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Unidirectional Influx of Phosphate Across the Mucosal Membrane of Rabbit Small Intestine*

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Abstract. The influx of phosphate across the mucosal border of different regions of rabbit small intestine was investigated using the technique of Schultz et al., J. Gen. Physiol. **50**, 1241–1260 (1967). In the duodenum, the phosphate influx consisted of two components: 1. a saturable part, inhibited competitively by the presence of arsenate in the mucosal solution and strongly dependent on the mucosal Na concentration, and 2. a Na-independent part, linearly related to the mucosal phosphate concentration. In the jejunum and the ileum, the phosphate influx was a linear function of the mucosal phosphate concentration. In these regions arsenate had no effect on the influx, supporting the idea of a diffusional transport. $HgCl_2$ (0.5 mM) reduced the phosphate inlflux in the duodenum, at 140 mM Na, to the levels under Na-free conditions. The Na-independent influx was only slightly decreased by $HgCl_2$, suggesting that this agent affects mainly the Na-dependent phosphate influx. In the ileum $HgCl_2$ decreased the influx by about the same amount as under Na-free conditions in the duodenum. Thus, in the rabbit, the duodenum only appears to have a Na-dependent, carrier mediated phosphate transport mechanism at the mucosal membrane. In the jejunum and the ileum the phosphate uptake seems to be by simple diffusion.

Key words: Small intestine – Phosphate transport – Brush border – Sodium – Mercury.

Introduction

Based on in vivo experiments McHardy and Parsons [14] suggested that the net absorption of phosphate in rat small intestine was due to a passive process. Later, Harrison and Harrison with everted sacs of rat small intestine [9, 10] and Helbock et al. [11] with short circuited preparations of the same tissue showed that, at least under some experimental conditions, part or all of the absorption can be mediated by an active process. More recently, experiments with brush border vesicles of rat small intestine [2] have confirmed that the uptake of phosphate at the mucosal border contains a saturable component which is dependent on the mucosal Na concentration. These results suggest that the transport of phosphate at this border is dependent on an active process at the expense of energy provided by the electrochemical gradient of Na ions across this membrane.

Recently large differences in net active transmural fluxes of phosphate between different regions of the short circuited rat small intestine have been reported [24]. These findings could be explained by different transport rates of phosphate across the mucosal membrane, if the transfer through this membrane is the rate limiting step during net absorption, as suggested for the kidney [22]. For example, more binding sites could be present in one segment than in another or, depending on pH, different ionic forms of phosphate could be transported across the mucosal border along the intestine.

In the present paper the unidirectional influx of phosphate across the mucosal border of the rabbit small intestine was investigated. In the rabbit, so far, a simple diffusion mechanism for phosphate transport across the mucosal border has been suggested for the ileum [8].

Methods and Materials

Determination of Influx. The unidirectional influx of phosphate (P_i) across the mucosal border of the intestinal epithelial cells was estimated by a technique similar to that originally developed by Schultz et al. [20] for the measurement of amino acid and sodium

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influxes. Briefly, rabbits were shot, the intestine quickly removed, washed free of intestinal contents and opened along the mesenteric border. The tissue was then mounted, mucosal surface upwards, between two blocks of lucite with 8 ports each, exposing 1.33 cm^2 of surface, and filled with approximately 2 ml of standard solution. Two chambers were used at a time so that 16 measurements under different conditions or on different parts of the small intestine were obtained from the same animal. The mucosal solution was stirred by bubbling with a humidified mixture of O_2/CO_2 (95%/5% v/v). After an equilibration period of 15–30 min, the mucosal solution was replaced by a "test" solution containing ^{14}C-polyethylene glycol (PEG), MW 4000, as an extracellular marker, and ^{32}P-H_3PO_4. After a suitable incubation time, the "test" solution was withdrawn and each tissue was then flushed with 3 ml isotonic cold mannitol solution (4° C) which was immediately removed. Each tissue sample was then punched out with a steel punch, blotted gently on filter paper and extracted with 2 ml distilled H_2O for 30 min at 100° C. Control experiments showed that longer extractions did not increase the amount of extracted radioactivity. The time between the injection of the "test" solution and the mannitol wash was considered as the time of exposure of the tissue to the isotopes. Aliquots of the "test" solution and the tissue extract were assayed for ^{14}C and ^{32}P in a liquid scintillation spectrometer. All experiments were performed in a warm room at 37° C.

