)$^{-1}$ ($n = 8$). To ensure that the vesicles prepared in this laboratory functioned as those reported in a prior study, the apparent K_m for L-ascorbic acid transport in rat renal vesicles was determined to be 399 ± 15 μM ($n = 6$), a result that agrees with that of Toggenburger et al. [5].

(5) Cis-inhibition

The uptake of dehydro-L-ascorbic acid was inhibited by the presence of L-ascorbic acid and D-isoascorbic acid (the epimer of L-ascorbic acid at C_5) in the external medium. The time course of dehydro-L-[^{14}C]ascorbic acid uptake in the presence of analogs is shown in Fig. 6. Dixon plot analysis of L-ascorbic acid inhibition of uptake at

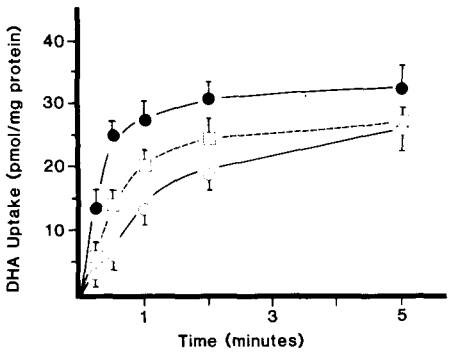

Fig. 6. The uptake of dehydro-L-ascorbic acid (DHA) in brush-border membranes in the presence and absence of L-ascorbic acid. Pre-equilibration conditions were as indicated in Fig. 2. Incubation was with 65 μM dehydro-L-[^{14}C]ascorbic acid at 20°C in 300 mM mannitol, 20 mM Hepes-Tris (pH 7.0) without (●) inhibitors and with (○) 500 μM L-ascorbic acid or 500 μM D-isoascorbic acid (□); ($n = 8$).

65 μM and 130 μM dehydro-L-[^{14}C]ascorbic acid indicates competitive inhibition with $K_i = 0.551 \pm 0.072$ mM ($n = 6$).

(6) Trans-stimulation

Trans-stimulation of dehydro-L-ascorbic acid transport was investigated by preloading 500 μM L-ascorbic acid inside the vesicles to act as an elicitor of counterflow. The presence of an outwardly directed L-ascorbic acid gradient allowed concentrative uptake of 65 μM dehydro-L-ascorbic acid with gradual efflux to an equilibrium value as shown in Fig. 7. Preloading the renal brush-border vesicles with 500 μM D-isoascorbic acid stimulated the uptake of 65 μM dehydro-L-ascorbic acid but did not result in concentrative uptake (results not shown).

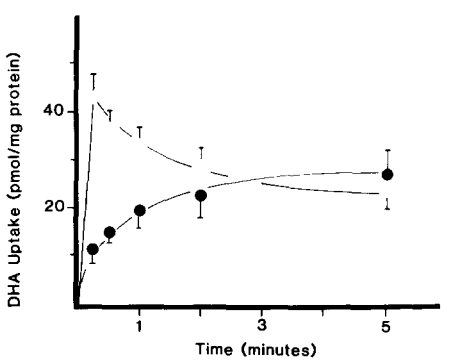

Fig. 7. The trans-stimulation of dehydro-L-ascorbic acid (DHA) transport in rat renal brush-border membranes by L-ascorbic acid. The vesicles were pre-equilibrated in 300 μM mannitol, 20 mM Hepes-Tris (pH 7.0) with (○) and without (●) 500 μM L-ascorbic acid. Incubation was at 20°C in 300 μM mannitol, 20 mM Hepes-Tris (pH 7.0), and 65 μM dehydro-L-[^{14}C] ascorbic acid ($n = 8$).

