J. exp. Biol. **106**, 163–180 (1983)
Printed in Great Britain © The Company of Biologists Limited 1983

CO- AND COUNTER-TRANSPORT MECHANISMS IN BRUSH BORDER MEMBRANES AND BASAL-LATERAL MEMBRANES OF INTESTINE AND KIDNEY

By H. MURER, G. AHEARN, J. BIBER, G. CASSANO, P. GMAJ and B. STIEGER

Department of Physiology, University of Zurich, Rämistrasse 69, CH-8028 Zurich, Switzerland

SUMMARY

One way to obtain a deeper understanding of the complex function of the small intestinal and renal proximal tubular epithelium is to dissect it into single components and then, having defined the components under well-controlled conditions, try to describe the behaviour of the whole system on the basis of the properties of the single components.

Brush border and basal-lateral membranes can be isolated by different methods, including free flow electrophoresis, differential and gradient centrifugation. Transport can be analysed in vesiculated membrane fractions by tracer techniques and spectrophotometric techniques.

Different sodium-solute co-transport mechanisms were identified in the brush border membrane. Until now, studies with vesicles failed to document a sodium-chloride co-transport mechanism satisfactorily. On the other hand, a sodium/proton and a chloride/hydroxyl exchange mechanism were documented. These two exchange mechanisms could represent partial reactions of the postulated electroneutral sodium-chloride co-transport. In addition to different sodium-independent transport systems, the basal-lateral membrane contains an ATP-driven transport system for calcium as well as a sodium/calcium exchange mechanism.

Studies with membrane vesicles isolated from animals which have been exposed to different dietary conditions or in which the parathyroid hormone or $1{\cdot}25(OH)_2$Vit D_3 level has been altered show altered transport of calcium and inorganic phosphate. Thereby, it might be possible to identify the biochemical mechanisms involved in transport regulation.

GENERAL REMARKS

Vectorial transcellular transport consists of at least three different steps: (a) transport across the luminal membrane, (b) transport across the cytosol and (c) transport across the basal-lateral (serosal) membrane. For vectorial transport processes at least quantitative differences in the transport properties have to exist between the luminal and basal-lateral membranes of the epithelial cell. From an energetic point of view, the transport across each of the two different plasma membranes of an epithelial cell can be active or passive. In primary-active transport an energy providing reaction

Key words: Kidney, intestine, membrane transport.

(e.g. ATP-hydrolysis) is directly linked to the transport process (e.g. *via* transp[illegible] ATPases). In secondary-active transport a primary-active transport pathway leads to a transmembrane electrochemical potential difference for a cosubstrate (Na or H). A flux-coupling mechanism (co-transport, symport, antiport, exchange) leads to the movement of a second substrate against its chemical or electrochemical potential difference.

In secondary-active transepithelial transport a spatial separation between the primary active sodium pump (Na-K-ATPase) and the Na coupled transport system for different solutes (flux coupling mechanisms) is frequently observed (for reviews see: Frizzell, Field & Schultz, 1979; Murer & Kinne, 1980; Sacktor, 1977; Schultz, 1981). As in most epithelia (with the exception of the choroid plexus), in the proximal tubule and small intestine the Na-K-ATPase is located in the basal-lateral membrane. Na/H-exchange, Na-glucose co-transport and Na-phosphate co-transport, examples of flux-coupling mechanisms, are located in the luminal membrane, whereas Na/Ca-exchange is located in the basal-lateral membrane. The co-transport systems in the luminal membrane lead to a substrate accumulation in the cell. Transcellular transport is completed by a passive efflux through the basal-lateral membrane. In Na-dependent secondary-active secretion, the polar distribution of the transport system would be reversed. If the cellular concentration is below equilibrium distribution in absorptive systems, the active transport mechanism(s) must be located in the basal-lateral membrane. Transepithelial transport of calcium is an example of this situation (see below).

The luminal membrane of the small intestinal and renal proximal tubular epithelial cell (brush border membrane) is a plasma membrane with highly specialized transport functions. At least with respect to transport functions, the basal-lateral membranes of these epithelial cells resemble plasma membranes of non-polarized cells (for review see: Murer & Kinne, 1980). Not only various Na-independent facilitated diffusion systems but also various Na-dependent transport systems are found in the basal-lateral membranes (e.g. Mircheff, Van Os & Wright, 1980). These transport systems are involved in the cellular uptake of different solutes from the interstitial fluid and can also represent the contraluminal exit mechanism in transcellular transport. The intracellular substrate concentrations would decide whether substrates would leave or be taken up by the cell.

Membrane mechanisms involved in transcellular transport can be analysed by various *in vivo* and *in vitro* techniques using intact epithelial preparations, cellular suspensions or membrane preparations, respectively. In this article we will restrict the discussion solely to studies with membrane vesicles.

Na-SOLUTE CO-TRANSPORT MECHANISMS

Na-solute co-transport mechanisms represent part of the secondary active (re)-absorptive transport mechanisms (see above). In studies with brush border membrane vesicles, co-transport mechanisms have been identified for sugars, different groups of amino acids, different di- and tricarboxylic acids, dipeptides, water-soluble vitamins and different inorganic anions (for review see: Murer & Kinne, 1980; Murer & Burckhardt, 1983). Affinity labelling-, membrane solubilization- and membra[illegible]

reconstitution-experiments have been performed in order to characterize the transport proteins more closely (Crane, Malathi & Preiser, 1976; Fairclough, Malathi, Preiser & Crane, 1979; Gibbs *et al.* 1982; Im, Ling & Faust, 1982; Kinne & Faust, 1977; Koepsell *et al.* 1980; Lin, da Cruz, Riedel & Kinne, 1981). A successful isolation of one of the transport molecules has not yet been reported.

A typical example of the study of Na-solute co-transport mechanisms by means of isolated membrane vesicles is presented in Fig. 1 (Stieger, Stange, Biber & Murer, 1983). The transport of L-cysteine by rat renal brush border membrane vesicles is stimulated by an inwardly directed Na-gradient as compared to an inwardly directed K-gradient. For a kinetic evaluation, linear initial velocity conditions are important (compare: Hopfer & Liedtke, 1981; Kaunitz, Gunther & Wright, 1982; Semenza, 1982; Turner & Silvermann, 1978). Solute dependent Na-flux, as well as the kinetic evaluation of Na-activation of solute transport (Hill analysis), provides information for the determination of the coupling coefficient between Na- and solute-fluxes (e.g. Kaunitz *et al.* 1982).

Electrophysiological studies on intact epithelia and tracer studies with isolated membrane vesicles using ion gradients (K and/or H) and ionophores (valinomycin and FCCP) documented a potential dependence of various Na-solute co-transport systems (for reviews see: Frömter, 1979; Murer & Kinne, 1980). In studies with vesicles, alterations in the transmembrane electrical potential difference can be recorded by using fluorescent cyanine dyes (Beck & Sacktor, 1978; Stieger *et al.* 1983; Wright, Krasne, Kippen & Wright, 1981). An example of this technique is presented in Fig. 2. Addition of D-glucose to Na-pre-equilibrated rat renal brush border membrane vesicles provokes a transient increase in the fluorescence of 3,3′-diethylthiadicarbocyanine. This refers to a transient increase of an inside positive transmembrane

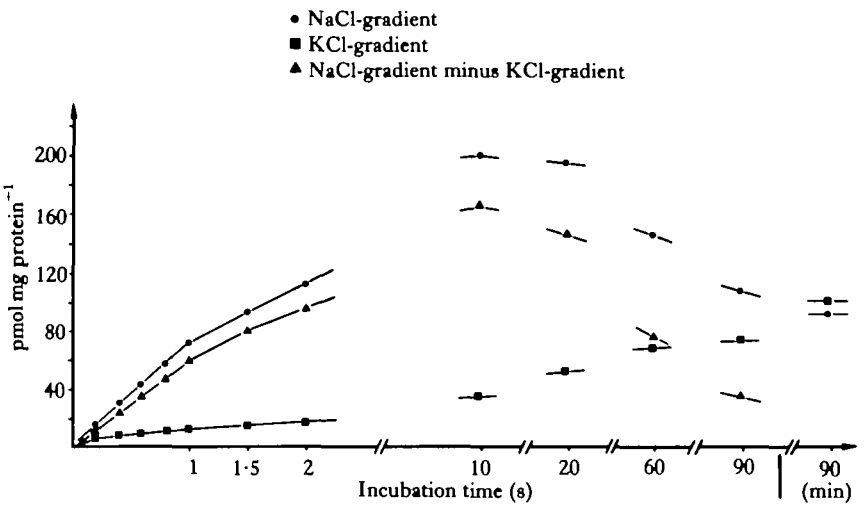

Fig. 1. Time course and sodium-dependence of cysteine-uptake by rat proximal tubular brush border membranes. Membranes were loaded with 300 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes adjusted with Tris to pH 7·4. The incubation media contained in addition (final concentrations): 100 mmol l^{-1} NaCl (●) or KCl (■), respectively; 0·1 mmol l^{-1} L-^{35}S-cysteine and 1·0 mmol dithiothreitol. This Figure is taken from Stieger, Stange, Biber & Murer (1983).

