Kinetics of Sodium Succinate Cotransport across Renal Brush-Border Membranes*

Stephen H. Wright†‡§, Bruce Hirayama†‡§, Jonathan D. Kaunitz†, Ian Kippen‡§, and Ernest M. Wright†‡§

From the †Department of Physiology, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, California 90024 and the §§Cedars-Sinai Medical Center, Los Angeles, California 90048

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The kinetics of Na/succinate cotransport across renal brush-borders was studied using membrane vesicles. Initial rates of succinate uptake (J) were estimated from 1-s uptakes measured under voltage-clamped conditions. Lowering the external (cis) sodium concentration reduced the succinate K, without affecting the Jmax. Increasing the intravesicular (trans) sodium concentration reduced both Jmax and K. This trans inhibition by Na was relieved by trans-succinate. Varying the membrane potential from -60 to +60 reduced succinate transport in an exponential manner, which was reflected by a K, effect, i.e. an increase in the K, with no change in Jmax. The membrane potential results suggest that the succinate/sodium-carrier complex bears a net positive charge. On the basis of these and earlier observations, we propose that Na/succinate cotransport across renal brush-borders is described by an iso ordered Quad Quad reaction kinetics in which three Na ions first bind to the carrier to increase its affinity for succinate.

Rabbit renal brush-border membranes have a specific Na+-dependent transport pathway for Krebs cycle intermediates (Kippen et al., 1979; Wright et al., 1980, 1981, 1982a, 1982b). This system transports both dicarboxylic acids, including succinate, and tricarboxylates such as citrate. However, both succinate and citrate are transported as divalent anions. We have shown that three Na ions are transported with each molecule of succinate and citrate, and that this causes a depolarization of the membrane potential. In the present study, we examine the kinetic basis for the effects of Na+ and membrane potential on succinate transport. Using techniques to measure initial rates of transport under well defined conditions, we provide evidence that succinate transport is an ordered process, in which the three Na+ ions bind first to increase the affinity of the transporter for succinate. We offer a quantitative kinetic model describing events associated with succinate transport.

EXPERIMENTAL PROCEDURES

Membrane Isolation—Brush-border membrane vesicles were prepared from rabbit renal cortex as described by Wright et al. (1980).

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† Recipient of a fellowship from the National Institutes of Health.
‡ Present address: Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724.
§ To whom correspondence should be addressed.

The brush-borders were enriched 10-fold with respect to the specific activity of trehalase compared to the initial cortical homogenate, while (Na,K)-ATPase was reduced at least 2-fold and succinate dehydrogenase was undetectable. Membranes were washed once in 25 ml of an appropriate preincubation buffer (detailed in the figure legends) containing valinomycin (25 µg/ml), pelleted, and resuspended in a small volume of the same buffer. Several minutes before the initiation of an uptake experiment, the proton ionophore, FCCP, 1  was added to the membranes to a final concentration of 75 µM (FCCP was omitted in experiments examining the effect of membrane potential on transport). Both valinomycin and FCCP were added from ethanolic stock solutions; the final concentration of ethanol was less than 1% and was constant in all experiments. Vesicles were usually used the day of preparation but in some cases were preloaded with solutes by incubation for 20 h at 4 °C; 10-25% of transport activity was lost after storage for 24 h at 4 °C.

Transport Measurements—Uptakes were determined using a rapid filtration procedure (Wright et al., 1982b). A 900 µl aliquot of transport buffer, containing [14C]succinate and appropriate concentrations of unlabeled succinate and salts, was rapidly mixed with a 10-µl aliquot of the brush-border suspension using a vortexing shaker. The transport reaction was stopped with an ice cold iso-osmotic sorbitol quench, the reaction mixture filtered, the filter assayed for radioactivity, and the uptake calculated in moles/(mg of protein). Control experiments showed that the activation energy for succinate transport ranged from 22 to 29 kcal/mol; i.e. the transport rate decreased 20-40 fold upon lowering the temperature from 22 to 2 °C. All experiments were performed at room temperature (20-23 °C).

The data presented here were selected from representative single experiments. Unless otherwise noted, all results were verified in at least three independent experiments. Determination of kinetic constants was made by either a least squares fit of data to a linear transformation of the Michaelis-Menten equation (Wooll-Augustins-Hofstee plot; Segel, 1975), or in some cases, by two-parameter nonlinear regression analysis (Duggleby, 1981) using a microcomputer (NorthStar Horizon II).

