J. Membrane Biol. 78, 119–127 (1984)

The Journal of
Membrane Biology

Ion Permeability of Rabbit Intestinal Brush Border Membrane Vesicles

Robert D. Gunther, Richard E. Schell and Ernest M. Wright
Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024

Summary. The ion permeability of rabbit jejunal brush border membrane vesicles was studied by measuring unidirectional fluxes with radioactive tracers and bi-ionic diffusion potentials with the potential-sensitive fluorescent dye, diS-C_3-(5). Tracer measurements provide estimates of the absolute magnitudes of permeability coefficients, while fluorescence measurements provide estimates of relative and absolute ion permeabilities. The magnitudes of the permeability coefficients for Na^+, K^+, Rb^+, and Br^- were approximately 5 nanoliters/(mg protein × sec) or 10^{-5} cm/sec as determined by radioactive tracer measurements. The apparent selectivity sequence, relative to Na^+, as determined by bi-ionic potential measurements was: F^-, isethionate, gluconate, choline (<0.1) < $Na^+(1.0)$ < $Cl^-(1.5) = NO_3^-(1.5)$ < $Br^-(2.3)$ < $K^+(2.4)$ < $Rb^+(2.5)$ < $Cs^+(2.6)$ < $Li^+(3.9)$ < $NH_4^+(12)$ < $I^-(40)$. The origin of this selectivity sequence and its relationship to the ion permeability of the brush border membrane in the intact epithelium are discussed.

Key Words ion permeability · potential-sensitive cyanine dye · intestinal brush border vesicles

Introduction

There are two possible pathways for ion movement across the intestinal epithelium: 1) the paracellular route, in which the ions cross the tight junctions and the lateral intercellular spaces without entering the cells, and 2) the transcellular route, in which the ions cross in turn, the brush border membrane, the cytoplasm and the basal-lateral membrane. Since the paracellular pathway is the major route for passive ion fluxes across the the intestine (Frizzell et al., 1973), it is difficult to measure the ion permeabilities of the plasma membranes in the intact tissue. One approach to this problem is to study ion transport in vesicles prepared from brush border and basal-lateral membranes.

This paper describes experiments designed to characterize the ion permeability of brush border membrane vesicles isolated from rabbit jejunum using radioactive tracer and bi-ionic potential measurements. It has become feasible to measure membrane potentials in small vesicles through the use of voltage-sensitive optical probes (*see* Cohen & Salzberg, 1978, and Waggoner, 1979, for reviews). A cyanine dye, diS-C_3-(5), has already been used to measure membrane potentials in both renal and intestinal brush border vesicles (Beck & Sacktor, 1978; Wright et al., 1981; Schell, Stevens & Wright, 1983). Our results indicate that the brush border membranes are selective (but not highly selective) for common ions. These results are useful in designing further transport studies on vesicular preparations and give an indication of the permeability of brush border membranes in the intact epithelium. The ability to control and calculate the membrane potentials in vesicle experiments should prove valuable in the study of transport systems in brush border membrane vesicles.

This work was published previously in preliminary form (Gunther et al., 1982).

Abbreviations

ETH 1097	N,N,N′,N′,-Tetrabenzyl-3,6-dioxaoctan-diamid
diS-C_3-(5)	3,3′-dipropylthiadicarbocyanine iodide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Tris	Tris(hydroxymethyl)aminomethane

Materials and Methods

Isolation of Brush Border Membranes

Jejunal brush border membrane vesicles (BBMV's) were prepared by calcium precipitation and differential centrifugation and stored in liquid N_2 as described by Stevens et al. (1982). The specific activity of alkaline phosphatase was typically enriched by 20-fold in the brush border fraction as compared to the initial homogenate. The membranes were suspended, before freezing, in 300 mM mannitol and 50 mM Tris/HEPES, pH 7.5, for radioactive tracer measurements or in 100 mM choline gluconate in 10 mM Tris/HEPES, pH 7.5 for fluorescence measurements.

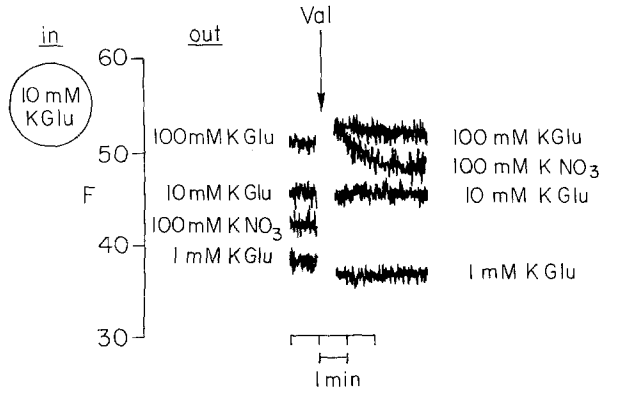

Fig. 1. Observed fluorescence of diS-C_3-(5) in the presence of jejunal BBMV's with various transmembrane ion gradients. Vesicles were loaded with 10 mM KGlu, 90 mM CholGlu, 100 mM D-mannitol and 10 mM Tris/HEPES (pH 7.5). The Figure shows 1:10, 1:1 and 10:1 K^+ gradients with Glu^- as the anion, and also a 10:1 (out/in) K^+ gradient with 100 mM NO_3^- outside. Total salt concentration was maintained at 100 mM with CholGlu. Valinomycin (4 μg/ml) was added at the arrow

Radioactive Tracer Uptake Measurements

These methods have been described previously (Gunther & Wright, 1983). The BBMV's were thawed in a 39° C water bath the day before the experiment, then allowed to equilibrate with a loading solution overnight on ice. The loading solutions are described in the Figure legends. All radioisotopes were obtained from commercial sources at the highest specific activity available. Apparent permeability coefficients (P_i) were determined by measuring ion uptakes (J_i pmol mg^{-1} sec^{-1}) as a function of ion activity (0 to 150 mM) and graphing the results as a Woolf-Augustinson-Hofstee plot (J_i *vs.* J_i/a_i, where a_i is the ion activity). Diffusive fluxes appear in this plot as a vertical line intersecting the abscissa at P'. In the presence of a carrier-mediated uptake the plot J_i *vs.* J_i/a_i approaches the vertical diffusive component at high ion activities, i.e. as $a_i > K_t$ where K_t is the half-saturation constant for the mediated flux (*see* Fig. 5). Activities were calculated using activity coefficients from Robinson and Stokes (1959).

