In vitro stimulation of phosphate uptake in isolated chick renal cells by 1,25-dihydroxycholecalciferol

(kidney transport regulation/Na⁺-dependent phosphate uptake/vitamin D₃ analogs/induction of transport/sugar uptake)

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ABSTRACT   Renal cells isolated from vitamin D-deficient chicks had an increased Na^+-dependent phosphate uptake when preincubated with 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$]. Phosphate uptake in the absence of Na^+ and methyl α-glucoside uptake dependent on Na^+ were not affected. Phosphate uptake was stimulated 15% by 0.010 pM 1,25-$(OH)_2D_3$. Maximal enhancement of 30% was obtained with 100 pM. The uptake when fully stimulated by preincubation *in vitro* approximated the uptake of cells isolated from chicks that were previously repleted with 1,25-$(OH)_2D_3$ *in vivo*. Cells from repleted chicks were not stimulated additionally when preincubated with 1,25-$(OH)_2D_3$ *in vitro*. The increase in phosphate uptake could be measured after a 1-hr preincubation period; full response required at least 2 hr. Phosphate uptake induced by 1,25-$(OH)_2D_3$ was blocked by cycloheximide and actinomycin D. Enhancement of phosphate uptake was relatively specific for the 1,25-$(OH)_2D_3$ analog of vitamin D₃. The potency order was 1,25-$(OH)_2D_3 \gg$ 25-$(OH)D_3 =$ 1-$(OH)D_3 >$ 24,25-$(OH)_2D_3 \gg D_3$. Kinetically, 1,25-$(OH)_2D_3$ increased the V_{max} of the phosphate uptake system; the affinity for phosphate was unaffected. ³H-Labeled 1,25-$(OH)_2D_3$ was taken up by the isolated renal cells. It was estimated that the stimulation of phosphate uptake might be initiated by very few molecules of 1,25-$(OH)_2D_3$ per cell. It is proposed that 1,25-$(OH)_2D_3$ contributes importantly to the mechanisms by which phosphate transport is regulated in the kidney.

Vitamin D is an important regulator of phosphate homeostasis (1). The active form of the vitamin, 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$], has been shown to enhance the intestinal absorption of phosphate (2–4). The effect of 1,25-$(OH)_2D_3$ on the reabsorption of phosphate in the kidney is in dispute. In some studies, infusion of the vitamin has been found to decrease phosphate excretion (5, 6), whereas in other investigations 1,25-$(OH)_2D_3$ has been reported to be phosphaturic (7) or without effect on urinary phosphate (8). Some of the difficulties in resolving the apparent discrepancies stem, in part, from whether 1,25-$(OH)_2D_3$ is administered chronically or acutely and from the complex interactions in the animal *in vivo* of the vitamin D metabolic and phosphate transport systems with various regulatory factors, including parathyroid hormone and other endocrines, dietary status, and calcium (9–11). To examine the question of the action of 1,25-$(OH)_2D_3$ on renal phosphate transport directly, we have recently initiated studies on the regulation of phosphate uptake in isolated renal cells (12).

Phosphate uptake by kidney cells isolated from the chick is stimulated markedly by Na^+ (12). When 1,25-$(OH)_2D_3$ is injected into vitamin D-deficient chicks and renal cells are isolated 3 hr later, Na^+-dependent phosphate uptake by the cells is increased about 40% (12). This effect is specific for the Na^+-dependent uptake of phosphate, because neither the uptake of phosphate in the absence of Na^+ nor the Na^+-dependent uptake of methyl α-glucoside is affected by 1,25-$(OH)_2D_3$ repletion. In the present communication, we report that 1,25-$(OH)_2D_3$ incubated *in vitro* with the cells from vitamin D-deficient chicks stimulates phosphate uptake. Phosphate uptake by cells derived from vitamin D-repleted animals is not enhanced. The stimulation by the vitamin is specific for 1,25-$(OH)_2D_3$ and is blocked by inhibitors of protein synthesis. These findings indicate that 1,25-$(OH)_2D_3$ regulates phosphate uptake by the renal cell.

