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Maturation of Jejunal Phosphate Transport by Rat Brush Border Membrane Vesicles

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ABSTRACT. The transport of phosphate into jejunal brush border membrane vesicles isolated from 14- to 42-day-old rats was investigated using a rapid filtration technique. The presence of a sodium gradient enhanced phosphate uptake at all ages. Both sodium-dependent phosphate uptake and passive phosphate uptake declined with increasing age. The activity of the sodium/phosphate cotransporter was significantly greater in the 14-day-old suckling animals than in the 42-day-old animals. Experiments with valinomycin in 14- and 42-day-old animals showed that at pH 7.4, phosphate uptake is electroneutral at both ages. These findings demonstrate there are maturational changes in the jejunal transport of phosphate. Suckling animals transport significantly more phosphate than adult animals. The increased uptake in the younger animals is related to an increase in the active uptake of phosphate and an increase in the passive permeability to phosphate. (*Pediatr Res* 19: 1308–1312, 1985)

The transport of phosphate across the small intestine of adult animals is an active process (1–7). In mature rats, jejunal phosphate transport takes place against an electrochemical gradient and is inhibited by arsenate (1, 8). This uptake of phosphate at the brush border consists of two components: a saturable, electroneutral sodium-dependent component, and an unsaturable, sodium-independent component (1, 2). The energy driving the sodium dependent entry of phosphate into the cell is provided by an extracellular to intracellular sodium gradient maintained by the Na^+-K^+-ATPase at the basolateral membrane (5, 9). A similar absorptive process has been described in the brush border membrane of the rat renal proximal tubule (10). The maximal tubular reabsorption of phosphate is significantly greater in young growing rats as compared in adults (11). We have utilized isolated jejunal apical brush border membrane vesicles from rats of differing ages to determine whether there are similar maturational changes in the intestinal uptake of phosphate.

MATERIALS AND METHODS

Sprague Dawley rats of varying ages were supplied by Sasco Industries (Omaha, NE). The animals were housed in the animal care facility and fed standard food *ad libitum* until their sacrifice by cervical dislocation. After sacrifice, the entire jejunum was removed, washed with ice cold 0.9% NaCl, and everted over a glass rod. Brush border membrane vesicles were prepared by sequential precipitation with 0.01 M $MgCl_2$ and differential centrifugation (12). The purity of the vesicle preparation for rats of varying ages has been previously demonstrated in our laboratory (12).

Phosphate uptake was measured by a rapid filtration technique (13) using Sartorius cellulose nitrate filters of 0.45 micrometer pore size (Sartorius Filters Inc., Hayward, CA) that had been presoaked in stop solution containing 100 mM mannitol, 100 mM choline chloride, 20 mM Hepes/Tris, and 50 mM magnesium chloride (pH 7.4). Incubations were carried out at room temperature using $KH_2{}^{32}PO_4$ (specific activity 1 Ci/mmol) as a label (New England Nuclear, Boston, MA). The composition of the incubation media for each individual experiment is described in the figure legends of the "Results" section. The amount of radioactive phosphate remaining on the filter was determined in a liquid scintillation counter (Beckman Instruments, Palo Alto, CA) using Bray's Solution (New England Nuclear) as liquid scintillant. The protein content of the vesicle solution was determined by the method of Lowry *et al.* (14) using bovine serum albumin 1 mg/ml as a standard.

Experiments described in this paper were performed three to seventeen times, and each transport measurement was done in triplicate. Each vesicle preparation involved a minimum of six adult or twelve suckling rats. All results comparing experimental groups are expressed as means ± SEM. The significances of differences were calculated using the two-sided Student's t test with the level of significance $p < 0.05$.

RESULTS

Validation Studies. Vesicle size. Mannitol uptake studies were performed to determine whether vesicle size is comparable in 14- and 42-day-old animals. Vesicle sizes as calculated from equilibrium values are 1.3 ± 0.12 μl/mg protein for the 14-day-old rats and 1.5 ± 0.15 μl/mg protein for the 42-day-old rats. Similar values can be calculated with glucose as the substrate (12). These vesicle sizes are similar to the sizes of intestinal and renal brush border membrane vesicles reported in the literature (11).

