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Phosphate Transport into Brush-Border Membrane Vesicles Isolated from Rat Small Intestine

By WINFRIED BERNER, ROLF KINNE and HEINI MURER
Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt (Main), Germany

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Uptake of P_i into brush-border membrane vesicles isolated from rat small intestine was investigated by a rapid filtration technique. The following results were obtained. 1. At pH 7.4 in the presence of a NaCl gradient across the membrane (sodium concentration in the medium higher than sodium concentration in the vesicles), phosphate was taken up by a saturable transport system, which was competitively inhibited by arsenate. Phosphate entered the same osmotically reactive space as D-glucose, which indicates that transport into the vesicles rather than binding to the membranes was determined. 2. The amount of phosphate taken up initially was increased about fourfold by lowering the pH from 7.4 to 6.0. 3. When Na^+ was replaced by K^+, Rb^+ or Cs^+, the initial rate of uptake decreased at pH 7.4 but was not altered at pH 6.0. 4. Experiments with different anions (SCN^-, Cl^-, SO_4^{2-}) and with ionophores (valinomycin, monactin) showed that at pH 7.4 phosphate transport in the presence of a Na^+ gradient is almost independent of the electrical potential across the vesicle membrane, whereas at pH 6.0 phosphate transport involves the transfer of negative charge. It is concluded that intestinal brush-border membranes contain a Na^+/phosphate co-transport system, which catalyses under physiological conditions an electroneutral entry of P_i and Na^+ into the intestinal epithelial cell. In contrast with the kidney, probably univalent phosphate and one Na^+ ion instead of bivalent phosphate and two Na^+ ions are transported together.

The absorption of P_i in the small intestine takes place against an electrochemical potential difference and is inhibited by metabolic inhibitors (Harrison & Harrison, 1961, 1963; Taylor, 1974) and therefore can be defined as active transepithelial transport (Frömter *et al.*, 1973; Sauer, 1973). Active transepithelial transport can be primary active or secondary active. In the former case the active transport step is directly coupled to cell metabolism, e.g. via transport ATPases*; in the latter case the transport of the substance is coupled to the flux of another substance whose transport is in this instance primary active. Such transport mechanisms are involved in the Na^+-dependent transepithelial transport of sugars and amino acids in the kidney (Kinne *et al.*, 1975; Evers *et al.*, 1976) and in the small intestine (Murer & Hopfer, 1976). In studies with everted loops (Harrison & Harrison, 1963), the intestinal phosphate uptake was enhanced in the presence of Na^+, similarly to the kidney (Baumann *et al.*, 1975), to nerves (Straub *et al.*, 1975), to cultured (SV3T3) cells (Brown & Lamb, 1975) and to the marine fungus *Thraustochytrium roseum* (Siegenthaler *et al.*, 1967), suggesting that phosphate transport is secondary active. In experiments with renal brush-border membrane vesicles, a coupling between Na^+ and phosphate flux, a prerequisite for secondary active transport, could be demonstrated (Hoffmann *et al.*, 1976).

In the present paper experiments with brush-border membrane vesicles isolated from rat small intestine are described which demonstrate that at pH 7.4 an electroneutral Na^+/phosphate co-transport system is operating in these membranes. This finding suggests that, at the luminal pole, phosphate entry into the epithelial cell is coupled to the entry of Na^+, and that the asymmetrical distribution of Na^+ across the plasma membrane (Na^+ gradient) provides the driving force for intestinal phosphate absorption.

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

Materials and Methods

Membrane isolation

Membranes were prepared from rat small intestine by the procedure of Schmitz *et al.* (1973), as modified by Sigrist-Nelson *et al.* (1975). Briefly, after homogenization of mucosal scrapings in a hypo-osmotic medium and addition of 10mM-$CaCl_2$, the brush-border membranes were purified by differential centrifugations. Ca^{2+}-free brush-border membrane

vesicles were isolated by the method of Hopfer *et al.* (1973).