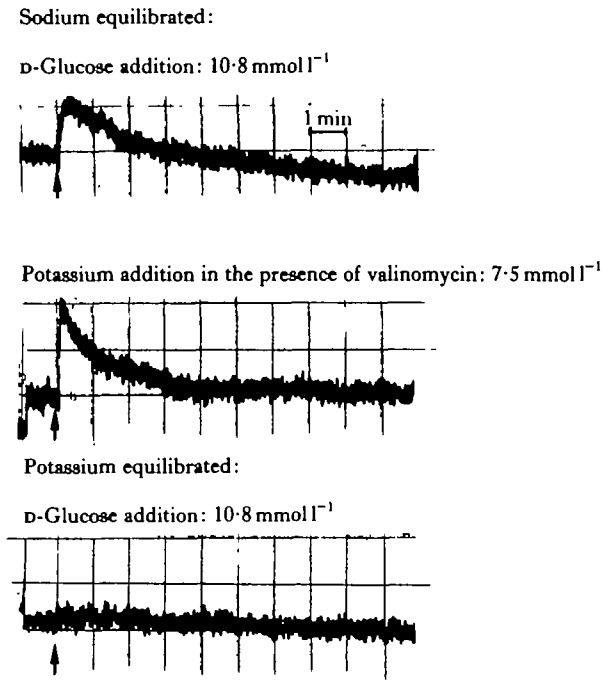

Fig. 2. Effect of Na-D-glucose co-transport on the fluorescence of 3'3-diethylthiadicarbocyanine iodide. Rat renal brush border membranes were prepared in either 74 mmol l^{-1} Na_2SO_4, 1 mmol l^{-1} K_2SO_4, 20 mmol l^{-1} Hepes-Tris pH 7·4 (sodium equilibrated) or in 75 mmol l^{-1} K_2SO_4, 20 mmol l^{-1} Hepes adjusted with Tris to pH 7·4 (potassium equilibrated). The solutes to be added during the experiment were suspended in the same buffer. For all experiments, 250 μg of membrane protein were used per cuvette (2 ml). Dye concentration was 3 μmol l^{-1}. This Figure is adapted from Stieger, Stange, Biber & Murer (1983).

electrical potential difference as also indicated by the observed increase in fluorescence after injection of K in the presence of valinomycin.

Na / H-EXCHANGE

In small intestinal and renal proximal tubular epithelia, uphill secretion of H is partially coupled to the downhill movement of Na (e.g. Chan & Giebisch, 1981; Malnic & Giebisch, 1979; Ullrich, Rumrich & Baumann, 1975). In studies with brush border membrane vesicles, the imposition of a Na-gradient ($Na_o > Na_i$) led to an acidification of the extravesicular medium. Likewise a ΔpH provided the driving force for the transient uptake of Na above the equilibrium value. Na/H exchange is electroneutral, accepts lithium and ammonium and is inhibited by amiloride (Kinsella & Aronson, 1981*a*,*b*; Liedtke & Hopfer, 1977; Murer, Hopfer & Kinne, 1976). These findings are in agreement with observations on the intact tubule (Chan & Giebisch, 1981; Ullrich *et al.* 1975). In studies with isolated proximal tubular brush border membrane vesicle preparations it has been shown that the rate of Na/H-exchange is under the influence of glucocorticoid hormones and allosterically regulated by an increase in the intracellular H-concentration (Aronson, Nee & Suhm, 1982; Freiberg, Kinsella & Sacktor, 1982).

Two different experimental approaches can be used to study Na/H-exchange by the acridine-orange fluorescence quench technique (Cohn, Hruska, Klahr & Hammerman, 1982; Reenstra, Warnock, Yee & Forte, 1981; Warnock, Reenstra & Yee, 1982). (1) The internal acidification occurring during Na-efflux can be monitored (Fig. 3). (2) A ΔpH can be preset and its breakdown can be studied under the influence of different Na concentrations (Fig. 4). In Figs 3 and 4 experiments with brush border membranes isolated from rat jejunum are presented. Na-efflux is accompanied by intravesicular acidification as indicated by the transient fluorescent quenching (Fig. 3). This quenching is partially due to indirect coupling between conductive Na-efflux (leak) and conductive H-entry (leak). The signal observed under conditions of 'short-circuited membrane potential' (K at equal concentrations on both membrane sides in the presence of valinomycin) is related to Na/H exchange only. In studies with brush border membrane vesicles, it is most important to analyse Na/H-exchange under conditions of a short-circuit membrane potential as significant non-specific conductive pathways have been observed (e.g. Reenstra *et al.* 1981). The use of amiloride as a potential inhibitor of Na/H exchange is complicated in these studies because of two facts: (1) amiloride at concentrations higher than 0·1 mmol l^{-1} shows strong quenching of the dye fluorescence (Sabolic & Burckhardt, 1983); (2) amiloride used at higher concentrations shunts the ΔpH and thus 'competes' for the

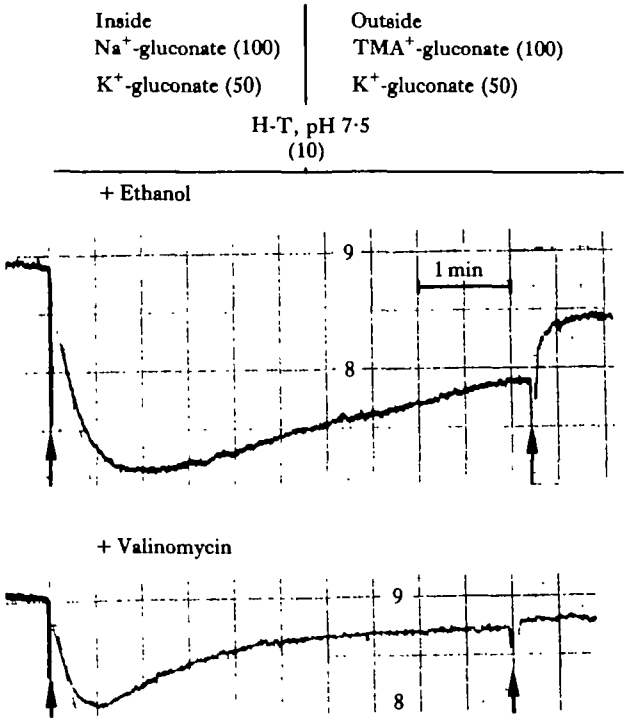

Fig. 3. Measurement of Na/H-exchange in rat small intestinal brush border membrane vesicles by the acridine orange fluorescence quench technique. The methods used for this experiment are similar to that described by Reenstra, Warnock, Yee & Forte (1981). Important experimental conditions are indicated in the Figure (G. Cassano & H. Murer, unpublished observations). Concentrations are given in mmol l^{-1}. H-T = Hepes adjusted with Tris, TMA = tetramethylammonium.

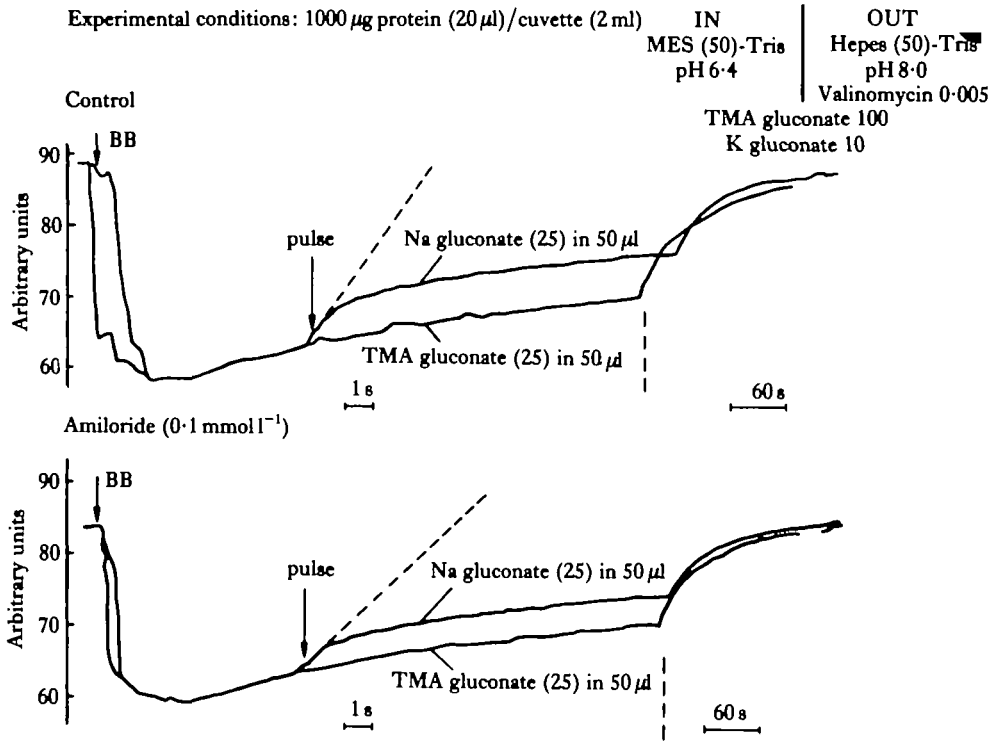

Fig. 4. Amiloride-inhibition of Na/H-exchange in brush border membranes from rat small intestine. The experimental condition is given in the Figure. The effect of sodium on the equilibration of a preset ΔpH was analysed in the presence and absence of amiloride (G. Cassano & H. Murer, unpublished observations).

dye-movement (Dubinsky & Frizzell, 1982). Thus, at high amiloride concentrations (above 0·1 mmol l^{-1}) the amiloride effect is not only related to Na/H-exchange but also observed for ionophore induced K-dependent H-movements (nigericin/valinomycin/FCCP). In the presence of only 0·1 mmol l^{-1} amiloride, the nonspecific effects mentioned above are small. There is a small quenching of fluorescence in the absence of brush border membranes (Fig. 4). The dissipation rate of a preset ΔpH in the absence of sodium is not altered by this low amiloride concentration. After the Na-pulse, however, the Na-dependent increase in rate is strongly reduced in the presence of 0·1 mmol l^{-1} amiloride. This experiment documents an inhibition of Na/H-exchange by amiloride (G. Cassano & H. Murer, unpublished observations).