METHODS AND MATERIALS

One-day-old White Leghorn cockerels were kept in a darkened room for 19–24 days and maintained on a vitamin D-deficient diet (Teklab test diet TD 75007, Madison, WI), containing 0.6% phosphorus and 0.6% calcium. The chicks had free access to deionized water. Repleted chicks were given orally 1 μg of 1,25-$(OH)_2D_3$ dissolved in 200 μl of propylene glycol.

Chick renal cells were isolated by a collagenase-hyaluronidase procedure described elsewhere (12). The final preparation of cells consisted almost exclusively of single cells or clusters of two to five cells. The isolated cells excluded trypan blue essentially completely, suggesting that the plasma membranes were intact (12). The metabolic state of the cells, reflected in terms of oxygen consumption, indicated that the cells were functionally active and no significant differences were found in the oxygen uptakes of cells from vitamin D-deficient and 1,25-$(OH)_2D_3$-repleted animals (12).

When the effects of 1,25-$(OH)_2D_3$ on the uptakes of [³²P]phosphate and methyl α-[¹⁴C]glucoside were determined *in vitro*, the isolated cells were resuspended in McCoy's medium. To each ml of cell suspension (3–5 mg of protein) 50 μl of fresh serum from vitamin D-deficient chicks was added. After 5 min at room temperature, 5 μl of 1,25-$(OH)_2D_3$ or other analogs of vitamin D₃, in ethanol, or ethanol alone, were added. Unless indicated otherwise, the cells were then preincubated for 2.5 hr, at 37°C, with constant shaking under an atmosphere of 95% O_2 and 5% CO_2. Afterwards, the cells were collected by centrifugation and washed twice with a medium containing 280 mM mannitol, 10 mM Hepes/KOH buffer at pH 7.2, 2.5 mM glutamine, 0.5 mM β-hydroxybutyrate, and 1 mM mannose. Uptakes of the labeled phosphate and sugar were determined by a centrifugation method (12). Briefly, a 100-μl aliquot of the cell suspension, about 1.6×10^6 cells, was equilibrated at 37°C. Uptake was initiated with the addition of 100 μl of a

Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxycholecalciferol; other vitamin D₃ analogs are abbreviated similarly.
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medium consisting of 140 mM NaCl or KCl, 10 mM Hepes/KOH buffer at pH 7.2, and either 0.2 mM [^{32}P]phosphate or 0.1 mM methyl α-[^{14}C]glucoside. Uptakes of phosphate, after 1 min of incubation, and the sugar, after 2 min of incubation, were terminated by removing 180 μl of the reaction mixture and adding the aliquot to a centrifuge tube containing 1 ml of ice-cold stopping solution containing 124 mM NaCl, 10 mM Na$_2$HAsO$_4$, and 1 mM Hepes/KOH buffer at pH 7.2. The cells were centrifuged for 1 min, the supernatant was discarded, and the pellet was washed with an additional 1 ml of stopping solution. The cells were pelleted and solubilized with alkali, and the radioactivity taken up was measured. Uptakes were linear with respect to cell number and protein within the range used in these experiments.

To determine the proportions of the accumulated ^{32}P that were in the inorganic and organic phosphate forms, the cell pellet was extracted with 5% perchloric acid. A 0.6-ml aliquot of the extract was added to 0.3 ml of a 2% suspension of acid-washed charcoal in water and the mixture was thoroughly mixed. After 10 min on ice, the charcoal was removed by centrifugation. Inorganic phosphate was in the supernatant. Control experiments showed that 100% of the inorganic phosphate was extracted by this procedure, whereas only 0.6% of the radioactivity from [γ-^{32}P]ATP was extracted.

