J. Physiol. (1983), **335**, *pp.* 307–318
With 4 text-figures
Printed in Great Britain

KINETICS OF SODIUM-DEPENDENT SOLUTE TRANSPORT BY RABBIT RENAL AND JEJUNAL BRUSH-BORDER VESICLES USING A FLUORESCENT DYE

BY RICHARD E. SCHELL, BRUCE R. STEVENS AND ERNEST M. WRIGHT
From the Department of Physiology, University of California Medical Center, Los Angeles, CA 90024, U.S.A.

(*Received 2 June 1982*)

SUMMARY

1. The kinetics of Na-coupled solute transport by renal and jejunal brush-border vesicles in the rabbit were examined using the potential-sensitive fluorescent dye diS-C_3-(5).

2. All organic solutes known to be transported across these membranes by Na-coupled mechanisms increase the fluorescence of the dye in the presence of Na, but not K. An increase in fluorescence (ΔF) corresponds to a depolarization of the electrical potential difference (5–60 mV) across the brush-border membrane in the intact cell. ΔF was independent of the valency of the transported solute.

3. The fluorescence response was saturable, and for twelve solutes the K_f, i.e. the concentration of the substrate generating 50 % of the maximal response, agreed quite closely with the K_t values reported from tracer studies.

4. For six solutes increasing the Na concentration decreased K_f, and this agrees with the effect of Na on the kinetics of succinate transport in renal vesicles.

5. We conclude that D-glucose, neutral amino acids and imino acids are co-transported with Na across both renal and jejunal brush-border membranes, and that carboxylic acids, β-amino acids, and dibasic amino acids are co-transported with Na across the renal, but not jejunal, membranes.

INTRODUCTION

Sugars and amino acids generate electrical potential differences across the epithelial cells of the small intestine and renal proximal tubule (Barry, Dikstein, Matthews, Smyth & Wright, 1964; Kohn, Smyth & Wright, 1968; Maruyama & Hoshi, 1972; Schultz & Zalusky, 1963, 1965). These potential differences originate with the co-transport of sodium with organic solutes across the brush-border membrane of these epithelial cells (White & Armstrong, 1971; Rose & Schultz, 1971; Maruyama & Hoshi, 1972; Samarzija, Molnar & Frömter, 1981). A novel approach to the kinetics of Na-coupled solute transport is to measure these transport potential differences in brush-border membrane vesicles using voltage-sensitive dyes (see Cohen & Salzberg, 1978, for a review of these dyes). Beck & Sacktor (1978) were the first to study glucose transport in renal brush-border vesicles using this procedure, and Wright, Krasne,

Kippen & Wright (1981) further exploited the technique to examine the transport of dicarboxylic acids. In these studies it was established that for D-glucose and succinate there was a good correlation between the transport kinetics measured by the fluorescent dye procedure and those obtained by more direct transport assays. We therefore set out in the present study to survey the specificity and kinetics of Na-dependent organic solute transport by renal and jejunal brush borders using a fluorescent dye. We also compared and contrasted the results with those obtained earlier for a limited selection of solutes using the more conventional radioactive tracer techniques (Mircheff, Kippen, Hirayama & Wright, 1982; Stevens, Ross & Wright, 1982*a*; Stevens, Wright, Hirayama, Gunther, Ross, Harms, Nord, Kippen & Wright, 1982*b*; Nord, Wright, Kippen & Wright, 1982). A preliminary account of this work has been presented recently (Wright, Schell & Krasne, 1982).

METHODS

Purified rabbit kidney and intestinal brush-border membrane vesicles were prepared by the Ca-precipitation procedure as described by Wright, Kippen, Klinenberg & Wright (1980) and Stevens *et al.* (1982*a*). Membranes prepared by these methods showed an increase in alkaline phosphatase specific activity of 10 (renal)- and 20 (intestinal)-fold over the initial homogenate. Vesicles were suspended in 300 mM-mannitol buffered to pH 7·5 with 10 mM-Tris-HEPES at a protein concentration of 10–15 mg/ml., and were preserved in liquid N_2 until use (Stevens *et al.* 1982*b*). The electrical potential difference (p.d.) across the plasma membrane of the brush-border vesicles was measured using the fluorescent dye 3,3′-dipropylthiadicarbocyanine iodide (diS-C_3-(5)) as described previously (Wright *et al.* 1981). The p.d.-dependent fluorescence changes (ΔF) are presented in arbitrary units where 10 units are equivalent to $\sim 35\%$ increase in total fluorescence. In renal and intestinal vesicles a valinomycin-induced potassium diffusion potential of +60 mV, intravesicular space positive with respect to the extravesicular space, produced an increase of 20–30 fluorescence units. In the intact epithelial cell this corresponds to a decrease in the magnitude of the negative intracellular p.d.