Uptake measurements

Uptakes of phosphate and, for comparison, D-glucose were measured by a Millipore filtration technique as follows. Membrane vesicles kept on ice were prepared in 0.1 M-mannitol/20 mM-Hepes/Tris (20 mM-Hepes adjusted to pH 6.0 or 7.4 with Tris base), and the uptake experiment was started by adding about 50 μg of membrane protein to the incubation medium, which was the above-mentioned medium with additions as indicated in the Figure legends. The buffer contained, as labelled substrate, 0.1 mM-$KH_2{}^{32}PO_4$ (500 Ci/mol) or 1 mM-D-[U-^{14}C]-glucose (750 Ci/mol). The uptake of phosphate or glucose was terminated by the removal of a portion which was diluted 20-fold with ice-cold buffer containing 0.1 M-mannitol, 20 mM-Hepes/Tris, 0.1 M-choline chloride, 50 mM-$MgCl_2$ and 5 mM-Na_2HAsO_4 or 1 mM-phlorrhizin. The membrane was immediately collected on a cellulose nitrate filter (pore size 0.6 μm; Sartorius, Göttingen, Germany) and washed once with 5 ml of the ice-cold buffer. The amount of labelled phosphate or glucose remaining on the filter was determined in a liquid-scintillation counter (Mark II, Nuclear-Chicago) by using Instagel (Packard Instrument Co.) as scintillation fluid. In general, single experiments are presented in this paper; however, all experiments were repeated at least three times with similar results.

The osmotic-shock procedure was carried out as follows. Before collection by filtration, phosphate-equilibrated vesicles were diluted in ice-cold double-distilled water instead of the iso-osmotic ice-cold buffer used as a routine for the dilution of the incubation medium.

The protein of the membranes was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid and complete solubilization with NaOH, by the method of Lowry *et al.* (1951), with bovine serum albumin as standard (Behringwerke, Marburg, Germany).

Materials

All chemicals used for the experiments were of the highest purity available. Tris, mannitol, KH_2PO_4, Na_2HAsO_4 and D-glucose were obtained from Merck (Darmstadt, Germany), Hepes was from Serva (Heidelberg, Germany), phlorrhizin was from Roth (Karlsruhe, Germany), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone was from Boehringer Mannheim (Mannheim, Germany) and valinomycin was from Sigma (St. Louis, MO, U.S.A.). Monactin was a gift from Ciba-Geigy A.G. (Basel, Switzerland). The labelled compounds were from New England Nuclear Corp. (Boston, MA, U.S.A.).

Results

General properties of the phosphate-transport system

When intestinal brush-border membrane vesicles were prepared in a Na^+-free solution and incubated in Na^+-containing buffer at pH 7.4, the amount of P_i taken up by the membrane vesicles increased with time and reached equilibrium after about 1 h (Fig. 1). In the presence of a choline chloride gradient, instead of a NaCl gradient, the initial rate of phosphate uptake decreased, but the same equilibrium value was reached after 2 h. When Na^+ was replaced by K^+, the phosphate uptake was lowered by a factor of two. The phosphate uptake in the presence of NaCl, but not that in the presence of choline chloride could be inhibited in part by arsenate (5 mM). The same general properties were observed with brush-border membrane vesicles prepared by the method of Hopfer *et al.* (1973) (Fig. 2). In this calcium-free membrane preparation, the effect of Ca^{2+} on phosphate uptake could also be investigated. Phosphate uptake in the presence of a NaCl gradient directed into the vesicles was identical in the absence and presence of 0.5 mM-$CaCl_2$. To decide whether phosphate uptake represents transport across the membrane into an intravesicular osmotically reactive space or only binding

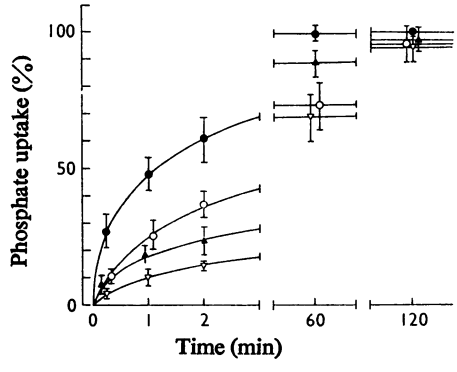

Fig. 1. *Phosphate uptake into intestinal brush-border membrane vesicles: cation-dependence and arsenate inhibition*