In experiments to determine whether the 1,25-(OH)$_2$D$_3$-induced increase in phosphate uptake was affected by inhibitors of protein and RNA synthesis, 1 ml of cell suspension was preincubated with actinomycin D (7.5 μg/ml) or cycloheximide (50 μg/ml) for 20 min at 37°C. 1,25-(OH)$_2$D$_3$ (2 pM) was then added and phosphate uptake by the cells was measured as described above. To monitor whether the inhibitors blocked protein and RNA synthesis in the renal cell system, 2 μCi (1 Ci = 3.7 × 10^{10} becquerels) of either ^{3}H-labeled L amino acid mixture or [^{3}H]uridine was added to the reaction mixture at the initiation of the 20-min preincubation period. After 2.5 hr of incubation, the cells (3 mg of protein per ml) were sedimented and washed in the buffered mannitol medium. To measure the total uptake of radioactivity, an aliquot of the washed cell suspension was placed in a centrifuge tube to which was then added 0.5 ml of 1 M NaOH. The partially solubilized cell suspension was transferred to a scintillation vial, together with 0.5 ml of water that was used to rinse the centrifuge tube, and radioactivity was measured. To another aliquot of the washed cell suspension, concentrated trichloroacetic acid (final concentration was 5%) was added. The acid-precipitable material was collected by centrifugation and solubilized in NaOH, and radioactivity was measured. The acid-precipitable radioactivity from incubations with labeled amino acids or uridine was taken to represent synthesized protein and RNA, respectively.

In measuring the uptake of 1,25-(OH)$_2$D$_3$, the cells were incubated with the vitamin as described. The concentration of 1,25-(OH)$_2$[^{3}H]D$_3$ was 42 pM. After 2.25 hr of incubation at 37°C, the cells were centrifuged, washed, and resuspended in the buffered mannitol medium. The cells were then washed again with unlabeled 1,25-(OH)$_2$D$_3$ to remove readily displaceable 1,25-(OH)$_2$[^{3}H]D$_3$. Radioactivity remaining with the cells was measured.

Synthetic 1,25-(OH)$_2$D$_3$, 24,25-(OH)$_2$D$_3$, and 25-(OH)D$_3$ were generously provided by M. Uskokovic of Hoffmann–La Roche. J. A. Campbell of Upjohn donated 1-(OH)D$_3$. [^{32}P]-Phosphate (carrier-free), methyl α-[^{14}C]glucoside (300 mCi/mmol), [5,6-^{3}H]uridine (38 Ci/mmol), and ^{3}H-labeled L amino acid mixture were purchased from New England Nuclear. 1,25-(OH)$_2$[23,24-^{3}H]D$_3$ (160 Ci/mmol) was obtained from Amersham. Collagenase (131 units/mg) was purchased from Worthington, and hyaluronidase (bovine testes, type 1-S) (300 units/

mg), vitamin D$_3$, and cycloheximide were from Sigma. Actinomycin D was obtained from Calbiochem. Other chemicals were reagent grade.