At the start of each experiment, vesicles (0·6–0·9 mg protein) were added to 3 ml. uptake buffer in polystyrene cuvettes. The uptake buffer contained either NaCl or KCl, 10 mM-Tris-HEPES pH 7·5, 1·5 μM-diS-C_3-(5), and sufficient mannitol to make the solution iso-osmotic with the intravesicular fluid. The base-line fluorescence in the absence of substrates differed by less than 20% between 100 mM-NaCl and 100 mM-KCl. In both renal and jejunal vesicles P_K/P_{Cl} and P_{Na}/P_{Cl} were in the range 0·3–1·3 (Schell, R. E., Gunther, R. D. & Wright, E. M., unpublished observations). Organic substrates were prepared in concentrated stock solutions (50–100 mM) containing 10 mM-Tris-HEPES pH 7·5 and sufficient mannitol to make the solution iso-osmotic with the uptake buffer. In the case of the organic acids the solutions were titrated to pH 7·5 with 1 N-KOH. The pH and osmolarity of all solutions were monitored using an Orion Model 801 pH meter and a Westcor 5100C vapour pressure osmometer. All experiments were carried out at room temperature (22–23 °C). Control experiments showed that, in the presence of a 100 mM-NaCl gradient, 1·2 μM-diS-C_3-(5) did not inhibit ($P > 0.05$) Na-coupled uptakes of L-[^{14}C]phenylalanine into intestinal vesicles. Similar observations were reported for D-glucose and succinate in renal brush-border vesicles (Beck & Sacktor, 1978; Wright *et al.* 1981). The fluorescent dye was generously donated by Dr Alan Waggoner, and all substrates were purchased from Sigma Chemical Co. The Na-dependent fluorescence responses of the dye to organic solutes were fitted to the equation

$$\Delta F = (\Delta F_{max} \times S)/(K_t + S), \quad (1)$$

where ΔF_{max} is the maximal fluorescent response produced by the substrate, K_t is the substrate concentration generating $0.5\Delta F_{max}$, and S is the substrate concentration. Over the solute concentration range tested (0·1–10 mM) this equation gave the best fit to the data (see Figs. 2–4).

All kinetic parameters, ΔF_{max} and K_t, were determined using a North Star computer (North Star, Inc., Berkeley, CA) and an iterative non-linear regression program. The program, which is a

modification of the Gauss–Newton method of non-linear regression described by Duggleby (1981), calculated ΔF_{max}, K_t and estimates of their errors from measurements of ΔF as a function of substrate concentration.

RESULTS

Renal brush-border membranes

The effect of phenylalanine on the potential-dependent fluorescence of diS-C_3-(5) in renal brush-border membranes is shown in Fig. 1. In both experiments the base-line fluorescence was recorded in the absence of the amino acid for 1 min, and then either

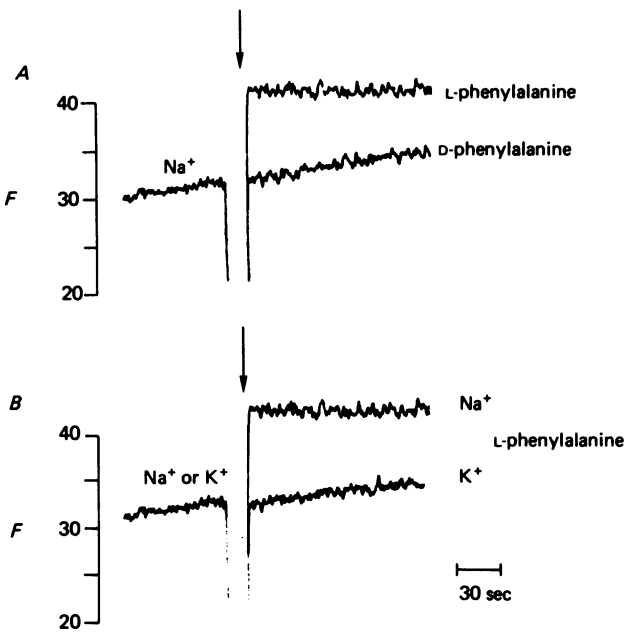

Fig. 1. The effect of L- and D-phenylalanine on the fluorescence of diS-C_3-(5) in renal brush-border membranes. In each experiment 0·60 mg of protein was added to 3 ml. buffer containing 100 mM-NaCl (or 100 mM-KCl), 100 mM-mannitol, 10 mM-Tris-HEPES pH 7·5, and 1·5 μM-diS-C_3-(5). The fluorescence signal was obtained by activating at 620 nm and recording the emission at 669 nm at a band width of 2 nm. *A* shows the addition of L- and D-phenylalanine (7 mM) 1 min after the start of the experiment, while *B* shows the effect of L-phenylalanine when the uptake buffer contained either 100 mM-NaCl or 100 mM-KCl. The arrow marks the time the amino acid was added to the membrane suspension.

L- or D-phenylalanine was added to give a final concentration of 7 mM. In the presence of an initial gradient of 100 mM-NaCl, L-phenylalanine produced an immediate increase in fluorescence of approximately 10 units (Fig. 1*A*,*B*). The D-stereoisomer produced no significant change in fluorescence compared to the base line. As shown in Fig. 1*B*, the increase in fluorescence with L-phenylalanine was observed when the buffer contained 100 mM-NaCl, but not 100 mM-KCl. This demonstrated that L-phenylalanine produced a Na-dependent increase in the fluorescence (p.d.) in renal

brush-border vesicles similar to that observed previously for glucose (Beck & Sacktor, 1978) and Krebs cycle intermediates (Wright *et al.* 1981).