Membrane vesicles isolated by the method of Sigrist-Nelson *et al.* (1975) and loaded with a buffer containing 0.1 M-mannitol and 20 mM-Hepes/Tris (pH 7.4) were incubated at 25°C in a medium containing 0.1 mM-P_i, 0.1 M-mannitol, 20 mM-Hepes/Tris (pH 7.4), 0.1 M-NaCl (●) or 0.1 M-NaCl plus 5 mM-K_2HAsO_4 (○) or 0.1 M-KCl (▲) or 0.1 M-choline chloride (▽). Phosphate uptake is expressed as percentage of the amount taken up by the vesicles after 120 min of incubation in the NaCl (●) medium, which amounted to 122.8 ± 8.7 pmol/mg of protein. Mean values ± S.E.M. derived from three experiments are given.

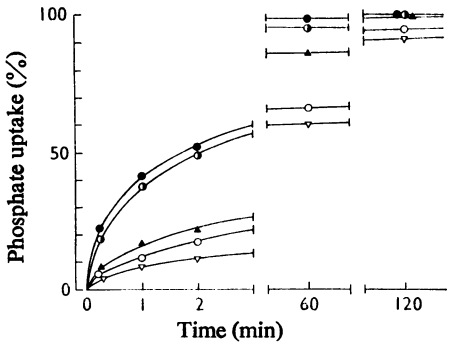

Fig. 2. *Phosphate uptake into intestinal brush-border membrane vesicles: effect of Ca^{2+} concentration*

Membrane vesicles isolated by the method of Hopfer *et al.* (1973) were loaded with a buffer containing 0.1 M-mannitol and 20mM-Hepes/Tris (pH7.4) and incubated at 25°C in the presence of NaCl (●) or NaCl+arsenate (○) or KCl (▲) or choline chloride (▽) or 0.5mM-$CaCl_2$+0.1M-NaCl (◑). The values are expressed as percentage of the phosphate taken up by the vesicles after 120min of incubation in the NaCl (●) medium, which amounted to 89.8pmol/mg of protein.

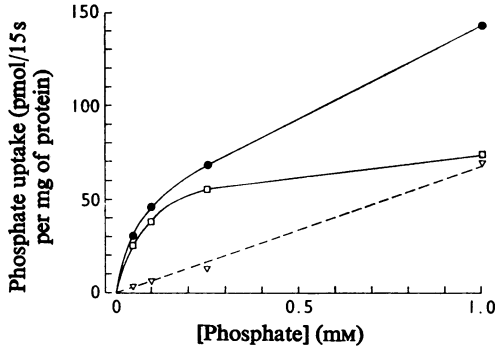

Fig. 3. *Phosphate uptake into intestinal brush-border membrane vesicles: effect of phosphate concentration*

Experiments were carried out as described in Fig. 1. The vesicles were incubated with increasing phosphate concentration in 0.1 M-NaCl (●) or 0.1 M-choline chloride (▽). □, Na^+-dependent phosphate uptake calculated as the uptake in the presence of NaCl minus the uptake in the presence of choline chloride.

to the membrane, the vesicles were subjected to an osmotic-shock procedure after pre-equilibration of the vesicles with phosphate. Thereby the phosphate content of the membranes was decreased to 11% of the control. Further evidence for transport was obtained when the uptake of phosphate was compared with that of D-glucose. From the equilibrium value of glucose, an intravesicular volume of 1.38 μl/mg of protein, and from the equilibrium value of P_i an intravesicular volume of 1.23 μl/mg of protein, was calculated. Hopfer *et al.* (1973) demonstrated that glucose uptake represents entirely transport into the vesicles and not binding to the membrane surface; thus it can also be concluded that phosphate uptake represents transport into an intravesicular space.