Measurement of Membrane Potential—Electrical potential differences across the brush-border vesicle membrane were measured using the potential-sensitive cyanine dye, diS-C3-(5), as described elsewhere (Wright et al., 1981).

Chemicals—[14C]Succinate (45 Ci/mol) was purchased from New England Nuclear. FCCP was obtained from Boehringer-Mannheim. All other chemicals were purchased from Sigma and were of the highest grade available.

RESULTS

Preliminary Experiments

Initial Rates of Transport—Before attempts were made to measure the kinetics of transport, studies were carried out to verify that our technical procedures provided reasonable estimates of initial rates of succinate transport. Fig. 1 presents time courses for succinate uptake into brush-border vesicles

1 The abbreviations used are: FCCP, carbonyl cyanide p-trifluoro- methoxyphenylhydrazone; HEPES, N-2-hydroxyethylpiperazine-N'- 2-ethanesulfonic acid; diS-C3-(5), 3,3’dipropylthiodicarbocyanine iodide; PD, potential difference.
at low and near maximal rates in the presence of an inwardly directed Na⁺ gradient (100 mM NaCl out, 0 mM in; the "zero-trans" condition). Transport of 22 μM succinate was a linear function of time from 0 to 3 s (Fig. 1, left), and so 1-s uptakes appear to produce adequate estimates of the initial rate of transport (initial rate equals J; expressed in nmol/(mg min)). Uptake of 5 mM succinate was not linear from 1 to 3 s (Fig. 1, right), but the time course is described by a quadratic equation: 
\[ U = a_0 + a_1 t + a_2 t^2 \]
where \( U \) is the uptake at time \( t \), and \( a_0 \) is the estimate of initial rate (Jacquez, 1980). Using this equation, the estimated initial rate of transport was 14% greater than that estimated from the 1-s uptake in this experiment. In five experiments, the calculated initial rate was 111 ± 5% of the measured 1-s rate. We have elected to use 1-s uptakes as estimates of the initial rate of succinate transport rather than to utilize "corrected data." As a result, our estimates of the kinetic parameters \( J_{\text{max}} \) (maximal rate of succinate transport) and \( K_i \) (Michaelis constant or half-saturation constant) are approximately 10% lower than the actual values of these constants (see below).

Control of Membrane PD—We reported previously that succinate transport is rhoeogenic (Wright et al., 1981). Hence, studies of the kinetics of succinate transport must be carried out under voltage-clamped conditions to avoid any PD effects on the process (Geek and Heinz, 1976; Hopfer, 1977; Carter-Su and Kimmich, 1980). In earlier studies we have used valinomycin to clamp membrane potential and control PD (Wright et al., 1981, 1982b). An experiment designed to confirm the effectiveness of this procedure is illustrated in Fig. 2. Transmembrane electrical potentials were determined by monitoring changes in fluorescence of a PD-sensitive cyanine dye, diS-C₃-(5). The addition of 2 mM succinate resulted in a 24% increase in fluorescence of the dye suspension (Fig. 2, upper trace), indicative of a transport-induced depolarization of the vesicle membrane (Wright et al., 1981; Schell et al., in press). The lower trace in Fig. 2 shows the same experiment in the presence of valinomycin. In this case, succinate had no effect on the fluorescence of the dye suspension, indicating that the membrane potential was short circuited by K⁺ in the presence of valinomycin. Neither valinomycin nor diS-C₃-(5) have direct effects on succinate transport (Wright et al., 1981). Recently, Turner and Moran (1982) also demonstrated the effectiveness of K⁺ and valinomycin to clamp membrane potential during measurement of the kinetics of D-glucose transport in renal BBMV. In the present report, K⁺ and valinomycin were used in all subsequent experiments to control membrane PD. Unless otherwise noted, we also included the proton ionophore FCCP to short circuit the PD and to prevent the formation of H⁺ gradients. Control experiments verified that FCCP has no direct effect on succinate transport.
Fig. 3. Effect of increasing substrate on the rate of succinate transport into BBMV. Data presented as a linear transformation of the Michaelis-Menten equation: $J$ represents the rate of transport determined via 1-s uptake at substrate concentration $[S]$. The units of the ordinate are in nmol/mg min; the units of the abcissa are in microliters/mg min. The y intercept of this plot represents the $J_{\text{max}}$ for transport, the slope is $-K_t$. The concentration range studied was 0.022 to 5.0 mM; experimental conditions are as described in Fig. 1. Each point represents a single determination of uptake. The solid line is a least squares regression of data uncorrected for Na"-independent uptake of succinate, or for the deviations from true "initial" rates of transport represented by 1-s uptakes. The dashed line provides the slope and intercept of the plot when these corrections are taken into account (see text).