The increment in fluorescence, ΔF, produced by L-phenylalanine in renal vesicles was dependent on the phenylalanine concentration (Fig. 2). ΔF saturated with increasing substrate concentration; the maximal increase in F, ΔF_{max}, was $8{\cdot}4 \pm 0{\cdot}1$ units, and the L-phenylalanine concentration producing half the ΔF_{max}, K_f, was

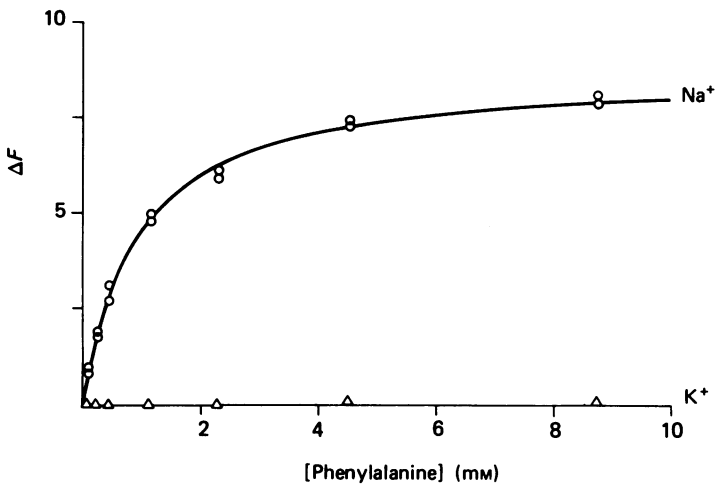

Fig. 2. The effect of L-phenylalanine concentration on ΔF in rabbit renal brush borders. Experimental conditions were identical to those in Fig. 1, except that the phenylalanine concentration was varied between 0·1 and 8·75 mM. At each concentration ΔF was estimated in duplicate, and ΔF was plotted against the phenylalanine concentration. The curve was drawn according to the expression

$$\Delta F = (\Delta F_{max} \times (S))/(K_f + (S)) \quad \text{(eqn. (1))},$$

where $\Delta F_{max} = 8{\cdot}4 \pm 0{\cdot}08$ units and $K_f = 0{\cdot}88 \pm 0{\cdot}03$ mM. Note that there was no increase in ΔF in the presence of KCl.

$0{\cdot}88 \pm 0{\cdot}03$ mM. In contrast, there was no increase in ΔF at any substrate concentration tested when the uptake buffer contained 100 mM-KCl instead of 100 mM-NaCl.

Table 1 summarizes the results obtained in experiments similar to those illustrated in Fig. 2 for a total of nineteen amino acids. It should be noted that all the amino acids tested, except D-phenylalanine, produced a saturable, Na-dependent increase in fluorescence. This included neutral and basic amino acids. Values of K_f ranged from 0·37 to 8·5 mM, and those of ΔF_{max} from 1·7 to 18·1 fluorescence units.

In these series of experiments we also tested the influence of D- glucose, L-glucose, and three metabolic intermediates (Table 2). Four Na-co-transported substrates produced saturable, Na-dependent increases in fluorescence, but L-glucose did not. The succinate K_f, 0·18 mM, is close to that obtained earlier by this procedure (0·10 mM; Wright *et al.* 1981).

The model amino acid for the L transport system, 2-amino-2-bornanecarboxylic acid hemihydrate (BCH), was tested in two experiments at a concentration of 4·3 mM. It gave a Na-dependent increase in fluorescence comparable to that observed for L-lysine at this concentration in those two experiments.

TABLE 1. ΔF_{max} and K_t values obtained for amino acids in renal brush-border membranes. For each substrate the kinetic parameters were measured in 100 mM-NaCl uptake medium as described in Fig. 2. In addition to the absolute values of ΔF_{max}, the values relative to that for L-phenylalanine (% ΔF_{max} L-phenylalanine) are given in parentheses: these were obtained by measuring ΔF_{max} for L-phenylalanine each day for each batch of membranes. When 100 mM-NaCl was replaced with 100 mM-KCl no ΔF was observed for any substance examined. ΔF_{max} values are quoted in arbitrary fluorescence units

	Kidney		
	ΔF_{max}	K_t (mM)	Number of observations
L-phenylalanine	11·1 ± 3·5 (100)	0·76 ± 0·05	4
L-methionine	4·5 ± 0·2 (95)	0·96 ± 0·01	1
L-isoleucine	8·9 ± 0·3 (150)	1·1 ± 0·1	1
L-valine	15·8 ± 0·2 (95)	0·65 ± 0·02	2
	2·6 ± 0·1 (26)	0·53 ± 0·05	
L-alanine	6·1 ± 0·7 (59)	2·6 ± 0·1	3
Glycine	8·6 ± 0·3 (147)	2·35 ± 0·2	1
L-proline	3·8 ± 0·05 (43)	0·38 ± 0·02	2
	1·7 ± 0·06 (17)	0·37 ± 0·04	
L-hydroxyproline	5·8 ± 0·1 (98)	0·69 ± 0·04	1
MeAIB	7·8 ± 0·2 (131)	2·6 ± 0·1	1
L-serine	18·1 ± 1·3 (194)	2·1 ± 0·1	1
L-threonine	6·2 ± 0·2 (106)	2·8 ± 0·2	1
L-homoserine	11·9 ± 0·8 (124)	1·4 ± 0·2	1
β-alanine	3·5 ± 1·2 (28)	2·5 ± 0·1	3
Taurine	5·0 ± 0·3 (52)	4·0 ± 0·4	1
D-lysine	2·9 ± 0·1 (30)	2·1 ± 0·1	1
L-lysine	2·7 ± 0·1 (28)	1·8 ± 0·2	1
L-histidine	15·3 ± 1·1 (167)	8·5 ± 1·0	1
L-glutamine	6·9 ± 0·2 (118)	2·2 ± 1·6	1
D-phenylalanine	0	∞	2

TABLE 2. ΔF_{max} and K_t values obtained for glucose and various carboxylic acids in renal brush-border membranes. Experimental conditions are as given in Fig. 2 and Table 1. ΔF_{max}'s are given in arbitrary fluorescence units

