*Biochem. J.* (1976) **154**, 597–604
*Printed in Great Britain*



# Sodium/Proton Antiport in Brush-Border-Membrane Vesicles Isolated from Rat Small Intestine and Kidney

By HEINI MURER, ULRICH HOPFER* and ROLF KINNE
*Max-Planck-Institut für Biophysik, Frankfurt/Main, German Federal Republic*

(*Received* 26 *August* 1975)

Studies on proton and $Na^+$ transport by isolated intestinal and renal brush-border-membrane vesicles were carried out to test for the presence of an $Na^+/H^+$-exchange system. Proton transport was evaluated as proton transfer from the intravesicular space to the incubation medium by monitoring pH changes in the membrane suspension induced by sudden addition of cations. $Na^+$ transport was determined as $Na^+$ uptake into the vesicles by a filtration technique. A sudden addition of sodium salts (but not choline) to the membrane suspension provokes an acidification of the incubation medium which is abolished by the addition of 0.5% Triton X-100. Pretreatment of the membranes with Triton X-100 prevents the acidification. The acidification is also not observed if the $[K^+]$ and proton conductance of the membranes have been increased by the simultaneous addition of valinomycin and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone to the $K^+$-rich incubation medium. Either valinomycin or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone when added alone do not alter the response of the membranes to the addition of $Na^+$. $Na^+$ uptake by brush-border microvilli is enhanced in the presence of a proton gradient directed from the intravesicular space to the incubation medium. Under these conditions a transient accumulation of $Na^+$ inside the vesicles is observed. It is concluded that intestinal and renal brush-border membranes contain a $Na^+/H^+$ antiport system which catalyses an electroneutral exchange of $Na^+$ against protons and consequently can produce a proton gradient in the presence of a concentration difference for $Na^+$. This system might be involved in the active proton secretion of the small intestine and the proximal tubule of the kidney.

In a variety of biological systems a $Na^+/H^+$ antiport mechanism has been described. Mitchell & Moyle (1967, 1969) and Brierley *et al.* (1968) demonstrated that the electroneutral transport of $Na^+$ through the cristae membranes of mammalian mitochondria occurs via an exchange of $Na^+$ for $H^+$ ions. A similar system was detected in *Escherichia coli* (West & Mitchell, 1974) and in *Streptococcus faecalis* (Harold & Pappineau, 1972).

$Na^+$/proton antiport has been postulated to be involved also in transepithelial electrolyte transport in the small intestine (Parsons, 1975; Turnberg *et al.*, 1970) and the renal proximal tubule (Pitts, 1961; Pitts *et al.*, 1949), as well as amphibian skin (Emilio & Menano, 1975), gills (Motais & Garcia-Romeu, 1972), gall bladder (Whitlock & Wheeler, 1969) and urinary bladder of the toad (Frazier & Vanatta, 1971) and turtle (Green *et al.*, 1968).

It was demonstrated in micropuncture studies that proton transport by the renal proximal tubule is dependent on the ambient $Na^+$ concentration (Ullrich *et al.*, 1971, 1975). This led to the assumption that by analogy with the $Na^+$-dependent sugar and amino acid-transport systems (Evers *et al.*, 1976; Kinne *et al.*, 1975; Murer & Hopfer, 1974; Murer *et al.*, 1975) a direct coupling between $Na^+$ and proton flux across the brush-border membrane might exist.

In the present paper the interrelation of proton and $Na^+$ fluxes was investigated by using isolated brush-border-microvilli vesicles. These preparations have been shown previously to be a very suitable model to study transport processes across the luminal membrane of epithelia without interference of intracellular metabolism and contraluminal transport systems (Evers *et al.*, 1976; Kinne *et al.*, 1975; Murer & Hopfer, 1974; Murer *et al.*, 1975; Hopfer *et al.*, 1973).

By measuring the acidification of the incubation medium during influx of $Na^+$ into the vesicles and by measuring $Na^+$ uptake during proton efflux out of the vesicles an $Na^+/H^+$ antiport could be demonstrated in the brush-border membranes and some of its properties could be studied.

We describe the $Na^+$-induced movement of $H^+$ in one direction and the movement of $OH^-$ in the

* Present address: Department of Anatomy, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

opposite direction as effective movements of $H^+$, as they cannot be distinguished in this type of study.

