

Role of 1,25-Dihydroxyvitamin D₃ on Intestinal Phosphate Absorption in Rats with a Normal Vitamin D Supply

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Research Article

In vitamin D-deficient rats, impaired intestinal phosphorus (P) absorption can be corrected by 1,25-dihydroxyvitamin D₃[1,25-(OH)₂D₃]. In the present study, it was investigated whether changes in 1,25-(OH)₂D₃ production can influence intestinal P transport also in animals with a normal supply of vitamin D. The intestinal P absorption was evaluated in rats using both the *in situ* duodenal loop technique and the determination of the overall gastrointestinal absorption under three conditions known to influence the production of 1,25-(OH)₂D₃: (a) variation in dietary P, (b) thyroparathyroidectomy (TPTX) with or without administration of parathyroid hormone (PTH), and (c) treatment with disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP). In all circumstances changes in duodenal absorption paralleled the changes in the overall fractional absorption. (a) Lowering dietary P stimulated P absorption. (b) TPTX decreased P absorption. This effect was corrected either by the administration of PTH or by the administration of 1,25-(OH)₂D₃. (c) EHDP, when given at a dose known to inhibit 1,25-(OH)₂D₃ formation, decreased the duodenal P absorption in both intact and TPTX animals. This effect was corrected by 1,25-(OH)₂D₃. In the TPTX-EHDP-treated animals, the administration of PTH did not rectify the low duodenal P absorption. These results support the thesis that, in rats with normal vitamin D supply, variations in the endogenous production of 1,25-(OH)₂D₃ change the rate of P absorption. However, these changes are in such magnitude [...]

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ABSTRACT In vitamin D-deficient rats, impaired intestinal phosphorus (P) absorption can be corrected by 1,25-dihydroxyvitamin D₃[1,25-(OH)₂D₃]. In the present study, it was investigated whether changes in 1,25-(OH)₂D₃ production can influence intestinal P transport also in animals with a normal supply of vitamin D. The intestinal P absorption was evaluated in rats using both the *in situ* duodenal loop technique and the determination of the overall gastrointestinal absorption under three conditions known to influence the production of 1,25-(OH)₂D₃: (a) variation in dietary P, (b) thyroparathyroidectomy (TPTX) with or without administration of parathyroid hormone (PTH), and (c) treatment with disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP). In all circumstances changes in duodenal absorption paralleled the changes in the overall fractional absorption. (a) Lowering dietary P stimulated P absorption. (b) TPTX decreased P absorption. This effect was corrected either by the administration of PTH or by the administration of 1,25-(OH)₂D₃. (c) EHDP, when given at a dose known to inhibit 1,25-(OH)₂D₃ formation, decreased the duodenal P absorption in both intact and TPTX animals. This effect was corrected by 1,25-(OH)₂D₃. In the TPTX-EHDP-treated animals, the administration of PTH did not rectify the low duodenal P absorption. These results support the thesis that, in rats with normal vitamin D supply, variations in the endogenous production of 1,25-(OH)₂D₃ change the rate of P absorption. However, these changes are in such magnitude that they are of relatively small importance when compared to the effect of variation in the dietary intake of P. These results also strongly suggest that the action of PTH on duodenal P transport is mediated by its effect on 1,25-(OH)₂D₃ production, inasmuch as the effect of the

hormone is abolished after blocking the renal 1-hydroxylation with EHDP.

INTRODUCTION

Vitamin D deficiency is associated with a decrease in intestinal phosphate absorption. This effect can be corrected by the administration of vitamin D₃ (1-7). More recently it was shown that, in vitamin D-deficient rats, a correction can also be obtained by 25-hydroxyvitamin D₃ (8) and 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃]¹ (8-10). Inasmuch as, in nephrectomized animals, 25-hydroxyvitamin D₃ had no effect, it was suggested that 1,25-(OH)₂D₃ is the metabolically active form for phosphate absorption (8). However, in D deficiency the intestinal wall is markedly altered both morphologically and functionally so that the effect on phosphorus (P) absorption could be part of a general abnormality. Hence, it has not been yet established whether variation in the endogenous production of 1,25-(OH)₂D₃ modulates intestinal phosphate absorption in animals receiving an adequate supply of vitamin D.

The aim of this paper is to investigate whether variations in the endogenous production of 1,25-(OH)₂D₃ in animals receiving an adequate supply of vitamin D leads to changes in the rate of intestinal phosphate absorption. High P intake (11-13), thyroparathyroidectomy (TPTX) (11, 14, 15), or treatment with large doses of the diphosphonate disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) (16, 17) have been shown to depress the production of the serum level of 1,25-(OH)₂D₃. Therefore, these procedures should promote a reduction in the capacity of the intestine to absorb P, which should be corrected or prevented by the administration of physiological dose of 1,25-(OH)₂D₃. We have investigated the intestinal absorp-

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¹Abbreviations used in this paper: 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D₃; EHDP, disodium ethane-1-hydroxy-1,1-diphosphonate; PTH, parathyroid hormone; SHAM, sham operation, -operated; TPTX, thyroparathyroidectomy, -ized.

tion of phosphate measured both in ligated duodenal segments and by balance technique in (a) intact rats fed low, middle, or high P diet. (b) sham-operated and TPTX rats with or without parathyroid hormone or 1,25-(OH)₂D₃ supplementation, and (c) intact and TPTX rats treated daily with 160 μmol/kg body wt of EHDP s.c. with or without 1,25-(OH)₂D₃ and parathyroid hormone (PTH) supplementation.