	Kidney		
	ΔF_{max}	K_t(mM)	Number of observations
D-glucose	5·3 ± 0·1	0·33 ± 0·01	2
	5·3 ± 0·1	0·33 ± 0·01	
L-glucose	0	∞	3
Succinic acid	5·8 ± 0·1	0·18 ± 0·01	1
Pyruvic acid	2·4 ± 0·1	0·25 ± 0·02	1
L-lactic acid	3·3 ± 0·1	0·19 ± 0·01	1

Intestinal brush-border membranes. Various organic substrates increased the fluorescence of diS-C_3-(5) in the intestinal vesicles. Fig. 3 shows the ΔF as a function of L-phenylalanine concentration in the presence of 100 mM-NaCl and 100 mM-KCl. There was a Na-dependent, saturable increase in ΔF with a ΔF_{max} of 3·5 ± 0·2 units and a K_t of 6·6 ± 0·6 mM. The results obtained in experiments with a total of twelve amino acids are summarized in Table 3. Seven substrates produced a Na-dependent increase in fluorescence; K_t values ranged from 0·3 to 8·1 mM, and ΔF_{max} from

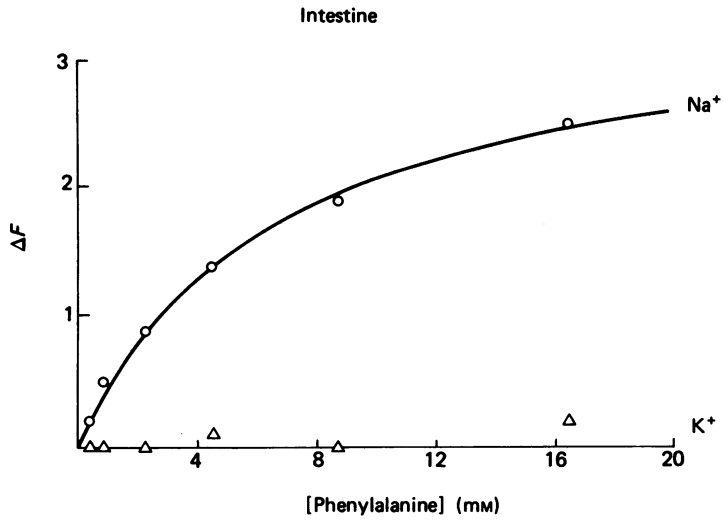

Fig. 3. The effect of L-phenylalanine concentration on ΔF in rabbit intestinal brush borders. The experimental conditions are identical to those described in Figs. 1 and 2. The ΔF_{max} was $3{\cdot}5 \pm 0{\cdot}2$ units and the K_t $6{\cdot}6 \pm 0{\cdot}6$ mM.

TABLE 3. ΔF_{max} and K_t values obtained for amino acids and various metabolites in rabbit intestinal brush borders. Experimental conditions are as given in Fig. 3 and Table 1. ΔF_{max} values are quoted in arbitrary fluorescence units

	Intestine		
	ΔF_{max}	K_t (mM)	Number of observations
L-phenylalanine	$3{\cdot}3 \pm 0{\cdot}3$ (100)	$6{\cdot}6 \pm 0{\cdot}2$	3
L-alanine	$2{\cdot}7 \pm 0{\cdot}3$ (79)	$8{\cdot}1 \pm 1{\cdot}5$	2
	$1{\cdot}3 \pm 0{\cdot}1$ (44)	$5{\cdot}2 \pm 0{\cdot}2$	
Glycine	$1{\cdot}7 \pm 0{\cdot}1$ (50)	$3{\cdot}5 \pm 0{\cdot}5$	1
L-proline	$2{\cdot}3 \pm 0{\cdot}1$ (68)	$0{\cdot}3 \pm 0{\cdot}05$	2
	$1{\cdot}7 \pm 0{\cdot}1$ (59)	$0{\cdot}3 \pm 0{\cdot}05$	
L-serine	$2{\cdot}3 \pm 0{\cdot}1$ (68)	$2{\cdot}0 \pm 0{\cdot}2$	1
L-homoserine	$2{\cdot}6 \pm 0{\cdot}1$ (76)	$2{\cdot}7 \pm 0{\cdot}3$	1
L-histidine	$2{\cdot}6 \pm 0{\cdot}1$ (76)	$5{\cdot}8 \pm 0{\cdot}5$	1
β-alanine	0	∞	2
Taurine	0	∞	2
L-lysine	0	∞	2
D-phenylalanine	0	∞	2
BCH	0	∞	2
D-glucose	$2{\cdot}7 \pm 0{\cdot}2$	$1{\cdot}1 \pm 0{\cdot}7$	1
L-glucose	0	∞	2
Succinic acid	0	∞	2
Pyruvic acid	0	∞	2
L-lactic acid	0	∞	2

1·7 to 3·4 fluorescence units. On the other hand, β-alanine, taurine, D-phenylalanine, lysine and BCH all failed to produce any detectable change in fluorescence (< 0·1 units). Similar experiments, not shown, revealed that L-threonine, L-hydroxyproline, L-methionine, L-valine and methyl aminoisobutyric acid (MeAIB) also increased the fluorescence of diS-C_3-(5) in the presence of Na, but the kinetics of these responses

