Parathyroidectomy Reduces 25-Hydroxyvitamin D_3-1α-Hydroxylase Activity in the Hypocalcemic Vitamin D-Deficient Chick

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ABSTRACT To test the hypothesis that in the vitamin D-deficient state the activity of 25-hydroxyvitamin D_3-1α-hydroxylase (25-OHD_3-1α-hydroxylase) is modulated by parathyroid hormone and the plasma concentration of phosphate only in the presence of small amounts of 1,25-dihydroxyvitamin D_3 (or some other metabolite of vitamin D), we measured the activity of this enzyme 24 h after parathyroidectomy (PTX) in frankly hypocalcemic, vitamin D-deficient chicks that were not supplemented with vitamin D or one of its metabolites. The otherwise predictable complications of PTX in this metabolic setting (hypocalcemia of increasing severity, tetany, moribundity, and death) were prevented by continuous intravenous administration of calcium (as a solution of calcium chloride/calcium gluconate 1:1) through a catheter in the external jugular vein placed at the time of PTX. The findings were as follows: (*a*) The activity of 25-OHD_3-1α-hydroxylase was significantly less in the parathyroidectomized group than in the sham-operated control chicks ($P < 0.001$). (*b*) The reductive effect of PTX on the activity of this enzyme was significantly attenuated when hypophosphatemia was increased in severity by administration of glucose. (*c*) In the post-PTX state the activity of 25-OHD_3-1α-hydroxylase and plasma concentration of phosphate were significantly, inversely related ($P < 0.001$). (*d*) In the sham-operated control group the activity of this enzyme and the plasma concentration of phosphate were not significantly correlated. These findings indicate that in the vitamin D-deficient state, both circulating parathyroid hormone and the plasma concentration of phosphate can significantly modulate the activity of 25-OHD_3-1α-hydroxylase in the absence of vitamin D or its metabolites. The findings also suggest that in the vitamin D-deficient state the plasma concentration of phosphate modulates the activity of this enzyme only when the concentration of circulating parathyroid hormone is not increased.

INTRODUCTION

1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$][1] is the most biologically active vitamin D metabolite known with respect to bone resorption and intestinal absorption of calcium (1–3). The synthesis of 1,25-$(OH)_2D_3$ from its only known endogenous precursor, 25-hydroxyvitamin D_3 (25-OHD_3) occurs uniquely in the renal cortex and is catalyzed by 25-hydroxyvitamin D_3-1α-hydroxylase (25-OHD_3-1α-hydroxylase) (4–6). Because of the apparent biological importance of 1,25-$(OH)_2D_3$ in calcium and phosphorus homeostasis the factors that might regulate the rate of its production and the activity of 25-OHD_3-1α-hydroxylase have been under intensive investigation (7–9).

A number of studies suggest that in the vitamin D-deficient state the plasma concentration of calcium might feed-back regulate the production of 1,25-$(OH)_2D_3$ through the parathyroid system (10–14). In vitamin D-deficient rats, the production of 1,25-$(OH)_2D_3$ was greater during hypocalcemia than during normocalcemia (induced by high calcium diets) (10). In vitamin D-deficient chicks the activity of 25-OHD_3-1α-hydroxylase varied inversely with dietary

Dr. Booth is a recipient of a Bank of America-Giannini Foundation Fellowship.

Received for publication 24 January 1977 and in revised form 1 August 1977.

[1] *Abbreviations used in this paper*: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 24,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; 25,26-$(OH)_2D_3$, 25,26-dihydroxyvitamin D_3; 25-OHD_3, 25 hydroxyvitamin D_3; 25-OHD_3-1α-hydroxylase, 25-hydroxyvitamin D_3-1α-hydroxylase; PTE, parathyroid extract; PTH, parathyroid hormone; PTX, parathyroidectomy; TPTX, thyroparathyroidectomy.

calcium (11). In rats raised on a vitamin D-deficient diet [but supplemented with 1,25-$(OH)_2D_3$ to ensure survival], thyroparathyroidectomy (TPTX) was attended in 24 h by a decrease in the production of 1,25-$(OH)_2D_3$, as judged from the amounts of tritiated 1,25-$(OH)_2D_3$ measured in serum, 12 h after intravenous administration of tritiated 25-OHD_3 (12). When parathyroid extract (PTE) was administered 48 h after TPTX, an increase in the amount of tritiated 1,25-$(OH)_2D_3$ in serum could be measured 12 h after initiation of PTE. It was concluded that parathyroid hormone (PTH) may be a trophic hormone that stimulates the production of 1,25-$(OH)_2D_3$ (12). In 4-wk-old chicks raised on a vitamin D-deficient diet but supplemented with vitamin D 16–18 h before parathyroidectomy (PTX), 25-OHD_3-1α-hydroxylase activity was decreased at 13 and 14 h after PTX (13). Accordingly, Fraser and Kodicek concluded that 25-OHD_3-1α-hydroxylase is regulated and stimulated by circulating PTH (13).