Fluorescence

BBMV's were thawed in a 39° C water bath the day before the experiment and allowed to equilibrate with a loading solution overnight on ice. The loading solutions are described in the Figure legends. The vesicles were diluted into solutions containing different concentrations of the ions under investigation. The transmembrane potential differences were measured using the fluorescent dye diS-C_3-(5) as described by Wright et al. (1981) and Schell et al. (1983). Briefly, 2 ml external solution was added to a polystyrene cuvette. DiS-C_3-(5) was added in ethanolic solution to 1.5 μM concentration. Loaded BBMV's were added to the cuvette to a final concentration of 0.2 to 0.3 mg/ml. The fluorescence change was expressed in arbitrary fluorescence units (F) where 10 units are equivalent to approximately 25% change in total fluorescence. ΔF was converted to mV using a calibration curve generated with K^+ gluconate gradients in the presence of 2 μM valinomycin. The voltage-fluorescence relationship was linear from 0 to +60 mV and from 0 to −60 mV with slopes ranging from 0.3 to 0.5% ΔF/mV (*see* Wright et al., 1981, Fig. 1). Similar relations were obtained with Na gluconate gradients in the presence of the Na-ionophore ETH 1097 (*see below*). Relative ion permeabilities were calculated from the membrane potential data using the Goldman-Hodgkin-Katz constant field equation (*see* Results). Ion concentrations may be used in these calculations instead of activities since activity coefficients may be factored out of the activity ratios when ionic strength is equal on both sides of the membrane.

The pH and osmolarity of all solutions were monitored using an Orion model 801 pH meter and a Wescor 5100C vapor pressure osmometer. All experiments were carried out at room temperature (22 to 23° C).

Both fluorescence and absorbance spectra of diS-C_3-(5) were examined in the presence of all of the ions studied. No changes in spectra were observed due to any of the ions mentioned in the results. Some ions did affect the spectra, however, (e.g. SCN^-, ClO_4^-), and were unsuitable for these types of experiments.

Results

Bi-ionic Potential Measurements

The electrical potential difference across the membrane of the brush border vesicles (E_m) was monitored using the voltage-dependent fluorescence of the cyanine dye diS-C_3-(5). The experimental protocol was to: i) calibrate the fluorescence response with imposed K-valinomycin diffusion potentials, i.e. $E_m = E_K = 58 \log K_i/K_o$, and ii) generate bi-ionic diffusion potentials (e.g. M^+X^- *vs.* N^+X^-) across the membrane in the absence of ionophores, and to estimate the relative ion permeability coefficients from the magnitude of the diffusion potential and the ion gradients using the constant field equation. Since the fluorescence of the dye is known to vary with ionic strength and pH all experiments are carried out in well-buffered solutions at constant ionic strength (100 mM) using choline and gluconate as impermeant ions. Increasing the buffer concentration in the solution from 10 to 50 mM Tris/HEPES, pH 7.5, had no significant effect on the magnitude of the fluorescence signals. In this series vesicles were preloaded with either 10 mM K gluconate, 10 mM Na gluconate or 10 mM choline chloride plus 90 mM choline gluconate. Bi-ionic potentials were usually generated by adding the vesicles to cuvettes containing 100 mM salts.

The protocol is illustrated by one experiment shown in Fig. 1. The vesicles, preloaded with 10 mM K gluconate + 90 mM choline gluconate, were added to cuvettes containing i) 100 mM K gluconate, ii) 10 mM K gluconate + 90 mM choline gluconate, iii) 100 mM KNO_3, or iv) 1 mM K gluconate + 99mM choline gluconate. The F recorded in the absence of ion gradients represents the baseline, i.e. $E_m = 0$ mV. Note that upon addition of valinomycin there is no change in F, i.e. $E_m = E_K =$

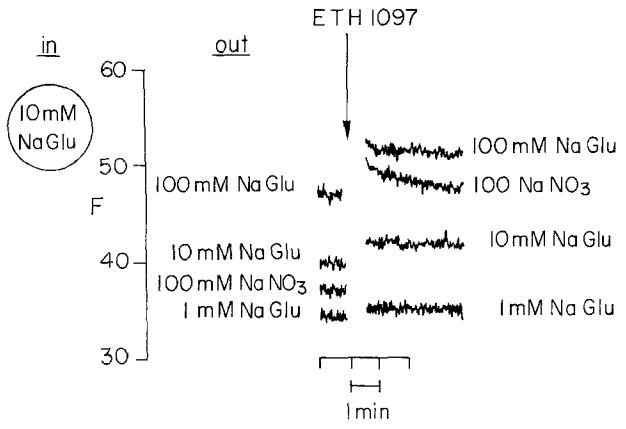

Fig. 2. Observed fluorescence of diS-C_3-(5) in the presence of jejunal BBMV's with various transmembrane ion gradients. Vesicles were loaded with 10 mM NaGlu, 90 mM CholGlu, 100 mM D-mannitol and 10 mM Tris/HEPES (pH 7.5). The Figure shows 1:10, 1:1 and 10:1 Na^+ gradients with Glu^- as the anion, and also a 10:1 (out/in) Na^+ gradient with 100 mM NO_3^- outside. Total salt concentration was maintained at 100 mM with CholGlu. ETH 1097 (4 μg/ml) was added at the arrow

0 mV. In the presence of 100 mM K gluconate F increased by 5.4 units before valinomycin and 8 units after valinomycin. In the presence of valinomycin $E_m = E_K = 58 \log 100/10 = 58$ mV (concentrations rather than activities are used because all solutions are at constant ionic strength). Thus in the absence of valinomycin the increase in F (5.4 units) corresponds to a membrane potential of +39 mV. In six experiments, each in quadruplicate, the mean potential under these conditions was 49 ± 1 (SEM) mV.

$$E_m = 49 \text{ mV} = 58 \log \frac{[K]_o + P_{gluc}/P_K [Gluc]_i}{[K]_i + P_{gluc}/P_K [Gluc]_o + P_{choline}/P_K [choline]_i} \quad (1)$$

Assuming that $P_{gluc}/P_K = P_{choline}/P_K$, or $P_{choline} \gg P_{gluc}$, or $P_{gluc} \gg P_{choline}$, solution of the constant field equation necessitates that P_{gluc}/P_K, $P_{choline}/P_K < 0.05$.