RESULTS

It was shown previously that the uptake of phosphate by isolated renal cells reflected the vitamin D status of the chick from which the cells were derived (12). The present experiments demonstrate that 1,25-(OH)$_2$D$_3$ incubated with the cells *in vitro* affects phosphate uptake. Fig. 1 shows that when cells obtained from vitamin D-deficient chicks were preincubated *in vitro* with 1,25-(OH)$_2$D$_3$ for 2.5 hr and then initial rates of phosphate uptake were determined a significant increase in uptake was found. Only Na$^+$-dependent phosphate uptake was enhanced; uptake was unaffected when K$^+$ or choline$^+$ was substituted for Na$^+$ in the extracellular medium (data not shown). Analysis of the ^{32}P accumulated by cells during the uptake period revealed that 82% of the label was as inorganic phosphate and 18% was in the organic phosphate form. This percent distribution was not altered in cells preincubated with 1,25-(OH)$_2$D$_3$. These findings argued against the possibility that the vitamin-induced increase in phosphate uptake resulted from the augmentation by 1,25-(OH)$_2$D$_3$ of the incorporation of free intracellular inorganic phosphate into organic phosphate pools. Other experiments (not illustrated) showed that the mean ($\pm$SEM) uptake of methyl α-glucoside (0.16 $\pm$ 0.03 nmol/2 min·mg of protein), which was also dependent on Na$^+$ (12), was not altered by these concentrations of the vitamin. The lack of an effect of 1,25-(OH)$_2$D$_3$ on the Na$^+$-dependent uptake of the sugar but the specific action of the vitamin on the Na$^+$-dependent uptake of phosphate suggests that the effect of 1,25-(OH)$_2$D$_3$ on phosphate uptake was not related to an alteration in the Na$^+$ gradient or in the membrane potential.
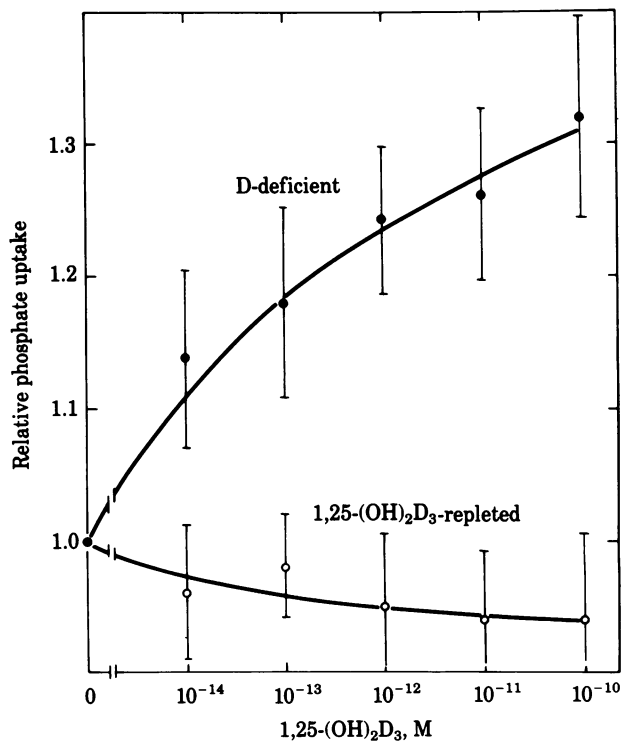

FIG. 1. Effects of the *in vitro* incubation of 1,25-(OH)$_2$D$_3$ with renal cells isolated from vitamin D-deficient and 1,25-(OH)$_2$D$_3$-repleted chicks. The values for D-deficient and 1,25-(OH)$_2$D$_3$-repleted chicks represent the mean $\pm$ SEM for 8–11 and 5 experiments, respectively.

Fig. 1 shows that the stimulatory effect of 1,25-(OH)$_2$D$_3$ on phosphate uptake was dependent on the concentration of the vitamin. A concentration as low as 10^{-14} M enhanced the uptake by about 15%, from 1.37 ± 0.13 nmol/min·mg of protein in the absence of the vitamin to 1.58 ± 0.18 nmol/min·mg of protein with 10^{-14} M. At the physiological concentration of 1,25-(OH)$_2$D$_3$, approximately 10^{-10} M, uptake was increased by 30%. Higher concentrations of 1,25-(OH)$_2$D$_3$ in the incubation medium did not stimulate uptake further. The rate attained with maximal concentrations of 1,25-(OH)$_2$D$_3$ in vitro—i.e., 1.8 nmol/min·mg of protein—approximated the rate of uptake of cells isolated from chicks repleted with 1,25-(OH)$_2$D$_3$ in vivo—i.e., 2.0 nmol/min·mg of protein (12).

In contrast to the increase in phosphate uptake found when 1,25-(OH)$_2$D$_3$ was incubated in vitro with cells from vitamin D-deficient animals, Fig. 1 also shows that the vitamin did not enhance uptake when incubated with cells derived from chicks injected with the vitamin 3 hr previously. In fact, a slight inhibition, perhaps of questionable statistical significance, was found. Thus, renal cells from vitamin D-deficient animals differed from cells from 1,25-(OH)$_2$D$_3$-repleted animals in their in vitro response to 1,25-(OH)$_2$D$_3$. This finding suggests that once stimulation of phosphate uptake was induced by 1,25-(OH)$_2$D$_3$, as was the case when the vitamin was administered to the vitamin D-deficient animal (12), the cell became refractory to further stimulation by the vitamin.