## Materials and Methods

Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone was obtained from Boehringer G.m.b.H. (Mannheim, West Germany). Valinomycin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The radioactive compounds were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). The other chemicals used for the experiments were of the highest grade available.

### *Membrane preparation*

Brush-border membranes of rat small intestine were prepared from male Wistar rats of 180–240g body wt. as described by Sigrist-Nelson *et al.* (1975). In essence, mucosal scrapings were homogenized in a hypo-osmotic medium; after addition of 10mM-$CaCl_2$ the brush-border membranes were purified by differential centrifugation. Renal brush-border membranes were prepared from the same animals as described by Heidrich *et al.* (1972) and by Kinne *et al.* (1975). The renal membranes were isolated after homogenization of the tissue in iso-osmotic buffered sucrose (10mM-triethanolamine/HCl and 250mM-sucrose, pH7.6) by differential centrifugation without the use of $CaCl_2$.

After isolation the membranes were suspended by homogenization with a glass/Teflon homogenizer (ten strokes, 1200rev./min) in 50ml of 500mM-D-mannitol, 1mM-Hepes†/Tris (1mM-Hepes adjusted with Tris to pH7.4) for $Na^+$-uptake experiments, or in 50ml of 100mM-mannitol, 1mM-Hepes/Tris, pH7.4, for proton-transport experiments, and centrifuged for 20min at 40000*g*.

For proton-transport experiments the membranes were then transferred into a buffer containing 150mM-potassium cyclamate(cyclohexylsulphamate), 5mM-glycylglycine and 2mM-$MgSO_4$ (adjusted with $H_2SO_4$ or KOH to pH6.15) and washed twice in this buffer by homogenization (ten strokes, 1200rev./min) and centrifugation (20min at 40000*g*).

For $Na^+$-uptake experiments the isolated brush-border vesicles were loaded with 50mM-Mes/Tris buffer (50mM-Mes adjusted with Tris to pH5.5 for intestinal membranes, and to pH5.9 for renal membranes) by mixing 5ml of membrane suspension with 25ml of 50mM-Mes/Tris buffer at 4°C. Then 10min later the vesicles were sonicated (Bandelin Sonorex, Bandelin Electronic KG, West Berlin, Germany) for 40s at 200mA; the sonication was repeated five times at 2min intervals. During this procedure the sample was cooled with ice-cold water. The brush-border membranes were then collected by centrifugation (40000*g* for 20min) and resuspended in a buffer containing 100mM-D-mannitol, 0.1mM-$MgSO_4$ and 50mM-Mes/Tris (pH5.5 for intestinal membranes, and pH5.9 for renal membranes).

† Abbreviations: Hepes, 2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid; Mes, 2-(*N*-morpholino)-ethanesulphonic acid; ATPase, adenosine triphosphatase.

### *pH measurements*

The pH determinations were performed in a closed, temperature-controlled (25°C) electrode vessel. A portion (20$\mu$l) of stock salt solutions or Triton X-100 solution was added to 200$\mu$l of membrane suspension through a lateral inlet by means of a Hamilton syringe. The samples were mixed continuously by a magnetic stirrer. A Radiometer pH-meter (model 26) connected with an Ingold pH-electrode (type 403/30/M8) and a Radiometer recorder (Servograph) was used to register the pH continuously. Solutions of added salts were 1.0M with respect to the cation and were prepared in cyclamate/glycylglycine buffer, pH6.15. Triton X-100 solution (5%, w/w) was used to dissolve the membrane vesicles. The pH of the salt solutions and of the Triton X-100 solution was adjusted to the pH of the membrane suspension (usually between pH6.3 and pH6.5) either with KOH or with $H_2SO_4$. Solutions of the ionophores were prepared in ethanol. The final ethanol concentration in the incubation medium after addition of the ionophores was 0.5%.

### *Uptake measurements*

*$Na^+$ uptake.* The uptake of $^{22}Na$ was measured by a filtration technique as described by Hopfer *et al.* (1973). The incubation medium contained 100mM-D-mannitol, 0.5mM-$Na_2SO_4$ and 50mM-Hepes/Tris, pH7.5, or 50mM-Mes/Tris, pH5.5, respectively.