DIRECTLY COUPLED TRANSPORT OF Na AND Cl

For transcellular Cl-flux in the small intestine, the luminal Cl-entry mechanism is Na-dependent. These findings suggested the existence of a Na/Cl-co-transport mechanism (for reviews see: Duffey, Thompson, Frizzell & Schultz, 1979; Frizzell *et al.* 1979; Schultz, 1979, 1981). An alternative mechanism is the coupled action of a Na/H and a Cl/HCO_3-exchange (Turnberg, Fordtran, Carter & Rector, 1970). In rat proximal tubular epithelial cells, the intracellular chloride activity was also found

be above equilibrium distribution (Cassola, Gebler & Frömter, 1981). The localization and nature of the active transport step, however, is at present not known for mammalian proximal tubule.

Liedtke & Hopfer (1982*a*) denied the existence of a Na/Cl-co-transporter in kinetic experiments with brush border membrane vesicles isolated from rat small intestine. Powell & Fan (1982) reported coupled transport of Na and Cl in intestinal brush border membranes isolated in the absence of calcium, although indirect coupling of Na/H- and Cl/OH-exchange could not be excluded. High intravesicular calcium concentrations inhibited 'coupled' entry of Na and Cl (Powell & Fan, 1982). As high calcium concentrations are normally used in the preparation of brush border membrane vesicles, this finding raises the possibility that a coupled mechanism could be inhibited during vesicle preparation. The existence of a Na-K-2Cl-co-transport [a co-transport mechanism observed in many different cells including epithelia (e.g. Greger & Schlatter, 1982; Murer & Greger, 1982; Oberleitner, Giebisch, Lang & Wenhui, 1982)] has never been rigorously tested in studies with vesicles. However, studies on intact epithelia did not provide evidence for the existence of a Na-K-2Cl co-transport in the luminal membrane of mammalian small intestine or proximal tubule.

TRANSPORT MECHANISMS FOR Cl

A Cl/OH-exchange (a possible component of electroneutral 'coupled' transport of sodium and chloride) was documented in tracer experiments for rat and rabbit small intestinal and proximal tubular brush border membrane vesicles (Liedtke & Hopfer, 1982*b*; Murer, Kinne-Saffran, Beauwens & Kinne, 1980*a*; Warnock & Yee, 1981; Warnock & Eveloff, 1982). In experiments using a pH-sensitive fluorescent dye no clear evidence could be obtained for a Cl/OH-exchange mechanism in studies with rat proximal tubular and rat jejunal brush border membrane vesicles (G. Cassano & H. Murer, unpublished observations). Surprisingly, measured under the corresponding conditions as Na/H-exchange (see Fig. 3) no internal acidification was obtained by applying an inwardly directed chloride gradient (Fig. 5). The simultaneous application of an outwardly directed chloride and sodium gradient was unable to reduce the internal acidification as compared to the simultaneous application of outwardly directed gradients for sodium and gluconate (G. Cassano & H. Murer, unpublished observations). If Na/H-exchange and Cl/OH-exchange mechanisms operated in the same membrane and with comparable rates, the signal in the outwardly directed Cl-gradient condition should be considerably lower than the signal in the presence of Na gluconate. Thus, it seems that the Cl/OH-exchange mechanism has a much smaller rate than the Na-H-exchange mechanism. It is likely, that HCO_3 is the preferentially accepted substrate of the anion exchanger (Knickelbein, Aronson & Dobbins, 1982).

Studies using proximal tubular and small intestinal membrane preparations provided evidence for a conductive pathway for chloride-entry (G. Cassano & H. Murer, unpublished observations; Reenstra *et al.* 1981). The physiological significance of this observation is not clear.

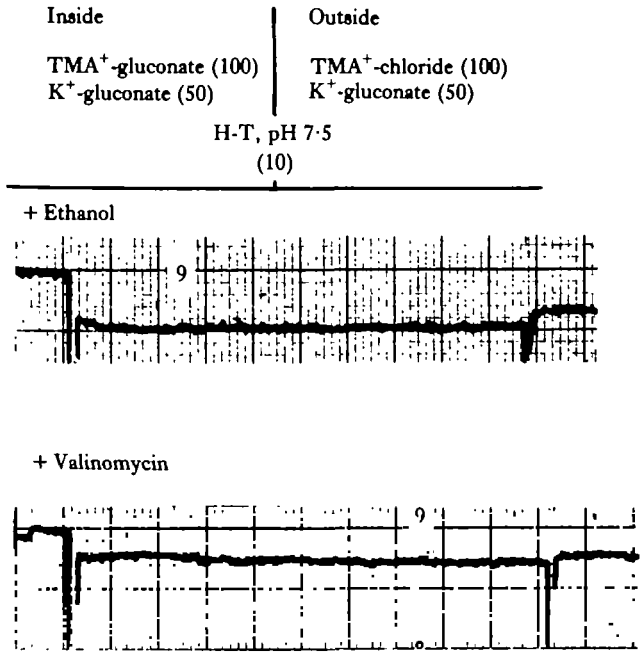

Fig. 5. Lack of Cl/OH-exchange in rat small intestinal brush border membrane vesicles (measured by acridine orange fluorescence quench technique). The experimental conditions are given in the Figure (G. Cassano & H. Murer, unpublished observations).

TRANSPORT MECHANISMS FOR Ca

Studies using intact tissue preparations revealed that transepithelial transport of Ca in the small intestine and renal proximal tubule are partially dependent on the presence of Na (for reviews see: Martin & DeLuca, 1969; Murer & Hildmann, 1981; Ng, Peraino & Suki, 1982; Ullrich, Rumrich & Klöss, 1976). Due to the presence of a high affinity Ca-ATPase in basal-lateral membranes isolated from renal proximal tubular and small intestinal epithelium (Ghijsen & Van Os, 1979; Ghijsen, De Jong & Van Os, 1982*a*; Gmaj, Murer & Carafoli, 1982; Van Os & Ghijsen, 1981), Ca can also be transported in a primary-active Na-independent manner. In view of the intra-/extracellular concentration difference for Ca, transport from the cytosol into the serosal interstitium represents an energy-requiring 'uphill' movement whereas influx into the cell from the lumen is a downhill movement.

In general, plasma membranes of non-excitable mammalian cells contain two types of Ca-transport mechanisms: (1) Ca/Mg-ATPase with high affinity sites for Ca and (2) Na/Ca-exchange systems. Evidence has been found for the existence of both mechanisms in basal-lateral membrane vesicles isolated from small intestinal and renal proximal tubular epithelia (Ghijsen & Van Os, 1982; Ghijsen *et al.* 1982*a*,*b*; Gmaj, Murer & Kinne, 1979; Hildmann, Schmidt & Murer, 1982; Murer & Hildmann, 1981). The addition of ATP leads to an acceleration of initial Ca-uptake as compared to the situation without ATP. Inhibition of ATP driven Ca-uptake by orthovanadate can be observed (Fig. 6B). It has been shown that the high affinity Ca-ATPase located in small intestinal and renal proximal tubular epithelia (De Jon

Membranes: 160 mmol l^{-1} KCl
20 mmol l^{-1} Hepes adjusted with Tris to pH 7·4
Incubation: 160 mmol l^{-1} KCl(NaCl), 20 mmol l^{-1} Hepes-Tris, 1 mmol l^{-1} $MgCl_2$, 2·5 mmol l^{-1} ouabain, 5 μg ml^{-1} oligomycin, 5 mmol l^{-1} MgATP, 56 μmol l^{-1} Mg – ATP