(7) Dependence of renal transport of dehydro-L-ascorbic acid on membrane potential

The electrical potential across the isolated membrane preparation was modified by three separate interventions: (a) an inwardly directed K^+-gradient in the presence and absence of valinomycin, (b) anion substitution of Cl^-, and (c) an outwardly directed K^+ gradient in the presence and absence of valinomycin. In case (a) the presence of the ionophore resulted in an exaggerated inside-positive diffusion potential due to a more rapid K^+ entry. In the type (b) study, the substitution of more lipophilic anions than Cl^- resulted in an increased inside-negative diffusion potential because these ions are more permeant. When the K^+ gradient is directed outwardly, (c), and valinomycin is present, an enhanced inside-negative potential results due to more rapid cation efflux.

For (a) the vesicles were preincubated with either 1% ethanol or 10 μg valinomycin/mg protein (1% ethanol) in 300 mm mannitol/20 mM Hepes-Tris, (pH 7.0). Transport studies were done in 100 mM NaCl/50 mM KCl/20 mM Hepes-Tris (pH 7.0). The results of such a study are shown in Table I. A decrease in the D-glucose overshoot was observed in our control studies (data not shown) which is consistent with previous observations [15]. There was a small inhibition of dehydro-L-ascorbic acid uptake due to valinomycin in the presence of an inwardly directed Na^+ gradient. This inhibition was not observed when there was no Na^+ gradient present.

For the experiments in case (b) an Na^+ gradient was initially established (100 mM out to in) and the anion was varied by the use of the Na^+ salts of SCN^-, N_3^-, NO_3^- and SO_4^{2-}. Where necessary, mannitol was used to maintain isosmotic conditions. The buffer was maintained at pH 7.0 by 20 mM Hepes-Tris. The effect of anionic substitution on dehydro-L-ascorbic acid transport was observed at 15 s (Table II). Observations obtained with D-glucose were consistent with those previously

TABLE I

THE EFFECT OF INWARDLY OR OUTWARDLY DIRECTED K^+-GRADIENTS ON THE UPTAKE OF DEHYDRO-L-ASCORBIC ACID IN RENAL BRUSH-BORDER VESICLES

The vesicles were pre-equilibrated in 300 mM mannitol, 20 mM Hepes-Tris (pH 7.0) with or without 10 μg valinomycin/mg protein. Incubation of these vesicles with 65 μM dehydro-L-[^{14}C]ascorbic acid was at 20°C in media containing 50 mM KCl and 20 mM Hepes-Tris (pH 7.0) along with either 100 mM NaCl or 200 mM mannitol ($n = 8$). Other vesicles were pre-equilibrated in 200 mM mannitol, 50 mM KCl, 20 mM Hepes-Tris (pH 7.0) with or without 10 μg valinomycin/mg protein. Incubation with 65 μM dehydro-L-[^{14}C]ascorbic acid was at 20°C in medium containing 100 mM NaCl and 100 mM mannitol, or 300 mM mannitol, 20 mM Hepes-Tris (pH 7.0). ($n = 8$). n.s., not significant; * $p < 0.01$.

Initial extravesicular concentrations (mM)		Initial intravesicular KCl concn. (mM)	Valino-mycin	Expected electrical change inside	Dehydro-L-ascorbic acid acid uptake (pmol/mg protein/15s)	% Change from control
NaCl	KCl					
100	50	0	−		15.9 ± 1.6	
100	50	0	+	+	11.1 ± 1.1	−30
0	50	0	+	+	14.9 ± 1.3	n.s.
100	8.3	50	−		15.0 ± 1.9	
100	8.3	50	+	−	20.2 ± 1.7	+35 *
0	8.3	50	+	−	15.8 ± 1.5	n.s.