Vesicle sizes as calculated from phosphate uptake experiments are 12.5 μl/mg protein for the 14-day-old animals and 2.4 ± 0.1 μl/mg protein for the 42-day-old animals. These values indicate there is significant intravesicular binding of phosphate at both ages; however, the binding is significantly greater in the 14-day-old rats. Significant internal binding of phosphate has also been reported in rabbit intestinal brush border membrane vesicles (15).

The effect of medium osmolality on phosphate uptake. To determine whether phosphate enters the intravesicular space, vesicles from 42- and 14-day-old animals were prepared in a Na^+-free solution and incubated in a Na^+-free buffer with mannitol concentrations varying from 60 to 560 mM (Fig. 1). At 60 min, uptake was inversely proportional to the medium osmolality. Extrapolation to infinite medium osmolality yields a phos-

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phate uptake of 1.0 nmol/mg of protein in the 42-day-old animals, and 0.9 nmol/mg of protein in the 14-day-old animals. These results suggest that in both 14 and 42-day-old rats, phosphate uptake represents transport into the intravesicular space, with a small fraction binding to the external surface of the membrane. The amount of phosphate bound to the external vesicle surface is similar in both age groups.

The effects of sodium on phosphate uptake. To define the effects of sodium on phosphate uptake, intestinal brush border membrane vesicles from 42- and 14-day-old animals were prepared in a Na^+-free solution and incubated in a Na^+-containing buffer at pH 7.4 (Fig. 2). When Na^+ in the incubation media was replaced with K^+, phosphate uptake at 20 s, 1, 2, and 5 min was significantly lowered. At 60 min, equilibrium seems to have been achieved in 42-day-old animals as evidenced by similar uptakes in the presence and absence of Na^+. In the 14-day-old animals, equilibrium did not occur until 480 min. At all time points, phosphate uptake in the presence and absence of sodium was five times greater in the vesicles prepared from the 14-day-old animals than in those prepared from the older animals.

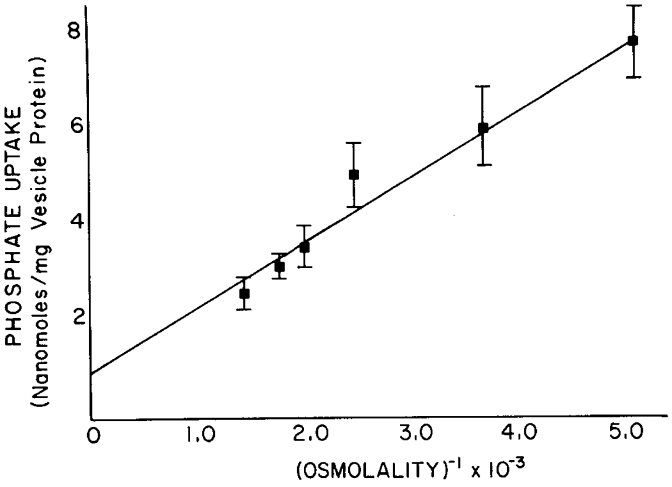

Fig. 1. Phosphate uptake into jejunal brush border membrane vesicles: effects of medium osmolality. Jejunal brush border membrane vesicles from 42-day-old rats were loaded with a buffer containing 0.1 M mannitol, 0.1 M choline chloride, and 20 mM Hepes/Tris (pH 7.4). The vesicles were incubated for 60 minutes at room temperature in a medium containing 20 mM Hepes/Tris (pH 7.4) and mannitol (60–600 mM). Phosphate uptake is expressed as nmol/mg of vesicle protein. The line is described y = 1.293x + 1.0 (correlation coefficient 0.99). Similar data were obtained for 14-day-old rats with the line described y = 6.60x + 0.96 (correlation coefficient 0.99).