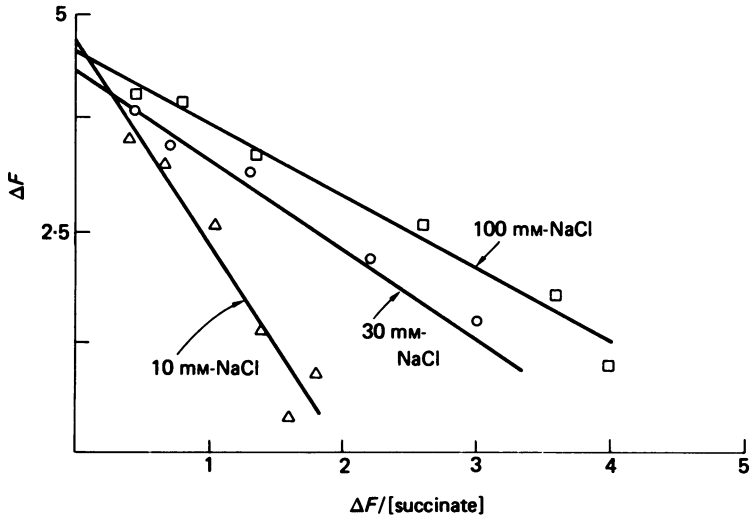

Fig. 4. The effect of Na concentration on the succinate-dependent changes in diS-C_3-(5) fluorescence in rabbit renal brush borders. At each sodium concentration (100 mM, □–□; 30 mM, ○–○; and 10 mM, △–△) ΔF was plotted against ΔF/[succinate]. Each point is the mean of three observations. In this plot of eqn. (1) ΔF_{max} is given by the intercept on the ordinate and $-K_t$ by the slope of the regression line. Computer analysis of the data showed that Na changed the succinate K_t with little change in ΔF_{max} (see Table 4). The regression coefficients were greater than 0·96. Note that all measurements in this Figure were carried out on one batch of membranes on the same day in random fashion.

were not recorded. D-glucose generated a Na-dependent ΔF with a K_t of 1·1 ± 0·7 mM and ΔF_{max} of 2·7 ± 0·2 units, but L-glucose, succinate, pyruvate and L-lactate did not (Table 3).

The effect of Na concentration

For six substrates we tested the effect of Na concentration on the kinetics of the fluorescence response. One succinate experiment is illustrated in Fig. 4. This shows ΔF plotted against ΔF/[succinate] at three different Na concentrations. At each Na concentration there was a linear relationship between ΔF and ΔF/[succinate]. There was no significant change in the intercept on the ordinate, ΔF_{max}, but there was an increase in the slope of the regression line, i.e., K_t decreased from 2·0 to 0·77 mM as the NaCl concentration increased from 10 to 100 mM. Similar results were obtained (Table 4) for all substrates tested in renal vesicles, and for L-phenylalanine in intestinal vesicles.

DISCUSSION

In this study we have demonstrated that all organic solutes with established Na-dependent co-transport across renal or jejunal brush-border membranes (or both) increase the fluorescence of the dye diS-C_3-(5) in the presence of Na. Since an increase in fluorescence of the voltage-sensitive dye corresponds to a depolarization of the

TABLE 4. Summary of the effects of NaCl concentration on kinetics parameters ΔF_{max} and K_f for various solutes in renal and intestinal brush-border membranes (b.b.m.). Kinetic parameters were obtained for each solute in one experiment as described in Fig. 4. ΔF_{max} values are quoted in arbitrary fluorescence units

	Sodium chloride concentration					
	100 mM		30 mM		10 mM	
Renal b.b.m. substrate	K_f (mM)	ΔF_{max}	K_f (mM)	ΔF_{max}	K_f (mM)	ΔF_{max}
Succinate	0·77 ± 0·05	4·5 ± 0·1	0·95 ± 0·06	4·3 ± 0·1	2·0 ± 0·22	4·5 ± 0·1
L-phenylalanine	1·3 ± 0·1	5·2 ± 0·1	—	—	3·5 ± 1·3	4·5 ± 0·7
	0·7 ± 0·1	10·3 ± 0·5	—	—	3·7 ± 0·4	7·7 ± 0·4
Serine	3·2 ± 0·2	12·3 ± 0·3	5·7 ± 1·3	12·6 ± 1·3	10·3 ± 4·0	13·0 ± 2·8
Alanine	3·3 ± 0·4	11·3 ± 0·6	6·2 ± 0·5	11·5 ± 0·4	7·3 ± 0·9	9·2 ± 0·6
Proline	0·52 ± 0·04	4·1 ± 0·1	—	—	1·3 ± 0·3	4·0 ± 0·2
D-glucose	0·38 ± 0·05	7·7 ± 0·2	0·51 ± 0·04	7·5 ± 0·1	0·95 ± 0·11	7·1 ± 0·2
Intestinal b.b.m. substrate						
L-phenylalanine	2·0 ± 0·4	3·7 ± 0·3	3·1 ± 0·8	3·6 ± 0·4	5·7 ± 1·1	3·7 ± 0·4

membrane potential (see Cohen & Salzberg, 1978; Beck & Sacktor, 1978; Wright *et al.* 1981), this observation suggests that all these solutes depolarize (i.e., make more positive) the electrical potential across renal and jejunal brush borders. This is consistent with electrophysiological experiments on the intact epithelia (see Hoshi, 1976; Samarzija, Hinton & Frömter, 1982; Samarzija & Frömter, 1982; Ullrich, 1979). The ΔF_{max} values observed in these studies, 3–18 fluorescent units, correspond to depolarizations in the range of 5–60 mV, which is compatible with those actually observed with micro-electrodes in kidney and intestine.