METHODS

From weaning, female Wistar rats from our own breeding colony were fed a commercial diet of Altromin 1314 (Altromin GmbH., Lage, West Germany), containing 1.1 g/100 g calcium, 1.0 g/100 g phosphorus, and 2,800 IU/kg vitamin D₃. At the weight of 150±10 g, they underwent one of the following investigations on intestinal P absorption.

Influence of a high or low P diet

Duodenal P absorption. The rats were switched to diets containing 0.2, 1.8, or 0.8 g/100 g P and pair-fed during 8 or 16 days. In all diets Ca content was 1.3 g/100 g. Diets were prepared from a vitamin D-poor diet (Altromin 1730) containing 0.12 g/100 g Ca and 0.22 g/100 g P to which calcium gluconate and a mixture of KH₂PO₄/K₂HPO₄ (ratio 3/7) were added. The three diets contained the same amount of K by adding KCl when necessary. All rats were supplemented twice weekly with vitamin D₃ given by gastric intubation at the dose of 25 IU dissolved in 0.1 ml of vegetable oil. At the end of the pair-feeding period, the rats were fasted overnight, and blood samples were taken from the tip of the tail for plasma calcium and phosphate determination. Then ³²P absorption was measured in duodenal segments as described below.

Overall intestinal P absorption. The influence of the dietary P intake on the fractional P absorption of the whole gastrointestinal tract was assessed by measuring as described below the intake and the fecal excretion of P in rats put into single metabolic cages. The rats were pair-fed with the low (0.2 g/100 g) and the high (1.8 g/100 g) P diets mentioned above over an 18-day period and also received 25 IU of vitamin D₃ twice weekly. The overall fractional absorption of P was measured during two 72-h periods: from day 8 to day 10 and from day 16 to day 18. The overall fractional absorption of P was also measured in a group of intact rats pair-fed for 11 days a 0.8-g/100 g P diet, treated or not with 2 × 13 pmol/day i.p. of synthetic 1,25-(OH)₂D₃ (kindly supplied by Hoffmann-La Roche & Co., Inc., Basel, Switzerland). In this experiment, all rats also received 25 IU of vitamin D₃ twice weekly. The measurement of P absorption was made from day 8 to day 11 of treatment.

Influence of PTH or 1,25-(OH)₂D₃ in TPTX rats

Duodenal P absorption. Throughout the experiment, rats were fed a diet containing 0.8 g/100 g P and supplemented with vitamin D₃ as described above. Surgical TPTX or a sham operation (SHAM) by exposing the trachea were performed under ether anesthesia. Plasma calcium was determined 48 h later after an overnight fast, and those animals displaying a value below 1.75 mM (7 mg/100 ml) were considered as TPTX. Then SHAM and TPTX animals were pair-fed for a 7–9 day period. The TPTX rats received daily s.c. 2 μg of L-thyroxine (Fluka AG., Buchs, Switzerland) dissolved in 10⁻³ N NaOH (18). The SHAM rats were injected with the vehicle alone.

In a first series of experiments, 40 IU of PTH was injected to one group of the TPTX rats every 8 h s.c. (trichloroacetic acid extract, The Wilson Laboratories, Chicago, Ill., 195 IU/mg) during the last 2 days before the measurement of duodenal ³²P absorption. The hormone extract was dissolved in 0.2 ml of a 10⁻³ N HCl solution containing 0.15 g/100 g bovine albumin (Biochemica, British Drug Houses, Ltd., Poole, England), 0.38 g/100 g NaCl, 0.85 g/100 g glucose, and 0.2 g/100 g glutathione. The last injection was made about 3 h before measuring the duodenal ³²P absorption. The rest of the TPTX rats and the SHAM animals were injected with the solvent alone.

In a second series of experiments, TPTX rats received via a tail vein either a single injection of 120 pmol of synthetic 1,25-(OH)₂D₃, dissolved in 50 μl of 95% ethanol or the vehicle alone. SHAM rats also were injected with the vehicle. ³²P absorption in the duodenal segment was determined 6 h after the i.v. injection.

Overall intestinal P absorption. Rats were either thyro-parathyroidectomized or sham-operated and then housed in metabolic cages. TPTX rats were supplemented with L-thyroxine as described above. 1 wk after the surgical procedure, half the TPTX rats received i.p. twice daily for 11 days 13 pmol/day of 1,25-(OH)₂D₃ dissolved in 25 μl of 95% ethanol. The rest of the TPTX and the SHAM rats were injected with the ethanol vehicle alone. All animals were pair-fed. During the last 3 days of treatment, the intake and the fecal excretion of P were measured, and the overall fractional intestinal absorption was calculated.