concentrations of substrate; i.e. we were unable to detect a diffusional component of uptake. The dashed line in Fig. 3 indicates a correction of the measured initial rates. The correction assumes that (i) 1-s uptakes of 22 $\mu$m succinate represent the true initial rate of transport (Fig. 1, left); (ii) 1-s uptakes of 5 mM succinate represent a 10% underestimate of the initial rate (Fig. 1, right); and (iii) a linear relationship exists between intervening substrate concentration and the error in the 1-s uptakes. Corrected in this fashion, the $J_{\text{max}}$ for succinate transport was increased by 8%, and the $K_t$ by 10%. Since the corrections are small, they are omitted in subsequent values for kinetic constants reported here. Inclusion of the correction in Fig. 3 serves to emphasize that 1-s uptakes represent reasonably accurate estimates of the initial rate of succinate transport. In eight experiments performed under conditions described in Fig. 3, the $J_{\text{max}}$ was $77 \pm 11$ (S.D.) nmol/(mg min) with a mean $K_t$ of 0.70 $\pm$ 0.06 mm.

Effect of cis-Na" on the Kinetics of Succinate Transport

Reduction of the cis (i.e. extravesicular) concentration of Na" results in a decrease in the rate of succinate transport (Kippen et al., 1979; Wright et al., 1980, 1982b). Consequently, we measured the kinetics of succinate transport at several concentrations of Na" (Fig. 4). When the cis concentration of Na" was reduced from 100 to 30 mM, there was a 3.7-fold increase in the apparent $K_t$ for succinate transport, with no change in $J_{\text{max}}$ (Table I). When Na" was completely replaced with choline, transport remained a saturable function of succinate concentration, but the apparent $K_t$ was increased to 69 mM compared to the control value of 0.8 mM. The $J_{\text{max}}$ was $46 \pm 12$ compared to the control value of 87 $\pm 2$ nmol/(mg min) (Table I).

Effect of trans-Na" on Succinate Transport

Increasing trans (i.e. intravesicular) concentrations of Na" caused a nonhyperbolic inhibition of succinate uptake (Fig. 5). When intravesicular Na" was increased to 100 mM (cis-Na" concentration constant at 100 mM), the initial rate of 0.02 mM succinate transport was inhibited by 90% ($3.3 \pm 0.19$ versus $0.3 \pm 0.02$ nmol/(mg min)). Transport was inhibited by 50% at a trans-Na" concentration of approximately 20 mM. Fig. 6 gives the results of an experiment examining the effect of trans-Na" on the kinetics of succinate transport. Both $J_{\text{max}}$ and $K_t$ for transport were reduced in the presence of a 30 mM trans concentration of Na", compared to the zero-trans control ($J_{\text{max}}$ of 88 reduced to 17 nmol/(mg min); $K_t$ of 1.06 reduced to 0.61 mm; see Table I). The effect of a trans concentration of succinate on succinate uptake was also examined. Under equilibrium Na" conditions (cis = trans = 100 mM), uptake of 0.23 mM succinate was reduced by 95% compared to the zero-trans condition (9.9 down to 0.5 nmol/(mg min); Fig. 7). When 2 mM succinate was included in the intravesicular solution, there was a 6-fold increase in transport under the Na" equilibrium condition, indicating that trans-succinate can relieve a portion of the inhibition caused by trans-Na" to 2 mM succinate alone produced no trans inhibitory effects and in fact, caused a slight stimulation (17%) of succinate influx (Fig. 7).