For the measurement of the uptake rate across the brush border it is necessary to have a good marker of the adherent mucosal fluid (AMF) space in order to correct for contamination by extracellular test substrate. In view of this correction we used labelled ^{14}C-PEG which has been shown not to permeate the intestine significantly [4, 19]. It was further assumed that the specific activity of ^{32}P in the ^{14}C-PEG space is equal to that in the bulk solution. The calculated ^{14}C-PEG space at different times of incubation is shown in Fig. 1A for duodenum. The values obtained at 4 min are not statistically different from those at 8 min, so that we could assume that the PEG was equilibrated in the AMF at that time. Figure 1B shows the estimated uptake of phosphate as a function of time, in duodenum, after correction for isotope present in the PEG space. The results in Fig. 1B show that the uptake is linear for at least 4 min and that the intercept is not significantly different from zero ($0.20 < P < 0.40$), suggesting that we are measuring initial rates of phosphate uptake. Similar results for ^{14}C PEG equilibration and P_i uptake were obtained for the ileum and for the jejunum. Therefore in subsequent experiments the tissue was exposed for 4–5 min to the "test" solution, i.e., during the linear portion of the phosphate uptake, after the equilibration of the AMF marker in the extracellular space.

Male and female rabbits obtained from local breeders were used indiscriminately. Duodenum, jejunum and ileum were examined separately; in our rabbits the duodenum was approximately 60 cm long, the first 20–40 cm distal to the duodeno-jejunal flexure were considered as jejunum, and the first 20–40 cm proximal to the ileo-caecal junction as ileum [1, 13].

In general the intestine was preincubated in the standard solution (140 mM Na, 2.8 mM P_i) and the P_i and Na concentrations of the mucosal solution were changed in the "test" solution only, after previous rinses (5 times) at the desired concentration. In the experiments under Na-free conditions the intestine was removed from the animal and preincubated in the absence of Na (Na substituted by choline), keeping the same P_i concentration as the standard solution.

Solutions and Materials. The standard solution had the following composition (in mM): NaCl, 115; $NaHCO_3$, 25; K_2HPO_4, 2.4; KH_2PO_4, 0.4; $CaCl_2$, 0.6 and $MgCl_2$, 0.6. Phosphate concentrations were changed by varying the amount of K_2HPO_4 and KH_2PO_4, keeping the K concentration constant by adding variable amounts of KCl. In some experiments NaCl was replaced by choline chloride and $NaHCO_3$ by choline bicarbonate. In the latter solutions, when the phosphate added in the form of K salts would cause the K concentration to exceed that of the standard solution, the excess phosphate salt was added as Tris-PO_4, prepared from phosphoric acid neutralized with Tris base to pH 7.4. The pH of all solutions was checked and was maintained at 7.4 (25° C). All reagents were obtained from Merck, except choline bicarbonate and choline chloride which were from Sigma. ^{32}P as H_3PO_4 (carrier and HCl free) and ^{14}C-PEG (0.1–1 mCi/g) were supplied by New England Nuclear.

Results

Preliminary experiments suggested that there are large differences in the mechanism of phosphate uptake at the mucosal membrane along the small intestine. Therefore the results in different regions will be treated separately.

Duodenum

The influx of P_i across the mucosal border of duodenum as a function of the mucosal P_i concentration can be seen in Fig. 2. Under control conditions (140 mM Na), the influx of P_i increases with concentration, showing a tendency to saturation. However, the behaviour at higher concentrations suggests that the uptake is composed of a linear component and a saturable one.