Membrane potentials in intact epithelia (see Kohn *et al.* 1968; Hoshi, 1976) and the fluorescence response in brush-border vesicles (Figs. 2–3; and Beck & Sacktor, 1978; Wright *et al.* 1981) vary with organic solute concentration in a saturable fashion. In both cases the change in p.d. (or fluorescence) follows a simple equation of the Michaelis–Menten type

$$\Delta F = (\Delta F_{max} \times S)/(K_f + S) \quad (1)$$

In twelve cases where it is possible to make comparisons, the values of K_f measured with the fluorescent dye and the K_t measured by radioactive tracer techniques (Table 5) agree quite closely. This suggests that the fluorescent dye provides a rapid, convenient technique for examining the specificity and affinity of Na-co-transport systems in renal and intestinal brush-border membranes. Despite differences in experimental technique, temperature and animal species, there is fairly good

agreement between the K_f values found in this study and those obtained for intact tissues using more direct electrical measurements (see, for example, Schultz & Zalusky, 1965; Kohn *et al.* 1968; Samarzija *et al.* 1982; Samarzija & Frömter, 1982).

Although there is a good correlation between K_t and K_f, there are two points to be borne in mind while interpreting our K_f values: the first is that the electrical potential generated across the membrane by the co-transported solute may influence

TABLE 5. Comparison of K_f and K_t values in renal and intestinal brush-border membranes. The values of K_f are those obtained in this study (Table 1–4) and K_t those obtained by this group for Na-dependent transport using radioactive tracer techniques

	Renal		Intestinal	
	K_f (mM)	K_t (mM)	K_f (mM)	K_t (mM)
L-phenylalanine	0·76	1 (*a*)	6·6	8·9 (*b*)
L-alanine	2·6	1 (*a*)	6·5	9·0 (*b*)
L-proline	0·38	0·5 (*a*)	0·31	0·55 (*b*)
MeAIB	2·6	1·5 (*a*)	—	—
D-glucose	0·33	0·86 (*c*)	1·1	0·5 (*d*)
Succinic acid	0·25	0·70 (*e*)	—	—
L-lactic acid	0·19	0·48 (*f*)	—	—
Pyruvic acid	0·25	0·12 (*f*)	—	—

All amino acid K_t values were estimated under open-circuit conditions, while all remaining K_t values were obtained when transmembrane voltages were short-circuited with K and valinomycin. K_t estimates were obtained from: (*a*) Mircheff *et al.* (1982); (*b*) Stevens *et al.* (1982*a*); (*c*) E. Nord (personal communication); (*d*) J. D. Kaunitz (personal communication); (*e*) Wright *et al.* (1982*b*); (*f*) Nord *et al.* (1982).

the transport kinetics. We have already established (Wright, Hirayama, Kippen & Wright, 1982*b*) that varying the membrane potential from −60 to +60 mV changes the succinate K_t by a factor of four; and, secondly, all our measurements of kinetics are made 1 min after setting up the 100 mM-NaCl gradient across the membrane. During this time the intravesicular Na concentration increases from 0 towards 100 mM (with half-times of 4 and 1 min respectively for jejunal and renal membranes (Gunther & Wright, 1982; Wright, Kippen & Wright, 1982*a*). The effect of *trans* sodium on the kinetics of solute uptake into vesicles is known only for succinate, where we find that it reduced V_{max} with no change in K_t (Wright *et al.* 1982*b*). Nevertheless, all our kinetic measurements are made under the same rigid protocol, and internal comparisons are quite valid.

The results in Tables 1, 2 and 3 confirm and extend our previous observations with radioactive tracer techniques (Mircheff *et al.* 1982; Stevens *et al.* 1982*a*, *b*; Nord *et al.* 1982). In general, there is a remarkable homology in the transport systems in renal and jejunal membranes, but clear differences have emerged: (1), neutral amino and imino acids are transported across both renal and jejunal brush borders by Na co-transport; (2), Na-dependent transport systems exist for β-amino acids (e.g. β-alanine and taurine) and dibasic amino acids (e.g. lysine) in renal, but not in jejunal brush borders; (3), the Na-coupled systems are quite stereospecific for glucose and some amino acids (e.g. phenylalanine), but not others (e.g. lysine) and (4), the

metabolic intermediates, succinate, pyruvate and L-lactate, are avidly transported by renal, but not jejunal, brush borders.

On the basis of this study two general conclusions can be drawn about organic solute transport via Na co-transport across brush borders. The first is that all co-transported solutes, irrespective of their charge, depolarize the brush-border membrane potential in the presence of Na. Neutral (e.g. glucose), zwitterion (e.g. amino acids), positive (e.g. basic amino acids), and negative (mono-, di-, and tricarboxylic acids) solutes all increase the fluorescence of diS-C_3-(5). The simplest explanation is that all classes of transported solutes produce a net flow of positive current, Na current, across the brush borders. Thus, we may expect to see variations in the Na/solute coupling ratios. In renal brush borders there is substantial evidence that the Na/succinate coupling ratio is 3 (Wright *et al.* 1982*a*), and in intestinal brush borders the Na/glucose ratio is at least 2 (Kaunitz, Gunther & Wright, 1982).

The second general conclusion is that extravesicular Na modulates the affinity (K_t) of each transport system tested (Table 4). Using radioactive tracers we have already established that extravesicular Na affects succinate uptake kinetics by decreasing the K_t (Wright *et al.* 1982*b*). The fluorescent-dye experiments (Fig. 4; Table 4) suggest that Na increases the affinity of the dicarboxylic acid, sugar and amino acid carriers in the kidney and the phenylalanine carrier in the intestine. In the intact rabbit ileum mucosal Na also decreases the K_t for alanine transport across the brush border without changing the V_{max}, but Na increases the 3-*O*-methylglucose V_{max} without altering K_t (see Schultz & Curran, 1970). We are currently using more direct transport assays to explore further the interaction of Na with carriers in renal and jejunal vesicles.