Effect of Membrane Potential on Succinate Transport

As indicated earlier, Na"-dependent succinate transport is electrogenic (Fig. 1). Fig. 8 presents the results of an experi-

TABLE I

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<th>Group</th>
<th>$[N\text{a}^+]_{\text{cis}}$</th>
<th>$[N\text{a}^+]_{\text{trans}}$</th>
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<th>$J_{\text{max}}$</th>
<th>$K_t$</th>
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constant by isomolar substitution of choline chloride for NaCl). The concentration with the addition of between 0 and 100 mM NaCl (ionic strength kept constant by isomolar substitution of choline chloride for NaCl). The transport buffer contained 100 mM NaCl, 100 mM KCl, 5 mM HEPES-Tris (pH 7.5), 25 μg/ml of valinomycin, 75 μM FCCP, and 22 μM [14C]succinate. The line describing the data was determined by a nonlinear regression fit to a “Hill-type” equation: apparent Hill coefficient = −1.75. $K_{Na^{+}}$ = 22.8 mM. Uptake at 0 intravesicular Na$^{+}$ was calculated to be 3.4 nmol/(mg min), and the regression coefficient = 0.99.

**FIG. 5.** Effect of increasing intravesicular (i.e., trans) concentration of Na$^{+}$ on the rate of succinate transport. BBMV were pre-equilibrated in solutions similar to that described in Fig. 1, with the addition of between 0 and 100 mM NaCl (ionic strength kept constant by isomolar substitution of choline chloride for NaCl). The transport buffer contained 100 mM NaCl, 100 mM KCl, 5 mM HEPES-Tris (pH 7.5), 25 μg/ml of valinomycin, 75 μM FCCP, and 22 μM [14C]succinate. The line describing the data was determined by a nonlinear regression fit to a “Hill-type” equation: apparent Hill coefficient = −1.75. $K_{Na^{+}}$ = 22.8 mM. Uptake at 0 intravesicular Na$^{+}$ was calculated to be 3.4 nmol/(mg min), and the regression coefficient = 0.99.

**FIG. 6.** Effect of trans-Na$^{+}$ on the kinetics of succinate transport. Vesicles were pre-equilibrated in solutions containing either 0 or 30 mM NaCl. Transport was measured and kinetic analysis performed as described in Figs. 1 and 3. Kinetic constants are listed in Table I.

In this study, we have demonstrated that the kinetics of succinate/C cystosol transport across the renal brush-border are dependent upon the Na$^{+}$ concentration on each side of the membrane and the membrane electrical potential difference. Both the cis-Na$^{+}$ concentration and the PD affect only the $K_{i}$ for succinate transport, while the trans concentration of Na$^{+}$ affects both $K_{i}$ and $J_{max}$. Based on these and earlier studies from this group (Kippen et al., 1979; Wright et al., 1980, 1981, 1982a, 1982b), we now propose a tentative kinetic model for Na$^{+}$-coupled cotransport of Krebs cycle intermediates across renal brush-border membranes. The model (Fig. 9) shows the transport of succinate (S) from the outside (cis) to the inside (trans) of brush-border membrane vesicles. The transporter (X) is depicted as a carrier that combines with three Na$^{+}$ ions and the divalent anionic species of succinate in an obligate

**FIG. 7.** Effect of intravesicular succinate and/or Na$^{+}$ on the rate of succinate uptake into BBMV. Vesicles contained KCl, valinomycin, and mannitol as described earlier. As indicated in the figure, in some cases vesicles also contained 100 mM NaCl (when absent, replaced with 100 mM choline chloride) and/or 2 mM succinate. The extravesicular solution contained 100 mM NaCl, KCl, valinomycin, and FCCP as described previously, and 0.2 mM [14C]succinate. Each bar represents the mean ± S.E. of a triplicate determination of uptake occurring in 1 s.

**FIG. 8.** Effect of membrane potential on succinate transport. Vesicles were pre-equilibrated in solutions containing either 50 mM K$_{2}$SO$_{4}$ (solid circles) or 5 mM K$_{2}$SO$_{4}$ (solid squares), 5 mM HEPES-Tris (pH 7.5), and valinomycin to maintain isomolarity with transport buffers. Extravesicular solutions contained 50 mM Na$_{2}$SO$_{4}$, 5 mM HEPES-Tris (pH 7.5), 25 μg/ml of valinomycin, and various concentrations of K$_{2}$SO$_{4}$ to produce the range of calculated Nernst K$^{+}$-equilibrium potentials indicated on the abscissa (mannitol is used to balance osmotic concentration). Each point is a single determination of uptake occurring in 1 s from a succinate concentration of 22 μM. Uptake is expressed in arbitrary units, normalized to the rate of uptake occurring at calculated PD values of 0 mM. The line was calculated as described under “Results.”
order to form a carrier-substrate complex. The fully loaded complex is able to translocate (i.e. cross) the membrane to complete the cycle. Although the transporter is represented as a mobile carrier, alternative mechanisms such as a gated channel are not eliminated by this formalism. The net result of 1 cycle of the transporter is the movement across the membrane of three Na⁺ ions and one molecule of succinate. In the terminology of enzyme kinetics, the system can be described as an Iso Ordered Quad reaction (Segel, 1975).