In the preceding experiment, increases in phosphate uptake by cells from vitamin D-deficient chicks were found after the isolated cells were preincubated with 1,25-(OH)$_2$D$_3$ for 2.5 hr. Stimulation of phosphate uptake was detectable after a preincubation period of only 1 hr (Fig. 2). However, the maximal response required at least 2 hr. This finding suggests that an interim period of time was necessary between exposure of the cells to the vitamin and the maximal response, expressed by an enhancement in phosphate uptake.

In addition to the observation that a lag period was needed before the full phosphate uptake response to 1,25-(OH)$_2$D$_3$ became evident, it was found that the increase in uptake induced by the vitamin could be blocked by cycloheximide and actinomycin D, respective inhibitors of protein and RNA synthesis. In the experiments described in Table 1, incubation of the cells with 2 pM 1,25-(OH)$_2$D$_3$ effected a 26% enhancement in Na$^+$-dependent phosphate uptake. This increase was suppressed by cycloheximide and actinomycin D. The inhibitors had no statistically significant affect on Na$^+$-dependent phosphate uptake by control cells not exposed to 1,25-(OH)$_2$D$_3$. The specificity of the actions of cycloheximide and actinomycin D was demonstrated additionally by their failure to alter Na$^+$-dependent methyl α-glucoside uptake by cells, incubated with or without
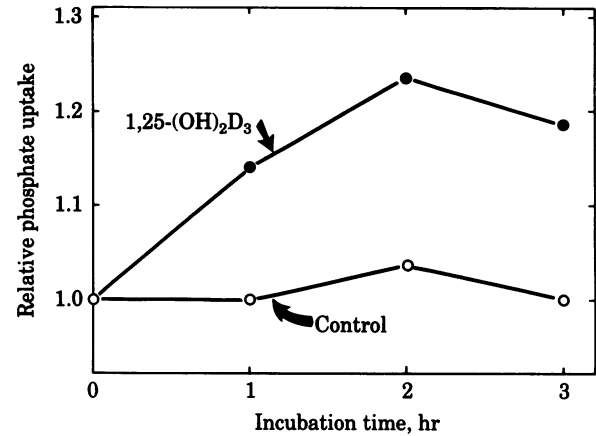

FIG. 2. Effect of the time of preincubation of 1,25-(OH)$_2$D$_3$ (200 pM) with cells from vitamin D-deficient chicks on the uptake of phosphate. The uptake at 0 min of preincubation was 1.85 ± 0.24 nmol/min·mg of protein and is given a relative value of 1.0. Each value represents the mean of four experiments.

1,25-(OH)$_2$D$_3$. Table 2 shows that in parallel experiments cycloheximide and actinomycin D did, in fact, inhibit the incorporation of labeled amino acids and uridine into the trichloroacetic acid-precipitable protein and RNA fractions of the renal cell. Cycloheximide inhibited labeled amino acid incorporation by 82% and actinomycin D inhibited labeled uridine incorporation by 84%. Radioactivity in the fractions not precipitated by trichloroacetic acid was not affected by the inhibitors.

The specificity of 1,25-(OH)$_2$D$_3$ in stimulating phosphate uptake in vitro is shown in Fig. 3. Cholecalciferol (vitamin D$_3$) was inactive at concentrations as high as 10^{-6} M. 24,25-(OH)$_2$D$_3$ showed slight activity in inducing the increases in phosphate uptake, but only at a concentration of about 10^{-10} M, 4 orders of magnitude greater than that found for 1,25-(OH)$_2$D$_3$ (Fig. 1). 1-(OH)D$_3$ was effective in stimulating phosphate uptake. However, 100 times the concentration of 1-(OH)D$_3$ was needed relative to 1,25-(OH)$_2$D$_3$. At high concentrations, 25-(OH)D$_3$ also produced an increase in phosphate uptake. Whether this stimulation was a direct response to 25-(OH)D$_3$ or required the conversion of 25-(OH)D$_3$ to 1,25-(OH)$_2$D$_3$ by the renal cells during the preincubation period was not ascertained.