Similar studies were performed in 18- and 28-day-old animals (Fig. 3). In the presence and absence of sodium, phosphate uptake in the 18-day-old weanling animals was significantly less than in the 14-day-old suckling animals. Uptake in the 28-day-old animals was intermediate between the weanling and 42-day-old animals. Both sodium-dependent and sodium-independent transport demonstrated a progressive decline with increasing postnatal age.

The effect of phosphate concentration on sodium-dependent uptake: kinetics of phosphate uptake. In an effort to define the kinetics of sodium-dependent phosphate transport, vesicles from 42- and 14-day-old animals were incubated in Na^+-containing and Na^+-free buffers with phosphate concentration varying from 0.05 to 2.5 mM (Fig. 4). Transport was measured at 10 s during the linear phase of uptake. In both groups, phosphate transported into the vesicles increased in the presence and absence of Na^+ as the concentration of phosphate in the media was increased. In the presence of Na^+, uptake was saturable. When Na^+ in the incubation media was replaced with K^+, uptake became linear. Lineweaver-Burk double reciprocal plots of the active component of phosphate transport (sodium-dependent uptake minus sodium-independent uptake) demonstrate a Vmax of 1.766 nmol phosphate/mg protein/10 s and a Km of 0.069 mM for the 42-day-old animals, and a Vmax of 4.679 nmol phosphate/mg protein/10 s and a Km of 0.210 mM for the 14-day-old animals (Fig. 5).

The effects of valinomycin on sodium-dependent phosphate uptake. Phosphate is an anion and its transport across the intestinal epithelium may depend on the electric potential across the cell membrane. Changes in this electric potential may account for the observed increase in phosphate uptake in the younger animals. The effects of membrane potential on phosphate transport were investigated with the ionophore valinomycin. Valino-

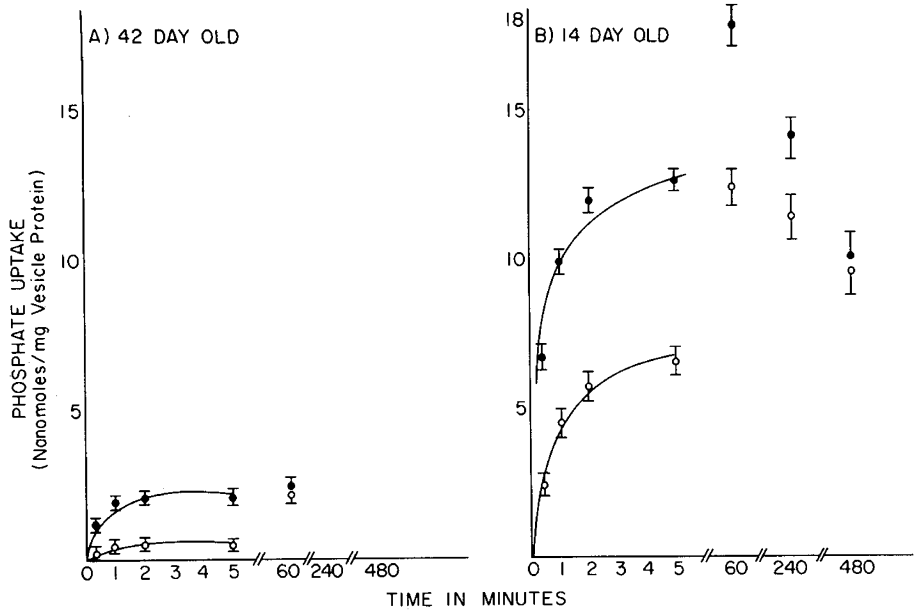

Fig. 2. Phosphate uptake into jejunal brush border membrane vesicles: effects of a sodium gradient. Jejunal brush border membrane vesicles from 42-day-old rats (*A*) or 14-day-old suckling rats (*B*) were loaded with a buffer containing 0.1 M mannitol, 0.1 M choline chloride, and 20 mM Hepes/Tris (pH 7.4). The vesicles were incubated at room temperature in a medium containing 0.8 mM phosphate, 0.1 M mannitol, 20 mM Hepes/Tris (pH 7.4), 0.1 M NaCl (●), or 0.1 M KCl (○). Phosphate uptake is expressed as nmol/mg of vesicle protein.