The experiment was started by addition of 20$\mu$l of membrane suspension [200–500$\mu$g of protein as measured by the method of Lowry *et al.* (1951)] to 200$\mu$l of incubation medium containing 10$\mu$Ci of $^{22}Na$, kept at 25°C. The uptake of substrate was terminated by the removal of 20$\mu$l of the incubation mixture and rapid dilution with 1ml of ice-cold buffer containing 150mM-$MgCl_2$, 100mM-D-mannitol and 10mM-Hepes/Tris, pH7.5; 2$\mu$Ci of D-[$^3$H]-mannitol was added to 1ml of dilution buffer. The diluted sample was immediately filtered through a filter (no. 11305, 0.6$\mu$m; Sartorius, Göttingen, West Germany) and the collected membranes were rinsed once with 4ml of buffer of the same composition as the dilution buffer except for omission of radioisotope. The radioisotope in the dilution buffer served as a correction for substrate retention by the filter due to insufficient washing. Usually this retention was negligible. The filters were then dissolved and counted for radioactivity in a liquid-scintillation counter as described previously (Hopfer *et al.*, 1973). Uptake experiments were carried out at 25°C.

*D-glucose uptake.* Uptake of D-glucose was measured by a similar method as that described for $Na^+$.

The composition of the incubation medium was 100mM-D-mannitol, 1mM-D-[U-$^{14}$C]glucose, 0.1mM-$MgSO_4$, 50mM-$Na_2SO_4$ and 50mM-Hepes/Tris, pH 7.5.

## Results and Discussion

### *Proton ejection induced by $Na^+$*

The effect of $Na^+$ on proton transfer across the membrane was studied by continuously monitoring the pH of the extravesicular space under the conditions of sudden injection of small volumes of highly concentrated cation solutions. The addition of 20$\mu$l of 500mM-$Na_2SO_4$ to 200$\mu$l of intestinal or renal brush-border-membrane vesicles (Figs. 1*a* and 2*a*, respectively) caused a rapid acidification of the incubation medium. This acidification was abolished by addition of 0.5% Triton X-100, which destroys the membrane vesicles. Addition of $Na^+$ after destruction of the vesicles (Figs. 1*b* and 2*b*) was ineffective. The addition of choline (Figs. 1*c* and 2*c*) causes a smaller and more rapid electrode response which, however, is not abolished by Triton X-100 and is also observed after disruption of the membrane vesicles. The effect of choline is probably due to an effect of the salt on the buffer and/or on the glass electrode. A similar, unspecific electrode response could be detected for choline in control experiments without membrane vesicles.

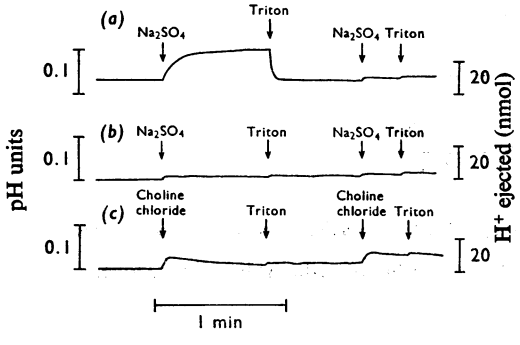


Fig. 1. *Proton ejection from intestinal brush-border-membrane vesicles induced by $Na^+$*

(*a*) and (*c*), intact membrane vesicles; (*b*) (0.5%)-Triton solubilized membrane vesicles. Additions: 20$\mu$l of 0.5M-$Na_2SO_4$; 20$\mu$l of 1.0M-choline chloride; 20$\mu$l of 5% Triton X-100; 200$\mu$l of membrane suspension contained 815$\mu$g of protein.

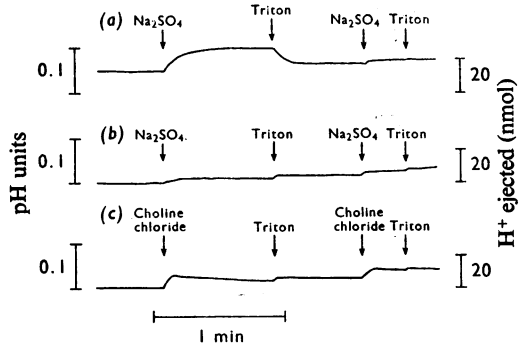


Fig. 2. *Proton ejection from renal brush-border-membrane vesicles induced by $Na^+$*

(*a*) and (*c*), intact membrane vesicles; (*b*) (0.5%) Triton-solubilized membrane vesicles. Additions were as indicated in Fig. 1; 200$\mu$l of membrane suspension contained 1530$\mu$g of protein.