Galante et al. concluded that, while PTH may increase production of 1,25-$(OH)_2D_3$ under certain circumstances, circulating PTH is not essential for the increased production of 1,25-$(OH)_2D_3$ characteristic of the vitamin D-deficient state (15). Their conclusions were based on the results of a study in vitamin D-deficient chicks in which 25-OHD_3-1α-hydroxylase activity remained elevated and statistically undiminished 14 h after PTX, unless supplements of vitamin D_3 had been administered 24 h beforehand. They argued that in the vitamin D-deficient chicks studied by Fraser and Kodicek (13) the fall in 25-OHD_3-1α-hydroxylase activity after PTX required not only a reduction in circulating PTH but also the supplements of vitamin D that were administered to ensure survival. Vitamin D_3 [as well as 25-OHD_3 and 1,25-$(OH)_2D_3$] is known to inhibit 25-OHD_3-1α-hydroxylase within 24 h of administration (11, 13, 16–18). In light of this fact, the observations of Galante et al. (15), and other considerations (10, 18, 19), DeLuca and his co-workers have proposed that the feed-back regulation of the biosynthesis of 1,25-$(OH)_2D_3$ by the serum concentration of calcium, through the parathyroid system (and by serum concentration of phosphate), requires vitamin D, probably as 1,25-$(OH)_2D_3$.

But in the vitamin D-deficient chicks studied by Galante et al. (15), circulating PTH may have been only minimally increased, if at all, since hypocalcemia obtained in none of the sham-operated animals. In vitamin D-deficient chicks that were hypocalcemic (and presumably, severely hyperparathyroid) and were not supplemented with vitamin D, Henry et al. (11) reported that the activity of 25-OHD_3-1α-hydroxylase was decreased 24 h after PTX, compared to the activity in sham-operated controls. The reported decrease in the enzyme activity, however, is difficult to interpret since the decrease might reflect a moribund status of the chicks, rather than an effect of the absence of circulating PTH. When PTX is performed in the vitamin D-deficient chick or rat that is already hypocalcemic, increasing hypocalcemia, tetany, and moribundity are predictable complications (12, 15, 20). Henry et al. (11) reported no attempt to prevent these complications.

Thus, the question still remains. In the state of vitamin D deficiency that is also characterized by prolonged hypocalcemia and presumably severe secondary hyperparathyroidism, does circulating PTH per se account for any of the increase in activity of 25-OHD_3-1α-hydroxylase? In the present study of hypocalcemic, vitamin D-deficient chicks that were not supplemented with vitamin D, we found that the activity of 25-OHD_3-1α-hydroxylase decreased significantly after PTX, and in the absence of increasingly severe hypocalcemia and moribundity. This finding provides a "yes" answer to the question posed, and indicates that at least part of the regulation of the activity of the enzyme by PTH does not require vitamin D or one of its metabolites.