Na-Dependence. Considering that, at pH 7.4, phosphate uptake in rat brush border intestinal vesicles in the absence of Na is a linear function of concentration and seems to represent simple diffusion [2], we have measured the phosphate influx as a function of mucosal P_i concentration in the duodenum when all Na in the solutions was replaced by choline. As can be seen also in Fig. 2 phosphate influx under Na-free conditions is proportional to the mucosal P_i concentration. This suggests that the linear component of P_i influx in both standard solution and in Na-free conditions is mediated by the same process. It could be argued that the observed linearity of the phosphate influx with concentration, after preincubation in choline chloride solution, may be due to some deleterious effect of this salt on the duodenum, as suggested for the guinea pig gut [16]. Therefore, the P_i influx at 140 mM Na was measured in duodenum previously exposed to choline-chloride solution. The observed P_i uptake did not appear to differ significantly from the values found in preparations kept in Na Ringer throughout the experiment. Thus, in a single experiment, in the presence of 140 mM Na, the P_i influx at 0.22 mM mucosal phosphate, was 25.9 nmole/cm^2h when the intestine was preincubated in the presence of NaCl and 38.6 nmole/cm^2h after preincubation with choline chloride (duplicate measurements in each condition, range 25.8–26.0 and 37.4–39.7, respectively). The corresponding values at 5.6 mM of mucosal P_i were 326 nmole/cm^2h and

337 nmole/cm^{2}h, respectively (duplicate measurements, range 306–346 and 258–417, respectively). Similarly, alanine influx at 140 mM does not appear to be decreased by preincubation in choline chloride [20].

Figure 2 shows the Na-dependent P_i influx estimated as the difference between the total uptake (in the presence of Na) and the uptake under Na-free conditions (in the presence of choline chloride). The Na-dependent P_i influx appears to follow Michaelis-Menten kinetics. This was confirmed by a Lineweaver-Burk plot of the data, which shows an apparent K_m of 1.16 mM and a maximal flux, J_{mc}^{max}, of 143 nmole/cm^{2}h ($r = 0.9995$).

In the experiments under Na-free conditions the intestine was preincubated in the absence of Na, which leads to a decrease in cellular Na [23]. Thus the lowering of the P_i uptake in the absence of Na could be due to a decrease in mucosal Na and/or to a lowering in intracellular Na. However in two experiments where the tissue was preincubated in the standard solution (140 mM Na, 2.8 mM P_i) and the mucosal solution was changed to 25 mM Na in the "test" solution only, the P_i influx decreased from 168 ± 13 to 92 ± 8 in one experiment and from 192 ± 9 to 112 ± 4 nmole/cm^{2}h in the other (mean ± SEM, 4 tissues per mean). Since during the short time used for the influx measurement the intracellular Na is not changed significantly [18], it is reasonable to conclude that the decrease in P_i influx after preincubation in the absence of Na is mainly due to the absence of mucosal Na.

Inhibitors. When arsenate, a competitive inhibitor of the phosphate transport in vivo [7] and in vitro [2, 12], was present in the "test" solution the P_i uptake across the brush border was affected differently depending on the concentration of phosphate present in the bathing solution. At the lower mucosal phosphate concentrations, 0.22 and 1.1 mM, the P_i uptake was decreased from 33.4 ± 5.15 to 14.3 ± 1.43 nmole/cm^{2}h and from 97.4 ± 9.57 to 64.6 ± 8.55 nmole/cm^{2}h, respectively, when arsenate was present. The values in the absence and in the presence of arsenate were statistically different (paired t test, $P < 0.025$ at least, mean ± SEM of 4 tissues in each condition, originating from 2 rabbits). At the higher phosphate concentrations the P_i influx was less affected by the presence of arsenate. Thus at 2.8 mM of mucosal phosphate, the phosphate uptake changed from 152 ± 13.5 nmole/cm^{2}h in the absence to 114 ± 18.7 nmole/cm^{2}h in the presence of arsenate. At 5.6 mM mucosal phosphate, the values of P_i influx were, respectively, 204 ± 22.8 and 187 ± 17.5 nmole/cm^{2}h. These values were not statistically different (paired t test, same number of tissues as above). When the Na-independent component was subtracted from the values of phosphate influx in the absence and in the presence of arsenate, it appeared that