In principle, two explanations for the acidification of the outer vesicular space after the addition of $Na^+$ are possible. Either protons have been replaced from protonated groups in the membrane surface, or protons have been translocated from the intra- to the extra-vesicular space. The former possibility is very unlikely, because under the experimental conditions used, those sites of the membrane surface which exchange unspecifically with cations should be preferentially occupied by $Mg^{2+}$ or $K^+$ present in the washing buffer and incubation medium. Further, replacement represents a net gain of protons. Therefore the acidification of the medium should not be abolished by destruction of the membrane vesicles. However, in the experiments with intestinal and renal brush-border membranes the pH of the medium returns to the initial value after addition of Triton X-100. Moreover, the response of the brush-border membranes to $Na^+$ can be prevented if the membranes are supplied with a system which allows proton flux in exchange with $K^+$ (see the experiments with the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and valinomycin described below). Thus it can be concluded that $Na^+$ (but not choline) induces a translocation of protons across the brush-border membranes.

This translocation can be caused by two different processes, which are represented schematically in Fig. 3. First, the two ions could use different transport systems, but their fluxes would influence each other via the membrane potential (Fig. 3*a*, 1). This might be detailed in terms of the following sequential processes: (1) movement of $Na^+$ into the brush-border-membrane vesicles is associated with a charge transfer; (2) in the presence of non-permeant anions, an inside positive diffusion potential is produced; (3) this potential exerts a driving force for $H^+$ movement out of the membrane vesicles. Secondly, a molecular coupling by counterflow via a transport system could occur similar to the antiporter described by West & Mitchell (1974) (Fig. 3*a*, 2). This antiporter catalyses

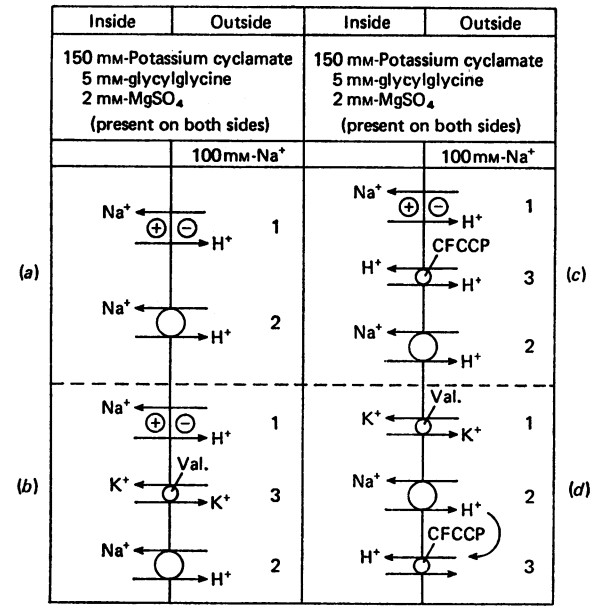


Fig. 3. *Experimental model to explain $Na^+$-induced proton ejection from brush-border-membrane vesicles*

Abbreviations: Val., valinomycin; CFCCP, carbonyl chloride *p*-trifluoromethoxyphenylhydrazone.