Interaction of the phosphate-transport system with phosphate and arsenate

The amount of phosphate taken up into the membrane vesicles increased when the phosphate concentration in the medium was augmented (Fig. 3). In the presence of choline chloride a linear increase was observed, whereas in the presence of NaCl a curvilinear relationship between initial uptake and phosphate concentration was found. The linear uptake might indicate that phosphate transport in the presence of choline chloride represents simple diffusion. This is also supported by the fact that phosphate uptake in the presence of choline chloride was not inhibited by arsenate at pH7.4 (Table 1). The specific Na^+-dependent phosphate-transport system (total uptake minus simple diffusion) seems to follow Michaelis–Menten kinetics, and from a Lineweaver–Burk plot an apparent Michaelis constant (K_m) of 1.1×10^{-4}M was obtained (Fig. 4). Arsenate, as a competitive inhibitor of phosphate transport *in vivo* (Ginsburg & Lotspeich, 1963), inhibited the phosphate uptake into the vesicles competitively. The inhibitor constant (K_i) for arsenate for the Na^+-dependent transport at pH7.4 was 3.4×10^{-4}M.

Influence of pH on the medium on phosphate uptake

One noteworthy aspect of the interaction of phosphate with the transport system is the question

Table 1. *Effect of arsenate on phosphate uptake into intestinal brush-border membrane vesicles in the presence of choline chloride*

Membrane vesicles isolated as described by Sigrist-Nelson *et al.* (1975) and loaded with a buffer containing 0.1 M-mannitol and 20mM-Hepes/Tris (pH6.0 or 7.4) were incubated in a medium containing 0.1 M-mannitol, 20mM-Hepes/Tris (pH6.0 or 7.4 respectively), 0.1mM-$^{32}P_i$ and 0.14mM-choline chloride in the absence and presence of 5mM-arsenate. The initial uptake (the amount taken up during the first 15s) is given.

	Phosphate uptake (pmol/15s per mg of protein)	
	pH6.0	pH7.4
Choline chloride	16.1	5.3
Choline chloride+arsenate	10.8	4.9

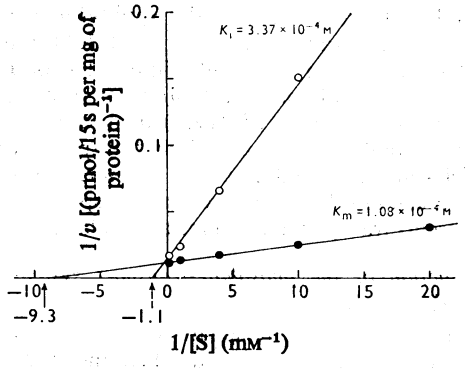

Fig. 4. *Determination of the Michaelis constant (K_m) and the inhibitor constant (K_i) of arsenate of Na^+-dependent phosphate uptake into intestinal brush-border membrane vesicles*

The experiments were carried out as described in Fig. 3 in a medium containing 0.1 M-mannitol, 20 mM-Hepes/Tris (pH 7.4), various phosphate concentrations and 0.1 M-NaCl in the absence (●) or in the presence (○) of 2.5 mM-K_2HAsO_4. The uptake values were corrected for uptake in the presence of 0.1 M-choline chloride. $V_{max.}$ was determined as 80 pmol/15 s per mg of protein.

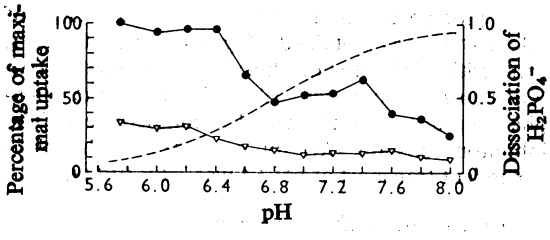

Fig. 5. *Phosphate uptake into intestinal brush-border membrane vesicles: influence of pH of the medium*