METHODS

Animals. 1-day-old white Leghorn cockerels (Pace/Setter Products, Cucamonga, Calif.) were raised on a vitamin D-deficient rachitogenic diet containing 0.5% calcium and 0.6% phosphorus (Grand Island Biological Co., Santa Clara, Calif.) for the duration of the study. All chicks were supplemented with vitamin D_3 dissolved in Wesson oil (Hunt Wesson Foods, Inc., Fullerton, Calif.), 100 IU three times/wk for the 1st 3 wk of life. Thereafter, the supplement of vitamin D_3 was withheld. The classic signs of vitamin D deficiency, i.e., hypocalcemia and growth failure, occurred by 7 wk of age. At 8–11 wk of age, the chicks were parathyroidectomized or sham operated in the early afternoon (1 to 3 p.m.); sodium pentobarbital (6 mg/100 g body wt) was administered intramuscularly for anesthesia. At the time of surgery, just before PTX or sham operation, an indwelling catheter was placed in the jugular vein for blood sampling and intravenous administration of various solutions. At this time, blood was drawn for measurement of plasma concentrations of calcium and phosphate. From the time of surgery until sacrifice, sham-operated chicks received a solution of 0.45% sodium chloride intravenously at a rate of 4 ml/kg per h; parathyroidectomized chicks received one of the following solutions at a rate of 4 ml/kg per h: a solution of 0.9% sodium chloride, a solution of 1.5% dextrose and 0.22% sodium chloride, or a solution of 5.0% dextrose and 0.22% sodium chloride. 3 h after surgery, blood was drawn for measurement of plasma concentrations of calcium and phosphate. At this time, in the parathyroidectomized chicks, calcium gluconate:calcium chloride (1:1) was added to the previously initiated solution in amounts calculated to deliver calcium at the rate of 15–20 mg/kg per h. In the earlier studies chicks were sedated overnight with intravenously administered sodium pentobarbital. With each successive study, the agent was administered at a lesser rate (1.0–0 mg/kg per h). Because it soon became apparent that sedation was unnecessary, the agent was not administered in the preponderance of studies. When administered, sodium

pentobarbital had no demonstrable effect on the plasma concentrations of calcium and phosphate or on the activity of 25-OHD_3-1α-hydroxylase. 18 h after surgery (6 h before sacrifice), blood was drawn to measure the plasma concentrations of calcium and phosphate. In some studies, the plasma concentrations of calcium and phosphate were determined every 2 h during the last 6 h of the study. Food and water were made available to the chicks during the last 6 h of the study. All drank and most ate heartily. At 23–25 h post-surgery, blood was drawn for determination of plasma concentrations of calcium and phosphate, animals were sacrificed by decapitation, and kidneys were rapidly removed and placed in an iced solution of 0.9% sodium chloride for determination of 25-OHD_3-1α-hydroxylase activity. Non-operated chicks that were allowed water ad libitum and food when the operated chicks began to eat served as additional controls.

To determine the effect of calcium administration on the plasma concentrations of calcium and phosphate of intact hypocalcemic vitamin D-deficient chicks, calcium gluconate: calcium chloride (1:1) was administered intravenously at a rate of 15 to 20 mg/kg per h to nonoperated vitamin D-deficient chicks.

Enzyme assay. The kidney tissue was pressed through a prechilled tissue press; the expressed tissue was homogenized in 15 ml of an ice-cold solution containing 200 mM sucrose, 10 mM sodium malate, 2 mM magnesium chloride, and 15 mM Tris-HCl (pH 7.4) by grinding in a Potter-Elvehjem homogenizer (Potter Instrument Co., Inc., Plainview, N. Y.) for three passes. A 3% (wt/vol) homogenate was prepared by addition of appropriate amounts of the above solution to the homogenate. 10 ml of the homogenate was set aside for determination of protein concentration by Biuret method. 10 ml of the homogenate was preincubated for 1 min in a water bath at 37°C before initiation of the reaction by the addition of 1.7 nmol of 25-OH[26,27-^{3}H]D_3 (Amersham/Searle Corp., Arlington Heights, Ill.; sp act 27.8–29.6 dpm/pm) in 50 μl of ethanol. 30 min after adding substrate the reaction was stopped by the addition of 50 ml of methanol:chloroform (2:1) to the incubation mixture. Total lipids were extracted by a modified method of Bligh and Dyer (21). After evaporation of chloroform under nitrogen, the extracted lipids were redissolved in chloroform: hexane (65:35) for column chromatography.

Column chromatography. 25-OH[26,27-^{3}H]D_3, and its metabolites were separated on a Sephadex LH-20 column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) (1.0 × 60 cm) with chloroform:hexane (65:35), a modification of the method of Holick and DeLuca (22). Each sample was co-chromatographed with [4-^{14}C]D_3 and its metabolites obtained from a lipid extract of the intestinal mucosa of 4-wk-old vitamin D-deficient chicks to which an intraperitoneal dose of 20 IU [4-^{14}C]D_3 had been administered 24 h before sacrifice. 90 fractions of 4 ml each were collected and dried under air in liquid scintillation vials. 10 ml of counting cocktail [7 g 2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxidiazole per liter of toluene] was added to each vial and the radioactivity content measured in a Tri-Carb 3385 liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.). Efficiency corrections were made by use of an external standard. Through use of a Hewlett-Packard 9810 calculator and plotter (Hewlett-Packard Co., Palo Alto, Calif.), disintegrations per minute of each fraction were calculated from counts per minute using the external standard. Picomoles of 1,25-$(OH)_2D_3$ produced were calculated from the disintegrations per minute under the peaks and specific activity, and are reported in picomoles per milligram protein/30 min incubation. Column recovery of tritium was 98.0 ±5.7%. Production of 1,25-$(OH)_2D_3$ was linear throughout 30 min of incubation.