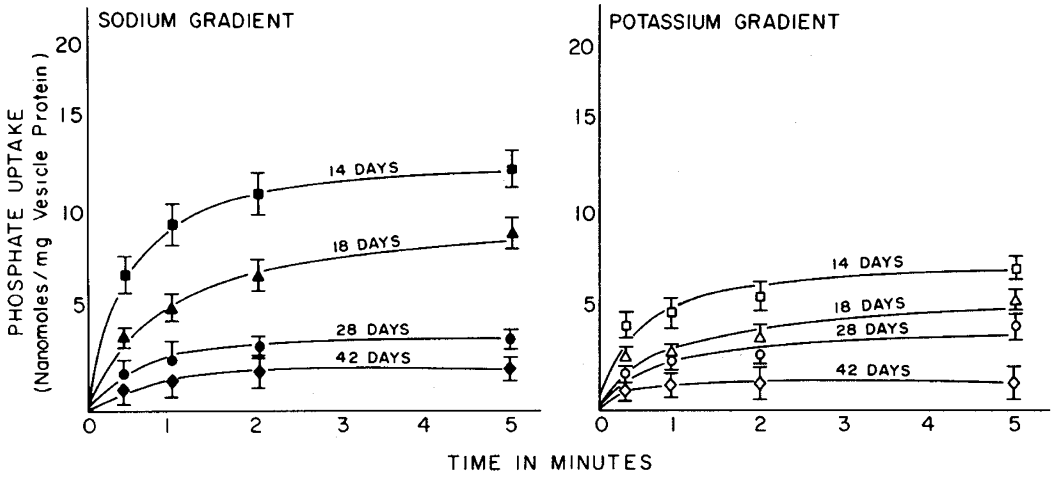

Fig. 3. Phosphate uptake into jejunal brush border membrane vesicles: effects of increasing age. Experiments were carried out as in Figure 2 using vesicles from 14-, 18-, 28-, and 42-day old rats. On the *left*, vesicles were incubated in a medium containing 0.1 M mannitol, 20 mM Hepes/Tris (pH 7.4), and 0.1 M NaCl. On the *right*, vesicles were incubated in a medium containing 0.1 M mannitol, 20 mM Hepes/Tris (pH 7.4), and 0.1 M KCl.

