JCI The Journal of Clinical Investigation

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J Clin Invest. 1963;42(12):1940-1946. https://doi.org/10.1172/JCI104880.

Research Article

Journal of Clinical Investigation
Vol. 42, No. 12, 1963

THE RELATIONSHIP BETWEEN VITAMIN D AND PARATHYROID HORMONE *

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(Submitted for publication July 25, 1963; accepted September 6, 1963)

Over three decades ago, it was established that deficiency of either vitamin D or parathyroid hormone may lead to tetany and hypocalcemia (1, 2). Since then, there has been continued interest in the relationship between the biologic effects of these two agents. At one time it was believed that the D vitamins exerted their effects by stimulating the parathyroid glands, but this concept became untenable after it was established that vitamin D could induce hypercalcemia in hypoparathyroid organisms. More recently it has been proposed that the hormone exerts its characteristic effects only in vitamin D-fed animals (3), and that the basis of this action depends upon a chemical interaction between the two (4). These proposals are not satisfactory because there is considerable clinical and pathological evidence (5) suggesting that parathyroid hyperfunction exists in vitamin D deficiency. Certainly complete synergism of action seems most unlikely because of the well-established difference in the syndromes produced by their separate deficiencies (5). On the other hand, there is no satisfactory explanation for the persistent hypocalcemia in D-deficient animals, in spite of apparent parathyroid overfunction, nor for the observations of Harrison, Harrison, and Park (3) that the administration of a standard dose of parathyroid hormone to a D-deficient rat results in little or no change in the concentration of either calcium or phosphate in the plasma.

Another problem that has remained unresolved is the explanation of the phosphate retention that occurs in a D-deficient animal or human shortly after the initiation of specific therapy (6). Harrison and Harrison (6) have favored the view that this retention is due to a direct action of vitamin D upon the renal tubular reabsorption of phosphate, whereas others (5) have considered it a consequence of decreased parathyroid gland activity.

A possible new insight into the nature of the vitamin D and parathyroid hormone relationship has come from mitochondrial studies (7–11). It has been found that vitamin D and parathyroid hormone promote the release of calcium (as a phosphate salt or ion pair) from isolated mitochondria (7), that they act synergistically, and that the presence of the vitamin is necessary for the hormone to exert this effect, although the converse is not true. The hormone has other effects upon the mitochondria that are neither produced by, nor dependent upon, the presence of vitamin D. These are the stimulation of phosphate (11), sulfate, and arsenate (9) accumulation, and a stimulation of respiration (8, 9) that appears to be coupled to the translocations of these ions. These effects are presented in schematic form in Figure 1.

Certain predictions concerning the physiological actions of vitamin D and parathyroid hormone can be made on the basis of these *in vitro* observations. The most important prediction, in the present context, is that parathyroid hormone will exert its effects upon calcium translocations only in the presence of vitamin D, but will continue to exert effects upon phosphate metabolism in the D-deficient organism.

The purpose of this paper is to report experiments designed to test this prediction. The results indicate that parathyroid hormone has a dramatic effect upon phosphate metabolism in the D-deficient rat, but has little apparent effect upon calcium mobilization, at least in moderate doses.

* Presented at the Fifty-fifth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 1963. This study has been supported by U. S. Public Health Service grants AM 05762–02 and AM 05800–02.

† Postdoctoral fellow of the U. S. Public Health Service.

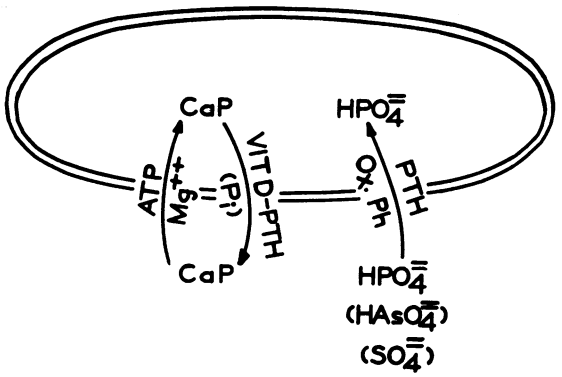

FIG. 1. THE EFFECT OF VITAMIN D AND PARATHYROID HORMONE (PTH) UPON CALCIUM AND PHOSPHATE MOVEMENTS IN MITOCHONDRIA. They both stimulate the release of calcium-phosphate, and the presence of the vitamin is required for this hormonal action. The hormone, but not the vitamin, stimulates phosphate ($HPO_4^=$) uptake. This is coupled to a stimulation of respiration. In addition to phosphate, parathyroid hormone stimulates sulfate ($SO_4^=$) and arsenate ($HAsO_4^=$) uptake.