When vesicles were added to cuvettes containing 1 mM K gluconate + 99 mM choline gluconate, the ΔF was −7.5 units before valinomycin and 9 units after addition of valinomycin, i.e. in the absence of ionophore the membrane potential hyperpolarized by 48 mV. In four experiments, each in duplicate, the mean E_m under these conditions was 44 ± 2 (SEM). Solution of the constant field equation, as above, necessitates that $P_{choline}/P_K$, $P_{gluc}/P_K < 0.005$. The apparent discrepancy between the P ratios estimated for hyperpolarizing and depolarizing membrane potentials may be due to voltage-dependent changes in K permeation, but further discussion is beyond the scope of the present study.

Finally, in this experiment when 100 mM KNO_3 was substituted for 100 mM K gluconate, F decreased by −3 units, i.e. the membrane hyperpolarized by 22 mV. Solving for P_{NO_3}/P_K yields a ratio of 2.4. In a total of 28 trials on 7 batches of vesicles the mean P_{NO_3}/P_K ratio was 0.9 ± 0.2 (SEM). The subsequent addition of valinomycin in the presence of 100 mM KNO_3 returned E_m to E_K (+58 mV) owing to the increase in the K conductance of the membrane. However, unlike the record in the absence of impermeable anions, E_m decays rapidly towards 0 mV, and this confirms that $P_{NO_3} \gg P_{gluconate}$, i.e. the net influx of K_{NO_3} dissipates the K gradient across the membrane.

An estimate of the absolute permeability coefficient can be obtained from the initial rate of decay of E_K in the presence of valinomycin (Fig. 1). This shows that E_K in the presence of NO_3 approaches 0 with time due to the net influx of KNO_3 dissipating the potassium gradient. In the presence of valinomycin the rate-limiting factor in determining the flux is the anion permeability. From the time course of change of E_K (9 mV/20 sec), the external $[NO_3]$ (100 mM), the membrane potential (E_K) and the unidirectional flux equation:

$$J_{NO_3} = P_{NO_3}[NO_3] \cdot (zFE_K/RT)/1 - \exp(zFE_K/RT) \quad (2)$$

we obtain a P_{NO_3} of 1 nanoliter/(mg × sec),

where

$J_{NO_3} = V(K_i^t - K_i)/(t \times \text{mg protein})$, $NO_3 = 100$ mM, F is the Faraday, V is the intravesicular volume (2 μl/mg protein), t is the number of seconds between the addition of valinomycin and the measurement of E_K^t; z, R and T have their usual meanings, K_i the internal K concentration at $t = 0$, and K_i^t the internal K concentration at time t (obtained from the Nernst equation). The NO_3 permeability obtained from these fluorescent measurements is in the range obtained for Br^- using radioactive tracers (*see* Table 4).

Figure 2 shows a similar experiment using Na^+ and ETH 1097 to illustrate that $Chol^+$ and Glu^- are relatively impermeant and that E_m before the addition of the ionophore was dependent on the relative permeabilities of the permeant ions. In the Na^+ experiment, a small increase in fluorescence following addition of the ionophore was observed in the absence of ion gradients. The cause of this nonspecific increase is not known. Notice, how-

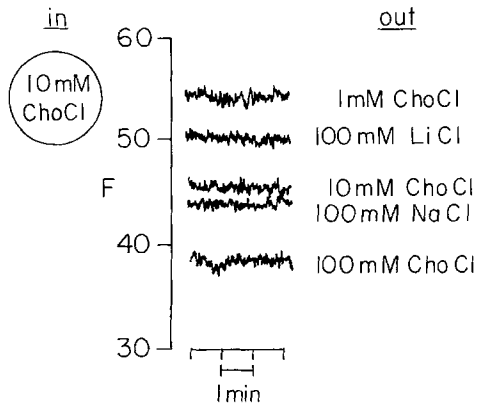

Fig. 3. Observed fluorescence of diS-C_3-(5) in the presence of jejunal BBMV's with various transmembrane ion gradients. Vesicles were loaded with 10 mM CholCl, 90 mM CholGlu, 100 mM D-mannitol and 10 mM Tris/HEPES (pH 7.5). The Figure shows 1:10, 1:1 and 10:1 Cl^- gradients with $Chol^+$ as the cation, and also 10:1 (out/in) Cl^- gradients with either 100 mM Li^+ or 100 mM Na^+ outside. Total salt concentration was maintained at 100 mM with CholGlu

ever, that the difference between the fluorescence levels with 10 mM NaGlu outside and either 1 mM or 100 mM NaGlu outside increases after the addition of ETH 1097. This increase indicates that the membrane potential was not at E_{Na} in the absence of the ionophore, demonstrating that Na^+ was not as overwhelmingly permeable compared to Glu^- and $Chol^+$ as K^+ (*see* Fig. 1). As in the K^+ experiment, replacing 100 mM NaGlu with 100 mM $NaNO_3$ yields a negative intravesicular potential (-5 ± 4 mV in 7 experiments), indicating that $P_{NO_3} > P_{Na}$. Furthermore, after addition of ETH 1097 E_m approximates E_{Na} and decayed more rapidly with NO_3^- than with Glu^-, again confirming that $P_{NO_3} > P_{Glu}$. The fact that E_m does not quite reach E_{Na} on addition of the ETH 1097 may be due to the low discrimination of this ionophore between Na^+ and NO_3^- ($P_{NO_3}/P_{Na} = 0.21$, Eisenman, Margalit & Kuo, 1981).