Membrane vesicles isolated by the method of Sigrist-Nelson *et al.* (1975) and loaded with a buffer containing 0.1 M-mannitol and 0.5 mM-Hepes/Tris (pH 7.4) were incubated in a medium containing 0.1 M-mannitol, 20 mM-Hepes (adjusted with Tris base to the pH indicated in the Figure), 0.1 mM-$^{32}P_i$ in the presence of 0.1 M-NaCl (●) or 0.1 M-choline chloride (▽). The phosphate uptake is given as a percentage of maximal uptake in the presence of Na^+ at pH 5.75. The incubation time was 15 s. ----, Dissociation curve of $H_2PO_4^-$.

whether univalent or bivalent phosphate is the anion species that is preferentially transported. Therefore the effect of pH of the incubation medium on the phosphate uptake was investigated.

As shown in Fig. 5, the phosphate uptake increased about fourfold in the presence of Na^+, and about 2.5-fold in the presence of choline, when the pH was lowered from 8.0 to 5.8. This increase is not due to a general alteration of membrane permeability, since arsenate inhibited phosphate transport at pH 6.0 and pH 7.4, and because the permeability of the membranes for L-glucose was not changed (W. Berner, R. Kinne & H. Murer, unpublished work). Thus the observed changes might be related to an effect of pH on the substrate (increased univalent phosphate concentration) or to an effect of pH on the transport system. Therefore in the subsequent experiments the properties of the transport system were investigated at both pH 7.4 and pH 6.0.

Interaction of cations with the phosphate-transport system

Fig. 6 shows the uptake of P_i in the presence of 0.1 mM-phosphate as a function of the Na^+ concentration in the incubation medium. At pH 7.4, phosphate uptake showed a saturation type of dependence on the Na^+ concentration. Maximal phosphate uptake was observed at 50 mM-Na^+ and 50% stimulation at about 27 mM. At pH 6.0 the relative increase in phosphate uptake caused by Na^+ was smaller (approximately twofold, compared with fivefold at pH 7.4). No saturation was apparent up to a Na^+ concentration of 0.14 M.

Other univalent cations such as K^+, Rb^+ and Cs^+ did not stimulate phosphate transport at pH 7.4 above the value observed in the presence of choline chloride (Table 2). Li^+ at a concentration of 100 mM could mimic the effect of Na^+ completely; at 50 mM, however, the rate of uptake was only 50% of that with 50 mM-NaCl. This indicates that the phosphate-transport system has a lower apparent affinity for Li^+ than for Na^+. The specificity for Na^+ of the cation

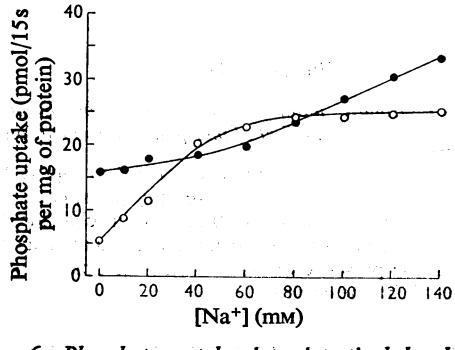

Fig. 6. *Phosphate uptake into intestinal brush-border membrane vesicles: influence of Na^+ concentration*

Experiments were carried out as described in Fig. 1. The vesicles were incubated in a medium containing 0.1 mM-$^{32}P_i$, 0.1 M-mannitol, 20 mM-Hepes/Tris, pH 7.4 (○) or pH 6.0 (●), and the various NaCl concentrations indicated in the Figure. Na^+ was replaced by choline to maintain osmolarity and ionic strength constant.

Table 2. *Effect of cations on phosphate uptake into intestinal brush-border membrane vesicles*

The experiments were carried out as described in Fig. 1 in an incubation medium containing 0.1 M-mannitol, 20 mM-Hepes/Tris (pH 6.0 or 7.4), 0.1 mM-$^{32}P_i$ and salts as indicated in the Table. The initial uptake (the amount taken up during the first 15 s) is given.