The effect of 1,25-(OH)$_2$D$_3$ on the kinetics of phosphate uptake, with respect to phosphate concentration, is shown in Fig. 4. In these experiments phosphate concentration was varied between 0.05 and 1.0 mM and the extracellular Na$^+$ concentration was 70 mM. Treatment of the cells with 1,25-(OH)$_2$D$_3$ increased the rate of phosphate uptake at all concentrations of

Table 1. Effects of cycloheximide and actinomycin D on the 1,25-(OH)$_2$D$_3$-induced increase in phosphate uptake by isolated renal cells

1,25-(OH)$_2$D$_3$, pM	Phosphate uptake, %			Methyl α-glucoside uptake, %		
	Control	With cyclo-heximide	With actino-mycin D	Control	With cyclo-heximide	With actino-mycin D
0	100	106 ± 6	110 ± 4	100	107 ± 4	114 ± 5
2	126 ± 6*	105 ± 9†	105 ± 4‡	96 ± 7	95 ± 8	106 ± 7

Uptakes of phosphate and methyl α-glucoside in the absence of 1,25-(OH)$_2$D$_3$ and inhibitors were 1.72 ± 0.16 nmol/min·mg of protein and 0.19 ± 0.03 nmol/2 min·mg of protein, respectively, and these are given relative uptake values of 100%. Each datum represents the mean ± SEM for four to eight experiments.
* Significantly different from uptake found in the absence of 1,25-(OH)$_2$D$_3$, $P < 0.005$.
† Significantly different from uptake found in the presence of 1,25-(OH)$_2$D$_3$, $P < 0.001$.
‡ Significantly different from uptake found in the presence of 1,25-(OH)$_2$D$_3$, $P < 0.025$.

Table 2. Effect of cycloheximide and actinomycin D on protein and RNA synthesis in isolated renal cells

Incorporation experiment	Inhibitor	Uptake, dpm $\times 10^{-3}$/mg of protein			Inhibition of acid-precipitable fraction, %
		Total	Acid-nonprecipitable	Acid-precipitable	
^{3}H-Labeled amino acids	Control	29.4 ± 2.0	15.2 ± 1.8	14.2 ± 0.7	—
	Cycloheximide	19.2 ± 1.8	16.6 ± 1.6	2.5 ± 0.2	82
[^{3}H]Uridine	Control	215 ± 18	145 ± 17	71 ± 2	—
	Actinomycin D	158 ± 17	147 ± 15	11 ± 0.5	84

Each datum is the mean ± SEM for four experiments.

substrate. Using the 1-min uptake value to represent an approximation of initial velocity (12), the apparent K_m was calculated to be about 0.2 mM. This did not change when the uptake was stimulated by 1,25-(OH)$_2$D$_3$. The V_{max}, however, was increased, from 5.0 to 6.4 nmol/min·mg of cell protein.

When the isolated renal cells were incubated with 1,25-(OH)$_2$[^{3}H]D$_3$ it was found that the labeled vitamin was taken up by the cells. For example, in four separate experiments in which the 2-ml incubation mixture contained 3×10^7 cells and 42 pM (36.6×10^3 dpm) 1,25-(OH)$_2$D$_3$, 17.0 ± 1.7% of the total radioactivity ($6.2 \pm 0.5 \times 10^3$ dpm) or 0.014 pmol of 1,25-(OH)$_2$D$_3$ was taken up in 2.5 hr. It could be calculated, assuming approximately equal distribution, that the average uptake per cell was 4.7×10^{-22} mol, indicating an average uptake of 280 molecules of 1,25-(OH)$_2$D$_3$ per cell. This value was likely to be an overestimation of the amount of 1,25-(OH)$_2$D$_3$ actually needed to induce the phosphate uptake response because in this experiment the concentration of 1,25-(OH)$_2$D$_3$ was far greater than that required to demonstrate an effect. It is not unreasonable to hypothesize that induction could have been initiated by the uptake of as little as one or at most very few molecules of 1,25-(OH)$_2$D$_3$.