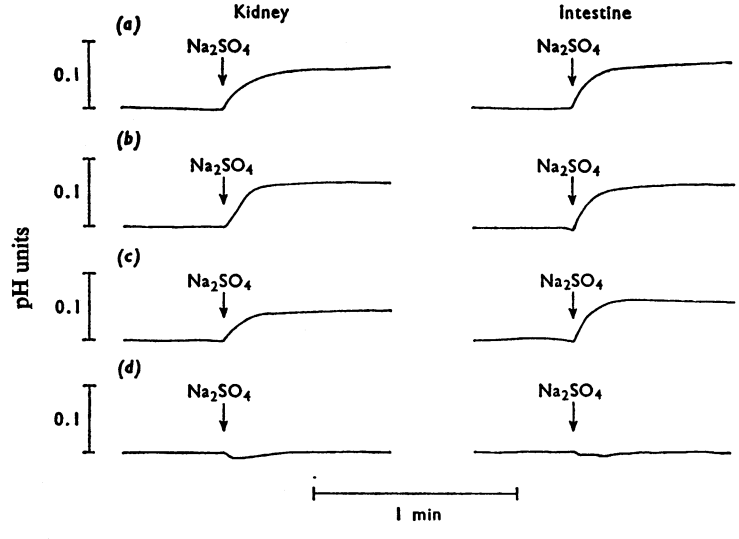


Fig. 4. *Effect of ionophores on proton ejection induced by $Na^+$*

The membrane vesicles (200 $\mu$l) were preincubated for 2 min with (*a*), 1 $\mu$l of ethanol; (*b*), 1 $\mu$l of valinomycin (10 $\mu$g); (*c*) 1 $\mu$l of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (5 $\mu$g); (*d*), 1 $\mu$l of valinomycin + 1 $\mu$l of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. A 200 $\mu$l portion of renal membranes contained 1530 $\mu$g of protein; a 200 $\mu$l portion of intestinal membranes contained 815 $\mu$g of protein.

the electroneutral exchange of $Na^+$ and protons, and the rate of transport therefore should not be dependent on the membrane potential.

The contribution of the membrane potential to the proton movements was evaluated by two experimental approaches. The first approach was designed to short-circuit the membrane potential by the introduction of the ionophore valinomycin into the membranes (Fig. 3*b*, 3). Valinomycin forms a charged complex with $K^+$ and increases the $K^+$ conductance (Fig. 3*b*) of artificial (Henderson *et al.*, 1969; Pressmann, 1968) and natural membranes without changing the characteristics of non-electrolyte permeability in the brush-border membranes (Murer & Hopfer, 1974; Sigrist-Nelson *et al.*, 1975; Hopfer, 1975). Thus if $Na^+$ and $H^+$ efflux would be coupled via the membrane potential (Fig. 3*b*, 1) the addition of valinomycin in the presence of equal $K^+$ concentration at both sides of the membrane should abolish the membrane potential and thereby the acidification. As shown in Fig. 4*b*, valinomycin did not alter the response of the brush-border-membrane vesicles to $Na^+$.

In a second approach the proton conductance of the membranes was increased by the addition of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (Fig. 3*c*). If the proton movement would be linked to the $Na^+$ movement electrically, one would expect that the acidification would be facilitated by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (Fig. 3*c*, 3), because now a very potent, potential-sensitive, proton-translocating system is present in the membranes (Henderson *et al.*, 1969). However, as shown in Fig. 4*c*, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone has no influence on the $Na^+$-induced proton ejection. On the contrary, however, the effect of $Na^+$ could not be observed when the proton ionophore and the $K^+$ ionophore were present simultaneously (Fig. 4*d*). This can be explained in terms of two overall electroneutral proton movements across the membranes which compensate each other (Fig. 3*d*). (*a*) Under the influence of $Na^+$ a proton gradient (medium > vesicle) would be created electroneutrally by the antiport system (Fig. 3*d*, 2). (*b*) Because of the lack of permeant anions this proton gradient can, however, only be dissipated by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (Fig. 3*d*, 3), if the $H^+$ movement is compensated in charge by the $K^+$ movement mediated by valinomycin (Fig. 3*d*, 1). The same result was obtained when nigericin instead of valinomycin plus carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone is present during the experiment. Nigericin as a $K^+/H^+$ antiporter combines the action of valinomycin ($K^+$ transfer) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone ($H^+$ transfer). The experiment with valinomycin plus carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone also supports our assumptions made for the experiments shown in Fig. 4*b* and 4*c*, namely that valinomycin short-circuits the membrane potential via an increased $K^+$ conductance and that carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone increases the proton permeability of the membrane.

From the experiments with the ionophore valinomycin and the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone we conclude that $Na^+$ influx and $H^+$ efflux are coupled directly via an antiport mechanism. These experiments can also be used to estimate the stoicheiometry of proton and $Na^+$ transfer via the exchange system. Under the experimental conditions used in this study mainly a 1:1 ratio is maintained, which renders the exchange electroneutral and thus insensitive to changes in the membrane potential (see Figs. 4*a* and 4*b*).

*Proton-induced $Na^+$ accumulation*

In earlier experiments (Sigrist-Nelson *et al.*, 1975; Kinne *et al.*, 1975) we demonstrated that isolated brush-border membranes from the small intestine and from the proximal tubule can be used to study $Na^+$ transport by means of tracer techniques. We found that $Na^+$ enters the same osmotically reactive space as transported non-electrolytes.