Several explicit models have already been presented for Na⁺-cotransport systems with coupling ratios of 1 (see Schultz and Curran, 1970; Heinz et al., 1972; Geck and Heinz, 1976; Hopfer and Groseclose, 1980; Turner, 1981) and 2 ( Vidaver and Shepherd, 1968). Although it is possible to derive explicit quantitative models for quadreactant systems (see Cornish-Bowden, 1979, p. 124), the number of conceivable mechanisms precludes meaningful analysis. Instead, we have used a more restrictive approach in which we sought to make simplifying assumptions. For example, Segel (1975, pp. 634–638) points out that in the absence of products (i.e. the zero-trans conditions where Naₐm = 0 and succinateₐm = 0), isomerization (i.e. the translocation steps) of the carrier does not affect the form of the velocity equation describing the forward reaction (J⁺ out → in). This experimental approach permits uptake to be treated as a simple quadreactant reaction. Further, in view of our experimental observation that changes in cis-Na⁺ concentrations affect only Kᵣ and not Jₘₐₓ (Fig. 4), the most likely reaction mechanism is a rapid equilibrium ordered system in which Na⁺ must bind to the transporter prior to succinate (Cuppoletti and Segel, 1975; Segel, 1975, p. 320). Under the zero-trans condition, the uptake of succinate and the effects of Na⁺ on this transport are described by:

$$J'/J_{\text{max}} = \frac{[S][Na]^n}{K_a K_c K_c^* (1 + [Na]/K_c) + [S][Na]^n K_c K_c^*}$$  \hspace{1cm} (1)

where Kᵣ is the apparent equilibrium dissociation constant for each Na⁺-binding site, Kᵣ is the apparent succinate-carrier dissociation constant, [S] is the concentration of substrate, and n is the number of Na⁺-binding sites. In this case, n = 3 and we make the assumption that the Na⁺ sites are equivalent and noninteracting (i.e. minimal cooperativity). The absolute magnitude of Jₘₐₓ is related to the total number of carriers [X] and the rate constants for the translocation of the loaded (Kᵣ) and unloaded (Kᵣ*) carriers across the membrane, i.e. Jₘₐₓ = [X] (Kᵣ * Kᵣ)/([Kᵣ * Kᵣ] + Kᵣ), and Kᵣ is related to the true succinate-carrier dissociation constant (Kᵣ) and the mobility of the carriers, i.e. Kᵣ = Kᵣ' (Kᵣ' + Kᵣ)(see Turner, 1981).

This equation is identical in form with that describing the effects of the ordered binding of multiple essential activators on the rate of an enzyme-mediated reaction (Segel, 1975, Equation VII-42). This model can account qualitatively and quantitatively for the observed kinetic interactions of cis-Na⁺ with succinate transport. The details of the model will be discussed in relation to the experimental findings.

**The Effect of Increasing Succinate Concentration on Transport**—The velocity equation can be rearranged to show the relationship between succinate concentration and rate of transport:

$$J' = \frac{J_{\text{max}}[S]}{K_a^3 + 3K_a^2K_c + 3K_aK_c^2 + K_c^3 + 1} K_c + [S]$$  \hspace{1cm} (2)

Inspection of Equation 2 reveals that at fixed Na⁺ concentration, transport of succinate should follow simple Michaelis-Menten type kinetics; i.e. J⁺ = Jₘₐₓ[S]/(Kᵣ + [S]), where:

$$K_c = \frac{K_a^3 + 3K_a^2K_c + 3K_aK_c^2 + K_c^3 + 1}{K_c^3} K_c \quad (3)$$

This was observed to be the case (Fig. 3).