Figure 3 shows a corresponding experiment for Cl^-. The ΔF was calibrated for the same vesicles on the day of the experiment using K^+ and valinomycin. The vesicles were then loaded with 10 mM CholCl. A 10-fold CholCl gradient (10 mM in; 100 mM out) yielded E_m of -48 ± 1 mV in 5 experiments. When Na^+ was substituted for $Chol^+$, 100 mV NaCl outside yielded E_m of -8 ± 2 mV in 5 experiments. 100 mM LiCl outside yielded E_m of 22 ± 1 mV in 5 experiments. These results indicate that $P_{Li} > P_{Cl} > P_{Na} > P_{Chol}$.

The values for E_m determined in quadruplicate for each ion pair in 5 to 7 experiments was used to calculate the relative permeabilities of the ions

Table 1. Permeabilities of anions compared to potassium[a]

Ion	E_m	P_i/P_K	n
Ise^-	44 ± 1	$<0.04\pm0.01$	5
F^-	39 ± 4	0.09 ± 0.04	5
Cl^-	2 ± 1	$0.8\ \pm0.1$	5
NO_3^-	3 ± 4	$0.9\ \pm0.2$	7
Br^-	$-\ 1\pm6$	$1.1\ \pm0.4$	7
I^-	-73 ± 3	$19\ \ \pm2$	5
Glu^-	49 ± 1	$<0.05\pm0.01$	6

[a] Permeabilities of various ions relative to K^+. Vesicles were loaded with 10 mM KGlu. K^+ salts of the various anions were present on the outside of the vesicles at 100 mM. Membrane potentials (E_m) were measured with diS-C_3-(5). P_i/P_K's were calculated from the E_m by the Goldman-Hodgkin-Katz constant field equation assuming $P_{choline} = 0$ and $P_{gluc}/P_K = 0.05$. Data are given as mean ± SEM of 5 to 7 experiments, and in each experiment the bi-ionic potentials were the mean of quadruplicate trials. All potentials are given with respect to the extravesicular fluid.

Table 2. Permeabilities of anions compared to sodium[a]

Ion	E_m	P_i/P_{Na}	n
Ise^-	39 ± 3	$<0.07\pm0.02$	5
F^-	57 ± 8	0.04 ± 0.02	5
NO_3^-	$-\ 5\pm4$	$1.2\ \pm0.2$	7
Cl^-	-13 ± 1	$1.5\ \pm0.1$	5
Br^-	-14 ± 4	$2.3\ \pm0.7$	7
I^-	-93 ± 2	$40\ \ \pm3$	5
Glu^-	44 ± 2	$<0.09\pm0.02$	7

[a] Permeabilities of various anions relative to Na^+. Vesicles were loaded with 10 mM NaGlu. Na^+ salts of the anions were present on the outside of the vesicles at 100 mM. Membrane potentials (E_m) were measured with DiS-C_3-(5). P_i/P_{Na}'s were calculated from the E_m by the Goldman-Hodgkin-Katz constant field equation assuming $P_{choline} = 0$ and $P_{gluc}/P_{Na} = 0.09$. Data are given as mean ± SEM of 5 to 7 experiments, and in each the bi-ionic potentials were the mean of quadruplicate trials.

Table 3. Permeabilities of cations relative to chloride[a]

Ion	E_m	P_i/P_{Cl}	n
NH_4^+	48 ± 2	$7\ \ \pm1$	5
Li^+	22 ± 1	$2.2\ \pm0.1$	5
Cs^+	13 ± 1	$1.5\ \pm0.1$	5
Rb^+	12 ± 1	$1.4\ \pm0.1$	5
K^+	11 ± 1	$1.4\ \pm0.1$	5
Na^+	$-\ 8\pm2$	$0.6\ \pm0.1$	5
$Chol^+$	-48 ± 1	$<0.06\pm0.01$	5

[a] Permeabilities of various cations relative to Cl^-. Vesicles were loaded with 10 mM CholCl. Cl^- salts of the cations were present on the outside of the vesicles at 100 mM. Membrane potentials were measured with diS-C_3-(5). P_i/P_{Cl}'s were calculated from the E_m by the Goldman-Hodgkin-Katz constant field equation assuming $P_{gluconate} = 0$, and $P_{chol}/P_{Cl} = 0.06$. Data are given as mean ± SEM of 5 experiments, and in each the bi-ionic potentials were the mean of quadruplicate trials.