Influence of PTH and 1,25-(OH)₂D₃ in EHDP-treated rats

Duodenal P absorption. For these experiments rats were fed throughout with the usual laboratory chow (Altromin 1314). In a first series of experiments, intact rats were treated with EHDP given s.c. at a daily dose of 160 μmol/kg body wt in 2 ml/kg of 0.15 M NaCl. Control rats received the same volume of 0.15 M NaCl s.c. After 7 days of treatment animals were fasted overnight. On the morning of the 8th day, i.e. 24 h after the last s.c. injection, half the EHDP-pretreated rats received a single tail-vein injection of 120 pmol of 1,25-(OH)₂D₃ dissolved in 0.05 ml of 95% ethanol. The rest of the EHDP-treated animals and the control rats received i.v. 0.05 ml of 95% ethanol. ³²P absorption was determined in a ligated duodenal segment 6 h after the i.v. injection.

In a second series of experiments, a similar protocol was followed for TPTX rats supplemented with 2 μg L-thyroxine daily. 48 h thereafter, TPTX rats were pair-fed and received either EHDP (160 μmol/kg body wt) or NaCl s.c., daily for 7 days. Among the rats injected with EHDP, a group received s.c. 7 × 40 IU of PTH over the last 48 h preceding the determination of ³²P absorption. Another group received one single i.v. injection of 120 pmol of 1,25-(OH)₂D₃ described above. The remaining rats were injected with the solvent vehicles used for dissolving PTH and 1,25-(OH)₂D₃.

Overall intestinal P absorption. Intact rats fed a diet containing 0.8 g/100 g P were injected daily with either EHDP (160 μmol/kg) or NaCl 0.15 M s.c. for 11 days. Half the EHDP-treated rats received twice daily 13 pmol of 1,25-(OH)₂D₃ i.p., the remainder of the animals being injected with the vehicle ethanol. The overall intestinal P absorption was measured over the last 3 days of treatment as described below.

Measurement of duodenal ³²P absorption

The rats were anesthetized with an i.p. injection of 30–40 mg/kg pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.). The abdomen was opened by a longitudinal

midline incision, and intestinal ^{32}P absorption was assessed by a modification (19) of the *in situ* ligated duodenal loop technique described by Wasserman and Taylor (6, 20). Just distally to the bile duct aperture, a glass cannula was inserted through a 1-cm incision in the intestinal lumen and tied in place. A second incision was made 8–14 cm distally, and the segment was washed with 3.5 ml of a 37°C warmed saline solution and drained off with 5 ml air. This segment (referred to as duodenum) was filled with 0.5 ml of a 2- or 5-mM sodium phosphate solution at pH 7.4, made isoosmolar (300 mosmol/liter with NaCl and containing 0.1 $\mu\text{Ci/ml}$ [^{32}P]orthophosphate (Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland)).

After a 15-min incubation, the rats were killed by sectioning the aorta abdominalis, and the ligated loop was quickly removed. The incubated solution was centrifuged for 6 min at 12,000 rpm. The recovered volume of the incubated solution was determined. This determination indicated that no apparent net movement of water occurred during the 15-min incubation period in these experimental conditions. The recovery was not different between control and experimental groups and ranged from 92.2 to 111.2%. Therefore, changes in the concentration of ^{32}P in the incubated solution reflect mostly alterations in phosphorus transfer. After centrifugation of the incubated solution, 50- μl aliquots of the supernate were added to 10 ml of a liquid scintillation solution prepared from 80 g naphthalene (E. Merck AG., Darmstadt, West Germany) and 7.0 g (2-(4-*tert*-butyl-phenyl)-5-(4-biphenyl)-1,3,4-oxadiazol (Ciba-Geigy Corporation, Basel) dissolved in 600 ml toluol and 400 ml ethylene glycol monoether. The ^{32}P radioactivity was measured in a Packard scintillation spectrometer (Packard Instrument International S. A., Zürich). The duodenal phosphate absorption was estimated by calculating the percentage of ^{32}P which disappeared from the incubated solution in 15 min. The intestinal walls were dried at 80°C for 12 h and then ashed for 18 h at 750°C. The ashed residue was dissolved with a 0.5-ml HCl concentration and then diluted 1:10 with distilled water. Radioactivity of this solution was counted as described above. The percentage of the initial dose of ^{32}P that left the luminal medium less the percentage remaining in the intestinal wall corresponds to the percentage of the ^{32}P dose transferred to the body.

Also of importance was to assess whether the amount of ^{32}P disappearing from the lumen reflects the transfer into the body. In all situations we have investigated, variations in the amount of ^{32}P absorbed from the intestinal lumen were always accompanied by parallel changes in the quantity transferred into the body. This also was the case when the luminal concentration of inorganic phosphate (P_i) was varied from 0.4 to 40 mM (Fig. 1). These two diagrams indicate that the change in the percentage of ^{32}P transferred to the body (B) parallels that leaving the intestinal lumen (A). They confirm the results obtained by Wasserman and Taylor in the chick ileum (6). Therefore, the disappearance rate of ^{32}P from the intestinal lumen was used to evaluate phosphate absorption at the duodenal level.

In the experiments concerning the influence of the P intake, the intraluminal concentration was set at 2 mM when the inhibitory effect of high P diet was expected to take place. Conversely the luminal medium was 5 mM, when a stimulation of ^{32}P absorption was expected, e.g., after dietary P restriction.