Identification of 1,25-$(OH)_2D_3$. In addition to co-chromatography with the ^{14}C-standard lipid which on Sephadex LH-20 separates only D_3, 25-OHD_3, and 1,25-$(OH)_2D_3$ as the major peaks (23), authentic nonradioactive 25-OHD_3 (through courtesy of The Upjohn Co., Kalamazoo, Mich.), 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$] and 1,25-$(OH)_2D_3$ (through courtesy of Hoffman-LaRoche, Inc., Nutley, N. J.) were co-chromatographed with the sample and ^{14}C-standard lipids on a Sephadex LH-20 column. The peaks of the nonradioactive compounds were detected by ultraviolet spectrophotometry (absorbancy at 265 nm on Beckman DU, Beckman Instruments, Inc., Fullerton, Calif.). The authentic 25-OHD_3 and 1,25-$(OH)_2D_3$ co-chromatographed with the ^{3}H-sample-^{14}C standard lipid peaks assumed to be 25-OHD_3 and 1,25-$(OH)_2D_3$. No detectable ^{3}H or ^{14}C peak was observed at the elution site of the authentic 24,25-$(OH)_2D_3$. Since 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2D_3$] and 1,25-$(OH)_2D_3$ will comprise a single peak on Sephadex LH-20, the fractions from the Sephadex LH-20 column that contained the radioactive peak believed to be 1,25-$(OH)_2D_3$ were rechromatographed on a celite liquid-liquid partition column according to the procedure of Haussler and Rasmussen (23). Only a single peak was observed. Additionally, lipid extracts of samples were treated with 5% sodium periodate according to Holick et al. (24) to detect the presence of 24,25-$(OH)_2D_3$ or 25,26-$(OH)_2D_3$. Less than 10% of the radioactivity was lost after this treatment.

Calcium, phosphorus. Plasma concentration of calcium was determined by atomic absorption spectrophotometry. Plasma concentration of inorganic phosphorus was determined by the Fiske-Subbarow method (25).

RESULTS

At the time of surgery in the vitamin D-deficient chicks, the plasma concentration of calcium was 6.1 ±1.2 mg/dl (mean±1 SD); the plasma concentration of phosphate was 5.0±1.4 mg/dl. The adequacy of parathyroidectomy was inferred by a fall in plasma concentration of calcium of at least 1.6 mg/dl, 2–3 h after PTX (mean fall of 2.2±0.4 mg/dl). During the same time period, in the sham-operated control group, plasma concentration of calcium fell, but the fall, 0.7±0.5 mg/dl, was significantly less ($P < 0.001$). A rise in plasma concentration of phosphate 2–3 h after PTX further indicated the adequacy of PTX. Plasma concentration of phosphate rose in all parathyroidectomized animals (mean rise 0.9±0.4 mg/dl) and fell in all sham-operated controls (mean fall 0.4±0.3 mg/dl). These responses were significantly different ($P < 0.001$ by Student's t test). In the parathyroidectomized group, the continuous administration of calcium at a rate of 10–20 mg/kg per h maintained the plasma concentration of calcium at the preexisting hypocalcemic level, 24 h after PTX. By contrast, in non-parathyroidectomized vitamin D-deficient chicks that received calcium at similar rates, the plasma concentration of calcium became normal or supernormal within 2 h. In the parathyroidectomized chicks, the plasma concentration of calcium at the time of sacri-

fice was not significantly different from that obtaining at the time of surgery or that of sham-operated control chicks, at the time of surgery or sacrifice. A steady state with respect to plasma levels of calcium and phosphate was obtained during the 6 h before sacrifice in both parathyroidectomized and sham-operated controls, as judged by the observation that the plasma concentrations of calcium and phosphate measured 6 h before sacrifice were not significantly different from those measured at sacrifice. In early studies blood for determination of plasma concentrations of calcium and phosphate was drawn every 2 h during the last 6 h of the experiment. Since significant changes in the plasma levels of these substances did not occur, blood was drawn less frequently thereafter.