METHODS

Young Holtzman rats [1] weighing 70 to 80 g were maintained upon a purified diet of varying calcium and phosphate that contained no vitamin D. The animals receiving vitamin D were given 50 U orally every 3 days. The diets employed were prepared as described by Steenbock and Herting (12) as modified by DeLuca and associates (13). They included diets of four main types: Diet I—.47% calcium and .3% phosphorus; Diet II—0.2% calcium and 0.3% phosphorus; Diet III— 1.2% calcium and 0.1% phosphorus; and Diet IV—0.02% calcium and 0.02% phosphorus. Animals grown on these diets are employed routinely in these laboratories. Objective criteria for D-deficiency have been established (12, 13). The rats were weighed every third day, and a lack of growth was taken as the criteria of vitamin D deficiency (12, 13). After 21 days on the test diets, the animals were divided into groups of six or eight. Alternate groups were subjected to parathyroidectomy by electric cautery or to a sham operation, and then were bled by heart puncture at appropriate times after operation. The experiments were repeated upon a second group of animals. The concentrations of calcium and phosphate in the plasma were determined by use of a Technicon autoanalyzer programmed to measure plasma calcium and phosphate simultaneously (14). Parathyroid hormone prepared as previously described (15) was suspended in oil and injected subcutaneously. The doses employed ranged from 25 to 2,000 U. In many cases a rather large dose, 100 to 150 U, was employed to produce significant hyperparathyroidism.

[1] Obtained from colonies maintained on diet containing minimal quantities of vitamin D. Holtzman Co., Madison, Wis.

RESULTS

The effect of the injection of parathyroid hormone. The data summarized in Table I confirm the observations of Harrison and co-workers (3). A dose of 100 U of purified parathyroid hormone caused an increase in plasma calcium concentration of the D-fed animal with intact parathyroid glands but, as noted by Stoerk and Silber (16), had little effect upon the phosphate concentration. A similar dose of hormone, administered to the D-deficient rat, had no effect upon the concentration of either calcium or phosphate. A dose of 500 U of hormone had little effect upon the plasma calcium of the D-deficient rat, but the administration of a massive dose of hormone, 2,000 U, to the D-deficient animal did induce a rise in the concentration of calcium (Table I). Necropsy and microscopic examination of renal tissue revealed no evidence of calcium deposition in the organs of these D-deficient animals injected with large doses of hormone. In contrast, the administration of 2,000 U of hormone to D-fed rats led to hypercalcemia, hyperphosphatemia (Table I), and severe nephrocalcinosis within 18 to 24 hours. A dose as small as 25 U had a significant effect upon plasma calcium in these D-fed animals.

Effect of parathyroidectomy. The effect of parathyroidectomy in rats maintained on a low-calcium diet supplemented with vitamin D (Diet

TABLE I

*The effect of parathyroid hormone (PTH) administration upon plasma calcium and phosphate in D-fed and D-deficient rats maintained on an adequate diet of calcium and phosphate**

	Plasma calcium	Plasma phosphate
	mg/100 ml†	*mg/100 ml*
+D Control	10.6 ± 0.8	10.8 ± 0.5
PTH,‡ 25 U	11.6 ± 0.6§	10.2 ± 0.5
PTH, 100 U	12.1 ± 0.8‖	10.2 ± 0.4
PTH, 2,000 U	17.5 ± 0.9‖	9.3 ± 0.5‖
−D Control	6.9 ± 0.3	12.9 ± 0.6
PTH, 100 U	6.8 ± 0.4	12.6 ± 0.7
PTH, 500 U	7.1 ± 0.4	11.9 ± 0.5§
PTH, 2,000 U	10.7 ± 0.2‖	11.6 ± 0.4‖

* The plasma was obtained 6 hours after hormone injection.
† ± SE.
‡ Eight animals in each group. Fed Diet I.
§ p value, < 0.05 when compared to controls.
‖ p value, < 0.01 when compared to controls.