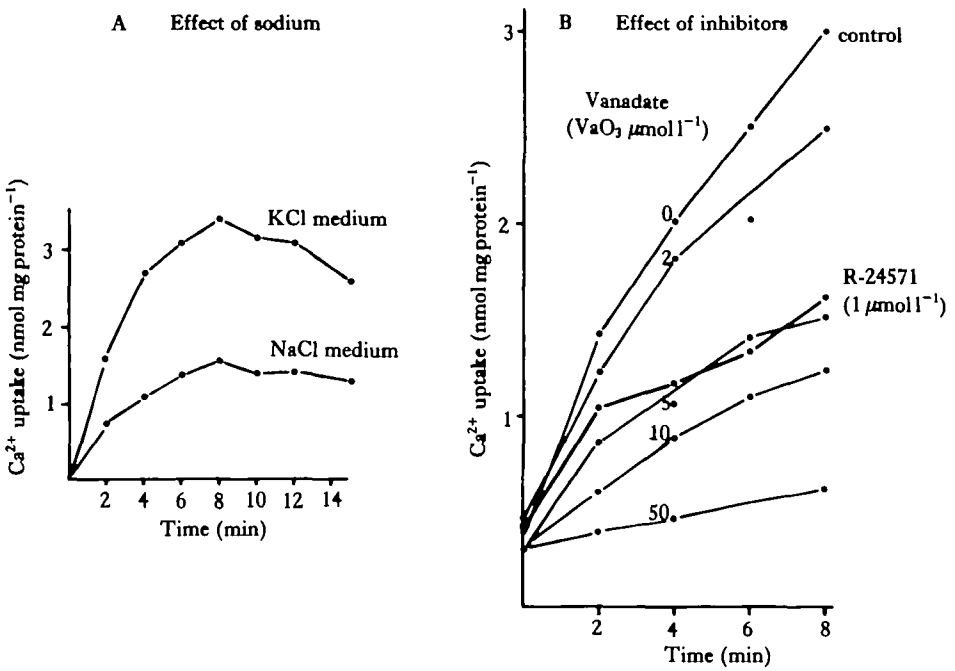

Fig. 6. Effect of 'inhibitors' on ATP-driven Ca-uptake by renal basal-lateral membrane vesicles. The experimental conditions are given in the Figure (P. Gmaj, E. Carafoli & H. Murer, unpublished observations).

Ghijsen & Van Os, 1981; Ghijsen, 1982; Gmaj *et al.* 1982) is inhibited by anti-calmodulin drugs and stimulated by the addition of calmodulin. Similarly, ATP-dependent Ca-uptake was inhibited by anti-calmodulin drugs (kidney, Fig. 6B) and stimulated by addition of calmodulin (Ghijsen, 1982; P. Gmaj, E. Carafoli & H. Murer, unpublished observations; Nellans & Popovitch, 1981).

Evidence for a Na/Ca-exchange mechanism is also available from studies with basal-lateral membrane vesicles isolated from renal proximal tubule and small intestine (e.g. Ghijsen, 1982; Gmaj *et al.* 1979; Hildmann *et al.* 1982). In both membrane preparations, ATP-driven Ca-uptake was maximal in a KCl-medium, i.e. in the complete absence of Na. Without inhibiting the Ca-ATPase activity, increased Na-concentration in the medium prevented ATP-driven intravesicular Ca-accumulation (see Fig. 6A). These findings suggested a Na-dependent pathway for Ca in the same vesicular structure that contained the ATP-driven Ca-transport mechanism. Further evidence for a Na/Ca-exchange mechanism was obtained from the experiments on the efflux of Ca from preloaded vesicles in the presence of different transmembrane ion gradients (Gmaj *et al.* 1979; Hildmann *et al.* 1982).

In agreement with observations mainly obtained on the intact intestinal epithelium, studies with isolated brush border membrane vesicles indicate the existence of a

passive, carrier-mediated entry mechanism for Ca at the luminal cell pole (e.g. Matsumoto, Fontaine & Rasmussen, 1980; Miller & Bronner, 1981; Murer & Hildmann, 1981). Studies with chicken brush border membrane vesicles demonstrated that the passive entry-mechanism is regulated by $1{\cdot}25(OH)_2$Vit D_3 level in the intact animal (Matsumoto *et al.* 1980; Rasmussen, Fontaine, Max & Goodman, 1979). This expression of the regulatory effect of $1{\cdot}25(OH)_2$Vit D_3 on the passive entry-mechanism in the luminal membrane has also been confirmed in studies with rat and rabbit small intestinal brush border membrane vesicles (Miller & Bronner, 1981; Murer & Hildmann, 1981).

More recently, it has been shown that the Ca-ATPase and the ATP-dependent Ca-uptake is higher in basal-lateral membranes isolated from $1{\cdot}25(OH)_2$Vit D_3 replete rats than in basal-lateral membranes from Vitamin D deficient rats (Ghijsen & Van Os, 1982). It remains to be determined whether this effect is the consequence of indirect effects (e.g. via the Ca-calmodulin complex or the Ca-binding protein) or whether it is a $1{\cdot}25(OH)_2$Vit D_3-induced direct effect on the 'Ca pump'. Na/Ca-exchange was not influenced by the $1{\cdot}25(OH)_2$Vit D_3 level in the intact animal (Ghijsen, 1982; Ghijsen & Van Os, 1982).

TRANSPORT MECHANISMS FOR PO_4

PO_4-transport against an electrochemical potential difference in the renal proximal tubule and the small intestine depends on the presence of Na, suggesting Na-PO_4 co-transport in the brush border membrane (for reviews see: Bikle, Morrissey, Zolock & Rasmussen, 1981; Dennis, Stead & Myers, 1979; Murer *et al.* 1980*b*; Murer & Hildmann, 1981; Ullrich & Murer, 1983). Na-PO_4 co-transport mechanisms have been identified in renal and intestinal brush border membrane preparations isolated from different animals (Berner, Kinne & Murer, 1976; Cheng & Sacktor, 1981; Danisi, Murer & Straub, 1982; Fuchs & Peterlik, 1980; Hoffmann, Thees & Kinne, 1976; Matsumoto *et al.* 1980; Tenenhouse & Scriver, 1978).

In both intact epithelia and isolated membrane vesicles, a decrease in luminal pH increased PO_4-transport in intestine and decreased it in the kidney (Baumann, Rumrich, Papavassiliou & Klöss, 1975; Berner *et al.* 1976; Burckhardt, Stern & Murer, 1981; Danisi *et al.* 1982; Dennis, Woodhall & Robinson, 1976; Hoffmann *et al.* 1976; Sacktor & Cheng, 1981; Ullrich *et al.* 1978). This has been taken as evidence for the preferential transport of monovalent or divalent PO_4, respectively (Baumann *et al.* 1975; Berner *et al.* 1976; Cheng & Sacktor, 1981; Hoffmann *et al.* 1976; Sacktor & Cheng, 1981). Three different lines of experimental evidence are available against a preferential transport of monovalent or divalent PO_4 in rat renal membrane preparations: (1) high sodium concentrations diminished the pH-sensitivity of the transport system (Burckhardt *et al.* 1981); (2) increasing pH from 6·3 to 6·9 increased the apparent K_m about twofold and V_{max} more than threefold. Assuming that the transport system accepts only divalent PO_4 and that pH has no direct effect on the transport system itself, an increase in pH should decrease K_m values in an analysis based on total PO_4 concentrations. (3) In electrophysiological experiments and in studies with rat renal brush border membranes, Na-PO_4 co-transport carries a positive charge only at acidic pH values (Barrett & Aronson, 1982; Burckhardt *et al.*

1; Samarzija, Molnar & Frömter, 1981). This is explained by the operation of a transport mechanism which always accepts two sodium ions but can accept monovalent and divalent PO_4. Experiments with rabbit small intestinal brush border membrane vesicles suggested that the pH-dependence of Na-PO_4 co-transport in small intestine is also provoked by a pH-sensitivity of the transporter and does not reflect preferential transport of monovalent PO_4 (Danisi *et al.* 1982).

Small intestinal and renal proximal tubular PO_4-transport is influenced by various control mechanisms. In the intestine regulatory phenomena are mediated by 1·25$(OH)_2$Vit D_3. The effect of 1·25$(OH)_2$Vit D_3 is expressed at the level of the brush border membrane and can be analysed in isolated brush border membrane vesicles (for reviews see: Bikle *et al.* 1981; Murer & Hildmann, 1981; Rasmussen, Matsumoto, Fontaine & Goodman, 1982; Fig. 7).

Proximal tubular PO_4-reabsorption is inhibited by parathyroid hormone. This effect is intracellularly mediated by cAMP (Aurbach & Heath, 1974; Ullrich *et al.* 1977). The regulatory effects are expressed at the level of the brush border membrane (Evers, Murer & Kinne, 1978; Hammerman, Karl & Hruska, 1980). It was suggested that cAMP-dependent membrane phosphorylation is responsible for a specific reduction of Na-dependent PO_4-transport (Hammerman, Hansen & Morrissey, 1982).