reported (data not shown) [16]. The initial rates obtained for the uptake of dehydro-L-ascorbic acid in the presence of various sodium salts reflect those observed by Toggenburger et al. [5] for L-ascorbic acid. The trend observed for L-ascorbic acid uptake is $N_3^- > NO_3^- > Cl^- > SCN^- > SO_4^{2-}$ and is unusual in that the azide and nitrate positions are exchanged with respect to D-glucose, and thiocyanate appears to be inhibitory. The azide effect was further evaluated to determine if it is due solely to the induced inside-negative diffusion potential as previously assessed for L-ascorbic acid [5]. Vesicles were pre-equilibrated in 50 mM KCl, 200 mM mannitol, 20 mM Hepes-Tris (pH 7.0) and 0.1% ethanol or 10 μg valinomycin/mg protein. These vesicles were incubated 15 s in a medium consisting of 100 mM NaN_3, 50 mM KCl, 20 mM Hepes-Tris and 65 μM dehydro-L-[^{14}C]ascorbic acid. With the azide diffusion potential short-circuited by the presence of K^+ and valinomycin, the uptake of dehydro-L-ascorbic acid was still enhanced when compared to the uptake in the absence of azide (28.6 ± 2.7 vs. 13.1 ± 1.8 pmol · (mg protein)$^{-1}$ · (15 s)$^{-1}$; $p < 0.01$, $n = 6$).

For case (c) vesicles were pre-equilibrated with 50 mM KCl, 200 mM mannitol, 20 mM Hepes-Tris (pH 7.0) and 1% ethanol or 10 μg valinomycin/mg protein in 1% ethanol. The external medium contained either 100 mM NaCl and 100 mM mannitol, or 300 mM mannitol, 20 mM Hepes-Tris (pH 7.0). The addition of the ionophore created an exaggerated intravesicular negative electrical potential due to an increased rate of K^+ efflux down its chemical gradient. The results are indi-

TABLE II

THE EFFECT OF ANION GRADIENTS ON DEHYDRO-L-ASCORBIC ACID UPTAKE

The vesicles, pre-equilibrated as in Fig. 2, were incubated with 65 μm dehydro-L-[^{14}C]ascorbic acid at 20°C for 15 s in media containing 100 mM Na^+ salts of the anions. The media also contained 20 mM Hepes-Tris (pH 7.0) and sufficient mannitol to maintain isosmotic conditions ($n = 8$; ** $p < 0.01$, * $p < 0.05$).

Na^+ salt	Dehydro-L-ascorbic acid uptake (pmol/mg protein / 15 s)
NaCl (control	14.9 ± 1.5
NaN_3	25.7 ± 2.0 **
$NaNO_3$	16.9 ± 1.4
NaSCN	11.3 ± 1.0 *
$NaSO_4$	10.9 ± 0.7 **

cated in Table I. In the presence of a Na^+ gradient and the K^+-ionophore valinomycin, the initial uptake of dehydro-L-ascorbic acid is increased. Such an increase was not observed in the absence of either the ionophore or an Na^+ gradient.

Discussion

The vesicles prepared from brush-border membranes of proximal tubules of the superficial renal cortex by cationic precipitation for use in this study were intact and capable of transport (Figs. 3 and 4). Substrate binding was demonstrated to be an insignificant portion of total dehydro-L-ascorbic acid uptake (Fig. 4). The mechanism of transport was demonstrated to have the characteristics of facilitated diffusion in that saturation (Fig. 5), cis-inhibition (Fig. 6), and trans-stimulation (Fig. 7) of solute uptake occur under appropriate conditions.

The determination of kinetic parameters of solute transport into an isolated membrane system requires important considerations to be satisfied. The intravesicular concentration of substrate must be small as the trans-effect can never be predicted. Some substrates may modify transport at one side of the membrane and not the other [19], or exhibit different modifying effects at each side [20]. The ideal situation is the determination of initial rate. In addition, the driving force should be kept constant. It therefore follows that incubations must be kept as short as possible.

In the present study the earliest time point that we were able to observe was 15 s, as shorter incubations gave radioactivity levels too low to yield reliable results due to both a low system capacity for the substrate and a low specific activity of the radiolabel. Even using protein levels greater than those reported by other investigators in studying hexose or amino acid transport [18] did not circumvent the problem.

Dehydro-L-ascorbic acid appears to cross the brush-border membrane of renal tubules by two mediated pathways. The primary one is a previously undescribed Na^+-independent, electrical potential-insensitive pathway. the secondary pathway is postulated to be the Na^+-dependent L-ascorbic acid system described by Toggenburger et al. [5]. Several observations suggest that these two pathways co-exist.