25-OHD_3-1α-hydroxylase activity decreased 45% 24 h after parathyroidectomy ($P < 0.001$): After parathyroidectomy the activity was 4.5±0.8 pmol/mg protein per 30 min; after sham operation the activity was 8.2±1.2 pmol/mg protein per 30 min (Table I). In contrast to the sham-operated controls in which no correlation obtained between 25-OHD_3-1α-hydroxylase activity and plasma concentration of phosphate, in the parathyroidectomized vitamin D-deficient group, the activity of 25-OHD_3-1α-hydroxylase was inversely correlated with the plasma concentration of phosphate and the correlation was highly significant ($P < 0.001$)

TABLE I

Effect of Parathyroidectomy on 25-OHD_3-1α-Hydroxylase Activity in Hypocalcemic Vitamin D-Deficient Chicks; the Counter Effect of Hypophosphatemia Induced by Glucose

	n	25-OHD_3-1α-hydroxylase	Plasma P_i B	Plasma P_i A	Plasma Ca A
		pmol/mg prot/30 min	*mg/dl*		*mg/dl*
Sham	6	8.2±1.2	5.0±2.0	2.8±1.3*	6.6±1.5
PTX	9	4.5±1.8‡	5.0±1.0	4.2±1.6	5.8±1.0
Alone	5	3.4±1.3‡	5.6±1.0	5.1±1.3§	5.3±0.5
Glucose	4	6.0±1.0$\$$‖	4.4±0.6	3.2±1.2	6.4±1.2

25-OHD_3-1α-hydroxylase activity was assayed 24 h after PTX or sham operation. Plasma concentrations of phosphorus and calcium were determined on blood drawn immediately before the surgical procedure (B) and at the time of the enzyme assay (A). When given, 5% glucose was administered from the time of PTX to the time of sacrifice. The plasma concentration of calcium in period A was not significantly different from that in period B (not shown). Values represent mean±1 SD.

* Vs. B, $P < 0.05$.

‡ Vs. sham, $P < 0.001$.

§ Vs. sham, $P < 0.02$.

‖ Vs. PTX alone, $P < 0.02$.

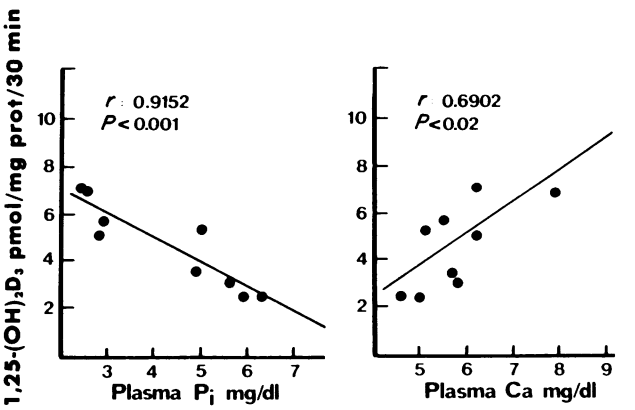

FIGURE 1 Relationship between 25-OHD_3-1α-hydroxylase activity and plasma concentrations of phosphate and calcium 24 h after PTX in hypocalcemic vitamin D-deficient chicks. 25-OHD_3-1α-hydroxylase activity was determined 24 h after surgery. Blood was drawn just before sacrifice for determination of plasma concentrations of phosphate and calcium. r = correlation coefficient; P value determined by Student's t test.

(Fig. 1). In the parathyroidectomized vitamin D-deficient chicks, in which the severity of hypophosphatemia was not increased by administration of glucose, the activity of 25-OHD_3-1α-hydroxylase was strikingly decreased 24 h after PTX whereas in those parathyroidectomized chicks in which hypophosphatemia of a degree similar to that of the sham-operated controls was induced by administration of glucose, activity of 25-OHD_3-1α-hydroxylase was only modestly decreased compared to that of the sham-operated controls (Table I).

In the parathyroidectomized group the activity of 25-OHD_3-1α-hydroxylase was directly correlated with plasma concentration of calcium (Fig. 1). In the sham-operated control group, the activity of 25-OHD_3-1α-hydroxylase was not correlated with the plasma concentrations of either calcium or phosphate.

In the nonglucose-treated parathyroidectomized chicks the plasma concentration of phosphate did not rise after surgery, but was greater than that of the sham-operated controls in which plasma phosphate concentration fell significantly 24 h after surgery (Table I).