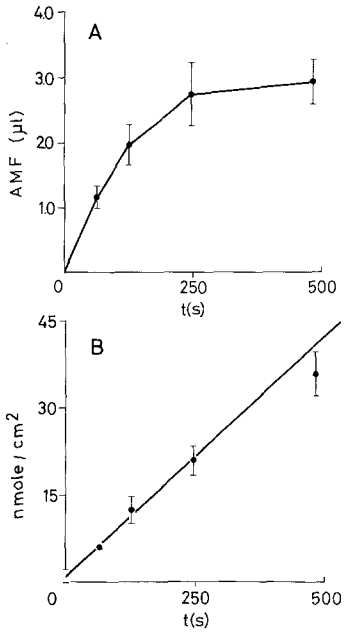

Fig. 1A and B. Validation of method. (**A**) Adherent mucosal fluid (*AMF*) as a function of incubation time in duodenum. AMF was measured with ^{14}C-PEG (MW 4000) present in the "test" solution. Points are means ± SEM of 12 determinations; tissue from three rabbits was used. (**B**) Mucosal phosphate uptake as a function of time in duodenum. The phosphate uptake at 5.6 mM P_i in the mucosal solution was corrected for the presence of phosphate in the PEG space. Data have been fitted by least-squares regression for individual points up to 4 min. Mean ± SEM of 4 tissues per point from a single rabbit. The slope corresponds to a J_{mc} of 290 nmole/cm^{2}h. The regression line is given by $y = (0.080 \pm 0.020)x + (1.300 \pm 1.123)$. $r = 0.87$

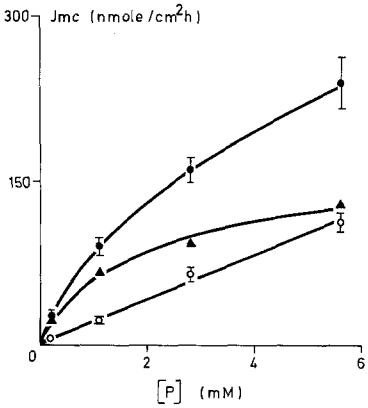

Fig. 2. Phosphate influx across the brush border of duodenum as a function of the mucosal phosphate concentration. Effect of the absence of Na. In the experiments in the presence of Na, tissues were preincubated in the standard solution (2.8 mM P_i, 140 mM Na). For the experiments in the absence of Na, tissues were preincubated in Na-free solution. Before the measurement of the phosphate influx at the desired mucosal phosphate concentration, each tissue was rinsed 5 times with solutions with the appropriate concentration of phosphate. (●) 140 mM Na; (○) 140 mM choline chloride; (▲) Na-dependent phosphate influx, calculated as the uptake in the presence of Na minus the uptake in the presence of choline chloride. Mean values ± SEM for 8 rabbits in the presence of Na and 5 rabbits in the absence of Na

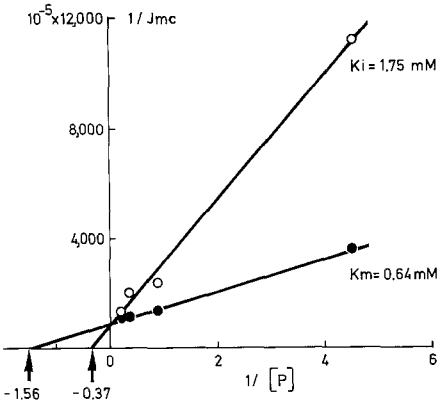

Fig. 3. Determination of the Michaelis constant (K_m) of the Na-dependent phosphate influx and the inhibition constant (K_i) of arsenate in duodenum. These paired experiments were carried out in the presence of Na as in Fig. 2. (●) control; (○) same conditions as control plus 5.6 mM arsenate. Mean ± SEM of 4 tissues in each condition. The influx values in the absence and in the presence of arsenate were corrected for the influx values under Na-free conditions taken from Fig. 4. Data have been fitted by least-squares regression for the four experimental points given in the kinetic plot. r, control = 0.998; r, arsenate = 0.997