An Na^+-independent pathway is quite apparent from the evidence of carrier-mediated uptake in the absence of Na^+. Evidence that the Na^+-dependent L-ascorbic acid system is also used is more subtle in that the initial uptake of dehydro-L-ascorbic acid is not significantly decreased due to the absence of Na^+ (Fig. 3). First, the uptake of dehydro-L-ascorbic acid is affected by the membrane potential only in the presence of Na^+. This is consistent with translocation of the cation with dehydro-L-ascorbic acid, which is not electrically charged under physiological conditions. The magnitude of the response of dehydro-L-ascorbic acid transport to membrane potential is not large, consistent with the hypothesis that only a small portion of this non-electrolyte transport is coupled with Na^+. Second, anion effects are similar on Na^+-dependent transport of L-ascorbic acid [5] and on dehydro-L-ascorbic acid. These effects include inhibited uptake of each substrate by thiocyanate and stimulated uptake of each by azide in a fashion that is not solely related to the change in the membrane potential induced by azide.

If dehydro-L-ascorbic acid uptake does proceed, in part, on the Na^+-dependent L-ascorbic acid carrier, our observation that concentrative uptake does not occur contrasts with observations on the uptake of L-ascorbic acid, and requires an explanation. This may be attributed to dissipation of the Na^+-gradient before the low capacity system can transport sufficient substrate to create a concentration gradient. The Na^+-independent transport system could also tend to prevent concentrative uptake of dehydro-L-ascorbic acid by the Na^+-dependent system because as soon as the intra-vesicular dehydro-L-ascorbic acid concentration began to exceed the external medium concentration, backflux on the former carrier system would be anticipated. It must be concluded, however, that the major form of vitamin C presented to the kidney for filtration (L-ascorbic acid), is the primary form available for conservation.

It is noted that the rat, a species that does not absorb L-ascorbic acid from the intestine, can reabsorb dehydro-L-ascorbic acid from the glomerular filtrate as does the guinea pig. The renal transport system for dehydro-L-ascorbic acid represents a conservation mechanism for total ascorbic acid.

Of potential pathological significance may be the competitive inhibition of dehydro-L-ascorbic acid uptake observed in the presence of D-isoascorbic acid. This compound is used as an antioxidant by the U.S. food industry and is absorbed by man. D-Isoascorbic acid does appear in the glomerular filtrate [21], and has been shown to inhibit L-ascorbic acid uptake in renal vesicles [5]. The presence of D-isoascorbic acid in the renal tubule may compromise complete reabsorption of dehydro-L-ascorbic acid.

In marked contrast with the present results, Toggenburger et al. [5] reported that renal brush-border vesicles transport very little, if any, dehydro-L-ascorbic acid. Details concerning preparation, storage, use and analysis of dehydro-L-ascorbic acid were not provided. This is of concern because of the hydrolytic lability of dehydro-L-ascorbic acid in vitro [6]. As dehydro-L-ascorbic acid, which appears in the plasma [8], has the same antiscorbutic activity in vivo as L-ascorbic acid [7] and as no dehydro-L-ascorbic acid or its metabolite 2,3-diketo-L-gulonic acid appear in urine of primates with or without L-ascorbic acid supplements in their diet [7,22], it would appear that the kidney does, indeed, have an uptake mechanism(s) for total L-ascorbic acid conservation.

A more complete picture of the renal handling of dehydro-L-ascorbic acid requires that transport in isolated basolateral membranes be studied. We have observed that both L-ascorbic acid and dehydro-L-ascorbic acid are transported by sodium-independent facilitated diffusion mechanisms at the serosal pole of the renal brush-border cell (Bianchi, J. and Rose, R.C., unpublished observations). Additionally, the metabolism of dehydro-L-ascorbic acid in the proximal tubule cells must be elucidated. In preliminary studies, it appears that dehydro-L-ascorbic acid is rapidly reduced to L-ascorbic acid in isolated renal tubules of the rat and guinea pig. Whether or not this reduction is quantitative remains to be determined.

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