

Evidence for a Direct Action of Cholecalciferol and 25-Hydroxycholecalciferol on the Renal Transport of Phosphate, Sodium, and Calcium

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ABSTRACT Acute clearance studies were performed in stable thyroparathyroidectomized dogs to evaluate the possibility of a direct renal action of vitamin D and its biologically active 25-hydroxylated metabolite. Alterations in renal hemodynamics and serum calcium concentration were minimized and attempts at vitamin D depletion were not undertaken. Steady-state volume expansion of modest degree was employed as the control experimental situation so that an effect of the vitamin to enhance phosphate reabsorption would not go undetected because of an already maximal phosphate reabsorptive rate secondary to parathyroidectomy. Under these experimental circumstances, 10,000 U of cholecalciferol and 25–120 U of 25-hydroxycholecalciferol (25-HCC) produced significant depressions in the percentage of filtered phosphate excreted (mean declines of 39 and 47%, respectively), which were not attributable to alterations in renal hemodynamics or to changes in the levels of serum calcium or phosphate. There was an accompanying decline in sodium and calcium excretion; mean percentage excretion rates for sodium fell by 38% with vitamin D and 26% with 25HCC, and for calcium this measurement declined by 46 and 23%, respectively. Furthermore, parathyroid hormone and 25HCC produced antagonistic effects on phosphate excretion. These observations provide the first conclusive evidence for a direct (proximal) tubular action of vitamin D to promote phosphate (as well as sodium and calcium) transport.

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INTRODUCTION

Investigations of the biological effects of vitamin D have been devoted largely to evaluations of its action on the skeleton and gastrointestinal tract. While not entirely ignored, the possibility of an additional effect of the vitamin on the transport of electrolytes by the kidney has been considered at the very least substantially unproven, and at best, controversial (1–6). This relative dearth of experimental inquiry as regards vitamin D and the kidney is all the more remarkable in view of the numerous suggestions from the clinical literature that, in fact, the vitamin might possess a renal action (7–11). Those reports which are available regarding alterations in renal phosphate (and calcium) reabsorption secondary to an action of the vitamin are difficult to interpret for one or more of the following reasons. First, in intact animals, there is the distinct possibility that the changes noted could have resulted from variations in circulating levels of parathyroid hormone (PTH)¹ and/or thyrocalcitonin (7, 11, 12). Secondly, the absolute level of the serum calcium concentration and the degree of vitamin D depletion appear to be important in and of themselves in establishing the level of excretion of phosphate by the kidney (13–16). Third, the employment of chronic periods of study allows time for other homeostatic mechanisms involved in calcium and phosphorous metabolism to come into play and renders difficult the separation of any renal effects of the vitamin from those on bone and gut

¹ Abbreviations used in this paper: 1,25DHCC, 1,25-dihydroxycholecalciferol; GFR, glomerular filtration rate; 25-HCC, 25-hydroxycholecalciferol; PAH, *p*-aminohippurate; PTH, parathyroid hormone; TPTX, thyroparathyroidectomized.

(6-15, 17); and, finally, there is evidence that variations in renal hemodynamics can produce major changes in the excretion of calcium and phosphate (18-22).

So that these impediments to data interpretation could be obviated, the studies to be described were conducted in stable thyroparathyroidectomized (TPTX) animals. *Acute* rather than chronic studies of vitamin D₃ (cholecalciferol) and 25-hydroxycholecalciferol (25-HCC) effects on nephron function were studied and no attempt was made to deplete the animals of vitamin D. Additionally, any potential effects of alterations in serum calcium concentration, glomerular filtration rate (GFR), and renal plasma flow on the experimental observations obtained were minimized.

The possibility has been entertained on numerous occasions that there might be important differences between the effects of physiologic as compared to pharmacologic doses of vitamin D (1, 5). The discovery, isolation, and recent availability of synthetic 25HCC (23, 24), an important biologically active metabolite of the parent compound, with demonstrated effectiveness on bone and gut (25, 26) has allowed us to substantially reduce the amount of material employed in studying the relationship of vitamin D to renal electrolyte transport. Because of the important actions of PTH and extracellular fluid volume status on renal transport (18, 27), the interactions of these agents with that of 25HCC on the kidney were also investigated.