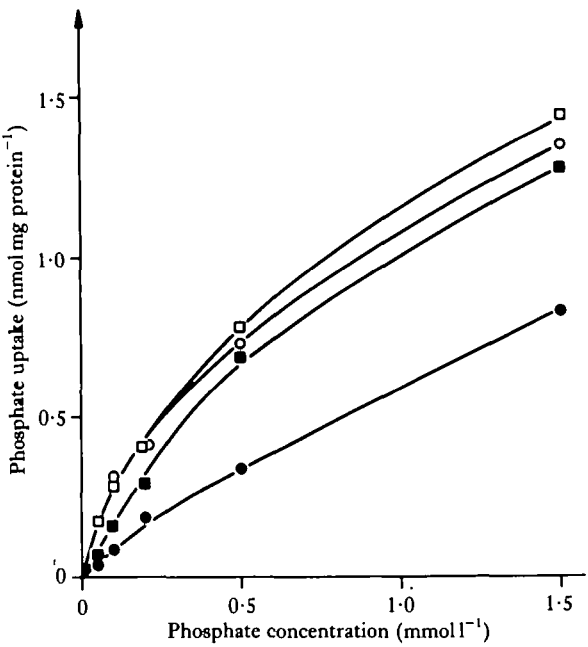

Fig. 7. Influence of 1·25$(OH)_2$Vit D_3 on the sodium gradient-dependent uptake of PO_4 by duodenal brush border vesicles. To induce 1·25 D_3-deficiency, two rabbits were injected s.c. with 40 mg kg^{-1} EHDP in the morning of three consecutive days. 8–10 h before killing them, one EHDP-treated rabbit and a sham-treated rabbit received an i.v. injection of 600 ng kg^{-1} 1·25 D_3. Membrane suspension prepared in 300 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes/Tris, pH 7·4 was added to incubation media containing in addition 100 mmol l^{-1} sodium chloride and different concentrations of KH_2PO_4. EHDP = disodium ethane-1-hydroxy-1-1-diphosphonate; this drug is known to lower plasma levels of 1·25-dihydroxy-vitamin D_3. This Figure is taken from Hildmann, Schmidt & Murer (1982). *In vivo* treatments: ■, control; ●, EHDP; □, 1·25$(OH)_2$Vit D_3; ○, EHDP + 1·25$(OH)_2$Vit D_3.

However, a causal link between cAMP-dependent membrane phosphorylation and inhibited Na-dependent PO_4-transport is missing (Biber, Murer & Mälstrom, 1983). The tubular transport mechanism for PO_4 responds in a parathyroid hormone-independent manner to alterations in the dietary PO_4-intake (Bonjour, Troehler, Preston & Fleisch, 1978; Steele & DeLuca, 1976; Ullrich *et al.* 1977). This effect is expressed at the level of the isolated brush border membranes (Caverzasio, Murer, Fleisch & Bonjour, 1982; Kempson *et al.* 1980; Stoll *et al.* 1979; Stoll, Murer, Fleisch & Bonjour, 1980).

An interesting theory attempts to relate the regulation of PO_4-transport in the proximal tubule to alterations in the cellular NAD/NADH ratio (Dousa & Kempson, 1982). In this respect, the inhibition of Na-dependent PO_4-transport in vesicles by NAD/NADH is interesting (Kempson *et al.* 1981). As NAD/NADH is hydrolysed very rapidly by brush border membrane preparations (Angielski, Zielkrewicz & Dzierzko, 1982), the inhibition of PO_4-transport by NAD/NADH seems to be indirect. It was shown that inhibition of alkaline phosphatase activity releases the inhibition of PO_4-transport by NAD/NADH (Tenenhouse & Chu, 1982). Hammerman *et al.* (1982) suggested a ribosylation reaction to be involved in inhibition of PO_4-transport by NAD. However, a causal link is missing between membrane protein-ribosylation and inhibition of PO_4-transport by NAD. Inhibition of transport is rather related to the hydrolytic breakdown of the NAD and to an indirect inhibition of PO_4-transport *via* released products (P. Gmaj, St. Angielski, J. Biber & H. Murer, unpublished observations).

The Na-PO_4-co-transport mechanism operates independently of Ca (Berner *et al.* 1976; B. Hildmann, C. Storelli & H. Murer, unpublished observations; Matsumoto *et al.* 1980). PO_4- and Ca-transport respond to stimulation by $1{\cdot}25(OH)_2$Vit D_3 with different time courses, and the polyene antibiotic filipin mimics only the $1{\cdot}25(OH)_2$ Vit D_3 stimulation of transmembrane Ca-flux, but not of transmembrane PO_4-flux (Matsumoto *et al.* 1980; Rasmussen *et al.* 1979; Rasmussen *et al.* 1982).

TRANSPORT MECHANISM FOR SO_4

SO_4 is reabsorbed in the ileum against its concentration gradient in a Na-dependent way (Anast, Kennedy, Volk & Adamson, 1965; Smith, Orella & Field, 1981). Proximal tubular SO_4-reabsorption is also Na-dependent (Brazy & Dennis, 1981; Ullrich & Murer, 1983; Ullrich *et al.* 1980).

Studies with brush border membrane vesicles isolated from rat ileum and rat and rabbit proximal tubule revealed a Na-SO_4-co-transport (Fig. 8 for rabbit ileum). Na-SO_4-co-transport is electroneutral (Lücke, Stange & Murer, 1979, 1981; Schneider, Durham & Sacktor, 1980) in agreement with electrophysiological data obtained with rabbit ileal epithelium (Smith *et al.* 1981) and proximal tubules *in vivo* (Samarzija *et al.* 1981). In isolated rabbit proximal tubules, the stilbene derivative SITS inhibited Na-dependent SO_4-transport across the luminal membrane (Brazy & Dennis, 1981). Langridge-Smith, Sellin & Field (1982) postulated a model for Na-sulphate co-transport in rabbit ileum with varying stoichiometry between Na- and SO_4-flux. At low SO_4-concentrations the stoichiometry factor is one; electroneutrality would then be obtained by the additional involvement of H-flux. At high SO_4-concentrations, two Na

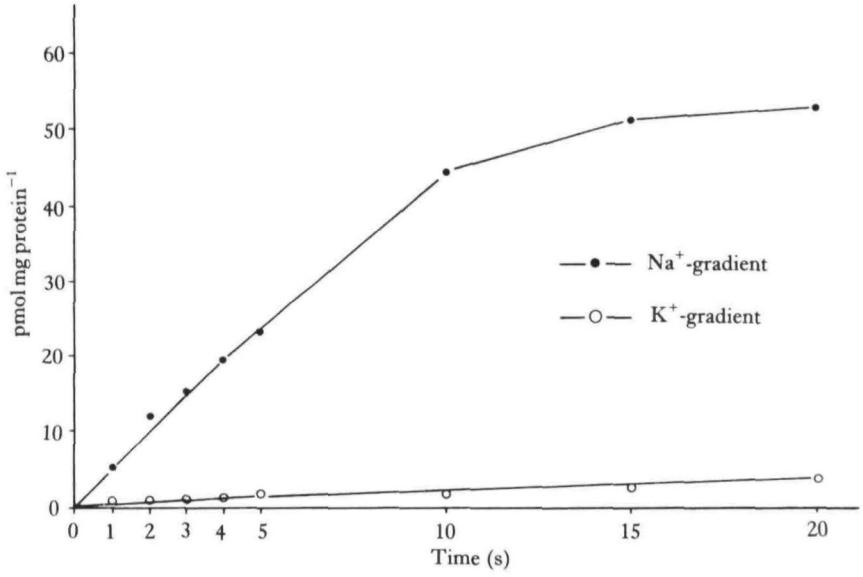

Fig. 8. Effect of sodium-gradient on initial linear uptake of sulphate by rabbit ileal brush border membranes. Vesicles were pre-loaded with 400 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes-Tris, pH 7·4 and at zero time were exposed to incubation media containing either 200 mmol l^{-1} NaCl or 200 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes-Tris, pH 7·4, and either 0·4 mmol l^{-1} Na_2SO_4 or 0·4 mmol l^{-1} K_2SO_4. Final cation gradients across membranes were 100 mmol l^{-1} Na^+ or 100 mmol l^{-1} K^+.

are involved in transmembrane SO_4-movement. Studies with rabbit ileal brush border vesicles should be useful to test this model (G. Ahearn & H. Murer, unpublished observations).

Langridge-Smith & Field (1981) provided evidence for a stilbene-sensitive anion exchange-mechanism for SO_4 in the basolateral membranes of isolated rabbit ileal epithelia. Similar findings were obtained in isolated rabbit proximal tubules (Brazy & Dennis, 1981). Stilbene derivatives also inhibited SO_4-flux in basolateral membrane vesicles isolated from rat small intestine and dog kidney cortex (Grinstein, Turner, Silverman & Rothstein, 1980), but were ineffective in rat proximal tubules *in vivo* (Ullrich *et al.* 1980). Most recently a stilbene derivative sensitive anion exchange mechanism was detected in isolated basal-lateral membrane vesicles from rat proximal tubule, (J. Löw & G. Burckhardt, unpublished observations.)

CONCLUDING REMARKS

In this brief review we have summarized experiments with membrane vesicles isolated from renal proximal tubules and small intestine. Such experiments represent useful tools for the analysis of membrane transport mechanisms involved in transepithelial transport. We have only given the examples related to the transport of inorganic ions.