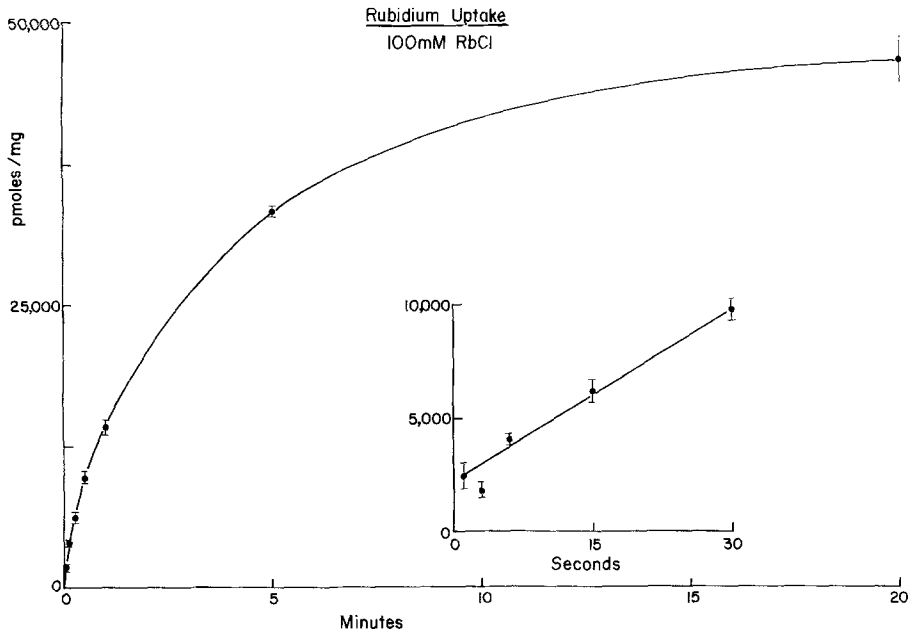

Fig. 4. Time course of $^{86}Rb^+$ uptake into jejunal BBMV's. BBMV's were pre-equilibrated in 50 mM Tris/HEPES (pH 7.5) and 300 mM D-mannitol. The extravesicular solution contained 100 mM RbCl and 50 mM Tris/HEPES (pH 7.5). Osmolarity of the extravesicular solution was maintained at 350 mOsm/liter with D-mannitol. The quench solution consisted of 175 mM KCl, 6 mM D-mannitol and 1 mM Tris/HEPES (pH 7.5). All points are the mean of 4 to 5 estimates ± the standard error of the mean. The linear regression of uptakes between 2 and 30 sec, shown in the inset, had a slope of 273 pmol/(mg × sec), an intercept of 1,850 pmol/mg and a regression coefficient of 0.95

using the Goldman-Hodgkin-Katz constant field equation. In each experiment increases and decreases in F from baseline were calibrated against 100/10 and 1/10 K + valinomycin signals, and all bi-ionic potentials were generated by adding vesicles to solutions containing 100 mM salts. These values of E_m and P_1/P_2 are presented in Tables 1, 2 and 3.

The results are internally consistent in all three sets of experiments. The relative permeabilities are the same regardless of the salts used in an individual experiment. For example, the ratio P_K/P_{Na} which can be deduced from the Cl^- data in Table 3 was 2.3. When the P_i/P_{Na} and P_i/P_K ratios from Tables 1 and 2 were used to calculate P_K/P_{Na}, it was 1.6 when Br^- was the anion, 1.7 for NO_3^-, 1.8 for Cl^-, Glu^-, and Ise^-, and 2.1 for I^-. As a consequence of this agreement, the same anion permeability sequence was obtained from either the Na^+ or the K^+ experiments. This agreement demonstrates that the fluorescence intensity is not specifically dependent upon which ions are present, but only upon the resulting membrane potential. The ion permeability sequence of jejunal brush border membranes as deduced by these experiments (relative to Na^+) was: F^-, Ise^-, Glu^-, $Chol^+(<0.1) < Na^+(1.0) < Cl^-(1.5) \leqq NO_3^-(1.5) < Br^-(2.3) < K^+(2.4) < Rb^+(2.5) < Cs^+(2.6) < Li^+(4) < NH_4^+(12) < I^-(40)$.

Radioactive Tracer Measurements

The uptakes of Br^-, Rb^+, Na^+ and K^+ were measured with radioactive tracers on the same batch of vesicles on which the fluorescence experiments were performed. Tracer measurements of SO_4^{2-} uptake were performed on different batches of membrane vesicles. Figure 4 shows the time courses of uptake of Rb^+. The inset shows the uptake during the first 30 sec. All ion uptakes were linear between 2 and 30 sec ($r > 0.9$). Time courses of uptake of Na^+ and Cl^- have been published previously (Gunther & Wright, 1983). Linear regressions of all uptakes between 2 and 30 sec were taken as the initial rates of transmembrane flux (J_i) of the substrates in all experiments. Kinetic parameters were determined by measuring J at various ion activities (a_i). The data were analyzed with Hofstee plots (J *vs.* J/a_i) to separate the J due to saturable mechanisms from simple electrodiffusive fluxes. Figure 5 shows two examples of Hofstee plots. Rb^+ shows a clear case of a saturable mechanism of uptake ($K_t = 3$ mM; $J_{max} = 75$ pmol/(mg protein × sec)) and a diffusive component ($P'_{Rb} =$ 7 nl/(mg protein × sec)), while Br^- had a pure diffusional flux ($P'_{Br} = 2.9$ nl/(mg protein × sec)). Once the existence of saturable mechanism(s) for each ion was determined, experiments were designed to measure P'_i at concentrations above the J_{max} of the saturable mechanisms.

Hofstee plots of three experiments from Gunther and Wright (1983) yielded P'_{Na} of 1.9, 2.2 and 2.5 nl/(mg × sec). The average P'_{Na} was 4.8 nl/(mg × sec) (±0.4 SE) in 8 experiments. P'_i's for five ions measured on a single preparation were all within a standard deviation of each other with the exception of Rb^+, which was approximately two-fold higher than the rest. The P'_i's were Na^+

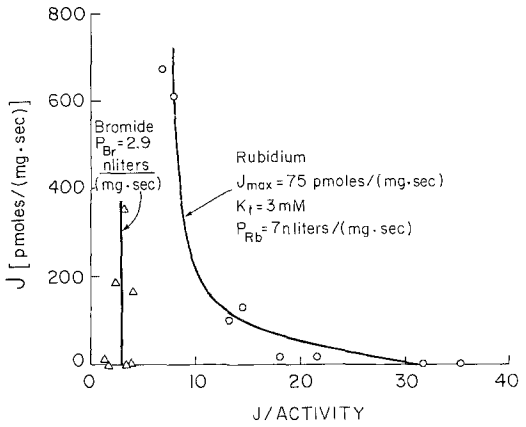

Fig. 5. Woolf-Augustinsson-Hofstee plots. BBMV's were pre-equilibrated as in Fig. 4. The extravesicular solutions were in 50 mM Tris/HEPES (pH 7.5). Osmolarity was maintained at 350 mOsm/liter with D-mannitol. Flux was determined as the slope of the linear regression through uptakes between 2 and 30 sec at each concentration. Rb^+: 8.5 μM – 130 mM RbCl, data from two experiments. Br^-: 10 μM – 150 mM KBr, data from one experiment