**The Effect of Na⁺ on the Kinetic Constants, Jₘₐₓ, and Kᵣ—** Implicit in Equation 2 is the prediction that the Jₘₐₓ for succinate transport should be independent of Na⁺ concentration; at infinitely large concentrations of succinate, the influence of Na⁺ on the reaction diminishes and uptake approaches Jₘₐₓ as a limit (Fig. 4). In contrast, the Kᵣ for succinate transport increases with decreasing Na⁺ concentration (Fig. 4). Values for Kᵣ and Kᵣ were estimated by analyzing the empirically determined relationship between Kᵣ and Na⁺ concentration (Fig. 4, Table I). Two-parameter nonlinear regression analysis produced values for Kᵣ of 32.5 ± 2.4 mM (S.D.) and Kᵣ of 0.30 ± 0.02 mM. Fig. 10 shows the relationship between Kᵣ and Na⁺ concentration; the solid line was drawn according to Equation 3, using the calculated values for Kᵣ and Kᵣ. The predicted relationship calls for an infinitely large Kᵣ at 0 cis-Na⁺. However, we found that when Na⁺ was completely replaced with choline⁺, the Kᵣ was ~69 mm (Table I); i.e. the Kᵣ was high but not infinite. This suggests that
port rates in the presence of choline, K', Rb', Cs+, and NH+, are about an order of magnitude greater than with mannitol (Kippen et al., 1979; Wright et al., 1982b; Wright et al., 1982c). We have also observed that Li+ is able to support dicarboxylate transport (Kippen et al., 1979; Wright et al., 1982b). J' in 100 mM LiCl was about twice that obtained with other nonNa+ cations. Recently, we reported that uptake of 0.08 mM succinate increased with Li+ concentration in a sigmoid fashion, with a Kt\textsubscript{i} of 200 mM (Wright et al., 1982c). At saturating Li+ concentrations (500 mM) the Kt for succinate transport was 22-29 mM, i.e. intermediate between that obtained with Na+ and choline+. This strongly suggests that Li+ can drive succinate transport at a finite rate by a mechanism analogous to Na+/succinate cotransport.

**Effect of Na+ Concentration on the Rate of Succinate Transport**—The relation shown in Equation 1 predicts a nonhyperbolic interaction between Na+ concentration and the rate of uptake at fixed concentrations of succinate. We showed previously that increasing the cis concentration of Na+ results in a sigmoid-stimulation of succinate transport (Fig. 4, Wright et al., 1982). Reanalysis of the data from three separate experiments using a two-parameter nonlinear regression fit to Equation 2 yielded a mean Kt of 31.7 ± 3.4 mM, and a K, of 0.037 ± 0.06 mM. These values are in reasonable agreement with the constants determined independently from the relationship shown in Equation 3.

The model discussed above is based upon several assumptions that warrant discussion. First, we have assumed a priori that three Na+ ions are transported/molecule of succinate. Three lines of evidence support this: (i) the ratio of the succinate-stimulated Na+ flux to the Na+-dependent succinate flux is between two and three (Wright et al., 1982b); (ii) kinetic analysis of the effect of Na+ on the transport of a fixed concentration of succinate using the Garay and Garrahan (1973) equation is consistent with three Na+ ions being involved in the transport process (Wright et al., 1982b); (iii) succinate is transported as a divalent anion, yet transport causes the depolarization of brush-border membrane (Wright et al., 1981; Samarzija et al., 1981). The independence of succinate transport on H+ concentration (Wright et al., 1982a) argues against proton cotransport, and indicates that three Na+ ions are the probable source of positive charge in succinate transport. With only the first two lines of evidence, a model involving the cotransport of two Na+ can be constructed that satisfactorily accounts for the kinetic effects of Na+ reported here. However, the third observation, that transport is electrogenic, is difficult to rationalize if only two cations are transported/molecule of succinate. We consider that an n value of three is necessary to explain all the available information.

An important consequence of the observation that the coupling coefficient appears to be fixed at 3 and is independent of Na+ concentration (Wright et al., 1982b) is that only the unloaded carrier and fully loaded substrate-carrier complex can undergo a translocation step, i.e. Kn, Kp ≫ KtNa, where KtNa is the mobility of the Na+ carrier complexes (XNa, X2Na, X3Na).

The second assumption is that the three Na+ sites are equal and noninteracting. This assumption is explicit in the Garay and Garrahan (1973) analysis used by Wright et al. (1982b). Analysis of the relationship between succinate transport and Na+ concentration using the Hill equation (Segel, 1975), which assumes infinite cooperativity between sodium-binding sites, resulted in an apparent n value of 2 (i.e. 2 Na+ sites). Since independent evidence presented above is consistent with an n of 3, any interaction between sites must be weak.