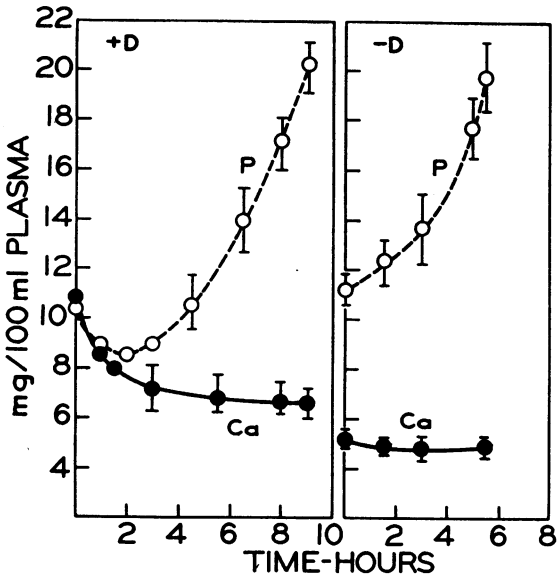

FIG. 2. THE SEQUENTIAL CHANGES IN PLASMA CALCIUM AND PHOSPHATE AFTER PARATHYROIDECTOMY OF D-FED RATS MAINTAINED ON A LOW-CALCIUM DIET (LEFT) AND OF D-DEFICIENT RATS MAINTAINED ON A NORMAL-CALCIUM DIET (RIGHT). The fall in plasma phosphate observed 2 hours after parathyroidectomy (left) in the D-fed rat was significant ($p < 0.01$) when compared to the initial value.

II) was compared to its effect in a separate group maintained on a regular diet (Diet I) without vitamin D. This dietary difference was employed in an effort to minimize the effect of vitamin D upon calcium intake in the D-fed animal and subject both groups to relative calcium deficiency. The results are shown in Figure 2. As shown in the left half of Figure 2, the most striking changes in the D-fed animals are the rapid and dramatic fall in calcium concentration, accompanied by a slight but significant fall in phosphate concentration, and the subsequent dramatic rise in plasma phosphate. In contrast, parathyroidectomy in the D-deficient animals resulted in a strikingly different time-course of events as seen in the right half of Figure 2. There was little change in the concentration of calcium, which was already quite low, but there was an immediate, as contrasted to a delayed (left half of Figure 2), rise in the concentration of phosphate. The administration of 40 to 70 U of hormone at the time of operation prevented the plasma electrolyte changes in the D-fed animals, but a dose of 125 to 150 U was required in the D-deficient animals to prevent the hyperphosphatemia. The parathyroid glands of the D-deficient rats were uniformly enlarged, and the onset of tetany in both groups was correlated with the onset of severe hyperphosphatemia, usually manifested only after the concentration of phosphate had risen above 14 to 15 mg per 100 ml.

TABLE II

Influence of diet, vitamin D, and parathyroid function upon plasma calcium and phosphate in the rat