Measurement of the fractional intestinal absorption of phosphorus

After a 4-day period of adaptation to the metabolic cages, the animals were pair-fed and treated as described above and

in Results. Over a 3-day period the food intake was monitored, and the feces were collected. To time the collection of feces, the rats were given two doses of carmine red, 0.5 ml of a 6% water solution, by gastric probe. The first dose was given at the beginning of the balance period and the second dose 72 h later. The feces stained with the first marker administration were collected, but not those stained after the second administration. 1-g dry wt samples of food and the collected feces were incinerated at 750°C for 18 h. The ash residue was dissolved in 25 ml of 1 N HCl and left at room temperature overnight before P_i determination. The net intestinal absorption over the amount of P ingested represents the overall fractional absorption of phosphorus.

Chemical method

Calcium was determined in plasma by titration with EGTA using calcein (2,7-bis[bis(carboxymethyl)-amino methyl]-fluorescein; E. Merck AG.) as indicator with a Corning calcium analyzer 940 (Corning Medical, Corning Glass Works, Medford, Mass.). P_i was determined in plasma and in ashed residue of food and feces according to the micromethod of Chen et al. (21).

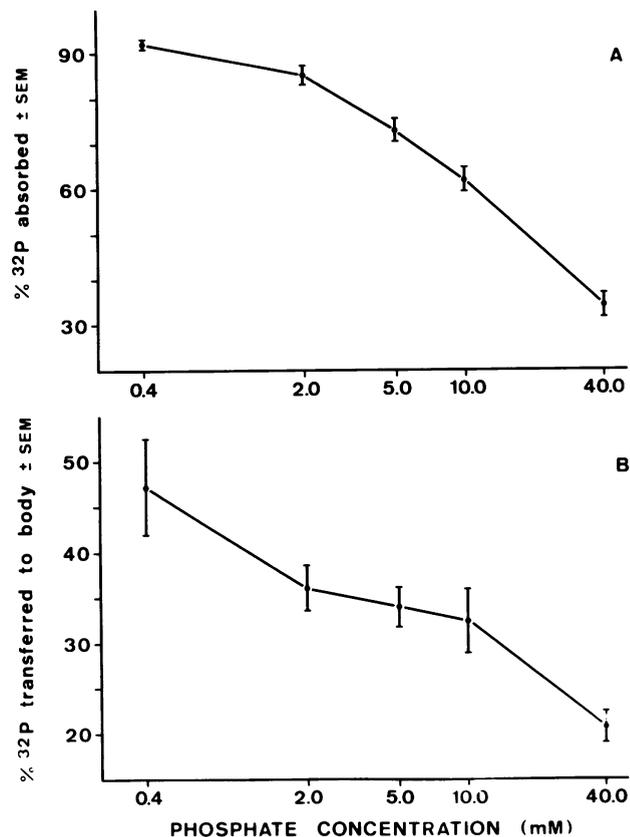


FIGURE 1 Effect of intraluminal P_i concentration in intact rats on (A) ^{32}P disappearance from the lumen of a tied duodenal segment and (B) ^{32}P transferred to the body from the duodenum segment. The intestinal segments were filled with a 0.5-ml solution containing the indicated concentrations of P_i made isoosmotic (300 mosmol/liter) with NaCl. The absorption period was 15 min. Each point represents the mean \pm SEM of four to six rats.

Statistical analysis

All results are expressed as mean \pm 1 SEM. The significance of difference between groups was determined by the two-sided Student's *t* test.

RESULTS

Dietary phosphorus. The influence of dietary phosphorus on the duodenal ^{32}P absorption is presented in Table I. Raising the P content from 0.8 to 1.8 g/100 g led to a decrease of ^{32}P absorption already detectable 8 days after switching the experiment diet. Decreasing the P content from 0.8 to 0.2 g/100 g had no significant effect after 8 days but enhanced ^{32}P absorption after 16 days. Despite these significant alterations observable in the first portion of the small intestine, the capability of the whole gastrointestinal tract for adapting to marked variation in P intake seems to be smaller. Indeed the difference in the overall fractional P absorption between groups of rats fed 0.2- and 1.8-g/100 g P diets was not $<5\%$ (Table II) 15 days after starting the experimental diet.

In another experiment, it was investigated whether the administration of physiological amounts of $1,25\text{-(OH)}_2\text{D}_3$ could mimic the effect produced by lowering dietary phosphorus. $1,25\text{-(OH)}_2\text{D}_3$ (2×13 pmol/day i.p.) was administered for 11 days to intact rats fed a 0.8-g/100 g P diet. Pair-fed control animals received the ethanol vehicle alone. The amount of phosphorus (milligram \pm SEM) ingested was 81.1 ± 1.5 ($n = 8$) and 83.0 ± 0 ($n = 9$) in the $1,25\text{-(OH)}_2\text{D}_3$ -treated and control groups, respectively. The overall fractional absorption

TABLE I
Influence of Dietary Phosphorus on Duodenal ^{32}P Absorption in Intact Rats

	Dietary P g/100 g	Days of pair-feeding	
		8	16
Exp. A	0.8	80.8 ± 2.7 (5)	—
	1.8	66.6 ± 3.1 (5)*	—
Exp. B	0.8	60.0 ± 1.8 (6)	57.5 ± 3.3 (13)
	0.2	62.7 ± 3.9 (6)	66.1 ± 2.2 (13)†

Rats were pair-fed the indicated diets, which contained 1.3 g/100 g Ca, for 8 or 16 days. After an overnight fast, ^{32}P absorption was measured in duodenal segments. The concentrations of P_i in the incubated solution were 2 and 5 mM in experiments A and B, respectively. All values are mean \pm 1 SEM. They correspond to the percentage of the initial doses of ^{32}P which has disappeared from the luminal site. Numbers in parentheses represent the number of animals.