We have paid particular attention to the kinetics and specificity of amino acid transport. Competition experiments with radioactive tracers have established that there are multiple Na-dependent amino acid carriers with overlapping specificities in renal and jejunal brush borders (Mircheff *et al.* 1982; Stevens *et al.* 1982*a*). In each membrane there are distinct systems for imino acids and neutral amino acids, and in the intestine, at least, the V_{max} for proline (166 p-mole/(mg . sec)) is significantly different from both L-phenylalanine (622 p-mole/(mg . sec)) and L-alanine (100 p-mole/(mg . sec)). In both membranes there is wide variation in the ΔF_{max} parameter (relative to that for L-phenylalanine measured on the same batch of membranes on the same day (see Tables 1 and 3). In renal membranes the relative ΔF_{max} for valine varies from 28 % (lysine and β-alanine) to 194 % (serine) of that for L-phenylalanine (100 %), while in the jejunum values range from 44 % (L-alanine) to 100 % (L-phenylalanine). We also observed that the relative values varied from one experiment to another for individual amino acids: in renal membranes the relative ΔF_{max} for valine was 26–95 % (Table 1), and in the intestine the estimate for alanine was 44–79 % (Table 3).

In renal membranes the amino acids appear to fall into three major groups (Table 1). (1) $\Delta F_{max} > 125\%\ \Delta F_{max}$ phenylalanine, consisting of isoleucine, glycine, MeAIB, serine and histidine. (2) $\Delta F_{max} = 75$–$125\%\ \Delta F_{max}$ phenylalanine, composed of methionine, valine, hydroxyproline, threonine, homoserine, and glutamine. (3) $\Delta F_{max} < 75\%\ \Delta F_{max}$ phenylalanine, consisting of valine, alanine, proline, β-alanine, taurine and lysine. In the jejunum there are two major groups: $\Delta F_{max} = 75$–125%

ΔF_{max} phenylalanine consisting of alanine, homoserine and histidine, and $\Delta F_{max} < 75\%$ ΔF_{max} phenylalanine composed of alanine, glycine, proline and serine. These results suggest that the V_{max} varies from one amino acid to another in the same preparation of membranes, and for one amino acid one preparation of membranes to another. Variations in relative ΔF_{max} may reflect different Na-dependent carrier systems with different V_{max} values, i.e. variations in carrier density, but other explanations are possible. For example, they may simply be due to variations in Na/solute coupling ratios similar to the case reported for amino acid transport in pigeon erythrocytes (see Schultz & Curran, 1970, p. 657).

It is expected that the presence of multiple amino acid carriers with overlapping specificities should display multiple component transport kinetics. However, we find that the kinetics of ΔF *vs.* concentration (0·1–10 mM) are approximated by a simple, one-carrier model, i.e. plots of ΔF *vs.* S, $1/\Delta F$ *vs.* $1/S$, and ΔF *vs.* $\Delta F/S$ are all fitted by a single Michaelis–Menten-type function (eqn. (1)). One explanation could be that, over the concentration range tested, each amino acid is predominantly transported by only one system. Similar comments apply to the transport of mono- and dicarboxylic acids in renal membranes (Table 2).

The final point to be considered is the magnitude of ΔF_{max} for substrates transported by both renal and jejunal brush borders. The D-glucose and L-phenylalanine ΔF_{max} values are both greater in renal than in jejunal membranes. Although the increase in fluorescence produced by valinomycin-induced K^+ diffusion potentials are comparable in both membranes, 0·3–0·6 fluorescence units/mV, it should be noted P_{Cl}/P_{Na} in renal vesicles is twice that observed in jejunal vesicles, 3·2 *vs.* 1·5 (Schnell, R. E., Gunther, R. D. & Wright, E. M., in preparation). This would require that in the presence of impermeable anions the discrepancy between the ΔF_{max} values should be even greater than that recorded here. The differences in ΔF_{max} may be due to either: (1) a higher density, or higher turnover number, of transport sites in the kidney, or (2) larger solute-linked Na currents in renal vesicles, or (3) a lower leak conductance of the renal vesicles. So far, there is preliminary evidence that the V_{max} of Na-dependent glucose transport is similar in renal and intestinal vesicles (E. Nord & J. Kaunitz, unpublished data).

We wish to thank Dr Sally Krasne for use of her spectrofluorometer and for valuable advice and criticisms throughout this study. Thanks are also due to Drs R. Gunther, J. Kaunitz, Y. Saito and S. Wright for valuable comments and reviews of the manuscript. This study was supported by grants from the U.S.P.H.S. (AM 19567 and NS 09666).

REFERENCES

BARRY, R. J. C., DIKSTEIN, S., MATTHEWS, J., SMYTH, D. H. & WRIGHT, E. M. (1964). Electrical potentials associated with intestinal sugar transport. *J. Physiol.* **171**, 316–338.

BECK, J. C. & SACKTOR, B. (1978). Membrane potential-sensitive fluorescence changes during Na^+-dependent D-glucose transport in renal brush border membrane vesicles. *J. biol. Chem.* **253**, 7158–7162.

COHEN, L. B. & SALZBERG, B. M. (1978). Optical measurement of membrane potential. *Rev. Physiol. Biochem. Pharmacol.* **83**, 35–88.

DUGGLEBY, R. G. (1981). A nonlinear regression program for small computers. *Analyt. Biochem.* **110**, 9–18.