There is one clue that the three Na+ sites may not be equivalent. We have observed that Li+ is a specific competitive inhibitor of Na+-dependent succinate transport (Wright et al., 1982c). The Li+ inhibitor constant of 1.2 mM suggests the presence of a site(s) with a high affinity for Li+. This is in contrast to the low apparent affinity of the transporter for Li+ in the absence of Na+ (Kt\textsubscript{i}; of 200; see above). Thus it appears that the sites presumed to have equal affinity for Na+ must have differing affinities for Li+. It is possible that the binding sites could be dissimilar with respect to Li+. If the binding sites are of equivalent field strengths, but differ in their structure, Li+ may polarize one site more than another (see Diamond and Wright, 1969). However, the observed Li+ effects may also indicate a degree of ordering or cooperativity among the activator sites that the present analysis is incapable of discerning.

The third assumption is the use of rapid equilibrium kinetics rather than the more general steady state kinetic approach. This simplifying assumption is justified by the observation that Na+ acts as a pure competitive activator of succinate transport under zero-trans conditions (Fig. 4). This observation is inconsistent with either a rapid equilibrium random, or a steady state ordered reaction (Segel, 1973), and is considered evidence for an ordered rapid equilibrium system (Fromm, 1975; Segel, 1975).

The fourth assumption is that our experimental procedures result in a zero-trans condition. This is justified by our previous observation (Wright et al., 1982b) that during the course of a 1-s uptake ([Na+]\textsubscript{t} = 0 at time 0), the intravesicular concentration of Na+ does not rise above 5 mM. This level of Na+ does not significantly affect the initial rate of succinate uptake (Wright et al., 1982b; present report, Fig. 5).

The model indicated in Equation 1 cannot account for the kinetic effects of trans concentrations of substrate because of the possible influences of isomerization (translocation) steps on the number of terms included in the rate constants. Thus we have elected to consider trans effects in terms of steady state models for multireactant iso-ordered reactions (Segel, 1975). The pattern of product inhibition observed (Figs. 6 and 7) can be described as representative of mixed-type inhibition, and is expected in the presence of products with an iso ordered multireactant system (Segel, 1975) in which the cis concentrations of substrates are not saturating. This is likely the case as Segel (1975) points out that saturating concentrations of reagents in product inhibition studies may exceed 100 times the Kp value. The inhibition pattern is consistent with either Na+ or succinate being released first from the transporter on the trans side of the membrane. However, as indicated in the model (Fig. 9), we suggest that Na+ is released first (i.e. "glide symmetry"). If succinate were released first, trans-Na+ should be an uncompetitive inhibitor of transport at saturating cis-Na+ concentrations (Segel, 1975), with Kt and J\textsubscript{max} decreased to the same degree. This was not the case; J\textsubscript{max} was reduced 5-fold, while Kt was decreased only 1.7-fold. This suggestion of ordered release is a testable hypothesis, requiring studies of the kinetics of succinate transport under a variety of trans versus cis concentrations of Na+ and succinate. Turner (1981) also suggests that glide versus mirror symmetry in cotransport systems (coupling coefficient = 1) may be distinguished by comparing activator effects on uptake under zero-trans and equilibrium conditions.

According to our model (Fig. 9), trans-Na+ inhibits transport by shifting trans-SX to tranS-SXNa and, in doing so, reduces the dissociation of succinate from SX. As expected, increasing the trans concentration of succinate relieves trans-Na+ inhibition (Fig. 7) by allowing exchange of unlabeled

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sucinate with the radioactively labeled substrate on SXNa3. In the absence of trans-Na+, trans-sucinate slightly stimulates J\textsuperscript{p} (Fig. 7), and this also may be due to isotopic exchange. Finally, the trans-Na+ inhibition of J\textsuperscript{p} also lends support to our assumption (see above) that the Na+-loaded forms of the carrier (XnNa) are virtually immobile. If the translocation rate constants for these intermediate complexes were significant, trans-Na\textsuperscript{+} should either have no effect or increase J\textsuperscript{p} (see Vidaver and Shephard, 1968).