* $P < 0.01$.

† $P < 0.05$ as compared to the 0.8-g/100 g P pair-fed group.

TABLE II
Influence of Dietary Phosphorus on the Overall Fractional Absorption of Phosphorus in Intact Rats

n	Dietary P g/100 g	Time of measurement after starting the experimental diets	
		Days 8-10	Days 16-18
		%	
6	0.2	61.7 ± 1.2	70.9 ± 1.4
6	1.8	64.0 ± 0.8	$66.7 \pm 0.7^*$

The fractional absorption of phosphorus was measured during two 3-day periods. n = number of animals. Values are mean \pm 1 SEM. Plasma P_i concentration measured on day 19 was 1.42 ± 0.15 and 2.50 ± 0.05 mM (mean \pm 1 SEM, $P < 0.001$), in the rats fed the low and the high P diet, respectively. * $P < 0.025$ as compared to the group fed the low (0.2 g/100 g) P diet.

of P (percent) measured from day 8 to day 11 was 74.5 ± 2.8 in the $1,25\text{-(OH)}_2\text{D}_3$ -treated group and 68.4 ± 1.1 in the control group ($P \leq 0.05$). This difference is of the same magnitude as that obtained between rats fed 0.2- and 1.8-g/100 g P diets for 15 days (Table II).

Thyroparathyroidectomy. Figs. 2 and 3 clearly illustrate that the removal of the thyroparathyroid glands brought about a small, but significant reduction in the rate of ^{32}P absorption at the duodenal level. Similarly the fractional P absorption measured for the

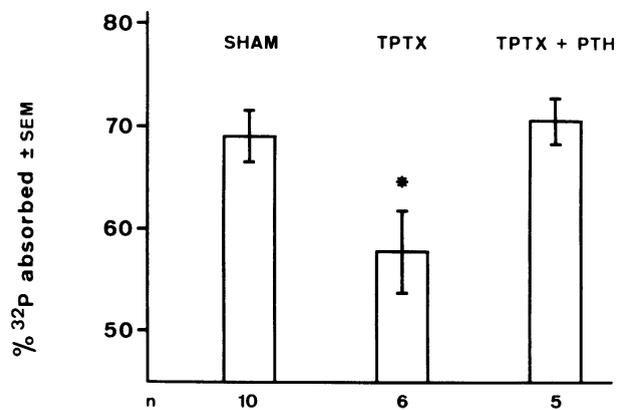


FIGURE 2 Reversal by PTH of the decreased ^{32}P duodenal absorption in TPTX rats. TPTX and SHAM rats were pair-fed a 0.8-g/100 g P diet. 40 IU of PTH was injected to half the TPTX rats every 8 h during the last 2 days of treatment. The last injection was given 3 h before measuring ^{32}P absorption. Duodenal ^{32}P absorption was measured with a solution containing 5 mM P_i . Each column represents the mean \pm SEM. * $P < 0.05$ as compared to the SHAM group. The plasma calcium measured just before the determination of ^{32}P absorption was 2.51 ± 0.05 mM in SHAM ($n = 10$), 1.77 ± 0.08 mM ($n = 6$) in TPTX rats, and 2.96 ± 0.13 mM ($n = 5$) in TPTX rats supplemented with PTH. The corresponding values of plasma P_i were 2.48 ± 0.11 mM in SHAM, 3.51 ± 0.15 mM in TPTX, and 2.74 ± 0.15 mM in TPTX + PTH.

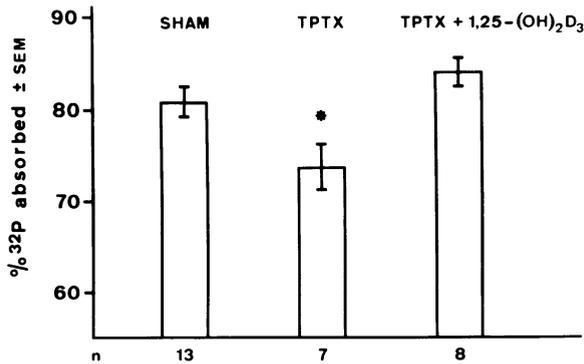


FIGURE 3 Reversal by 1,25-(OH)₂D₃ of the decreased ³²P absorption in TPTX rats. 120 pmol of 1,25-(OH)₂D₃ was injected i.v. 6 h before measuring ³²P absorption. Duodenal ³²P absorption was measured with a solution containing 5 mM P_i. Each column represents the mean ± SEM. * P < 0.05 as compared to the SHAM group.

whole gastrointestinal tract was also reduced in TPTX rats (Table III).

Parathyroid hormone in TPTX rats. As shown in Fig. 2 when TPTX rats were supplemented with PTH (3 × 40 IU/day s.c.), the low ³²P absorption in duodenum was corrected. As indicated in the legend of Fig. 2, the calcemia and the phosphatemia of TPTX rats were corrected by the supplement of PTH.