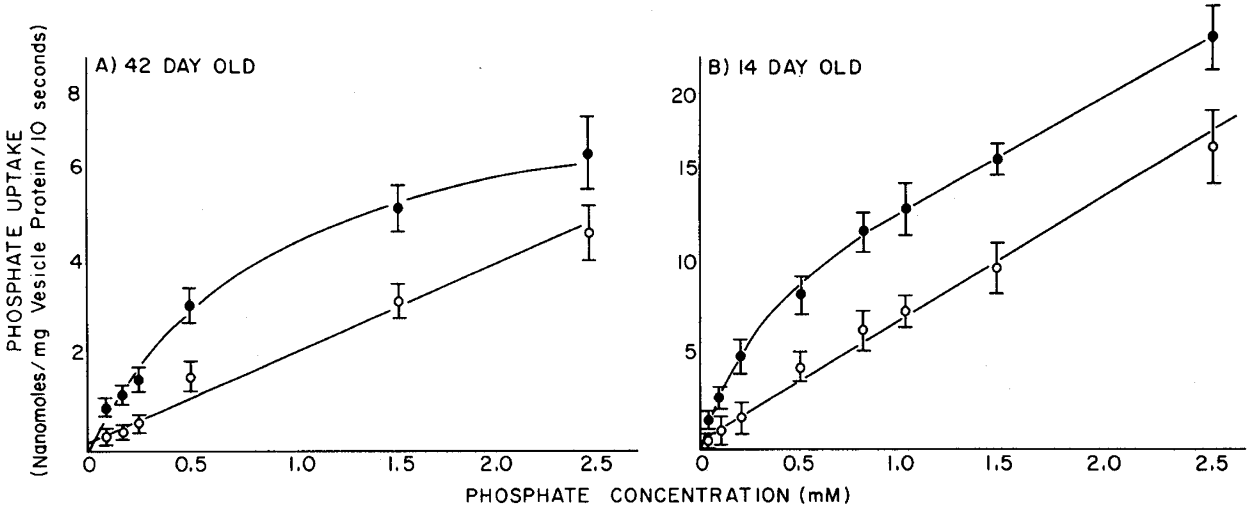

Fig. 4. Phosphate uptake into jejunal brush border membrane vesicles: effects of phosphate concentration. Jejunal brush border membrane vesicles from 42-day-old rats (*A*) and 14-day-old suckling rats (*B*) were loaded with a buffer containing 0.1 M mannitol, 0.1 M choline chloride, and 20 mM Hepes/Tris (pH 7.4). The vesicles were incubated with increasing phosphate concentrations in 0.1 M mannitol, 20 mM Hepes/Tris (pH 7.4), and 0.1 M NaCl (●) or 0.1 M KCl (○). Uptake was measured at 10 s. Note the differences in the scales between the two ages.

mycin facilitates potassium flow down its concentration gradient therefore increasing the inside negative potential of the vesicle (16).

Vesicles from the 14-day-old suckling animals and 42-day-old adult animals were prepared in a K^+-containing buffer (pH 7.4) and incubated in a Na^+-containing buffer with valinomycin (8 μg/mg vesicle protein). At both ages, uptake in the presence and absence of valinomycin was identical (Fig. 6). Similar experiments were performed with K^+ present in the incubation media with and without valinomycin, with the same results. The results of these studies suggest phosphate uptake is electroneutral at both ages.

Effects of age on isotope exchange uptake of phosphate. Isotope exchange studies were performed to determine whether the observed difference in phosphate uptake between the 14- and 42-day-old animals relates to a change in the activity of the sodium/phosphate cotransporter (Fig. 7). Vesicles were prepared in a Na^+-containing buffer with 0.8 mM potassium monophosphate and 6 μg/ml gramicidin (pH 7.4) and were incubated in an identical buffer containing a tracer amount of radioactive phosphate. The ionophore gramicidin increases the cation conductance of membranes (16) nullifying all electrochemical gradients across the vesicle membrane. Phosphate uptake occurring under these experimental conditions is due to activity of the sodium/phosphate cotransporter. At all time points, uptake in the 14-day-old animals was significantly greater than uptake in the 42-day-old animals, suggesting there is an increase in the activity of the brush border membrane sodium/phosphate cotransporter in the younger animals.

DISCUSSION

These studies demonstrate that in the rat, developmental changes occur in the jejunal transport of phosphate between 14 and 42 days of postnatal age.

The uptake of phosphate across the apical membrane of the jejunal enterocyte has a sodium-dependent component and a sodium-independent component at all ages studied. In both 14-day-old suckling rats and 42-day-old adult rats, the sodium-dependent component of phosphate uptake across the apical

enterocyte membrane is saturable and demonstrates Michaelis Menton kinetics, fulfilling the criteria for a carrier-mediated process. Studies with valinomycin demonstrate that at pH 7.4, this sodium-dependent transport of phosphate is electroneutral resulting in no net transfer of charge across the membrane in either the adult or suckling animals. Isotope exchange studies demonstrate stimulation of phosphate uptake in the presence of sodium without a sodium gradient. This provides further evidence that sodium stimulation of phosphate uptake is not due to electrical coupling.