Although we have no direct information on the relative translocation rates of the fully loaded versus the unloaded carrier, \( k_t \) and \( k_{p} \), the arguments put forward by Schultz and Curran (1970) and Geck and Heinz (1976) suggest that \( k_t \) must be greater than \( k_{p} \). Clearly, more much information about the kinetics of Na+-sucinate cotransport may be gained by expanding studies of trans substrate interactions.

The effects of membrane potential on transport kinetics can be predicted on the basis of recent models of cotransport processes (e.g. Geck and Heinz, 1976). We found that the kinetic basis for the effect of PD on sucinate transport is through changes in the \( K_t \), while \( J_{\text{max}} \) was largely unchanged. Geck and Heinz (1976), working on the premise that only translocation rates are voltage-sensitive, demonstrated that for cotransport processes in which Na\textsuperscript{+} acts as a pure competitive activator ("affinity-type" systems), PD can have a primary effect on \( K_t \) only if the fully loaded carrier is charged while the unloaded carrier is neutral. We stress that this type of analysis is limited by the number of assumptions made (Turner, 1981), but it does suggest that the fully loaded carrier (SX3Na) has a valency of +1.

There are several similarities, and an apparent difference in the qualitative characteristics of Na\textsuperscript{+}-dependent sucinate transport and the Na\textsuperscript{+}-dependent transport of glucose. As shown here for sucinate, Na\textsuperscript{+} appears to be a pure competitive activator for glucose transport.\textsuperscript{2} There is also increasing evidence that coupling coefficients for Na\textsuperscript{+}-glucose cotransport can exceed unity (Kimmich and Randles, 1960; Kaunitz et al., 1982; Turner and Moran, 1982). However, in contrast to the present finding with sucinate, the only study examining the effect of PD on Na\textsuperscript{+}-dependent glucose transport (Carter-Su and Kimmich, 1980; ATP-depleted avian enterocytes) demonstrated that membrane potential exerts its principal effect on the \( J_{\text{max}} \) rather than on the \( K_t \). Whether this reflects a real difference in the fundamental mechanisms of the Na\textsuperscript{+}-dependent transports of sucinate and glucose, or merely species differences, remains to be seen.

There is a striking parallelism between sucinate transport in renal brush-borders and glycine transport in pigeon erythrocytes (Vidaver, 1964; Vidaver and Shephard, 1968). Glycine transport is an Iso Ordered Ter Ter reaction system in which two Na\textsuperscript{+} ions bind first to the carrier to decrease the \( K_t \) for glycine transport. trans-Na\textsuperscript{+} inhibits transport, and this is relieved by trans-glucose. As in the case of sucinate, this was interpreted as indicating that the Na\textsuperscript{+}-carrier complexes are immobile. Quantitative analysis, in fact, suggested that the translocation rate constants were in the sequence \( K_{\text{NaG}} \gg K_{\text{NaP}} \gg K_{\text{NaH}} \). Furthermore, Vidaver and Shephard (1968) obtained evidence that the system was asymmetrical under zero-trans conditions; i.e. the \( J_{\text{max}} \) for glycine efflux was greater than that for influx. So far, we have neither tackled the question of symmetry in renal BBMV, nor attempted to evaluate kinetic parameters under equilium conditions (see also Turner, 1981).

The results of the present study indicate that the reabsorption of sucinate, and thus of other Krebs cycle intermediates, across the proximal tubule can be expected to be sensitive to the luminal and intracellular concentrations of Na\textsuperscript{+}, and to the potential difference across the brush-border of the proximal tubular cell. The use of zero-trans conditions, in combination with clamp-membrane PD values, represents a non-physiological situation. Physiological variations in intracellular Na\textsuperscript{+} concentration and in membrane PD are thus expected to have considerable influence on the rate of uptake of dicarboxylates, and, perhaps, of other solutes.

In summary, the transport of succinate across the renal brush-border is influenced by both the cis and trans concentrations of Na\textsuperscript{+}, and by membrane potential. cis-Na\textsuperscript{+} and PD affect the \( K_t \) for transport, while trans-Na\textsuperscript{+} influences both \( J_{\text{max}} \) and \( K_t \). The transport process can be described as an iso Ordered Quad Quad reactant system, involving the ordered binding of three Na\textsuperscript{+} ions to a transporter to increase its affinity for the subsequent binding of succinate. The overall transport reaction may involve glide symmetry, in which Na\textsuperscript{+} is released from the trans respect of the carrier prior to the release of succinate. Further experimental work is required to test and obtain a fuller quantitative description of this model.

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