Table 4. Radioactive tracer permeability data[a]

Ion	P_i'	Ion	P_i
Na^+	4.8±0.9 (n=7)	Br^-	2.9, 4.7
K^+	4.6±1.2 (n=4)	SO_4^{2-}	1.1, 1.0
Rb^+	7.0±3.6 (n=3)		

[a] Ion permeability coefficients determined by radioactive tracer uptake experiments as described in Fig. 5. P_i''s are given as nl/(mg protein × sec). Errors, where noted, are standard errors of the mean. If less than three measurements were available, data from individual experiments is given. The counter ions were: Cl^- for Na^+, K^+ and Rb^+ experiments, Na^+ or K^+ for Br^- experiments, and Rb^+ for SO_4^{2-} experiments.

$(4.7) = Br^- < K^+(5.2) < Rb^+(11.5)$ nl/(mg protein × sec).

The results obtained with different batches of BBMV are summarized in Table 4. The P_i' sequence relative to Na^+ was: $SO_4^{2-}(0.2) < Br^-(0.8) \leqq K^+ = Na^+(1.0) \leqq Rb^+(1.5)$.

Results are presented as apparent permeability coefficients (P_i') because the E_m was not controlled in these experiments. However, there are two reasons to believe that they are close to the absolute permeability coefficients (P_i). The first is that the membranes exhibit little selectivity between the ions used in these flux measurements (with the exception of SO_4^{2-}) so that membrane potentials are likely to be small. The second is that in a single experiment in which P_{Na}' was measured at activities from 122 to 568 mM and E_m was minimized by having 1 M Cl^- both inside and outside of the vesicles, the same apparent permeability was obtained, 4.8±0.9 (n=7) for the open circuit experiment and 4.7±0.9 (n=1) for the voltage-clamped experiment.

There are saturable mechanisms for Na^+, K^+ and Rb^+ (Gunther & Wright, 1983), but the K_t's are low enough (~5 mM) that the saturable mechanisms contribute very little to the total flux in the activity range in which the apparent permeability coefficients were measured.

Discussion

These experiments were designed to determine the ion permeability characteristics of jejunal brush border membrane vesicles isolated from rabbit. Fluorescence measurements of membrane potential using diS-C_3-(5) provide a method for determination of relative electrodiffusive ion permeabilities. With this technique, small differences in permeability between two ions can be assessed and, at least under certain circumstances, absolute values of ion permeability can be obtained. P_i''s can also be determined with measurements of radioactive tracer fluxes into membrane vesicles. Radioactive tracer measurements are not as sensitive as fluorescence measurements for detecting small differences in P_i between two ions because the variability in the measurement is too great (the average standard deviation for a tracer permeability measurement was 23±9% of the P_i') and the diffusive flux must be separated from the saturable flux. However, they do provide an estimate of an absolute permeability coefficient, and confirm the fluorescence results that the ion selectivity is low.

Saturable components of ion flux can be detected using radioactive tracer measurements. For example, jejunal brush borders appear to have a Na^+/H^+ exchange mechanism (Gunther & Wright, 1983) and several Na^+ cotransport systems, including Na^+/glucose (Kaunitz, Gunther & Wright, 1982). The absolute magnitudes of permeability coefficients can be obtained from either radioactive tracer or fluorescence measurements. All ions tested had permeability coefficients in the range of 1 to 7 nl/(mg × sec). The sequence of electrodiffusive permeabilities obtained with fluorescence measurements of diffusion potentials also indicate that jejunal brush borders do not discriminate markedly between the common monovalent ions (e.g. $P_K/P_{Cl}/P_{Na} = 2.4:1.5:1.0$). These values for relative permeability predict that the membrane potential across the brush border should be −23 mV (*see below*). These results are consistent with data available from experiments on intact tissue.

Techniques

DiS-C_3-(5) is a membrane-potential-sensitive cyanine dye. In isolated brush border membrane vesicle suspensions diS-C_3-(5) apparently acts as a "slow dye" (redistribution or accumulation dye) (Cohen & Salzberg, 1978). In other words, the fluorescence changes observed when membrane potential alters are due to a potential-dependent redistribution of the positively charged dye between the external medium and the vesicle interior. The dye is accumulated in the vesicles when the interiors become more negative and released when the interiors become more positive. The emission from the dye decreases as the dye is accumulated intravesicularly, presumably due to the formation of dye aggregates, which have reduced fluorescence. DiS-C_3-(5) may also act as a "fast dye," which shows a change in fluorescence due to potential-dependent changes in dye molecules located on or in the membrane. However, the signal from the fast mode of ΔF is very much smaller than the slow response, and is unlikely to be observed with the experimental system described here.

DiS-C_3-(5) has recently been used to measure membrane potentials in renal and intestinal brush border membrane vesicles (Beck & Saktor, 1978; Wright et al., 1981; Schell et al., 1983). Both of these studies used K^+/valinomycin-induced membrane potentials to calculate the voltage response of the dye. In our study, the response of the dye was similar when either K^+/valinomycin or Na^+/ETH 1097 was used to produce known E_m. This is taken as evidence that the dye is monitoring E_m, rather than specific interaction of the dye with the substrates.

The internal consistency of the measurements of permeability ratios in jejunal vesicles is remarkable. The fact that the observed P ratios for Na^+ and K^+ are the same, regardless of the anion they are individually compared to, suggests that this method for determining the ratios is valid. As noted in the Results section, P_K/P_{Na} varied between 1.6 and 2.3 for all anions they were paired with (with the exception of F^-, whose permeability was too low compared to either to make the comparison meaningful).