DISCUSSION

The present results show that 1,25-(OH)$_2$D$_3$ incubated *in vitro* with renal cells isolated from vitamin D-deficient chicks induced an increase in the Na$^+$-dependent uptake of phosphate. The specificity of the effect is demonstrated by the findings that the uptake of phosphate, in the absence of extracellular Na$^+$, and the uptake of sugar, in the presence or absence of Na$^+$, were unaffected. These results are in accord with our earlier observations that the initial response to repletion of vitamin D-deficient chicks *in vivo* was to increase the renal cell Na$^+$-depen-

dent uptake of phosphate (12) and with reports of others that the acute effect of infusion of 1,25-(OH)$_2$D$_3$ in the intact animal was to decrease phosphate excretion (5, 6). The present findings also agree with the previously reported effect of the vitamin in increasing phosphate uptake by organ culture preparations of chick ileum explants and isolated intestinal cells (13, 14).

The 1,25-(OH)$_2$D$_3$-induced increase in Na$^+$-dependent phosphate uptake may be attributable to changes in several cell properties. One possibility is that the vitamin enhanced the Na$^+$ gradient driving force. Because the Na$^+$-dependent uptake of methyl α-glucoside was not affected by 1,25-(OH)$_2$D$_3$, this alternative is precluded. A second possibility is that 1,25-(OH)$_2$D$_3$ stimulated the conversion of intracellular inorganic phosphate to organic phosphate. The finding that the proportion of ^{32}P that was distributed into inorganic and organic pools was unaltered by 1,25-(OH)$_2$D$_3$ makes this possibility unlikely. A third possibility is that the phosphate leak through pathways other than the Na$^+$-phosphate cotransport system is the greatest in the cells from vitamin D-deficient chicks and the lowest in the cells incubated with 1,25-(OH)$_2$D$_3$. The former condition would then represent a "pump–high back leak" mechanism, whereas the latter situation would represent a "pump–low back leak" mechanism. Because the uptakes of phosphate were the same in the absence of Na$^+$, independent of the vitamin D status of the cells, this third possibility seems unlikely. A fourth possibility, and one that we currently support, is that 1,25-(OH)$_2$D$_3$ induces a change in the Na$^+$/phosphate cotransport system. Kinetically, the action of 1,25-(OH)$_2$D$_3$ in enhancing
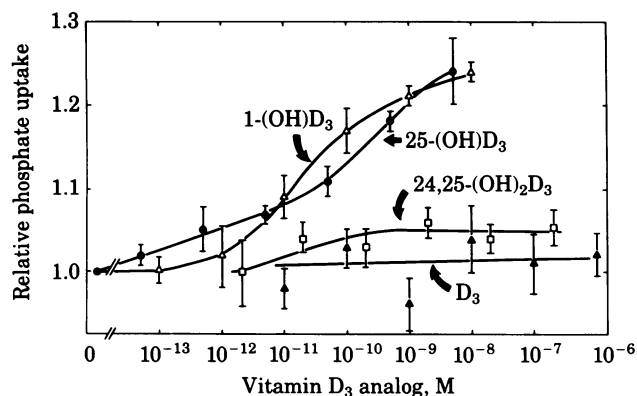
FIG. 3. Specificity of vitamin D$_3$ analogs in stimulating phosphate uptake of isolated renal cells *in vitro*. Each datum represents the mean ± SEM for four experiments.
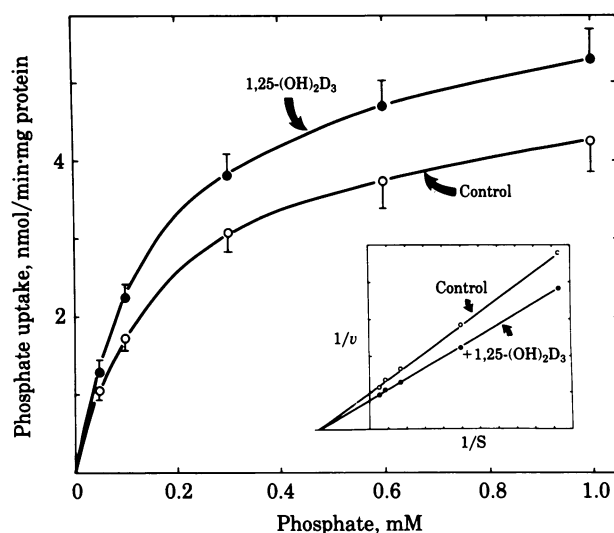
FIG. 4. Effect of 1,25-(OH)$_2$D$_3$ (0.1 pM) on the kinetics of phosphate uptake, with respect to phosphate concentration. (*Inset*) Lineweaver–Burk transformation of the data. Each value represents the mean ± SEM of four experiments.