For Ca it has been observed that the basal-lateral membrane contains two 'uphill' transport mechanisms, whereas influx into the cell is a passive process. For the transport of Na the passive entry into the cell across the luminal membrane is coupled

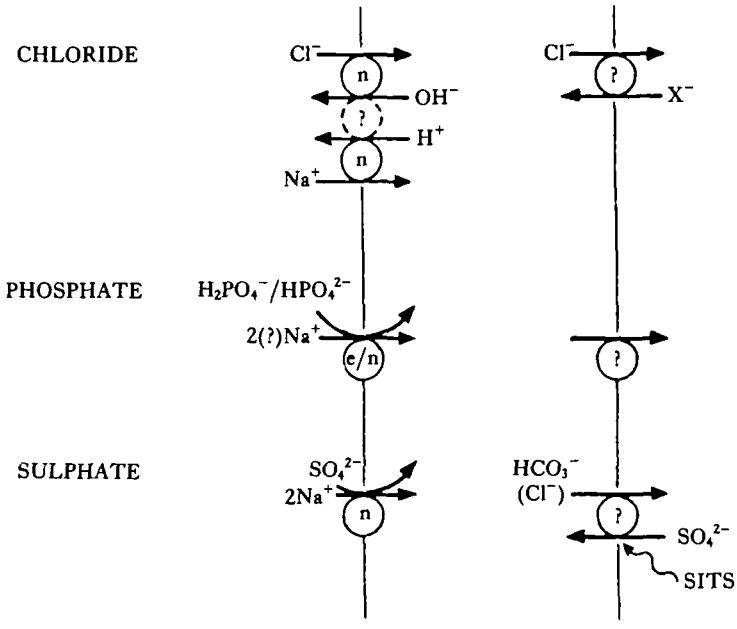

Fig. 9. Schematic representation of membrane transport mechanisms involved in the intestinal absorption of anions. For extensive discussions see: Murer & Burckhardt (1983).

to the uphill movement of a second substrate (co-transport). This was discussed for an antiport mechanism, namely Na/H-exchange, as well as for different Na-coupled movements of anions as summarized in Fig. 9. Inspection of the Figure may indicate where future efforts can complete our knowledge of the mechanisms involved in transepithelial inorganic anion transport.

In more recent time, studies with vesicles have also been used to determine regulatory mechanisms involved in the regulation of transepithelial transport. As discussed above, regulation of transcellular PO_4- and Ca-flux most probably proceeds *via* altered membrane transport (luminal influx only?). Certainly, studies with vesicles can help to define the cellular mechanisms leading to the final event of altered membrane transport.

REFERENCES

ANAST, C., KENNEDY, R., VOLK, G. & ADAMSON, L. (1965). *In vitro* studies of sulfate transport by the small intestine of the rat, rabbit and hamster. *J. Lab. clin. Med.* **65**, 903–911.

ANGIELSKI, ST., ZIELKREWICZ, J. & DZIERZKO, G. (1982). Metabolism of NAD by isolated rat renal brush border membranes. *Pflügers Arch. ges. Physiol.* **395**, 159–161.

ARONSON, P. S., NEE, J. & SUHM, M. A. (1982). Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Nature, Lond.* **299**, 161–163.

AURBACH, G. & HEATH, D. A. (1974). The parathyroid hormone in regulation of renal function. *Kidney Int.* **6**, 331–345.

BARRETT, P. Q. & ARONSON, P. S. (1982). Glucose and alanine inhibition of phosphate transport in renal microvillous membrane vesicles. *Am. J. Physiol.* **242**, 126–131.

BAUMANN, K., RUMRICH, G., PAPAVASSILIOU, F. & KLÖSS, S. (1975). pH-dependence of phosphate reabsorption in the proximal tubule of rat kidney. *Pflügers Arch. ges. Physiol.* **360**, 183–187.

BECK, J. C. & SACKTOR, B. (1978). The sodium electrochemical potential mediated uphill transport of D-glucose in renal brush border membrane vesicles. *J. biol. Chem.* **253**, 5531–5535.

NER, W. R., KINNE, R. & MURER, H. (1976). Phosphate transport into brush border membrane vesicles isolated from small intestine. *Biochem. J.* **160**, 467–474.

BIBER, J., MURER, H. & MALMSTRÖM, K. (1983). The role of membrane phosphorylation in PTH dependent inhibition of sodium dependent phosphate transport. *Pflügers Arch. ges. Physiol.* (in press).

BIKLE, D. D., MORRISSEY, R. L., ZOLOCK, D. T. & RASMUSSEN, H. (1981). The intestinal response to vitamin D. *Rev. Physiol. Biochem. Pharmacol.* **89**, 63–142.

BONJOUR, J.-P., TROEHLER, U., PRESTON, C. & FLEISCH, H. (1978). Parathyroid hormone and renal handling of P_i: effect of dietary P_i and diphosphonates. *Am. J. Physiol.* **234**, F497–F505.

BRAZY, P. C. & DENNIS, V. W. (1981). Sulfate transport in rabbit proximal convoluted tubules: presence of anion exchange. *Am. J. Physiol.* **241**, F300–F307.

BURCKHARDT, G., STERN, H. & MURER, H. (1981). The influence of pH on phosphate transport into rat renal brush border membrane vesicles. *Pflügers Arch. ges. Physiol.* **340**, 191–197.

CASSOLA, A. C., GEBLER, B. & FRÖMTER, E. (1981). Measurements of intracellular chloride activity in proximal convolution of rat kidney. *Pflügers Arch. ges. Physiol.* **391**, R17.

CAVERZASIO, J., MURER, H., FLEISCH, H. & BONJOUR, J.-P. (1982). Phosphate transport in brush border membrane vesicles isolated from renal cortex of young growing and adult rats. Comparison with whole kidney data. *Pflügers Arch. ges. Physiol.* **399**, 217–221.

CHAN, Y. L. & GIEBISCH, G. (1981). Relationship between sodium and bicarbonate transport in the rat proximal convoluted tubule. *Am. J. Physiol.* **240**, F222–F230.

CHENG, L. & SACKTOR, B. (1981). Sodium gradient-dependent phosphate transport in renal brush border membrane vesicles. *J. biol. Chem.* **256**, 1556–1564.

COHN, E. D., HRUSKA, K. A., KLAHR, S. & HAMMERMAN, M. R. (1982). Increased Na^+-H^+ exchange in brush border vesicles from dogs with renal failure. *Am. J. Physiol.* **243**, F293–F299.

CRANE, R. K., MALATHI, P. & PREISER, H. (1976). Reconstitution of specific Na^+-dependent D-glucose transport in liposomes by Triton X-100 extracted proteins from purified brush border membranes of rabbit kidney cortex. *FEBS Lett.* **67**, 214–216.

DANISI, G., MURER, H. & STRAUB, R. (1982). Effect of pH on rabbit intestinal phosphate transport. *Experientia* **388**, 712.

DE JONGE, H. R., GHIJSEN, W. E. J. M. & VAN OS, C. H. (1981). Phosphorylated intermediates of Ca^{2+}-ATPase and alkaline phosphatase in plasma membranes from rat duodenal epithelium. *Biochim. biophys. Acta* **647**, 140–149.

DENNIS, V. W., STEAD, W. W. & MYERS, J. L. (1979). Renal handling of phosphate and calcium. *Ann. Rev. Physiol.* **41**, 257–271.

DENNIS, V. W., WOODHALL, P. B. & ROBINSON, R. R. (1976). Characteristics of phosphate transport in isolated proximal tubule. *Am. J. Physiol.* **231**, 979–985.

DOUSA, J. P. & KEMPSON, S. A. (1982). Regulation of renal brush border membrane transport of phosphate. *Mineral Electrolyte Metab.* **7**, 113–121.

DUBINSKY, W. & FRIZZELL, R. A. (1982). Mechanism of amiloride inhibition of an intestinal brush border Na^+/H^+ antiporter. *Fedn Proc. Fedn Am. Socs exp. Biol.* **41**, 1261.

DUFFEY, M. E., THOMPSON, S. M., FRIZZELL, R. A. & SCHULTZ, S. G. (1979). Intracellular chloride activities and active chloride absorption in the intestinal epithelium of the winter flounder. *J. Membr. Biol.* **50**, 331–341.

EVERS, C., MURER, H. & KINNE, R. (1978). Effect of parathyrin on the properties of isolated renal brush border vesicles. *Biochem. J.* **172**, 49–56.

FAIRCLOUGH, P., MALATHI, P., PREISER, H. & CRANE, R. K. (1979). Reconstitution into liposomes of glucose active transport from the rabbit renal proximal tubule. *Biochim. biophys. Acta* **553**, 295–306.

FREIBERG, J. M., KINSELLA, J. & SACKTOR, B. (1982). Glucocorticoids increase the Na^+-H^+ exchange and decrease the Na^+-gradient dependent phosphate uptake systems in renal brush border membrane vesicles. *Proc. Natn Acad. Sci. U.S.A.* **79**, 4932–4936.

FRIZZELL, R. A., FIELD, M. & SCHULTZ, S. G. (1979). Sodium-coupled transport by epithelial tissues. *Am. J. Physiol.* **236**, F1–F8.

FRÖMTER, E. (1979). Solute transport across epithelia: What can we learn from micropuncture studies on kidney tubules? *J. Physiol., Lond.* **288**, 1–31.

FUCHS, R. & PETERLIK, M. (1980). Vitamin D induced phosphate transport in intestinal brush border membrane vesicles. *Biochem. biophys. Res. Commun.* **93**, 87–92.

GHIJSEN, W. E. J. M. (1982). Mechanisms of active calcium transport in small intestinal epithelium. Thesis, University Nijmegen, The Netherlands.