A knowledge of the relative permeabilities of ions in JBBMV makes it possible to design experiments for radioactive tracer measurements of permeability where the membrane potential is known. For example, although the Na^+ activities in one experiment varied from 122 to 568 mM, the calculated E_m varied only between 1 and 9 mV. This was accomplished by taking advantage of the high permeability of the vesicles to Cl^-. One molar Cl^- inside and out served to shunt any membrane potential induced by Na^+ gradients. Under these conditions the contribution of the electrical component of the driving force for Na^+ was less than 16% of the total driving force. A knowledge of the E_m allows an estimation of the error in P_i'. In the Na^+ experiment mentioned above, the E_m was positive at all concentrations, resulting in an underestimate of the actual P_{Na} by $<25\%$.

The validity of the procedure for determination of initial rates of flux with tracers has been discussed previously (Gunther & Wright, 1983). Briefly, a linear regression analysis of time points between 2 and 30 sec was shown to yield J (the slope) and an external binding component (the intercept on the ordinate). Maneuvers expected to change the rate of sodium transport (e.g. inhibition of transport with amiloride or harmaline or stimulation with glucose, amino acids, or pH gradients) caused changes in the slope of the regression, but not in the intercept.

Comparison to Literature Values

Sequences

The permeability sequence for JBBMV's measured by diffusion potential experiments was (relative to Na^+): $F^-(0.04) < Na^+(1.0) < Cl^- = NO_3^-(1.5) < Br^-(2.3) \leqq K^+(2.4) \leqq Rb^+(2.5) \leqq Cs^+(2.6) < Li^+(3.9) < NH_4^+(12) < I^-(40)$. Although the membranes are not as selective as had previously been assumed, an estimate of the relevance of this data to the *in vivo* situation can be made by calculation of the membrane potential expected across the brush border using these values of relative permeability and estimates of ion concentrations. The above values yield an intracellular potential of -23 mV at 22° C when the Goldman-Hodgkin-Katz constant field equation is used and the concentrations are assumed to be: [Na] = 140 mM, [K] = 4 mM, and [Cl] = 145 mM in the lumen, and [Na] = 15 mM, [K] = 140 mM and [Cl] = 55 mM in the cell. This is approximately one-half of the measured potential in intact tissue at 37° C (-40 to -60 mV).

Ion permeability sequences for apical membranes of various tissues have been measured by several investigators. Reuss, Chung and Grady (1981) have determined that the ratio of cation permeabilities in *Necturus* gall bladder apical membranes is pH dependent, but the sequence at all pH's studied was $K^+ > Na^+ > Li^+$. In contrast, Li^+ was more permeable than K^+ or Na^+ in

JBBMV, as measured by the fluorescence technique. Baerentsen et al. (1982) determined that in *Necturus* gallbladder apical membranes, the permeability ratios were $K^+/Cl^-/Na^+$, 1.1:0.7:0.1. This is similar to what is observed in JBBMV with fluorescence, although the jejunal membranes seem less selective. Gögelein and van Driessche (1981) measured the selectivity of a K^+ channel in *Necturus* gallbladder apical membranes using noise analysis and observed the sequence: $K^+ > Rb^+ > Cs^+$. In JBBMV there was very little discrimination between these cations.

Magnitudes of P_i

The average ion permeability coefficient of JBBMV's measured with radioactive tracer uptake was approximately 5 nl/(mg protein × sec) or 10^{-5} cm/sec. In order to compare the magnitudes of the permeability coefficients measured in these experiments with measurements of uptake across the brush border in whole tissue preparations, it is necessary to convert flux per mg brush border protein to flux per cm^2 of whole tissue. This can be done since the enrichment (mg total protein/mg brush border protein), cell density (cells/cm^2 of mucosal area) and mg protein/cell can be estimated. Two different estimates of cell density were used. The first was from cell counts of photomicrographs of small intestine. There were approximately 10^8 cells/cm^2 (Harms & Wright, 1980). The second method involved measurement of the amount of mucosal protein scraped off a known serosal surface area of intestine (6 mg/cm^2) and the amount of protein per cell ($\sim 4 \times 10^{-7}$ mg/cell; Harms, *personal communication*). This method yielded a value of 1.6×10^7 cells/cm^2. The mean of these two estimates of cell density was used in the following calculations.

If J/mg brush border protein is measured, dividing this by the enrichment gives J/mg mucosal protein. Multiplying this rate by protein/cell and cells/cm^2 gives J/cm^2 serosal area. The permeability of the monovalent ions corresponds to about 10^{-5} cm/sec. This is in the range of values that can be extracted from studies on intact tissues (e.g., Goldner, Schultz & Curran, 1969; Bindslev, 1979).

Implications of the Sequences

The selectivity sequence for alkali cations: $Li^+ > Cs^+ > Rb^+ > K^+ > Na^+$ does not correspond exactly to any of the sequences predicted by the model described by Eisenman which was based on a simple coulombic interaction of the ions with a selectivity filter and the hydration energies of the ions (*see* Diamond & Wright, 1969). It is similar to Eisenman's sequence I, with the exception of Li^+, which is more permeable than expected. Sequence I is obtained with a selectivity filter with a low field strength (weakly negative) so that the binding energy between the ions and the filter is small compared to the energy of hydration of the cations. There are a number of explanations for the high Li^+ permeability. For example, there may be more than one cation permeation pathway, multiple energy barriers across a single pathway, or the ions may enter the selectivity filter in different states of hydration. The data is consistent with a model involving polarization of the ions as they contact the selectivity filter. The observed sequence is one of the ones predicted for an ion polarization model (sequence *Vp*; Krasne, 1978), where both NH_4^+ and Li^+ are highly permeant.