Hours after operation		Plasma calcium			Plasma phosphate		
		Control	Sham operation	Parathyroidectomy	Control	Sham operation	Parathyroidectomy
		mg/100 ml			*mg/100 ml*		
		Diet I, normal calcium-normal phosphate					
4	−D	5.2 ± 0.3*	5.9 ± 0.3	5.8 ± 0.4	11.2 ± 0.5	10.9 ± 0.6	13.1 ± 0.5†
4	+D	10.4 ± 0.5	10.6 ± 0.4	7.7 ± 0.4†	10.8 ± 0.5	9.9 ± 0.7	11.1 ± 0.6†
6‡	+D+PTH	12.1 ± 0.8	11.9 ± 0.6	13.2 ± 0.7	10.8 ± 0.5	10.0 ± 0.4	9.8 ± 0.6
		Diet II, low calcium-normal phosphate					
4	−D	4.7 ± 0.3	4.3 ± 0.4	4.1 ± 0.4	10.8 ± 0.5	10.0 ± 0.4	14.0 ± 0.6†
4	+D	9.6 ± 0.4	9.3 ± 0.5	6.6 ± 0.3†	11.8 ± 0.6	10.2 ± 0.6	12.4 ± 0.8†
		Diet III, high calcium-low phosphate					
2	−D	10.2 ± 0.6	9.1 ± 0.7	9.0 ± 0.5	2.0 ± 0.3	2.3 ± 0.4	2.6 ± 0.4
6	−D	—	8.8 ± 0.6	8.6 ± 0.4	—	2.7 ± 0.3	2.6 ± 0.4
24	−D	9.3 ± 0.5	8.6 ± 0.5	9.3 ± 0.6	2.8 ± 0.3	2.5 ± 0.4	2.6 ± 0.4
6‡	−D+PTH	9.1 ± 0.6	8.8 ± 0.4	9.5 ± 0.6	2.7 ± 0.3	2.5 ± 0.4	2.5 ± 0.3
6	+D	11.2 ± 0.8	12.1 ± 0.7	11.5 ± 0.6	4.1 ± 0.3	3.5 ± 0.4	4.1 ± 0.5
6‡	+D+PTH	11.2 ± 0.8	12.0 ± 0.6	12.7 ± 0.3	4.1 ± 0.3	3.5 ± 0.4	4.9 ± 0.4

* Each value represents the mean and standard error obtained from 14 to 18 individual rats.
† A significant difference ($p < 0.01$) from appropriate sham or control groups.
‡ Each rat was given 150 U purified parathyroid hormone 1 hour before parathyroidectomy.

TABLE III

Minimal vitamin D requirements for parathyroid responsiveness

	Plasma Sham operation	Calcium Parathyroid-ectomy	Plasma Sham operation	Phosphate Parathyroid-ectomy
		mg/100 ml		
−D	4.9	4.8	10.9	12.9*
+D, 0.05 U × 6	7.4†	4.6‡	9.3	10.8*
+D, 0.50 U × 6	10.5†	8.7‡	9.5	11.2*
+D, 100 U§	10.6†	8.6‡	9.9	11.6*

* A significant ($p < 0.01$) rise in plasma phosphate after parathyroidectomy in all groups. Each group contained 8 animals.

† Significant ($p < 0.01$) rise in plasma calcium as compared to −D animal.

‡ Significant ($p < 0.01$) fall in plasma calcium as a result of parathyroidectomy.

§ The 100 U of vitamin D was administered by mouth 48 hours before blood was withdrawn. All animals were fed Diet I.

The effect of dietary calcium and phosphate. Because of the above results, the interrelation among dietary calcium and phosphate, dietary vitamin D, and parathyroid gland activity was determined. The results are shown in Table II. Of particular interest are the results obtained in animals maintained upon a high-calcium–low-phosphate diet. In these animals, parathyroidectomy had little effect upon either plasma calcium or phosphate in either the D-deficient or D-fed animals. Exogenous hormone, 150 U, given to a D-fed animal resulted in a rise in both phosphate and calcium, but had no effect in the D-deficient animal. Vitamin D caused an increase in both plasma calcium and plasma phosphate (compare plasma phosphate values in control groups given vitamin D with the control group maintained without vitamin D).

Studies with diets low in both calcium and phosphate were carried out, but because of the extreme fluctuations in plasma calcium and phosphate induced by sham operation, no definite conclusions could be drawn. This problem, however, deserves further study because D-deficient animals maintained on this diet have a high plasma calcium and low plasma phosphate (12, 13).

Vitamin D and hormone responsiveness. An effort was made to establish the minimal dose of vitamin D necessary to restore the responsiveness of D-deficient animals to endogenous parathyroid hormone. Groups of D-deficient animals were given 0.05 and 0.5 U of vitamin D each day for a period of 6 days. Appropriate groups were parathyroidectomized or subjected to sham operation, and blood was obtained 4.5 hours later. The results are shown in Table III. A total dose of 0.30 U of vitamin D given orally in divided doses is sufficient to induce a state of partial responsiveness to endogenous hormone, and a dose of 3 U is enough to correct the altered electrolyte pattern of D-deficiency.