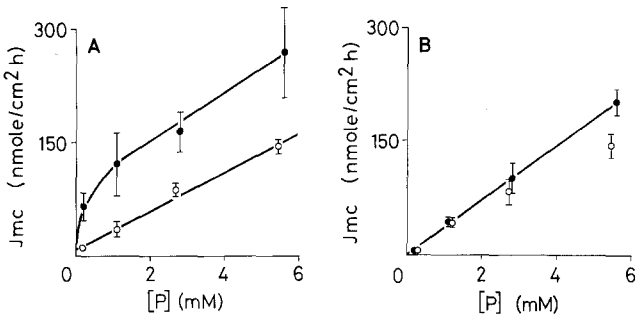

Fig. 4A and B. Effect of 0.5 mM $HgCl_2$ on phosphate influx in duodenum. (**A**) Effect of $HgCl_2$ on phosphate influx under control conditions. Tissues were preincubated in the presence of Na as in Fig. 2. $HgCl_2$ was present in the rinse and "test" solution only. (●) control; (○) same conditions as control plus $HgCl_2$. Mean values ± SEM of 3 rabbits. (**B**) Effect of $HgCl_2$ on the Na-independent phosphate influx. Tissues were preincubated in the presence of choline chloride as in Fig. 2. $HgCl_2$ was present in the rinse and "test" solution only. (●) control Na-free; (○) same conditions as control Na-free plus $HgCl_2$. Mean values ± SEM of 4 rabbits

this anion is a competitive inhibitor of the Na-dependent P_i influx in rabbit duodenum. The J_{mc}^{max} was not affected (109 and 118 nmole/cm²h for controls and in the presence of arsenate, respectively) while the K_m increased from 0.64 mM in controls to 2.69 mM with arsenate (Fig. 3). In another set of experiments, the effect of $HgCl_2$, an inhibitor of Na-dependent transport processes [3, 5,21], on P_i influx was investigated. The effect of 0.5 mM $HgCl_2$, in the "test" solution, on P_i influx in the duodenum can be seen in Fig. 4A. There is striking decrease in P_i influx, which in the presence of $HgCl_2$ becomes a linear function of the substrate concentration, which resembles the behaviour in the absence of mucosal Na. The lowering of P_i influx by $HgCl_2$ suggests an inhibition of the Na-dependent P_i flux. The P_i influx under Na-free conditions in the presence of 0.5 mM $HgCl_2$ is seen in Fig. 4B. This agent does not seem to affect the Na-independent P_i influx at low P_i concentrations. At 5.5 mM mucosal phosphate, however, the P_i influx decreased by 29% which corresponds to a significant lowering (paired t test $0.025 < P < 0.05$).

Jejunum

The results obtained in the jejunum were quite different from those in the duodenum, as shown in Fig. 5. The influx of phosphate in this segment was smaller than in the duodenum, at all external P_i concentrations tested, and hardly distinguishable from a straight line. These data could suggest that P_i transport across the mucosal border in the jejunum is by simple diffusion. In order to test this possibility, experiments were carried out where the P_i uptake at 0.22 mM mucosal phosphate was measured in the presence of 25 mM Na and in the presence of 5.6 mM arsenate (140 mM Na). The results were the following. In one experiment, the P_i uptake under control conditions was 9.78 ± 1.77 nmole/cm²h, in the presence of 25 mM Na it averaged 4.85 ± 0.95 nmole/cm²h; when arsenate was present the uptake was 6.74 ± 1.06 nmole/cm²h (mean ± SEM of 7, 4 and 4 tissues, respectively). In another experiment the corresponding values were 8.15 ± 0.82, 5.59 ± 1.03 and 7.27 ± 0.29 nmole/cm²h (mean ± SEM of 8, 4 and 4 tissues, respectively). The values in the presence of 25 mM Na and in the presence of arsenate were not statistically different from the respective control levels.