The anion selectivity sequence for the halides conforms to sequence I as described by Wright and Diamond (1977): $I^- > Br^- > Cl^- > F^-$. It corresponds to a model of a selectivity filter described by a simple coulombic interaction of the ion with the filter and the energies of hydration of the ions where the filter is positively charged with a weak field strength. From the permeability ratio of I^-/Cl^- it is possible to predict the relative permeabilities of other anions from the selectivity isotherms produced by Wright and Diamond (1977). The sequence of anion permeability predicted by these isotherms, based on the P_I/P_{Cl} of ~ 25 is (relative to Cl^-): $F^-(0.1) < IO_3^-(0.5) < Cl^-(1.0) < HCO_3^-(2) < BrO_3^-(3) < Br^-(5) < NO_3^-(7) \leqq ClO_3^-(7) < BF_4^-(25) \leqq I^-(25) < SCN^-(50) < ClO_4^-(100)$. At present it is not clear whether there are nonselective anion and cation channels in this membrane, or whether there are multiple highly selective channels.

We wish to thank Professor W. Simon of the Swiss Federal Institute of Technology, Zurich, for his generous gift of ETH 1097, Dr. Alan Waggoner, for his generous gift of diS-C_3-(5) and Dr. Sally Krasne for the use of her spectrofluorometer and her invaluable advice on this project. Supported by USPHS AM 19567 and AM 07270.

References

Baerentsen, H.J., Christensen, O., Thomsen, P.G., Zeuthen, T. 1982. Steady states and the effects of ouabain in the *Necturus* gallbladder epithelium. *J. Membrane Biol.* **68**:215–226

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

Bindslev, N. 1979. Sodium transport in the hen lower intestine. Induction of sodium sites in the brush border by a low sodium diet. *J. Physiol. (London)* **288**:449–466

Cohen, L.B., Salzberg, B.M. 1978. Optical measurement of membrane potential. *Rev. Physiol. Biochem. Pharmacol.* **83**:35–88

Diamond, J.M., Wright, E.M. 1969. Biological membranes: The physical basis of ion and non-electrolyte selectivity. *Annu. Rev. Physiol.* **31**:581–646

Eisenman, G., Margalit, R., Kuo, K.H. 1981. The ability of the peptide backbone to bind anions as well as cations – implications for peptide carriers, channels and electrodes. *In:* Progress in Enzyme and Ion-Selective Electrodes. D.W. Lübbers, H. Acker, R.P. Buck, G. Eisenman, M. Kessler and W. Simon editors. pp.1–8. Springer-Verlag, Berlin

Frizzell, R.A., Nellans, H.N., Rose, R.C., Markscheid-Kaspi, L., Schultz, S.G. 1973. Intracellular Cl concentrations and influxes across the brush border of rat ileum. *Am. J. Physiol.* **224**:328–337

Gögelein, H., Driessche, W. van 1981. Noise analysis of the K^+ current through the apical membrane of *Necturus* gallbladder. *J. Membrane Biol.* **63**:243–254

Goldner, A.M., Schultz, S.G., Curran, P.F. 1969. Sodium and sugar fluxes across the brush border of rabbit ileum. *J. Gen. Physiol* **53**:362–383

Gunther, R.D., Schell, R., Krasne, S., Wright, E.M. 1982. Ion permeability of jejunal brush border membranes measured with a potential sensitive dye and with radioactive tracers. *Physiologist* **25**:335

Gunther, R.D., Wright, E.M. 1983. Na^+, Li^+ and Cl^- transport by brush border membranes from rabbit jejunum. *J. Membrane Biol* **74**:85–94

Harms, V., Wright, E.M. 1980. Some characteristics of Na/K-ATPase from rat intestinal basal lateral membranes. *J. Membrane Biol.* **53**:119–128

Kaunitz, J.D., Gunther, R.D., Wright, E.M. 1982. Involvement of multiple sodium ions in intestinal D-glucose transport. *Proc. Natl. Acad. Sci. USA* **79**:2315–2318

Krasne, S. 1978. Ion selectivity in membrane permeation. *In:* Physiology of Membrane Disorders. T.E. Andreoli, J.F. Hoffman and D.D. Fanestil, editors. pp 217–242. Plenum, New York, London

Reuss, L., Chung, L.Y., Grady, T.P. 1981. Mechanisms of cation permeation across apical cell membrane of *Necturus* gallbladder: Effects of luminal pH and divalent cations on K^+ and Na^+ permeability. *J. Membrane Biol.* **59**:211–224

Robinson, R.A., Stokes, R.H. 1959. Electrolyte Solutions. Butterworths, London

Schell, R.E., Stevens, B.R., Wright, E.M. 1983. Kinetics of Na-dependent solute transport by rabbit renal and jejunal brush border vesicles using a fluorescent dye. *J. Physiol. (London)* **335**:307–318

Stevens, B.R., Wright, S.H., Hirayama, B., Ross, H.J., Gunther, R.D., Nord, E., Kippen, I., Harms, V.K., Wright, E.M. 1982. Liquid nitrogen preservation of organic and inorganic solute transport in renal and intestinal membrane vesicles. *Membr. Biochem.* **4**:271–281

Waggoner, A.S. 1979. Dye indicators of membrane potential. *Annu. Rev. Biophys. Bioeng.* **8**:47–68

Wright, E.M., Diamond, J.M. 1977. Anion selectivity in biological systems. *Physiol. Rev.* **57**:109–156

Wright, S.H., Krasne, S., Kippen, I., Wright, E.M. 1981. Na^+-dependent transport of tricarboxylic acid cycle intermediates by renal brush border membranes. Effects on fluorescence of a potential sensitive cyanine dye. *Biochim. Biophys. Acta* **640**:767–778

Received 30 June 1983; revised 13 September 1983

Note Added in Proof

More recent experiments with DiS-C_3-(5) have revealed that the ion permeability of rabbit intestinal BBMV's were not significantly altered by either increasing the temperature from 22 to 37 °C or by the addition of K-channel blockers such as tetraethylammonium. However, we (Mandel, K.G., et al., 1984. Influence of calcium on properties of intestinal brush borders. *Fed. Proc. (Abstr.) (In Press)* have shown that membranes prepared by Ca-precipitation are more permeable to Cl (relative to Na, K, Br, choline, and gluconate) than membranes prepared in the absence of divalent cations.