Adequacy of the vitamin D-deficient state was supported by the following observations: (*a*) The enzyme activity of the sham-operated chicks (8.2±1.2 pmol/mg protein per 30 min) was as high as that measured in 4-wk-old vitamin D-deficient chicks that were raised from day 1 on a vitamin D-deficient diet (7.7±0.4 pmol/mg protein per 30 min). In this classic model of the vitamin D-deficient state (the 4-wk-old vitamin D-deficient chicks), 1,25-$(OH)_2D_3$ and 25-OHD_3 were recently reported to be undetectable in serum (26). (*b*) In the sham-operated chicks the mean activity of 25-OHD_3-1α-hydroxylase and the mean plasma

concentration of calcium of those operated at 8 wk of age were not significantly different from those at 11 wk. If the chicks were less deficient in vitamin D at 8 than at 11 wk, one might expect a lower enzyme activity and a higher plasma concentration of calcium at the younger age. (*c*) The relationship of 25-OHD_3-1α-hydroxylase to plasma concentration of phosphate in those chicks parathyroidectomized at 8 wk was not different from that in the chicks parathyroidectomized at 11 wk of age: in the 8-wk-old group, the slope of the line of plasma concentration of phosphate vs. 25-OHD_3-1α-hydroxylase activity (slope = -1.2069, $r = 0.9074$) was not significantly different from that of the 11-wk-old group (slope = -1.2110, $r = 0.9954$). These findings, that in the parathyroidectomized chicks modulation of 25-OHD_3-1α-hydroxylase by plasma concentration of phosphate did not change with time, and that in the sham-operated chicks the activity of 25-OHD_3-1α-hydroxylase and plasma concentration of calcium did not change with time, indicate that either the degree of vitamin D deficiency did not change during the 4-wk period of concern, or any change in the vitamin D status that did occur had no effect on 25-OHD_3-1α-hydroxylase.

DISCUSSION

This study provides the first documentation that in a vitamin D-deficient state, 25-OHD_3-1α-hydroxylase activity significantly decreases after PTX in the absence of either supplementation of vitamin D (or a metabolite) or worsening hypocalcemia and its associated moribundity. 24 h after PTX in hypocalcemic vitamin D-deficient chicks in which worsening hypocalcemia was prevented by continuous intravenous administration of calcium, the activity of 25-OHD_3-1α-hydroxylase was significantly decreased compared to that in sham-operated controls. The reductive effect of PTX on the activity of this enzyme was significantly attenuated when the severity of hypophosphatemia was experimentally increased. In the post-PTX state the activity of 25-OHD_3-1α-hydroxylase and plasma concentration of phosphate were significantly, inversely related. These findings indicate that in the vitamin D-deficient state both circulating PTH and the plasma concentration of phosphate can significantly modulate the activity of 25-OHD_3-1α-hydroxylase in the absence of vitamin D or one of its metabolites. Hence, these observations do not accord with the formulation of DeLuca and co-workers that in the vitamin D-deficient state, modulation of 25-OHD_3-1α-hydroxylase activity by circulating PTH, or by the serum concentration of phosphate, can occur only in the presence of 1,25-$(OH)_2D_3$ (9, 18, 19).

The observations of the present study are not, however, inconsistent with observations inferred to support the formulation of Boyle et al. (10) and Galante et al. (15). The observations of the present study and those of previously published studies are, in fact, consistent with the suggestion made by Henry et al. (11) and by Tanaka et al. (18) that the extant activity of 25-OHD_3-1α-hydroxylase reflects a critical balance between the stimulating effect of PTH and the dampening effect of vitamin D. In the normocalcemic, minimally vitamin D-deficient chicks studied by Galante et al. (15) in which the activity of 25-OHD_3-1α-hydroxylase decreased after PTX only when the chicks were supplemented with vitamin D, the level of circulating PTH obtaining at the time of PTX was presumably not nearly so high as that obtaining in the frankly hypocalcemic chicks of the present study. Thus, in the study of Galante et al., the failure of 25-OHD_3-1α-hydroxylase to decrease after PTX alone could be more a function of the relatively low levels of circulating PTH and enzyme activity obtaining at the time of PTX, rather than an indication of the incapacity of PTH to modulate the activity of 25-OHD_3-1α-hydroxylase in the absence of small amounts of vitamin D.