In a postulated $Na^+/H^+$ antiport system, not only stimulation of proton flux by $Na^+$ but also stimulation of $Na^+$ flux by protons should be demonstrable. When brush-border-membrane vesicles are preloaded

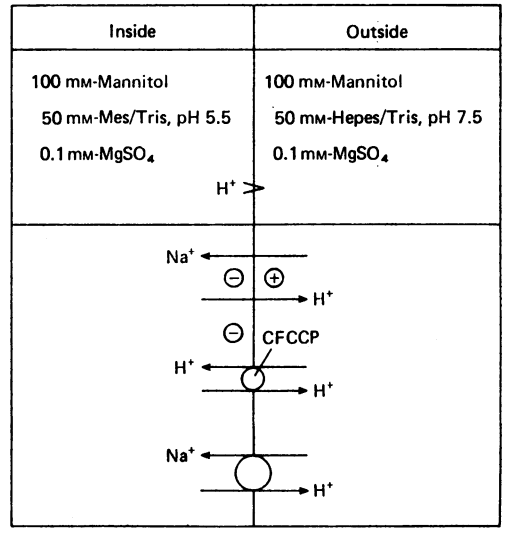


Fig. 5. *Experimental model to explain $H^+$-induced $Na^+$ uptake by brush-border-membrane vesicles*

Abbreviation: CFCCP, carbonyl chloride *p*-trifluoromethoxyphenylhydrazone.

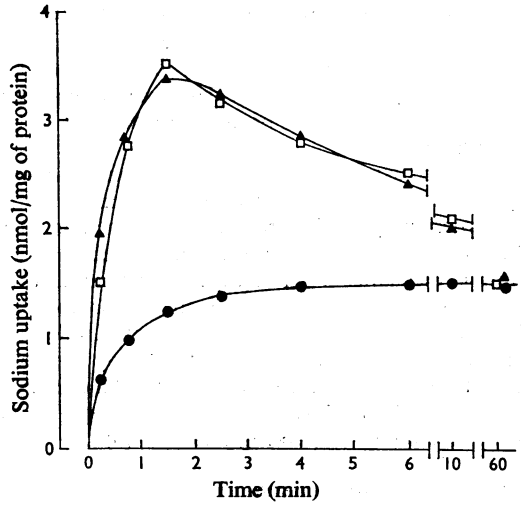


Fig. 6. *Uptake of $Na^+$ by intestinal brush-border-membrane vesicles: influence of a pH gradient and carbonyl cyanide p-trifluoromethoxyphenylhydrazone*

The membranes were preloaded with 50mM-Mes/Tris buffer, pH5.5, followed by incubation in a medium containing 0.5mM-$Na_2SO_4$ and: (●), 50mM-Mes/Tris, pH5.5; (□), 50mM-Hepes/Tris, pH7.5; (▲), 50mM-Hepes/Tris, pH7.5, +1 $\mu$l of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (5 $\mu$g). Other components of the incubation medium are given in the Materials and Methods section.

with a buffer of low pH and incubated in a buffer with a high pH the influence of a pH gradient on $Na^+$ uptake can be studied. In addition the influence of the membrane potential on transport processes can be investigated by increasing the proton diffusion potential by the addition of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (see Fig. 5 for schematic representation). As shown in Fig. 6 for intestinal and Fig. 7 for renal membranes, a proton gradient is able to accomplish a transient overshoot in the uptake of $Na^+$. The overshoot is not observed under non-gradient conditions at either pH7.5 or 5.5. A small stimulation of $Na^+$ uptake can be observed in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. This stimulation is most probably due to an effect of the potential on an electrogenic movement of a small proportion of $Na^+$ ions through a system not identical with the electroneutral $Na^+/H^+$-exchange system.