Salt in the incubation medium (0.1 M)	Phosphate uptake (pmol/15 s per mg of protein)	
	pH 6.0	pH 7.4
Choline chloride	13.3	7.4
LiCl	25.4	20.8
NaCl	27.9	20.0
KCl	25.3	10.1
RbCl	22.9	11.0
CsCl	24.2	9.5

stimulation of phosphate transport and the saturability of this Na^+ effect point to a direct interaction (co-transport) of the Na^+ ion with the phosphate-transport system at pH 7.4.

At pH 6.0 the ability of other cations to stimulate phosphate transport was almost identical with the effect of Na^+. The lack of specificity and saturability at pH 6.0 could be an indication of an unspecific effect of cation/salt gradients. Thereby inside-positive diffusion potentials could stimulate the translocation of the negatively charged univalent phosphate. A cation-independent phosphate movement at pH 6.0 is also evident from the inhibition of phosphate transport by arsenate in the choline chloride incubation medium (Table 1).

Influence of membrane potential on phosphate uptake

Since phosphate is an electrolyte, its transport might depend on the electrical potential difference across the membrane. The effect of membrane potential on phosphate uptake was investigated first by anion-replacement experiments (Table 3). When a NaCl gradient was replaced by a sodium thiocyanate gradient (increased negativity inside the vesicle), at pH 7.4 phosphate transport was not affected, whereas at pH 6.0 phosphate transport was inhibited by 30%. Decreased negativity inside the vesicle (replacement of the NaCl gradient by a Na_2SO_4 gradient) stimulated the uptake at pH 6.0 but not at pH 7.4.

Similar results were obtained when the effect of valinomycin in the presence of RbCl and KCl gradients was studied. Addition of valinomycin (increased inside-positive diffusion potential) stimulated phosphate uptake at pH 6.0 by 30% (Table 4).

These results indicate that at pH 6.0 phosphate transfer is sensitive to potential and involves a translocation of negative charges. At pH 7.4, however, most of the phosphate seems to be transported electroneutrally, probably via Na^+ phosphate co-transport.

For a Na^+/solute co-transport system one would postulate that Na^+ also influences the transport of the solute in the absence of a cation gradient. This could be demonstrated for the phosphate transport at pH 7.4, as shown in Fig. 7. The relatively small stimulation (30%) when Na^+ but no gradient was present, compared with the threefold stimulation in the presence of a Na^+ gradient, suggests that the Na^+ gradient can act as an additional driving force for the phosphate transport. This assumption can be tested by the use of monactin, which increases the permeability of membranes to Na^+ (Henderson *et al.*, 1969). When monactin is used in the presence of a Na^+ gradient, this gradient is dissipated faster than under control conditions. Therefore the rate of dissipation depends on the permeability of the accompanying anions, since the transfer of Na^+ by monactin involves the transfer of positive charges. In agreement with these considerations, phosphate uptake was strikingly decreased by the addition of monactin in

Table 3. *Effect of anion replacement on phosphate uptake into intestinal brush-border membrane vesicles*

The experiments were carried out as described in Fig. 1 except for replacement of NaCl (0.1 M) by NaSCN (0.1 M) or by Na_2SO_4 (50 mM)+mannitol (50 M). The initial uptake (the amount taken up during the first 15 s) is given.

Salt in the incubation medium	Phosphate uptake (%)	
	pH 6.0	pH 7.4
NaSCN	68.3	96.8
NaCl	100	100
Na_2SO_4	115	106

Table 4. *Influence of valinomycin-induced diffusion potentials on phosphate uptake into intestinal brush-border membrane vesicles*

Membranes were loaded with 0.1 M-mannitol, 20 mM-Hepes/Tris at pH 6.0 and incubated in a medium containing 0.1 mM-$^{32}P_i$, 0.1 M-mannitol, 20 mM-Hepes/Tris, pH 6.0, and 0.1 M-KCl or 0.1 M-RbCl. Valinomycin (10 mg/ml in ethanol) was added at a dose of 10 μg/mg of protein (final concentration of ethanol 1%). The controls contained the ethanol only. Initial uptake (the amount taken up during the first 15 s) is given.