1,25-dihydroxyvitamin D₃ in TPTX rats. The low ³²P absorption observed in duodenum of TPTX rats was also corrected by 1,25-(OH)₂D₃, 6 h after the i.v. injection of 120 pmol of the vitamin D₃ metabolite. The chronic administration of 1,25-(OH)₂D₃ (2 × 13 pmol/day i.p.) also prevented the fall in the fractional P absorption of the whole gastrointestinal tract, as measured by monitoring the intake and the excretion

TABLE III
Reversal of the Decrease of the Overall Fractional Absorption of Phosphorus in TPTX Rats by 1,25-(OH)₂D₃

	SHAM (n = 5)	TPTX (n = 7)	TPTX + 1,25- (OH) ₂ D ₃ (n = 8)
Fractional phosphorus absorption, %	65.0 ± 1.8	55.6 ± 1.1*	63.4 ± 1.6†
Plasma P _i concentration, mM	2.51 ± 0.04	4.73 ± 0.26*	3.58 ± 0.22‡

TPTX rats supplemented with thyroxine were injected i.p. with 13 pmol of 1,25-(OH)₂D₃ twice daily or with the same volume of ethanol for 10 days. The overall fractional absorption of phosphorus was measured during the last 3 days of treatment by monitoring the intake and the fecal excretion of phosphorus. P_i in plasma was determined on the 11th day of treatment.

* P < 0.001 as compared to pair-fed, ethanol-injected sham-operated rats.

† P < 0.01 as compared to TPTX rats injected with ethanol vehicles only.

‡ P < 0.005 as compared to SHAM rats.

of phosphorus in TPTX rats (Table III). Note that, in spite of the rise in the inflow of phosphate from the intestine, plasma P_i decreased significantly in the TPTX rats supplemented with 1,25-(OH)₂D₃.

Treatment with EHDP. In intact rats, the administration of EHDP in doses (160 μmol/kg body wt per day s.c.), which inhibit the production of 1,25-(OH)₂D₃ in the kidney (16, 17), reduced ³²P absorption in the duodenum significantly (Fig. 4). This reduction was completely corrected 6 h after injecting i.v. 120 pmol of 1,25-(OH)₂D₃ (Fig. 4). The study concerning the overall intestinal P absorption corroborates the result obtained at the duodenal level. Indeed, the reduction in the fractional absorption of P observed in EHDP-treated rats was prevented by the concomitant administration of 1,25-(OH)₂D₃ (2 × 13 pmol/day i.p.) (Table IV). In this experiment the values of plasma P_i did not significantly differ one from another.

In TPTX rats treated with EHDP, ³²P absorption was also reduced (Fig. 5). As in the intact animals, a single dose of 120 pmol of 1,25-(OH)₂D₃ reversed the effect of EHDP. In sharp contrast, such a correction could not be achieved by PTH given in doses which normalized the intestinal P absorption, the calcemia, and the phosphatemia of untreated TPTX rats, as shown in the experiment presented above. This dose of PTH also exerted a significant effect on the plasma level of calcium and phosphate in the EHDP-treated TPTX rats (Table V). This effect was somewhat blunted, since EHDP given at 160 μmol/kg body wt per day s.c. tends in itself to normalize both the level of plasma Ca and

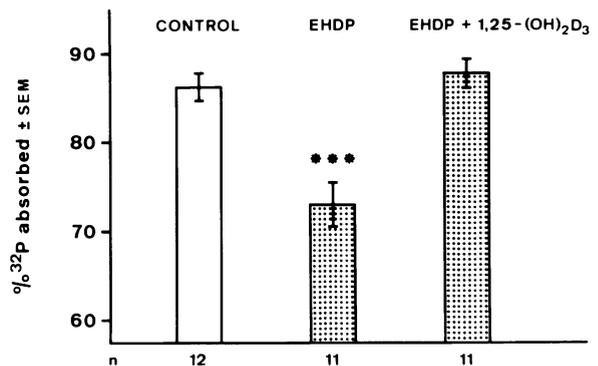


FIGURE 4 Reversal by 1,25-(OH)₂D₃ of the EHDP-induced inhibition of duodenal ³²P absorption. The rats received EHDP (160 μmol/kg body wt per day s.c.) for 7 days, the controls receiving the same volume of 0.15 M NaCl. On the 8th day, 24 h after the last injection and after an overnight fast, half the EHDP-pretreated rats were injected with 120 pmol of 1,25-(OH)₂D₃ in a tail vein. The other animals received 0.05 ml of ethanol. 6 h later, ³²P absorption was measured by the *in situ* duodenal loop technique. Each column is the mean ± SEM. The incubation solution contains 2 mM phosphate. ***P < 0.001 as compared to the control group.

TABLE IV
Influence of EHDP on the Overall Fractional Absorption of Phosphorus in Intact Rats: Preventive Effect of 1,25-(OH)₂D₃

	Control	EHDP*	EHDP* + 1,25-(OH) ₂ D ₃ †
No. of animals	6	6	5
Fractional phosphorus absorption, %§	79.8±1.2	69.0±0.9¶	79.8±0.8
Plasma P _i concentration, † mM	2.71±0.13	2.48±0.10	2.81±0.13

Results are mean±1 SEM.

* 160 μmol/kg body wt per day s.c. for 10 days.

† 2 × 13 pmol/day i.p. for 10 days.