The second component of phosphate transport is sodium independent and nonsaturable at all ages studied. This component of phosphate transport most likely represent passive and/or facilitated diffusion of phosphate across the brush border membrane (9, 17, 18).

These studies demonstrate that both the passive and active components of jejunal phosphate uptake decline progressively with increasing postnatal age. Moreover, the weaning process causes a decline in jejunal phosphate uptake independent of postnatal age. These age-related changes in uptake are not associated with changes in the electrical characteristics of phosphate uptake.

The Km value for the active uptake of phosphate in 14-day-old suckling animals is nearly twice that of the 42-day-old adult animals. This suggests that the carriers of the older animals have greater affinity for phosphate than those of the suckling animals. In contrast, the Vmax value is nearly three times greater in the younger animals than in the adults. This suggests that the increased uptake observed in the younger animals is in part due to an increase in the number of sodium/phosphate cotransporters available in the brush border membrane.

This conclusion is further supported by isotope exchange studies in which all electrochemical gradients were nullified. Under these conditions, any transport taking place must be carrier mediated. Isotope exchange uptake is significantly greater in the 14-day-old animals than in the 42-day-old animals sug-

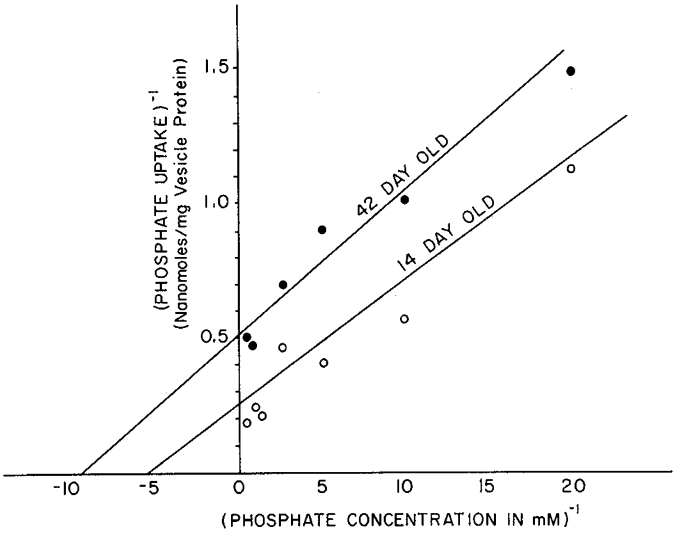

Fig. 5. Lineweaver-Burk plots of active phosphate transport. Lineweaver-Burk double reciprocal plots were constructed for the active component of phosphate uptake (sodium-dependent minus sodium-independent uptakes) from 42-day-old animals (●) and 14-day-old animals (○). For the 42-day-old animals the line y = 0.39x + 5.66 has a correlation coefficient of 0.96. Km is 0.07 mM and Vmax is 1.766 nmol/mg protein/10 s. For the 14-day-old animals, the line y = 0.45x + 2.14 has a correlation coefficient of 0.97. Km is 0.210 mM and Vmax is 4.673 nmol/mg protein/10 s.