Figs. 8 and 9 show D-glucose uptake by intestinal and renal brush-border-membrane vesicles under the condition of a pH gradient ([$H^+$] inside the vesicles higher than outside) in the absence and presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. In contrast with $Na^+$ transport, the uptake of D-glucose is markedly stimulated by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. $Na^+$-dependent D-glucose transport across the brush-border membrane is known to be potential-sensitive (Murer & Hopfer, 1974) and to involve a net transfer

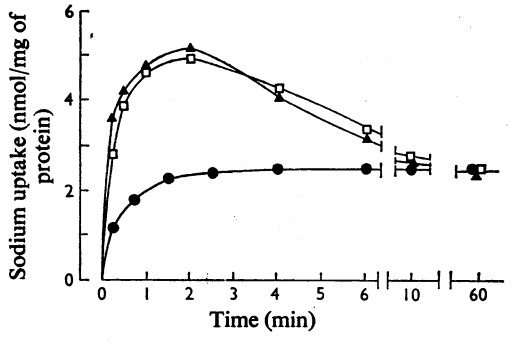


Fig. 7. *Uptake of $Na^+$ by renal brush-border-membrane vesicles: influence of a pH gradient and carbonyl cyanide p-trifluoromethoxyphenylhydrazone*

The experiment was carried out as described in Fig. 6 for intestinal membranes except for preloading the vesicles with 50mM-Mes/Tris, pH5.9; pH of the incubation medium: (●) pH5.9; (□), pH7.5; (▲), pH7.5 + 1 $\mu$l (5 $\mu$g) of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

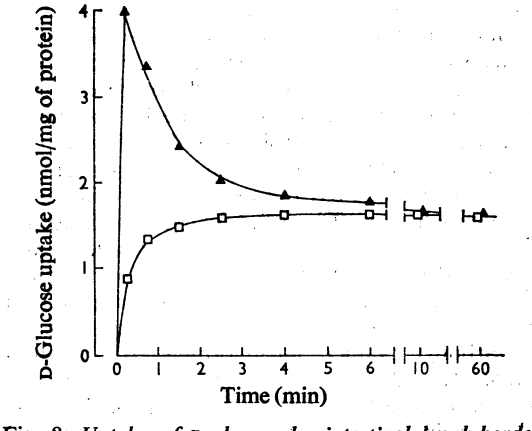


Fig. 8. *Uptake of D-glucose by intestinal brush-border-membrane vesicles: influence of a pH gradient and carbonyl cyanide p-trifluoromethoxyphenylhydrazone*

The membranes were preloaded with 50mM-Mes/Tris buffer, pH5.5, followed by incubation in a medium containing 1mM-D-[U-$^{14}$C]glucose, 50mM-$Na_2SO_4$ and (□), 50mM-Hepes/Tris, pH7.5; (▲), 50mM-Hepes/Tris, pH7.5 + 1 $\mu$l (5 $\mu$g) of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

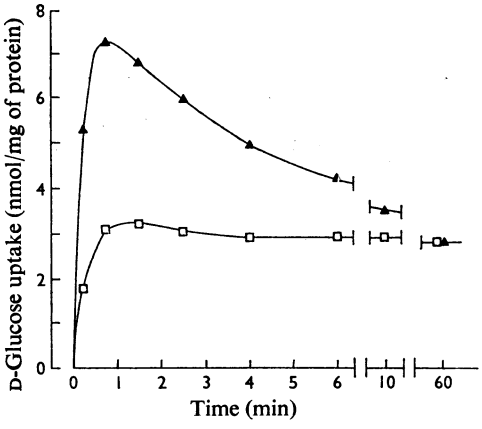


Fig. 9. *Uptake of* D-*glucose by renal brush-border-membrane vesicles: influence of a pH gradient and carbonyl cyanide p-trifluoromethoxyphenylhydrazone*

The experiment was carried out as indicated in Fig. 8 for intestinal membranes except for replacement of the 50mM-Mes/Tris buffer, pH5.5, by a 50mM-Mes/Tris buffer, pH5.9: (□), without carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; (▲), with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

of positive charges. Thus the observed stimulation indicates that carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone under the experimental conditions used increases the inside negative membrane potential.

The observation of a counterflow for $Na^+$ and protons in the absence of significant $H^+$ conductance provides further evidence for the presence of an $Na^+$/proton antiport system in the brush-border membrane.

*Conclusions*

Two consequences of the operation of an electroneutral $Na^+/H^+$ antiport mechanism have been demonstrated in intestinal and renal brush-border membranes. First, addition of $Na^+$ to a membrane suspension causes an expulsion of protons from an intravesicular space. Secondly, a proton gradient stimulates the uptake of $Na^+$ into the intravesicular space.