Ileum

In the ileum the phosphate uptake across the mucosal border was linearly related to the applied phosphate concentration (Fig. 6), suggesting simple diffusion.

Na-Dependence. Altering the Na concentration in the mucosal solution had only a small effect on the P_i influx in this segment. Thus at 2.8 mM of mucosal P_i, the P_i influx was 115 ± 16.5 nmole/cm²h in the presence of 140 mM Na and 79.3 ± 10.2 at 25 mM Na (mean ± SEM of 3 ilea, 3–8 determinations in each condition per rabbit). These values were statistically different (paired t test, $0.025 < P < 0.05$).

Inhibitors. The effect of 5.5 mM arsenate on the P_i influx as a function of different substrate concen-

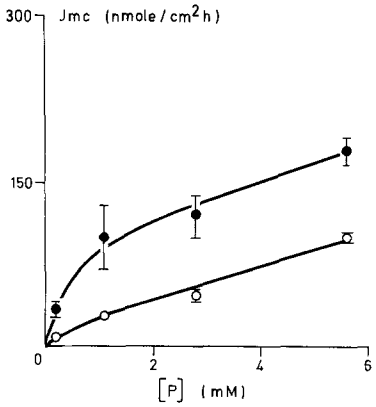

Fig. 5. Mucosal phosphate influx in duodenum and jejunum as a function of the mucosal phosphate concentration. Tissues were pre-incubated in the presence of Na as in Fig. 2. (●) duodenum; (○) jejunum. Paired experiments. Mean values ± SEM of 3 rabbits

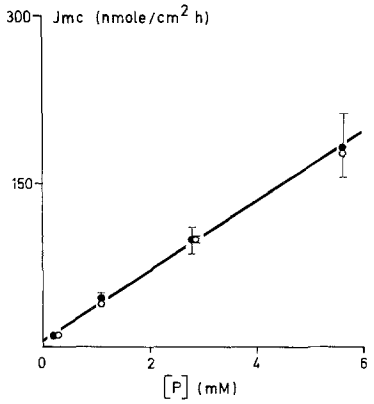

Fig. 6. Phosphate influx across the brush border of rabbit ileum as a function of the mucosal phosphate concentration. Effect of arsenate. Tissues were pre-incubated in the standard solution as in Fig. 2. (●) control; (○) same conditions as control plus 5.6 mM arsenate. Mean values ± SEM of 3 rabbits

trations in the mucosal solution can also be seen in Fig. 6. Contrarily to what happens in the duodenum, arsenate did not lower the P_i influx in the ileum.

The effect of $HgCl_2$ was also investigated. At a concentration of 0.22 mM phosphate, the influx was reduced from 9.8 ± 2.51 to 7.6 ± 10.6 nmole/cm²h by 0.5 mM $HgCl_2$ (mean ± SEM of 4 rabbits, 2–4 tissues in each condition per rabbit). This difference was not statistically significant. However, when the phosphate concentration was raised to 5.5 mM, the inhibitor caused a significant reduction ($P < 0.01$), from 286 ± 37.2 to 175 ± 42.2 nmole/cm²h (mean ± SEM of 4 rabbits, 2–4 tissues in each condition per rabbit).

Discussion

The present results show that there are large regional differences in the mechanism of P_i uptake along the rabbit small intestine. In the duodenum a large uptake is found which is to a considerable extent due to a Na-dependent mechanism. In the jejunum (Fig. 5) and the ileum (Fig. 6) on the other hand, the influx is smaller, especially at the low mucosal P_i concentrations, and appears to be diffusional (see below).