That an increased concentration of PTH of the magnitude obtaining in frank hypocalcemia can stimulate 25-OHD_3-1α-hydroxylase in the absence of 1,25-$(OH)_2D_3$ is strongly supported by observations made in a study of vitamin D-deficient rats in which graded degrees of hypocalcemia were achieved by systematically varying the dietary intake of calcium (10). One group of rats was supplemented with vitamin D, the other group was not. In the nonsupplemented group, 1,25-$(OH)_2D_3$ remained the major serum metabolite of tritiated 25-OHD_3 until dietary calcium was 3.0% or greater. By contrast, in the supplemented group, the major serum metabolite of 25-OHD_3 was already 24, 25-$(OH)_2D_3$ when the dietary calcium content was only 0.47%. Although these results have been interpreted as being consistent with the possibility that vitamin D per se, or one of its metabolites, is required for dietary calcium to dampen 25-OHD_3-1α-hydroxylase activity (9, 18), 1,25-$(OH)_2D_3$ was the major serum metabolite of 25-OHD_3 at serum concentrations of calcium below 9.1 mg/100 ml, and 24,25-$(OH)_2D_3$ was the major serum metabolite at serum concentrations of calcium above 9.1 mg/100 ml, irrespective of the state of supplementation of vitamin D. Since the release of PTH is regulated by serum calcium level, the observed inverse relationship between serum calcium level and the apparent production of 1,25-$(OH)_2D_3$ constitutes evidence that circulating PTH can stimulate 25-OHD_3-1α-hydroxylase irrespective of the presence or absence of vitamin D supplementation. Indeed, the observation suggests that in the frankly hypocalcemic, vitamin D-deficient animal, the stimulating effect of

PTH on 25-OHD_3-1α-hydroxylase can override the dampening effect of small amounts of vitamin D (or one of its metabolites) on the activity of this enzyme.

The observations of this study are in accord with those of Tanaka and DeLuca demonstrating that the suppressive effect of PTX on 25-OHD_3-1α-hydroxylase activity can be largely if not completely reversed by the presence of hypophosphatemia (20). In contrast to Tanaka and DeLuca's investigation, our animals were not supplemented with vitamin D, thus providing further evidence that 25-OHD_3-1α-hydroxylase can be modulated in the vitamin D-deficient state in the absence of small amounts of supplemental vitamin D. Furthermore, the inverse correlation of 25-OHD_3-1α-hydroxylase activity with plasma concentration of phosphate is the first such demonstration in parathyroidectomized vitamin D-deficient animals not supplemented with vitamin D. In the sham-operated controls, which were hypocalcemic and presumably hyperparathyroid, the plasma concentration of phosphorus and production of 1,25-$(OH)_2D_3$ were not correlated. Others have made similar observations: in intact vitamin D-deficient animals no correlation between production of 1,25-$(OH)_2D_3$ and plasma concentration of phosphate has been observed (11). In fact, in intact vitamin D-deficient animals, an inverse correlation between production of 1,25-$(OH)_2D_3$ and plasma concentration of phosphate has been observed only in vitamin D-supplemented animals with normal plasma levels of calcium (27). The inverse relationship between serum concentration of phosphate and 1,25-$(OH)_2D_3$ production might then obtain only with reduced amounts of circulating PTH.

In the present study the failure of the plasma concentration of phosphate to increase by 24 h after parathyroidectomy in the non-glucose-treated chicks, and the frank fall in plasma concentration of phosphate in the sham-operated controls, may be a reflection not only of calcium administration (in the parathyroidectomized group), and the possible phosphaturic effects of sodium chloride administration, but also of the vitamin D status of the animal. Since it has been shown that 1,25-$(OH)_2D_3$ raises serum concentration of phosphate in hypophosphatemic vitamin D-deficient rats, even in the parathyroidectomized state (18), it is possible that in the vitamin D-deficient animal absolute hyperphosphatemia occurs after parathyroidectomy only when vitamin D or some other metabolite of vitamin D, namely 1,25-$(OH)_2D_3$, has been supplemented. Published data permit no conclusion in this regard.

ACKNOWLEDGMENTS

We thank Bernadine Serena and Lisbeth Streiff for technical assistance and Susan Curley for help in preparation of this manuscript.

This work was supported by grants from the National Institutes of Health: National Institute of Arthritis, Metabolism, and Digestive Diseases, no. AM 16764, and the Division of Research Resources, General Clinical Research Center, no. RR-00079.

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