phosphate uptake by the renal cells appears to be due to an increase in the V_{max} of the system without a change in the apparent affinity for phosphate. A similar suggestion was made for the effect of the vitamin in stimulating phosphate uptake in intestinal preparations (13, 15, 16).

The findings (i) a lag period of about 2 hr before maximal phosphate uptake was elicited by 1,25-$(OH)_2D_3$; (ii) the induced increase in phosphate uptake was blocked by inhibitors of RNA and protein synthesis; and (iii) 1,25-$(OH)_2D_3$ was taken up by the renal cells are consistent with the hypothesis that the action of 1,25-$(OH)_2D_3$ is mediated by specific RNA and protein syntheses (17). An essential component of this process is the binding of 1,25-$(OH)_2D_3$ to a cytosolic receptor and the transfer of the receptor to the nucleus (18, 19). Of particular interest is the report that the receptor macromolecule in chick kidney had the binding potency of 1,25-$(OH)_2D_3$ > 25-$(OH)D_3$ > 1-$(OH)D_3$ > 24,25-$(OH)_2D_3$ >> D_3 (20), precisely the order of potency found here for the ability of the analogs to stimulate phosphate uptake in the isolated renal cell.

The precise mechanism by which 1,25-$(OH)_2D_3$ functions in the kidney is unknown and remains to be elucidated. The results of this paper, that 1,25-$(OH)_2D_3$ stimulated Na^+-dependent phosphate uptake by renal cells *in vitro*, may suggest that the vitamin influences a crucial component in the overall renal transport of phosphate. Tubular reabsorption of phosphate was shown to be increased by Na^+ in the perfusing fluid (21, 22) and a Na^+/phosphate cotransport system was demonstrated in the renal brush border membrane (23, 24). In contrast, phosphate transport across the basolateral membrane of the renal tubular cell was reported to be Na^+ independent (23). Therefore, the findings that the uptake of phosphate by the chick renal cell was greatly enhanced by extracellular Na^+ (12) and the Na^+-dependent phosphate uptake by the cell was specifically enhanced by 1,25-$(OH)_2D_3$ (Fig. 1) suggest that the vitamin stimulated the entry of phosphate across the luminal membrane of the cell. The low level of phosphate uptake by the isolated cell when Na^+ was omitted from the extracellular medium probably included nonspecific diffusional pathways and the solute that entered the cell across the contraluminal membrane. These pathways were not affected by 1,25-$(OH)_2D_3$. Because the rate-limiting and regulated (diet, parathyroid hormone) step in net phosphate reabsorption by the nephron was reported to be the translocation of the ion across the luminal membrane (23, 25), it is hypothesized that 1,25-$(OH)_2D_3$ acting as described in the present

study also contributes importantly to the regulation of renal phosphate transport.

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