Differences in phosphate transport have also been observed in the rat small intestine [24]. In young, vitamin D_3-treated rachitic animals, which may be comparable to our normal rabbits, active P_i absorption was highest in the jejunum. Vitamin D_3 stimulated the J/ms in the duodenum, jejunum and ileum. Since Vitamin D_3 seems to act on the Na-dependent transport system (ref. [15] and unpublished results) these observations suggest that in the rat all these regions possess a Na-dependent phosphate transport at the brush border membrane. In the rabbit, on the contrary, only the duodenum appears to have a Na-dependent, carrier mediated uptake of phosphate at this border. In the jejunum and the ileum, P_i influx shows a linear dependence on the mucosal phosphate concentration, suggesting simple diffusion. Furthermore in these regions, P_i influx is not affected by the presence of arsenate in the mucosal solution, an agent that at pH 7.4 inhibits the Na-dependent phosphate uptake (Fig. 3 and ref. [2]). but has no effect on the Na-independent one [2]. The slight decrease observed in the ileum when the mucosal Na concentration is decreased to 25 mM may suggest that phosphate influx in these regions is sensitive to potential as the mucosal membrane hyperpolarizes when the mucosal Na is decreased [17]. Na-dependent phosphate uptake in the intestine is not sensitive to potential as it appears to be electroneutral [2]. All these observations are evidence for diffusional P_i uptake in the ileum. If the results found in the rat are confirmed with techniques similar to ours, a species difference may become apparent. Our results in the ileum are in accordance with those of Harms and Stirling [8] for the same region of rabbit small intestine. These authors observed a permeability coefficient for P_i of 0.85×10^{-3} cm/min whereas we obtained the similar value of 0.61×10^{-3} cm/min (Fig. 6).

In rabbit duodenum the P_i influx across the brush border is made up of two components: 1) A saturable component, inhibited competitively by arsenate and strongly dependent on mucosal Na concentration (Fig. 2 and 3) and 2) a Na-independent component, apparently diffusional as it is linearly related to substrate concentration and not inhibited by arsenate (Fig. 2 and ref. [2]). Our results in isolated rabbit duodenum confirm those obtained with isolated and brush border vesicles of rat small intestine [25, 2] and point to the existence of a specific mechanism for P_i transport at this membrane. $HgCl_2$ appears to mainly

act on the Na-dependent P_i influx in the duodenum as the P_i uptake in the presence of this agent is similar to the P_i uptake in the Na-free solution (Fig. 4, A and B). In rabbit ileum Na influx does not seem to be affected by 30 min preincubation with 0.1 mM $HgCl_2$, suggesting that membrane leakiness is not generally increased in these conditions [21]. If the same applies to our experiments, the results of $HgCl_2$ on P_i influx in the duodenum could suggest that the binding of P_i to its carrier site is affected.

In conclusion we have characterized the uptake of phosphate across the mucosal border in rabbit small intestine. Large regional differences were observed. The isolated rabbit duodenum appears to be a very suitable model for the investigation of phosphate transport and could be used in the study of the regulation of this transport by hormones.