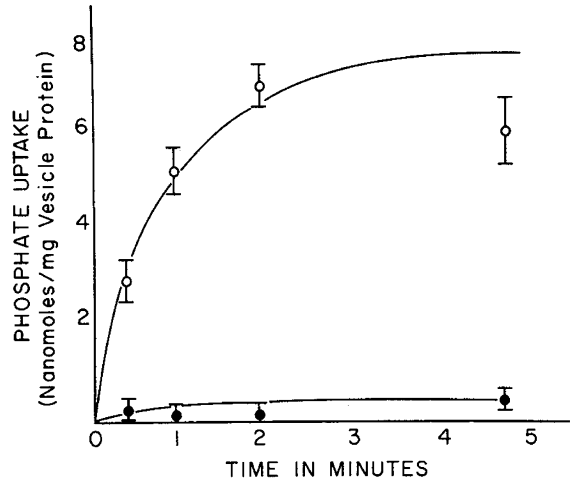

Fig. 7. Isotope exchange uptake of phosphate into jejunal brush border membrane vesicles. Jejunal brush border membrane vesicles from 42-day-old rats (●) or 14-day-old rats (○) were loaded with 0.1 M mannitol, 0.1 M NaCl, 0.8 mM KH_2PO_4, 20 mM Hepes/Tris (pH 7.4), and gramicidin (6 μg/ml). The vesicles were incubated in a medium containing 0.1 M mannitol, 0.1 NaCl, 0.8 mM KH_2PO_4, and 20 mM Hepes/Tris (pH 7.4).

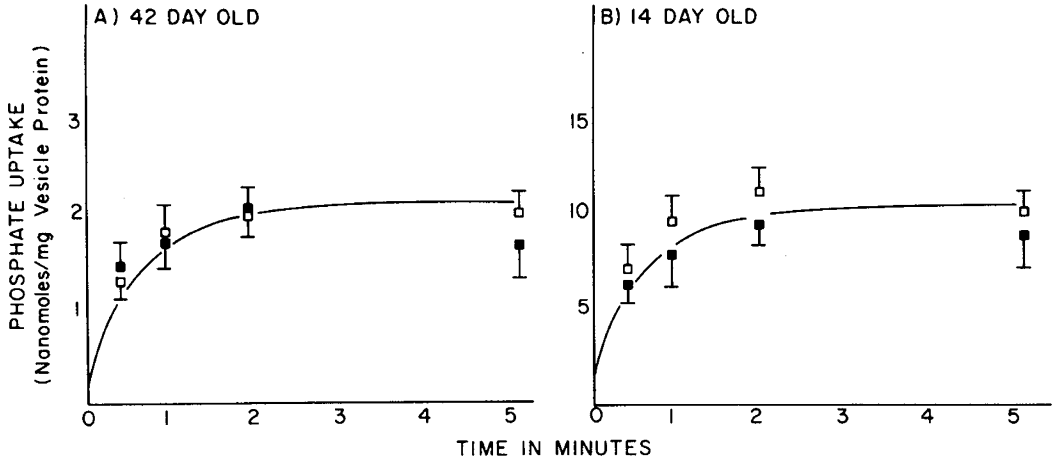

Fig. 6. Influence of valinomycin-induced electric potential on phosphate uptake into jejunal brush border membrane vesicles. Jejunal brush border membrane vesicles from 42-day-old rats (*A*) or 14-day-old rats (*B*) were loaded with 0.1 M mannitol, 0.1 M KCl, and 20 mM Hepes/Tris (pH 7.4). The vesicles were incubated in a medium containing 0.1 M mannitol, 0.1 M NaCl, 20 mM Hepes/Tris (pH 7.4) with (■) or without (□) valinomycin (8 μg/mg of vesicle protein). Similar results were obtained when 0.1 M KCl was added to the incubation media.

gesting there is an increase in the activity of the sodium/phosphate cotransporter in the younger animals.

A decrease in the number of sodium/phosphate cotransporters cannot entirely explain the observed decrease in phosphate uptake in the older animals. There is also a progressive decline in sodium-independent (passive) phosphate uptake with increasing postnatal age.

It is unlikely that changes in vitamin D status during development account for the observed changes in phosphate uptake. Investigators have shown that in adult rats, 1,25 dihydroxycholecalciferol exerts its effects only on the sodium-dependent component of phosphate transport. When adult animals are supplemented with 1,25 dihydroxycholecalciferol, the Vmax value of phosphate uptake is increased and the Km value is unaffected. The passive component of phosphate uptake does not change in adult animals treated with, or deprived of 1,25 dihydroxycholecalciferol (15, 18, 19).

A decrease in the intestinal permeability to small molecules with advancing postnatal age could explain the decrease in passive phosphate uptake observed in the older animals. This hypothesis is supported by studies in our laboratory which demonstrate a significantly greater uptake of sodium into apical brush border membrane vesicles from 14-day-old animals than in vesicles from 42-day-old animals (12).