GUNTHER, R. D. & WRIGHT, E. M. (1982). Na^+, Li^+ and Cl^- transport by brush border membranes from rabbit jejunum. *J. Membrane Biol.* (Submitted for publication.)

HOSHI, T. (1976). Electrophysiological studies on amino acid transport across the luminal membrane of the proximal tubular cells of *Triturus* kidneys. In *Amino Acid Transport and Uric Acid Transport*, ed. SILBERNAGL, S., LANG, F. & GREGER, R., pp. 96–104. Stuttgart: Thieme.

KAUNITZ, J. D., GUNTHER, R. D. & WRIGHT, E. M. (1982). Involvement of multiple sodium ions in intestinal D-glucose transport. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2315–2318.

KOHN, P. G., SMYTH, D. H. & WRIGHT, E. M. (1968). Effects of amino acids, dipeptides and disaccharides on the electrical potential across rat small intestine. *J. Physiol.* **196**, 723–746.

MARUYAMA, T. & HOSHI, T. (1972). The effect of D-glucose on the electrical potential profile across the proximal tubule of Newt kidney. *Biochim. biophys. Acta* **282**, 214–225.

MIRCHEFF, A. K., KIPPEN, I., HIRAYAMA, B. & WRIGHT, E. M. (1982). Delineation of sodium-stimulated amino acid transport pathways in rabbit kidney brush border vesicles. *J. Membrane Biol.* **64**, 113–122.

NORD, E., WRIGHT, S. H., KIPPEN, I. & WRIGHT, E. M. (1982). Pathways for carboxylic acid transport by rabbit renal brush border membrane vesicles. *Am. J. Physiol.* (in the Press).

ROSE, R. C. & SCHULTZ, S. G. (1971). Studies on the electrical potential profile across rabbit ileum. Effect of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. gen. Physiol.* **57**, 639–663.

SAMARZIJA, I. & FRÖMTER, E. (1982). Electrophysiological analysis of rat renal sugar and amino acid transport. III. Neutral amino acids. *Pflügers Arch.* **393**, 199–209.

SAMARZIJA, I., HINTON, B. T. & FRÖMTER, E. (1982). Electrophysiological analysis of rat renal sugar and amino acid transport. II. Dependence on various transport parameters and inhibitors. *Pflügers Arch.* **393**, 190–197.

SAMARZIJA, I., MOLNAR, V. & FRÖMTER, E. (1981). The stoichiometry of Na^+ coupled anion absorption across the brush border membrane of rat renal proximale tubule. In *Advances in Physiological Sciences*, vol. II, *Kidney and Body Fluids*, ed. TAKACS, L., pp. 419–423. Oxford: Pergamon.

SCHULTZ, S. G. & CURRAN, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637–718.

SCHULTZ, S. G. & ZALUSKY, R. (1963). The interaction between active sodium transport and active sugar transport in the isolated rabbit ileum. *Biochim. biophys. Acta* **71**, 503–505.

SCHULTZ, S. G. & ZALUSKY, R. (1965). Interactions between active sodium transport and active amino-acid transport in isolated rabbit ileum. *Nature, Lond.* **205**, 292–294.

STEVENS, B. R., ROSS, H. J. & WRIGHT, E. M. (1982*a*). Multiple pathways for neutral amino acids in rabbit jejunal brush border vesicles. *J. Membrane Biol.* **66**, 213–225.

STEVENS, B. R., WRIGHT, S. H., HIRAYAMA, B. S., GUNTHER, R. D., ROSS, H. J., HARMS, V., NORD, E., KIPPEN, I. & WRIGHT, E. M. (1982*b*). Organic and inorganic solute transport in renal and intestinal membrane vesicles preserved in liquid nitrogen. *Membrane Biochemistry* **4**, 271–281.

ULLRICH, K. J. (1979). Renal transport of organic solutes. In *Membrane Transport in Biology*, vol. IV B, ed. GIEBISCH, G., TOSTESON, D. C. & USSING, H. H., pp. 413–443. Berlin: Springer-Verlag.

WHITE, J. F. & ARMSTRONG, W. McD. (1971). Effect of transported solutes on membrane potentials in bullfrog small intestine. *Am. J. Physiol.* **221**, 194–201.

WRIGHT, E. M., SCHELL, R. E. & KRASNE, S. (1982). Studies of amino acid transport across renal brush border membrane vesicles using a fluorescent dye. *Fedn Proc.* **41**, 1596.

WRIGHT, S. H., HIRAYAMA, B., KIPPEN, I. & WRIGHT, E. M. (1982*b*). Effect of Na^+ and membrane potential on kinetics of succinate transport in renal brush border membranes. *Fedn Proc.* **41**, 1264.

WRIGHT, S. H., KIPPEN, I., KLINENBERG, J. R. & WRIGHT, E. M. (1980). Specificity of the transport system for tricarboxylic acid cycle intermediates in renal brush borders. *J. Membrane Biol.* **57**, 73–82.

WRIGHT, S. H., KIPPEN, I. & WRIGHT, E. M. (1982*a*). Stoichiometry of Na^+-succinate cotransport in renal brush border membranes. *J. biol. Chem.* **257**, 1773–1778.

WRIGHT, S. H., KRASNE, S., KIPPEN, I. & WRIGHT, E. M. (1981). Na^+-dependent transport of tricarboxylic acid cycle intermediates by renal brush border membranes. Effect on fluorescence of a potential-sensitive cyanine dye. *Biochim. biophys. Acta* **640**, 767–778.