DISCUSSION

The present data are in agreement with those of Harrison and co-workers (3) in that ordinary doses of parathyroid hormone do not alter the concentration of either calcium or phosphate in the plasma of the D-deficient rat. The present study, however, reveals that removal of the parathyroid glands from a D-deficient rat is followed by a dramatic rise in the concentration of phosphate in its plasma. The lack of change in the phosphate concentration of the plasma of the D-deficient rat given parathyroid hormone can be explained by the fact that phosphate excretion is maximally stimulated in this animal owing to the heightened production of endogenous hormone. It seems clear that parathyroid hyperplasia and hyperparathyroidism do exist in the D-deficient rat and that sufficient endogenous hormone is produced to maintain a high degree of control over phosphate metabolism. This amount of hormone, however, exerts little calcium mobilizing activity (Figure 2). Likewise, the administration of a relatively large dose, 200 to 500 U, of purified bovine hormone, while inducing severe hyperparathyroidism in the D-fed rat, has little or no effect upon the concentrations of plasma calcium or phosphate of the D-deficient animal.

The lack of change in plasma calcium after

parathyroid hormone administration or parathyroidectomy in the D-deficient rat (Figure 2, Tables I and III) might be due to depletion of bone calcium. However, this is clearly not the case. Rats maintained on Diet I without vitamin D fail to grow optimally but do not have rickets (12), and their bone ash is 51.5% compared to a 51% bone ash in rats grown on a low-calcium diet (Diet II) supplemented with vitamin D (12). Thus the D-deficient and D-fed animals employed in the study depicted in Figure 2 had comparable bone ash, although their response to parathyroidectomy was strikingly different. The important point is that by the selection of the proper diet, it is possible to study, in the rat, the effects of D-deficiency without concomitant rickets.

Nichols, Schartum, and Vaes (17) raised the question of whether or not this situation is unique to the rat. They found that D-deficient mice given parathyroid hormone respond with a rise in plasma calcium. Before coming to the conclusion that relative unresponsiveness to parathyroid hormone is unique to the D-deficient rat, a critical evaluation of this question is needed. Nichols and associates (17) present no objective criteria to establish that their mice were truly D-deficient. As shown in the present study, the unresponsiveness to hormone in D-deficiency is a relative matter. The amount of vitamin D needed to induce a state of hormone-responsiveness is quite small (Table III).

This question of the adequacy of D-deficiency should no longer be a matter of uncertainty. One of the lasting contributions of Steenbock and his co-workers was the development of procedures for the routine preparation of D-deficient animals (12, 13). The two most important features of these methods were *1*) the employment of progeny from animals maintained for generations on diets containing minimal amounts of vitamin D and *2*) the use of standardized purified diets. With these methods, it is possible to procure, with considerable assurance, vitamin D-deficient animals. The importance of employing animals with true and rather complete deficiency of vitamin D for studies concerned with defining the nature of its relationship with parathyroid gland activity cannot be overemphasized.

The question must also be raised as to the applicability of the present data to the human situation. Bernstein, Kleeman, Dowling, and Maxwell have described a patient with steatorrhea, hypocalcemia, and normal bone histology (18) in whom the administration of parathyroid extract produced no change in serum calcium or phosphate, but did increase urinary phosphate excretion. After treatment with vitamin D, the injection of the same amount of parathyroid extract resulted in a significant rise in serum calcium. Undoubtedly this patient suffered from a combined deficiency of calcium and vitamin D, but the important point is that he exhibited a relative lack of responsiveness to parathyroid hormone (as measured by serum calcium) when in the D-deficient state, but nevertheless exhibited a phosphaturia. These results are directly analogous to those in the present study, but must be interpreted with caution. It seems unlikely that D-deficiency in humans is usually as complete as that obtained in the laboratory. If this is the case, then compensatory hyperparathyroidism is sufficient to bring about the observed changes in plasma calcium and phosphate.