Salts in the incubation medium	Phosphate uptake (pmol/15 s per mg of protein)
KCl	24.9
KCl+valinomycin	32.2
RbCl	21.6
RbCl+valinomycin	27.1

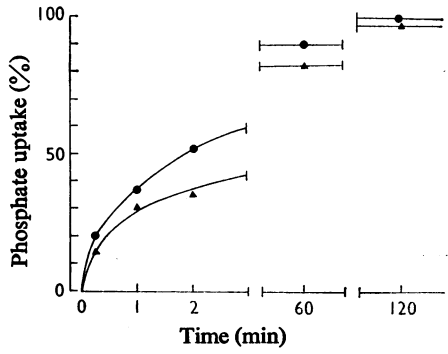

Fig. 7. *Phosphate uptake into intestinal brush-border membrane vesicles: influence of Na^+ and K^+ under non-salt-gradient conditions*

The membrane vesicles, isolated by the method of Sigrist-Nelson *et al.* (1975) and loaded with a buffer containing 0.1 M-mannitol, 20 mM-Hepes/Tris (pH 7.4), were pre-incubated with 0.1 M-NaCl (●) or -KCl (▲) at 25°C for 30 min. Phosphate uptake was started at 25°C by adding labelled substrate (0.1 mM final concentration) to the incubation medium. Phosphate uptake is given as a percentage of the amount taken up after 120 min in the presence of NaCl, which amounted to 129.7 pmol/mg of protein.

Table 5. *Influence of monactin-induced Na^+ permeability on phosphate transport into intestinal brush-border membrane vesicles*

The experiments were carried out as indicated in Table 4 at pH 7.4. Monactin (10 mg/ml in ethanol) was added at a dose of 10 μg/mg of protein. Initial uptake (the amount taken up during the first 15 s) is given.

Salt in the incubation medium	Phosphate uptake (pmol/15 s per mg of protein)	
	Control	+Monactin
NaSCN	24.3	14.4
NaCl	25.1	19.6
Na_2SO_4	26.6	24.7

the presence of a sodium thiocyanate gradient but only slightly influenced in the presence of a Na_2SO_4 gradient (Table 5).

Discussion

Phosphate-transport mechanism across the brush-border membrane

The results presented above show that phosphate transport is saturable and competitively inhibited by arsenate. This indicates that a specific mechanism for P_i transport is present in the brush-border membrane. At pH 7.4 phosphate transport by this system involves co-transport with Na^+, as suggested by the stimulation of phosphate transport by Na^+ under gradient as well as under non-gradient conditions. The observed electroneutrality of the Na^+/phosphate co-transport and the fact that phosphate transfer is dependent in a simple hyperbolic fashion on the Na^+ concentration might be evidence of a co-transport of the univalent phosphate ion together with one Na^+ ion, although electroneutrality could also be explained by a co-transport of the bivalent phosphate ion with two Na^+ ions. In this case a sigmoid dependence on the Na^+ concentration would be expected, as has been demonstrated in cultured cells (Brown & Lamb, 1975) and in renal brush-border membranes (Hoffmann *et al.*, 1976).

Further evidence for the transport of univalent phosphate might be obtained by the pH-dependence of the phosphate transport, which seems to be similar to the dissociation curve for bivalent phosphate. So the observed increased uptake of P_i, when the pH is lowered, would be due to an increased concentration of univalent phosphate as the substrate. Another possible explanation for the pH-dependence could be that intestinal brush-border membranes contain a phosphate/OH^- exchange system, as has been described in mitochondria (Chappell & Crofts, 1966; Mitchell & Moyle, 1969; Papa *et al.*, 1969; Klingenberg, 1970) and bacterial systems (Hirata *et al.*, 1974; Burnell *et al.*, 1975; Cockburn *et al.*, 1975). Indeed, in our experiments a OH^- gradient (vesicles > medium) existed across the microvillus membrane, because only the pH in the extravesicular space was altered and because the microvillus membrane has a low proton conductance (Murer & Hopfer, 1974; Murer *et al.*, 1976). Thus at low pH the OH^- gradient (vesicle > medium) could be an additional driving force for phosphate uptake. However, in experiments with a K^+ gradient (medium > vesicles), nigericin, which catalyses K^+/H^+ exchange, and renders the intravesicular space alkaline, stimulated phosphate transport at neither pH 6.0 nor pH 7.4 (results not shown). Therefore we have no evidence that phosphate/OH^- exchange occurs in the brush-border membrane.