§ Measured during the last 3 days of treatment.

¶ Determined on the last day of treatment.

‡ P < 0.001 as compared to the control group.

P_i (Table V) (22, 23). Thus, the action of PTH on the transport of calcium and phosphate elsewhere than in the intestine is not blocked by EHDP.

DISCUSSION

Measurement of intestinal phosphorus absorption.

The present work confirms previous results obtained by Wasserman and Taylor (6) in the chick showing that the rate of ³²P absorption in the small intestine tends

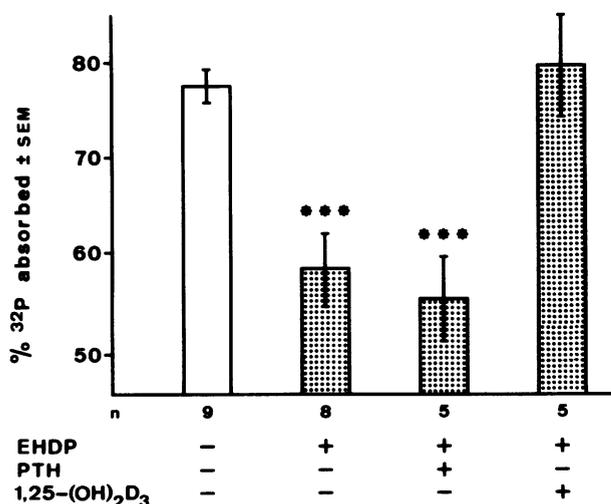


FIGURE 5 Comparative study of the effect of PTH and 1,25-(OH)₂D₃ on ³²P duodenal absorption in TPTX rats treated with EHDP. EHDP was administered at the dose of 160 μmol/kg body wt per day s.c. for 7 days. ³²P absorption was determined on the 8th day. PTH (40 IU) was injected s.c. every 8 h during the last 2 days of treatment, the last injection occurring 3 h before the tied loop experiment. 1,25-(OH)₂D₃ (120 pmol) was injected i.v. 6 h before measuring ³²P absorption. The control rats received the vehicle only. Each column is the mean±SEM. ***P < 0.001 as compared to the control group.

to decrease with increasing concentration of stable P_i in the intestinal lumen. These results are consistent with the existence of a saturable component for P_i transport system in the intestine. Our results indicate that changes in ³²P absorption in the duodenal loop are always associated with a parallel alteration in the overall net intestinal absorption measured by oral intake and fecal excretion. Therefore, the ligated loop assay at the duodenal level can be considered as a reliable technique in the rat for assessing variations in the intestinal absorption of P.

Influence of dietary phosphorus. The results presented indicate that the capacity of the P transport system in the intestine can be altered by the prior dietary supply of P in rats receiving a normal supply of vitamin D₃. This alteration is effected according to homeostatic requirements inasmuch as a high P diet leads to a decrease, whereas a low P supply promotes a rise in the intestinal fractional absorption of phosphate. However, the change in the capacity to absorb phosphate is small in comparison with the changes in fluxes induced by variation in the dietary P content. Furthermore, the influence of the diet on the overall fractional absorption can be detected only 16 days after the animals have been exposed to new P supply. The magnitude and the induction time of the intestinal response contrast strikingly with that observed at the kidney level. Indeed, variations in the P supply of the same magnitude as those employed in the present study have been shown to change markedly the capacity of the renal tubule to transport phosphate (24–26). Furthermore, the renal response to dietary P appears to be quickly induced in that it can be detected as soon as 72 h after altering the P intake (24).

The intestinal response could be explained by the stimulation of 1,25-(OH)₂D₃ production which has been reported to occur in rats fed a low P diet (11, 12). In our experiments the effect of a 0.2-g/100 g P diet on the intestinal phosphorus transport was matched by giving a small supplement of 1,25-(OH)₂D₃ (2 × 13

TABLE V
Plasma Ca and P_i in TPTX Rats Treated with EHDP: Effect of PTH and 1,25-(OH)₂D₃

	Control	EHDP	EHDP + PTH	EHDP + 1,25-(OH) ₂ D ₃
No. of rats	8	8	4	5
Plasma Ca, mM	1.22±0.05	2.27±0.05*	2.65±0.06*†	2.26±0.06*
Plasma P _i , mM	4.96±0.13	3.21±0.11*	2.33±0.06*†	3.31±0.18*

The values (mean±SEM) apply to the rats of which the results of ³²P absorption are presented in Fig. 5. Blood samples were taken about 3 h before the measurement of ³²P absorption and about 3 h after an injection of PTH (40 IU s.c.) or of 1,25-(OH)₂D₃ (13 pmol i.p.).

* P < 0.001 as compared to the control group.

† P < 0.001 as compared to the group treated with EHDP (160 μmol/kg body wt per day s.c. for 10 days).

pmol/day i.p.) to rats maintained on a 0.8-g/100 g P diet. The question whether such exogenous supplement would approximate the increased amount of 1,25-(OH)₂D₃ produced in response to a 0.2-g/100 g P diet cannot be easily answered. Indeed, it is possible that the influence of a low P diet on the intestinal Ca (12) and perhaps also on phosphate transport is not entirely due to variations in the endogenous production of 1,25-(OH)₂D₃ (27, 28).