In conclusion, the characteristics of jejunal phosphate uptake in 14-day-old suckling rats and 42-day-old adult rats are similar. At both ages, uptake consists of two components: a saturable, electroneutral sodium-mediated component and an unsaturable, sodium-independent, passive component. The younger animals demonstrate five times the phosphate uptake the older animals do. This increased uptake in the younger animals is related to both an increase in the passive permeability of the brush border membrane to phosphate and an increase in the active uptake of phosphate. Associated with these changes in the active component of phosphate uptake is an increase in the number of sodium/phosphate cotransporters in the brush border membrane of the younger animals.

REFERENCES

1. Berner WR, Kinne R, Murer H 1976 Phosphate transport into brush border membrane vesicles isolated from rat small intestine. Biochem J 160:467–474
2. Danisi G, Straub R 1980 Unidirectional influx of phosphate across the mucosal membrane of rabbit small intestine. Pflugers Arch 385:117–122
3. Harrison HE, Harrison HC 1961 Intestinal transport of phosphate: action of vitamin D calcium, and potassium. Am J Physiol 201:1007–1012
4. Harrison HE, Harrison HC 1963 Sodium, potassium, and intestinal transport of glucose, l-tyrosine, phosphate, and calcium. Am J Physiol 205:107–111
5. Murer H, Hildmann B 1981 Transcellular transport of calcium and inorganic phosphate in the small intestinal epithelium. Am J Physiol 240:G409–G416
6. Rizzoli R, Fleisch H, Bonjour JP 1977 Role of 1,25-dihydroxycholecalciferol on intestinal phosphate absorption in rats with a normal vitamin D supply. J Clin Invest 60:639–647
7. Taylor A 1974 *In vitro* phosphate transport in chick ileum, effect of cholecalciferol, calcium, sodium and metabolic inhibitors. J Nutr 104:489–494
8. Birge S, Miller R 1977 The role of phosphate in the action of vitamin D on the intestine. J Clin Invest 60:980–988.
9. Hildmann B, Storelli C, Danisi G, Murer H 1982 Regulation of Na^+-Pi cotransport by 1,25-dihydroxyvitamin D_3 in rabbit duodenal brush border membrane. Am J Physiol 242:G533–G539
10. Cheng L, Sacktor B 1981 Sodium gradient-dependent phosphate transport in renal brush border membrane vesicles. J Biol Chem 256:1556–1564
11. Caverzasio J, Murer H, Fleisch H, Bonjour JP 1982 Phosphate transport in brush border membrane vesicles isolated from renal cortex of young growing and adult rats. Pflugers Arch 394:217–221
12. Ghishan FK, Wilson FA 1985 Developmental maturation of D-glucose transport by jejunal brush border membrane vesicles. Am J Physiol 248:G87–G92
13. Hopfer U, Nelson K, Penotto J, Isselbacker KJ 1973 Glucose transport in isolated brush border membrane from rat small intestine. J Biol Chem 248:25–32
14. Lowry DH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
15. Danisi G, Murer H, Straub R 1984 Effect of pH on phosphate transport into intestinal brush border membrane vesicles. Am J Physiol 246:G180–G186
16. Pressman B 1968 Ionophorous antibiotics as models for biological transport. Fed Proc Am Soc Exp Biol 27:1238–1288
17. Danisi G, Straub R 1980 Unidirectional influx of phosphate across the mucosal membrane of rabbit small intestine. Pflugers Arch 385:117–122
18. Fuchs R, Peterlik M 1980 Vitamin D-induced phosphate transport in intestinal brush border membrane vesicles. Bioch Biophys Res Comm 93:87–92
19. Walling M 1977 Intestinal Ca and phosphate transport: Differential responses to vitamin D_3 metabolites. Am J Physiol 233:E488–E494