The observation that parathyroidectomy has little influence upon phosphate metabolism in rats fed a low-phosphorus diet confirms the data of Shikita, Tsurufuji, and Ito (19). Even large doses of hormone will not lower the plasma phosphate below 7.0 to 10.0 mg per 100 ml in rats maintained on ordinary diets, but the plasma phosphate will fall to 1.0 to 3.0 mg per 100 ml in rats on a low-phosphate diet. In this latter case, the data recorded in Table II indicate that the hypophosphatemia is not a result of parathyroid hyperactivity. In fact, in rats on a high-calcium–low-phosphate diet, indications are that the parathyroid glands exhibit minimal activity. A similar conclusion was reached by Shelling nearly 30 years ago (2).

The data in Table II do bring out one point of interest. Vitamin D given to rats on a high-calcium–low-phosphate diet produces a significant increase in plasma phosphate (in a situation of minimal parathyroid activity). This finding possibly supports the thesis of Harrison and Harrison that vitamin D has a direct effect upon the renal retention of phosphate (6), which in our present understanding of the mitochondrial systems implies that physiological concentrations of vitamin D increase the tubular reabsorption of calcium-

phosphate (similar but less striking than its effects upon promoting calcium-phosphate absorption from the gastrointestinal tract). Gran (20) has recently suggested that vitamin D promotes renal tubular reabsorption of calcium.

The most important aspect of the present study is that concerned with the relationship between the *in vivo* observations and the alterations in mitochondrial function produced by vitamin D and parathyroid hormone *in vitro*. The prediction made upon the basis of the mitochondrial responses has been verified in this study (Figure 2). The nature of the time course of events depicted in Figure 2 can be directly related to the two mitochondrial systems (Figure 1). The initial fall in plasma calcium and phosphate in the D-fed rat could be considered a reflection of calcium and phosphate movement out of the extracellular compartments owing to a decrease in the activity of a vitamin D- and hormone-dependent calcium-phosphate transport system. The subsequent rise in plasma phosphate might result from a suppression of a hormone-dependent, vitamin D-independent phosphate transport system. In the D-deficient rat the activity of the release system is already suppressed, so that a change in only the latter system occurs after parathyroidectomy. This does not imply that changes in the activities of these mitochondrial membrane systems are solely responsible for the changes *in vivo*. There are a number of reasons that make it likely that the plasma membrane of some cells possesses similar transport systems (9).

The only unexpected result was that obtained by the administration of a massive dose of hormone to a D-deficient animal (Table I). Here the mitochondrial studies would have predicted no change in plasma calcium, but there was a significant rise in this value after the administration of 2,000 U of hormone. Neither endogenous hormone (Figure 2) nor lesser amounts of exogenous hormone, 200 to 500 U, had any significant effect upon plasma calcium. Thus for these latter doses, the prediction was verified. The result with the massive dose has prompted a reinvestigation of the response to hormone of mitochondria from D-deficient rats.

The fact that a massive dose of hormone will return the plasma calcium concentration to a normal value even in the D-deficient rat is of considerable interest because it raises the question of why the parathyroid glands of the D-deficient hypocalcemic rat do not hypertrophy and increase hormone production sufficiently to compensate for the lack of vitamin D. One obvious possibility is that the demand is greater than their ability to compensate. Another is that vitamin D may play a direct role in the glands' response to hypocalcemia.

SUMMARY

The effect of parathyroidectomy and parathyroid hormone administration upon the concentrations of plasma calcium and phosphate have been measured in vitamin D-deficient rats and the results compared to those observed in D-fed animals. Also, the influence of dietary calcium and phosphate content upon these responses has been investigated. Parathyroidectomy in the D-fed animal results in a rapid fall in plasma calcium and a biphasic plasma phosphate response with an initial fall followed by a striking rise. In contrast, parathyroidectomy in the D-deficient rat is followed by no significant change in plasma calcium and an immediate rise in plasma phosphate that can be prevented by the administration of parathyroid hormone. Dietary phosphate restriction abolished the effect of parathyroidectomy in both groups of animals.

Administration of a dose of 500 U of hormone to the intact D-deficient rat had little influence upon plasma calcium; a dose of 2,000 U did increase plasma calcium. A dose of 25 U was sufficient to increase the plasma calcium of the D-fed animal. These results are discussed in relation to the effects of vitamin D and parathyroid hormone upon mitochondrial metabolism.

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