The direct demonstration of $Na^+/H^+$ antiport in the luminal membrane offers a new explanation for the molecular basis of active proton transport by the proximal tubule and the small intestine. Since in the intact cell an $Na^+$ concentration gradient exists across the luminal membrane (the extracellular $Na^+$ concentration is higher than the intracellular) an active proton excretion could be envisaged to occur by a coupling of the $Na^+$ entry with a proton ejection via the $Na^+/H^+$ antiport system.

On the basis of its cellular localization and its sensitivity to bicarbonate also, a $HCO_3^-$-stimulated ATPase has been postulated to be involved in active proton secretion (Kinne-Saffran & Kinne, 1974) and one could speculate that $Na^+/H^+$ exchange might be a partial reaction of the enzyme which is independent of the presence of ATP. However, at the moment, apart from an identical cellular localization, there is no evidence of an identity of the $Na^+/H^+$ antiport system and the $HCO_3^-$-stimulated ATPase. Moreover, the $Na^+$-independence of the ATPase might argue against any relation of the two systems.

We are grateful to Professor Dr. K. J. Ullrich for valuable discussion during the preparation of the manuscript. We also express our gratitude to Mrs. I. Rentel and R. Petzold for the excellent art work of the Figures.

## References

Brierley, G. P., Settlemire, C. T. & Knight, V. A. (1968) *Arch. Biochem. Biophys.* **126**, 276–288
Emilio, M. G. & Menano, H. P. (1975) *Biochim. Biophys. Acta* **382**, 344–352
Evers, J., Murer, H. & Kinne, R. (1976) *Biochim. Biophys. Acta*, in the press
Frazier, L. W. & Vanatta, J. C. (1971) *Biochim. Biophys. Acta* **241**, 20–29
Green, H. H., Steinmetz, P. R. & Frazier, H. S. (1968) *J. Clin. Invest.* **47**, 43a
Harold, F. M. & Pappineau, D. (1972) *J. Membr. Biol.* **8**, 45–62
Heidrich, H. G., Kinne, R., Kinne-Saffran, E. & Hannig, K. (1972) *J. Cell Biol.* **54**, 232–245
Henderson, P. J. F., McGivan, J. D. & Chappell, J. B. (1969) *Biochem. J.* **111**, 521–535
Hopfer, U. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2027–2031
Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M. & Sachs, G. (1975) *J. Membr. Biol.* **21**, 375–395
Kinne-Saffran, E. & Kinne, R. (1974) *Proc. Soc. Exp. Biol. Med.* **146**, 751–753
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Mitchell, P. & Moyle, J. (1967) *Biochem. J.* **105**, 1147–1162
Mitchell, P. & Moyle, J. (1969) *Eur. J. Biochem.* **9**, 149–155
Motais, R. & Garcia-Romeu, F. (1972) *Annu. Rev. Physiol.* **34**, 141–176
Murer, H. & Hopfer, U. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 484–488
Murer, H., Sigrist-Nelson, K. & Hopfer, U. (1975) *J. Biol. Chem.* **250**, 7392–7396
Parsons, D. S. (1976) in *Intestinal Ion Transport* (Robinson, J. W. L., ed.), Medical and Technical Publishing Co., Lancaster, in the press
Pitts, R. F. (1961) *Progr. Cardiovasc. Dis.* **3**, 537–563
Pitts, R. F., Ayer, J. L. & Schiess, W. A. (1949) *J. Clin. Invest.* **28**, 35–44

Pressmann, B. (1968) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **27**, 1283–1288

Sigrist-Nelson, K., Murer, H. & Hopfer, U. (1975) *J. Biol. Chem.* **250**, 5674–5680

Turnberg, L. A., Bieberdorf, F. A., Morawski, S. G. & Fordtran, J. S. (1970) *J. Clin. Invest.* **49**, 557–567

Ullrich, K. J., Radtke, H. W. & Rumrich, G. (1971) *Pflügers Arch.* **330**, 149–161

Ullrich, K. J., Rumrich, G. & Baumann, K. (1975) *Pflügers Arch.* **357**, 149–163

West, I. C. & Mitchell, P. (1974) *Biochem. J.* **144**, 87–90

Whitlock, R. T. & Wheeler, H. O. (1969) *Am. J. Physiol.* **217**, 310–316