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References

1. Barone, R., Pavaux, C., Blin, P. C., Cug, P.: Atlas d'anatomie du lapin. Paris: Masson et Cie. 1973
2. Berner, W., Kinne, R., Murer, H.: Phosphate transport into brush-border membrane vesicles isolated from rat small intestine. Biochem. J. **160**, 467–474 (1976)
3. Bihler, I., Cybulsky, R.: Sugar transport at the basal and lateral aspect of the small intestinal cell. Biochim. Biophys. Acta **298**, 429–437 (1973)
4. Esposito, G., Csáky, T. Z.: Extracellular space in the epithelium of rats' small intestine. Am. J. Physiol. **226**, 50–55 (1974)
5. Frizzell, R. A., Schultz, S. G.: Effects of monovalent cations on the sodium-alanine interaction in rabbit ileum. J. Gen. Physiol. **56**, 462–490 (1970)
6. Fuchs, R., Peterlik, M.: Pathways of phosphate transport in chick jejunum. Influence of vitamin D and extracellular sodium. Pflügers Arch. **381**, 217–222 (1979)
7. Ginsburg, J. M., Lotspeich, W. D.: Interrelations of arsenate and phosphate transport in the dog kidney. Am. J. Physiol. **205**, 707–714 (1963)
8. Harms, V., Stirling, C. E.: Transport of purine nucleotides and nucleosides by in vitro rabbit ileum. Am. J. Physiol. **233**, E47–E55 (1977)
9. Harrison, H. E., Harrison, H. C.: Intestinal transport of phosphate: action of vitamin D, calcium and potassium. Am. J. Physiol. **201**, 1007–1012 (1961)
10. Harrison, H. E., Harrison, H. C.: Sodium, potassium, and intestinal transport of glucose, l-tyrosine, phosphate, and calcium. Am. J. Physiol. **205**, 107–111 (1963)
11. Helbock, H. J., Forte, J. G., Saltman, P.: The mechanism of calcium transport by rat intestine. Biochim. Biophys. Acta **126**, 81–93 (1966)
12. Hoffmann, N., Thees, M., Kinne, R.: Phosphate transport by isolated renal brush border vesicles. Pflügers Arch. **362**, 147–156 (1976)
13. Kozma, C., Macklin, W., Cummins, L. M., Maurer, R.: The anatomy, physiology, and the biochemistry of the rabbit. In: The Biology of the laboratory rabbit. S. H. Weisbroth, R. E. Flatt, A. L. Kraus, eds.), pp. 50–72. New York: Academic Press 1974
14. McHardy, G. J. R., Parsons, D. S.: The absorption of inorganic phosphate from the small intestine of the rat. Quart. J. Exp. Physiol. **41**, 398–409 (1956)
15. Peterlik, M.: Phosphate transport by embryonic chick duodenum. Stimulation by vitamin D_3. Biochim. Biophys. Acta **514**, 164–171 (1978)
16. Robinson, J. W. L.: Comparative aspects of the response of the intestine to its ionic environment. Comp. Biochem. Physiol. **34**, 641–655 (1970)
17. Rose, R. C., Schultz, S. G.: Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. J. Gen. Physiol. **57**, 639–663 (1971)
18. Rose, R. C., Nahrwold, D. L., Koch, M. J.: Electrical potential profile in rabbit ileum: role of rheogenic Na transport. Am. J. Physiol. **232**, E5–E12 (1977)
19. Sallee, V. L., Wilson, F. A., Dietschy, J. M.: Determination of unidirectional uptake rates for lipids across the intestinal brush border. J. Lipid Res. **13**, 184–192 (1972)
20. Schultz, S. G., Curran, P. F., Chez, R. A., Fuisz, R. E.: Alanine and sodium fluxes across mucosal border of rabbit ileum. J. Gen. Physiol. **50**, 1241–1260 (1967)
21. Stirling, C. E.: Mercurial perturbation of brush border membrane permeability in rabbit ileum. J. Membr. Biol. **23**, 33–56 (1975)
22. Stoll, R., Kinne, R., Murer, H., Fleisch, H., Bonjour, J.-P.: Phosphate transport by rat renal brush border membrane vesicles: Influence of dietary phosphate, thyroparathyroidectomy, and 1,25-dihydroxyvitamin D_3. Pflügers Arch. **380**, 47–52 (1979)
32. Tai, Y. H., Desjeux, J. F., Danisi, G., Curran, P. F.: Na and Cl transport and short-circuit current in rabbit ileum. J. Membr. Biol. **31**, 189–208 (1977)
24. Walling, M. W.: Intestinal Ca and phosphate transport: differential responses to vitamin D_3 metabolites. Am. J. Physiol. **233**, E488–E494 (1977)
25. Walling, M. W.: Effects of 1,25-dihydroxyvitamin D_3 on active intestinal inorganic phosphate absorption. In: Vitamin D, biochemical, chemical and clinical aspects related to calcium metabolism (A. W. Norman, K. Schaefer, J. W. Coburn, H. F. De Luca, D. Frazer, H. G. Grigoleit, D. v. Herrath, eds.), pp. 321–330. Berlin: de Gruyter 1977

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