GHIJSEN, W. E. J. M., DE JONG, M. D. & VAN OS, C. H. (1982*a*). Properties of Na^+-Ca^{2+} exchange in basolateral plasma membranes of rat duodenum. *Biochim. biophys. Acta* (in press).

GHIJSEN, W. E. J. M., DE JONG, M. D. & VAN OS, C. H. (1982*b*). ATP-dependent calcium transport and its correlation with Ca^{2+}-ATPase activity in basolateral plasma membranes of rat duodenum. *Biochim. biophys. Acta* **689**, 327–336.

GHIJSEN, W. E. J. M. & VAN OS, C. H. (1979). Ca-stimulated ATPase in brush border and basolateral membranes of rat duodenum with high affinity sites for Ca ions. *Nature, Lond.* **379**, 802–803.

GHIJSEN, W. E. J. M. & VAN OS, C. H. (1982). 1α,25-dihydroxy-vitamin D_3 regulates ATP-dependent calci[redacted] transport in basolateral plasma membranes of rat enterocytes. *Biochim. biophys. Acta* **689**, 170–172.

GIBBS, E. M., HOSANG, M., REBER, B. F. X., SEMENZA, G. & DIEDRICH, D. F. (1982). 4-Adizophlorizin, a high affinity probe and photoaffinity label for the glucose transporter in brush border membranes. *Biochim. biophys. Acta* **668**, 547–556.

GMAJ, P., MURER, H. & CARAFOLI, E. (1982). Localization and properties of a high affinity (Ca^{2+}-Mg^{2+})-ATPase in isolated kidney cortex plasma membranes. *FEBS Lett.* **144**, 226–230.

GMAJ, P., MURER, H. & KINNE, R. (1979). Calcium ion transport across plasma membranes from rat kidney cortex. *Biochem. J.* **172**, 57–62.

GREGER, R. & SCHLATTER, E. (1981). Presence of luminal K^+, a prerequisite of active NaCl transport in the cortical thick ascending limb of Henle's loop. *Pflügers Arch. ges. Physiol.* **392**, 92–94.

GRINSTEIN, S., TURNER, R. J., SILVERMAN, M. & ROTHSTEIN, A. (1980). Inorganic anion transport in kidney and intestinal brush border and basolateral membranes. *Am. J. Physiol.* **238**, F452–F460.

HAMMERMAN, M. R., HANSEN, V. A. & MORRISSEY, J. J. (1982). ADP-Ribosylation of canine renal brush border membrane vesicle protein is associated with decreased phosphate transport. *J. biol. Chem.* **257**, 12380–12386.

HAMMERMAN, M. R., KARL, J. E. & HRUSKA, K. A. (1980). Regulation of canine renal vesicle P_i transport by growth hormone and parathyroid hormone. *Biochim. biophys. Acta* **603**, 322–335.

HILDMANN, B., SCHMIDT, A. & MURER, H. (1982). Ca^{2+}-transport across basal-lateral plasma membranes from rat small intestinal epithelial cells. *J. Membr. Biol.* **65**, 55–62.

HOFFMANN, N., THEES, M. & KINNE, R. (1976). Phosphate transport by isolated brush border membrane vesicles. *Pflügers Arch. ges. Physiol.* **362**, 147–156.

HOPFER, U. & LIEDTKE, C. M. (1981). Kinetic features of cotransport mechanisms under isotope exchange conditions. *Membr. Biochem.* **4**, 11–29.

IM, W. B., LING, K. J. & FAUST, R. G. (1982). Partial purification of the Na^+-dependent D-glucose transport system from renal brush border membranes. *J. Membr. Biol.* **65**, 131–137.

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

KEMPSON, S. A., COLON-OTERO, G., OU, S.-YL., TURNER, S. T. & DOUSA, T. P. (1981). Possible role of nicotinamide adenine dinucleotide as an intracellular regulator of renal transport of phosphate in the rat. *J. clin. Invest.* **67**, 1347–1360.

KEMPSON, A. S., SHAH, S. V., WERNES, P. G., BERNDT, T., LEE, P. H., SMITH, L. H., KNOX, F. G. & DOUSA, T. P. (1980). Renal brush border membrane adaptation to phosphorus deprivation: effects of fasting versus low-phosphorus diet. *Kidney Int.* **18**, 36–47.

KINNE, R. & FAUST, R. G. (1977). Incorporation of D-glucose, L-alanine and phosphate transport systems from rat renal brush border membranes into liposomes. *Biochem. J.* **168**, 311–314.

KINSELLA, J. L. & ARONSON, P. S. (1981*a*). Amiloride inhibition of the Na^+-H^+ exchange in renal microvillus membrane vesicles. *Am. J. Physiol.* **241**, F374–F379.

KINSELLA, J. L. & ARONSON, P. S. (1981*b*). Interaction of NH_4^+ and Li^+ with the renal microvillus membrane Na^+-H^+ exchanger. *Am. J. Physiol.* **241**, C220–C226.

KNICKELBEIN, R. G., ARONSON, P. S. & DOBBINS, J. W. (1982). Na-H and Cl-HCO_3 exchange in rabbit ileal brush border membrane vesicles. *Fedn Proc. Fedn Am. Socs exp. Biol.* **41**, 1494. (Abstract).

KOEPSELL, H., MENUHR, H., WISSMÜLLER, T. F., DUCIS, J. & HAASE, W. (1980). Reconstitution of D-glucose transport and high affinity phlorizin binding after solubilization of kidney brush border proteins. *Ann. N.Y. Acad. Sci.* **385**, 267–276.

LANGRIDGE-SMITH, J. L. & FIELD, M. (1981). Sulfate transport in rabbit ileum: characterization of the serosal border anion exchange process. *J. Membr. Biol.* **63**, 207–214.

LANGRIDGE-SMITH, J. L., SELLIN, J. E. & FIELD, M. (1982). Sulfate influx across the rabbit ileal brush border membrane: sodium and proton dependence, and substract specificity. *J. Membr. Biol.* (in press).

LIEDTKE, C. & HOPFER, U. (1977). Anion transport in brush border membranes isolated from rat small intestine. *Biochem. biophys. Res. Commun.* **76**, 579–585.

LIEDTKE, C. & HOPFER, U. (1982*a*). Mechanism of Cl^- translocation across the small intestinal brush border membrane. I. Absence of NaCl cotransport. *Am. J. Physiol.* **242**, G263–G271.

LIEDTKE, C. & HOPFER, U. (1982*b*). Mechanism of Cl^- translocation across the small intestinal brush border membrane. II. Demonstration of Cl^-/OH^- exchange and Cl^- conductance. *Am. J. Physiol.* **242**, 272–280.

LIN, J. T., DA CRUZ, M. E. M., RIEDEL, S. & KINNE, R. (1981). Partial purification of hog kidney sodium D-glucose cotransport system by affinity chromatography on a phlorizin polymer. *Biochim. biophys. Acta* **640**, 43–54.

LÜCKE, H., STANGE, G. & MURER, H. (1979). Sulfate-ion/sodium-ion cotransport by brush border membrane vesicles from rat kidney cortex. *Biochem. J.* **182**, 223–229.

LÜCKE, H., STANGE, G. & MURER, H. (1981). Sulfate sodium cotransport by brush border membrane vesicles isolated from rat ileum. *Gastroenterology* **80**, 22–30.

MALNIC, G. & GIEBISCH, G. (1979). Cellular aspects of renal tubular acidification. In *Membrane Transport in Biology*, (eds G. Giebisch, D. C. Tosteson & D. D. Ussing), pp. 299–356. Berlin, Heidelberg, New York: Springer.

[illegible]TIN, D. L. & DELUCA, H. F. (1969). Influence of sodium on calcium transport by rat small intestine. *Am. J. Physiol.* **216**, 1351–1359.

MATSUMOTO, T., FONTAINE, O. & RASMUSSEN, H. (1980). Effect of 1,25-dihydroxy-vitamin D_3 on phosphate uptake into chick intestinal brush border vesicles. *Biochim. biophys. Acta* **599**, 13–23.

MILLER, A. & BRONNER, F. (1981). Calcium uptake in isolated brush-border vesicles from rat small intestine. *Biochem. J.* **169**, 391–401.

MIRCHEFF, A. K., VAN OS, C. H. & WRIGHT, E. M. (1980). Pathways for alanine transport in intestinal basal lateral membrane vesicles. *J. Membr. Biol.* **52**, 83–92.

MURER, H. & BURCKHARDT, G. (1983). Membrane transport of anions across epithelia of mammalian small intestine and kidney proximal tubule. In *Reviews of Physiology, Biochemistry and Pharmacology*, Vol. 96, pp. 1–51. Berlin, Heidelberg, New York: Springer.

MURER, H. & GREGER, R. (1982). Membrane transport in the proximal tubule and thick ascending limb of Henle's loop: Mechanisms and their alterations. *Klin. Wschr.* **60**, 1103–1113.

MURER, H. & HILDMANN, B. (1981). Transcellular transport of calcium and inorganic phosphate in the small intestinal epithelium. *Am. J. Physiol.* **240**, G409–G416.

MURER, H., HOPFER, U. & KINNE, R. (1976). Sodium/proton antiport in brush border membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* **154**, 597–604.