Also a direct effect of pH on the phosphate-transport system itself was observed. In contrast with that at pH 7.4, phosphate transport at pH 6.0 seems not to involve co-transport with Na^+. This can be concluded from the observation that phosphate transport at pH 6.0 is inhibited by arsenate even in the absence of Na^+ (Table 1). In addition, phosphate transport at pH 6.0 involves translocation of a negative charge and is influenced by the electrical potential difference across the membrane. This sensitivity to potential could explain the stimulatory effect of the NaCl gradient, assuming the generation of a diffusion potential (vesicle interior positive). This assumption is also supported by lack of saturability

and specificity of cation stimulation of phosphate transport at pH 6.0.

Considering the different properties of phosphate transport at pH 7.4 and 6.0, one might speculate that protonation of one or more protein side groups of the phosphate-transport system prevents the binding of Na^+ to its specific binding site and provokes a similar effect to that of Na^+ on the mobility of the carrier. This hypothesis might explain the lack of co-transport phenomena, the sensitivity to potential differences and the inhibition by arsenate in the absence of Na^+ at pH 6.0. It should be noted, however, that an equally feasible explanation for our finding would be the presence of two different transport mechanisms, one being a phosphate/Na^+ co-transport and the other one representing a Na^+-independent anion-transport system.

Involvement of Na^+/phosphate co-transport in intestinal phosphate absorption

The membrane potential, cell interior negative, across the brush-border membrane (−40 mV; Rose & Schultz, 1971) inhibits transfer of negatively charged ions. However, with an electroneutral coupling of P_i and Na^+ flux in the brush border the inhibitory effect of the membrane potential is cancelled. Further, the concentration difference for Na^+ across the mucosal membrane provides an additional driving force for the Na^+/phosphate co-transport. Assuming a luminal Na^+ concentration of 140 mM and cellular concentration of 35–50 mM (Schultz & Curran, 1968), a concentration difference for phosphate (cell > lumen) across the brush-border membrane could be built up. The effective accumulation of P_i in the epithelial cell, however, also depends on the rate of simple back-diffusion of phosphate across the luminal brush-border membrane and on the transport capacity at the contraluminal cell border.

Assuming that, by analogy with the cellular distribution of non-electrolyte-transport systems (Murer *et al.*, 1974; Kinne & Murer, 1976; Murer & Hopfer, 1976), phosphate transport at the serosal (contraluminal) cell side is facilitated by a Na^+-independent transport system, the driving forces at the serosal cell side would be the concentration gradient for phosphate and the membrane potential (−40 mV). In the presence of a potential difference of 40 mV, cell interior negative, bivalent phosphate could be accumulated eightfold in the extracellular fluid compared with the intracellular compartment. A 32-fold difference in P_i concentration across the intestinal epithelium (serosal > mucosal), as has been described in experiments by Harrison & Harrison (1961), could therefore be explained by the combined operation of an electroneutral Na^+/phosphate co-transport in the brush-border membrane and by a potential-sensitive outflow of P_i at the serosal cell side. A prerequisite to such calculations, however, is the fact that the transfer rate across the contraluminal membrane must be high compared with the rate of phosphate entry across the brush border and that the paracellular shunts (tight junctions) are relatively tight for phosphate. The latter is important because the transmural (transepithelial) potential difference is low (+5 to +10 mV) and is not able to maintain a concentration difference of 32 for P_i across the epithelium.

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