Influence of TPTX. Studies upon the effects of PTH on intestinal P absorption in rats have led to conflicting results. Wasserman and Comar (29) did not find any change in ³²P absorption after parathyroidectomy, using the ligated-tied-loop technique. Similarly Clark and Rivera-Cordero (30-32) did not observe a difference in the overall P absorption between intact and TPTX rats. In contrast, Lifshitz et al. (33) reported a decreased intestinal phosphate transport in TPTX rats. In agreement with this latter study (33), our results indicate that removal of the parathyroid glands leads to a decrease in the intestinal phosphate absorption. In both Lifshitz's and our own studies, intestinal absorption was assessed >6 days after the TPTX. However, in the experiments reported by Wasserman and Comar (29), phosphate absorption was measured only 5 and 24 h after the removal of the parathyroid glands. This difference in the time elapsing between the operation and the transport measurement could explain these conflicting results. If, as it will be discussed later, the action of PTH on P absorption is mediated by 1,25-(OH)₂D₃, one could expect that more than a 24-h period is required for obtaining the change in the intestinal transport.

Borle et al. (34) and Lifshitz et al. (33) have shown that injection of parathyroid extract to intact (34) and TPTX (33) rats stimulates the intestinal phosphate transport, measured *in vitro* in everted intestinal loops. In addition, Lifshitz et al. (33) did not observe a stimulation of phosphate transport when PTH was added *in vitro*. Our results confirm that parathyroid extract given *in vivo* affects the intestinal P transport. The decreased intestinal phosphate absorption of TPTX rats is reversed by PTH given in doses which correct the plasma level of calcium and phosphate (33). Furthermore, our experiments demonstrate that 1,25-(OH)₂D₃ given in small dosage can also correct the low phosphate absorption of TPTX rats. This suggests that the influence of PTH on the intestinal phosphate absorption is mediated through its action on the production of 1,25-(OH)₂D₃ (14).

Influence of EHDP. The present study indicates that rats treated with EHDP at doses which inhibit the formation of 1,25-(OH)₂D₃ display a lower intestinal phosphate absorption. This effect of EHDP is still present in TPTX rats. This suggests that, in the present experimental conditions, EHDP treatment has a

stronger inhibitory effect on the formation of 1,25-(OH)₂D₃ than removal of the thyroparathyroid glands. The action of EHDP on phosphate absorption can be counteracted by small amounts of 1,25-(OH)₂D₃. This indicates that it is very likely that EHDP affects not only Ca (19, 35) but also P absorption by decreasing the formation of 1,25-(OH)₂D₃ (16, 17). Of particular interest is the observation that doses of PTH which normalize the intestinal P transport, calcemia, and phosphatemia of TPTX rats, have no action on intestinal P absorption when the rats are treated with EHDP at doses which are known to block the formation of 1,25-(OH)₂D₃ (16, 17). Recent observations in our laboratory also indicate that in EHDP-treated rats, PTH (3 × 40 IU/day s.c.), unlike 1,25-(OH)₂D₃, cannot overcome the reduced intestinal Ca absorption, whereas the same doses of PTH normalize the intestinal Ca absorption of TPTX rats (23). This absence of response to PTH at the gut level in EHDP-treated rats does not just represent one aspect of a generalized blockage of the action of this hormone. Indeed, at the kidney level the phosphaturic tubular response to PTH has been shown to be enhanced rather than depressed (36). Furthermore, in the present experiment the rise in the calcemia and the fall in the phosphatemia observed in the response to PTH in TPTX-EHDP-treated rats indicate that other target organs than the intestine are still responding to the hormone. For these reasons, it appears much more likely that under EHDP treatment, the intestinal response to PTH is blocked, because the diphosphonate prevents the stimulatory action of PTH on the renal production of 1,25-(OH)₂D₃. If so, our results represent the first clear-cut evidence that the action of PTH on intestinal phosphate and Ca transport is indirectly mediated by an increased formation of 1,25-(OH)₂D₃. Thus, they bring support to the recent suggestion made by Garabedian et al. (37) that 1,25-(OH)₂D₃ would mediate the effect of PTH on intestinal calcium absorption.

In summary, the results presented in this work support the thesis that, in rats with a normal vitamin D supply, variations in the endogenous production of 1,25-(OH)₂D₃ affect the rate of intestinal P absorption. A recent clinical study in uremic man (38) lends support to this thesis. Indeed, in chronic renal failure, wherein a malabsorption of phosphorus (39) is associated with a low or undetectable level of serum 1,25-(OH)₂D₃ (40), small doses of 1,25-(OH)₂D₃ can enhance the intestinal phosphate absorption (38). Our results also show that the changes in the intestinal P transport capacity observed under conditions which alter 1,25-(OH)₂D₃ production are relatively small. These changes can modify the absolute intestinal P absorption only slightly when compared to the effect of variations in the dietary intake of P. Therefore, the regulation of intestinal P transport does not appear to play a major role in the control

of the overall P_i balance in animals receiving a normal supply of vitamin D. This emphasizes the importance of the kidney for the homeostasis of phosphate. Finally, our experiment strongly suggests that the action of PTH on duodenal P transport is mediated by its effect on 1,25-(OH)₂D₃ production, inasmuch as the effect of the hormone is abolished after blockage of the renal 1-hydroxylation by the diphosphonate EHDP.

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