MURER, H. & KINNE, R. (1980). The use of isolated vesicles to study epithelial transport processes. *J. Membr. Biol.* **55**, 81–95.

MURER, H., KINNE-SAFFRAN, E., BEAUWENS, R. & KINNE, R. (1980*a*). Proton fluxes in isolated renal and intestinal brush border membranes. In *Hydrogen Ion Transport in Epithelia*, (eds I. Schulz, J. G. Forte & K. J. Ullrich), pp. 267–285. Elsevier/North-Holland Biomedical Press.

MURER, H., STERN, H., BURCKHARDT, G., STORELLI, C. & KINNE, R. (1980*b*). Sodium-dependent transport of inorganic phosphate across the renal brush border membrane. In *Phosphate and Minerals in Health and Disease*, (eds S. G. Massry & H. Jahn), pp. 11–23. New York, London: Plenum.

NELLANS, H. N. & POPOVITCH, J. E. (1981). Calmodulin-regulated, ATP-driven calcium transport by basolateral membranes of rat small intestine. *J. biol. Chem.* **256**, 9932–9936.

NG, R. C. K., PERAINO, R. A. & SUKI, W. N. (1982). Divalent cation transport in isolated tubules. *Kidney Int.* **22**, 492–497.

OBERLEITHNER, H., GIEBISCH, G., LANG, F. & WENHUI, W. (1982). Cellular mechanism of the furosemide sensitive transport system in the kidney. *Klin. Wschr.* **60**, 1173–1179.

POWELL, D. W. & FAN, C. C. (1982). Calcium regulation of intestinal Na and Ca transport in *Oryctolagus cuniculus*. Abstracts Symposia Intestinal Transport European Society for comparative Physiology and Biochemistry, Bielefeld 1982, pp. 34–35.

RASMUSSEN, H., FONTAINE, O., MAX, E. E. & GOODMAN, D. B. P. (1979). The effect of 1-α hydroxyvitamin D_3 administration on calcium transport in chick intestine brush border vesicles. *J. biol. Chem.* **254**, 2993–2999.

RASMUSSEN, H., MATSUMOTO, T., FONTAINE, D. & GOODMAN, D. B. P. (1982). Role of changes in membrane lipid structure in the action of 1·25-dihydroxyvitamin D_3. *Fedn Proc. Fedn Am. Socs exp. Biol.* **41**, 72–77.

REENSTRA, W. W., WARNOCK, D. G., YEE, V. J. & FORTE, J. G. (1981). Proton gradients in renal cortex brush-border membrane vesicles. Demonstration of a rheogenic proton flux with acridine orange. *J. biol. Chem.* **256**, 11663–11666.

SABOLIC, J. & BURCKHARDT, G. (1983). Apparent inhibition of Na^+/H^+ exchange by amiloride and harmaline in acridine orange studies. *Biochim. biophys. Acta* (in press).

SACKTOR, B. (1977). Transport in membrane vesicles isolated from the mammalian kidney and intestine. *Curr. Top. Bioenerg.* **6**, 39–81.

SACKTOR, B. & CHENG, L. (1981). Sodium gradient dependent phosphate transport in renal brush border membrane vesicles. Effect of an intravesicular to extravesicular proton gradient. *J. biol. Chem.* **256**, 8080–8084.

SAMARZIJA, I., MOLNAR, V. & FRÖMTER, E. (1981). The stoichiometry of Na^+ coupled anion absorption across the brush border membrane of rat renal proximal tubule. In *Kidney and Body Fluids* (Adv. Physiol. Sci. **11**), (ed. L. Takács), pp. 419–423. Oxford: Pergamon Press; Budapest: Akadémiai Kiadó.

SCHNEIDER, E. G., DURHAM, J. C. & SACKTOR, B. (1980). The sodium-dependent transport of inorganic sulfate by rabbit renal brush border membranes. *Fedn Proc. Fedn Am. Socs exp. Biol.* **39**, 1711.

SCHULTZ, S. G. (1979). Transport across small intestine. In *Membrane Transport in Biology*, (eds G. Giebisch, D. C. Tosteson & H. H. Ussing), pp. 749–777. Berlin, Heidelberg, New York: Springer Verlag.

SCHULTZ, S. G. (1981). Salt and water absorption by mammalian small intestine. In *Physiology of the Gastrointestinal Tract*, (ed. L. R. Johnson), pp. 983–990. New York: Raven Press.

SEMENZA, G. (1982). The small intestinal Na^+/D-glucose carrier is inserted asymmetrically with respect to the plane of the brush border membrane. A model. In *Membranes and Transport: A critical Review*, (ed. A. Martonosi). New York: Plenum Press (in press).

SMITH, P. L., ORELLA, S. A. & FIELD, M. (1981). Active sulfate absorption in rabbit ileum: dependence on sodium and chloride and effects of agents that alter chloride transport. *J. Membr. Biol.* **63**, 199–200.

STEELE, T. H. & DELUCA, H. (1976). Influence of dietary phosphorus on renal phosphate reabsorption in the [illegible]arathyroidectomized rat. *J. clin. Invest.* **57**, 867–874.

STIEGER, B., STANGE, G., BIBER, J. & MURER, H. (1983). Transport of L-cysteine by rat renal brush border membrane vesicles. *J. Membr. Biol.* (in press).

STOLL, R., KINNE, R., MURER, H., FLEISCH, H. & BONJOUR, J.-P. (1979). Phosphate transport by renal brush border membrane vesicles: influence of dietary phosphate, thyroparathyroidectomy, and 1·25-dihydroxyvitamin D_3. *Pflügers Arch. ges. Physiol.* **380**, 47–52.

STOLL, R., MURER, H., FLEISCH, H. & BONJOUR, J.-P. (1980). Effect of diphosphonate treatment on phosphate transport by renal brush border membrane vesicles. *Am. J. Physiol.* **239**, F13–F16.

TENENHOUSE, H. S. & CHU, J. L. (1982). Hydrolysis of nicotinamide-adenine dinucleotide by purified renal brush border membranes. Mechanism of NAD^+ inhibition of brush border membrane phosphate transport. *Biochem. J.* **204**, 635–638.

TENENHOUSE, H. S. & SCRIVER, C. R. (1978). The defect in transcellular transport of phosphate in the nephron is located in brush border membranes in X-linked hypophosphataemia. *Can. J. Biochem.* **56**, 640–646.

TURNBERG, L. A., FORDTRAN, J. S., CARTER, N. W. & RECTOR, F. C. (1970). Mechanism of bicarbonate absorption and its relation to sodium transport in the human jejunum. *J. clin. Invest.* **49**, 557–567.

TURNER, R. J. & SILVERMAN, M. (1978). Sugar uptake into brush border vesicles from dog kidney. II. Kinetics. *Biochim. biophys. Acta* **511**, 470–486.

ULLRICH, K. J. & MURER, H. (1983). Sulfate and phosphate transport in the renal proximal tubule. *Phil. Trans. R. Soc. Lond. Ser. B.* **299**, 549–558.

ULLRICH, K. J., RUMRICH, G. & BAUMANN, K. (1975). Renal proximal tubular buffer (glycodiazine) transport. Inhomogeneity of local transport rate, dependence on sodium, effect of inhibitors and chronic adaptation. *Pflügers Arch. ges. Physiol.* **357**, 149–163.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1976). Active Ca^{2+}-reabsorption in the proximale tubule of the rat kidney: dependence on sodium and buffer transport. *Pflügers Arch. ges. Physiol.* **364**, 223–228.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1977). Phosphate transport in the proximal convolution of the rat kidney. I. Tubular heterogeneity, effect of parathyroid hormone in acute and chronic parathyroidectomized animals and effect of phosphate diet. *Pflügers Arch. Eur. J. Physiol.* **372**, 269–274.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1978). Phosphate transport in the proximal convolution of rat kidney. III. Effect of extracellular and intracellular pH. *Pflügers Arch. Eur. J. Physiol.* **377**, 33–42.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1980). Active sulfate reabsorption in the proximal convolution of the rat kidney: specificity, Na^+ and HCO_3^- dependence. *Pflügers Arch. ges. Physiol.* **383**, 159–163.

VAN OS, C. H. & GHIJSEN, W. E. J. M. (1981). High affinity Ca-ATPase in basolateral plasma membranes of rat duodenum and kidney cortex. In *Calcium and Phosphate Transport across Biomembranes*, (eds F. Bronner & M. Peterlik), pp. 159–162. New York: Academic Press.

WARNOCK, D. G. & EVELOFF, J. (1982). NaCl entry mechanisms in the luminal membrane of the renal tubule. *Am. J. Physiol.* **242**, F561–F574.

WARNOCK, D. G., REENSTRA, W. W. & YEE, V. J. (1982). Na^+/H^+ antiporter of brush border vesicles: studies with acridine orange uptake. *Am. J. Physiol.* **242**, F733–F739.

WARNOCK, D. G. & YEE, V. J. (1981). Chloride uptake by brush border membrane vesicles isolated from rabbit renal cortex. Coupling to proton gradients and K^+ diffusion potentials. *J. clin. Invest.* **67**, 103–115.

WRIGHT, S. H., KRASNE, S. K., KIPPEN, J. & 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.