METHODS

28 acute clearance studies were performed on TPTX female mongrel dogs weighing 18-23 kg. Completeness of the removal of the parathyroid glands was verified by determination of the serum calcium concentration 2-4 days post-operatively, and just before each study. Animals were selected for study only if there had been at least a 30% fall in serum calcium concentration. Several of the animals developed tetany which was treated with parathyroid extract or calcium infusion, or both. When this occurred, no animal was utilized for study within 48 hr of the cessation of such therapy, and most often, at least a week elapsed during which neither agent was administered before a study was attempted. The animals were allowed to recover from the neck surgery for at least 3 days, and usually 6-7 days before clearance experiments were performed. They were maintained on a diet adequate in calcium, phosphorus, sodium, and potassium and were given 100 mg of synthroid or 60 mg of desiccated thyroid every other day.

Experimental protocol. The animals were fasted and thirsted for 16 hr before the study and received 1 cc of vasopressin tannate in oil (pitressin, Parke, Davis & Co., Detroit, Mich.) the evening before the experiment. Light anesthesia was induced with sodium pentothal (25 mg/kg) and maintained by the subsequent intermittent injection of supplemental doses. A cuffed endotracheal tube was inserted and the animals were ventilated with a Harvard respirator. After the percutaneous insertion of an intravenous cannula (Intracath, 17 gauge, Becton-Dickinson & Co., Rutherford, N. J.) in a hind leg vein, priming doses of inulin and *p*-

aminohippurate (PAH) were injected and a sustaining infusion of these substances dissolved in physiological saline solution in concentrations suitable for the performance of clearance determinations was begun. This sustaining solution, to which was added aqueous vasopressin in amounts calculated to deliver 20 mU of antidiuretic hormone per min, was administered at a rate of 1 ml/min by a Holter constant infusion pump. A polyethylene catheter was placed in the external jugular vein for blood sampling and a Foley catheter was inserted into the bladder. Urine collections were begun after an equilibration period of 45-60 min and air washout of the bladder was employed to insure completeness of collection. Volume expansion was then performed by infusing 25 ml/kg of physiological saline solution containing 4-4.5 mEq/liter calcium gluconate at 20-25 ml/min, after which urinary losses were replaced by constantly adjusting the rate of the infusion solution. Steady-state volume expansion was assumed to have been achieved when urine flow rates for five to six consecutive collection periods did not vary by more than $\pm 5\%$. At this point, one of the following experimental maneuvers was initiated.

In five animals, propylene glycol was infused with a Harvard infusion pump for periods of 25-120 min, simulating the total amounts of this vehicle administered with the vitamin or its metabolite, while the rate of the calcium-containing saline solution continued to be readjusted to match that of the urine flow. These experiments were continued for about 3 hr after the propylene glycol had been started. The total volume of propylene glycol given varied from 1.0 to 5.0 ml.

In five dogs, crystalline vitamin D₃ (obtained from Philips-Roxane Inc., Columbus, Ohio) dissolved in propylene glycol to a final concentration of 4000 U/ml was given at a rate of 0.2 ml/min until a total of 10,000 U had been infused (approximately 12.5 min). Again, urine collections were continued for 2-3 hr.

Two dogs received 10-12 U of 25HCC^a dissolved in propylene glycol, administered intravenously over a period of 3-5 min. To four dogs, 25 U of 25HCC was given, and in six animals a total of 100-120 U of 25HCC was administered. In each case the rate of infusion was always substantially less than 1 ml/min, and the total volume of vitamin-containing propylene glycol infused was never greater than 5 ml in 2 hr.

In six of the studies in which 25HCC (50-60 U/hr) was superimposed upon steady-state volume expansion, a third experimental maneuver was then employed. In these studies, PTH^b was administered intravenously in doses ranging from 15 to 60 U/hr, and an additional period of about 2 hr was allowed to elapse during which clearance determinations were continued. In six additional studies, the infusion of PTH in doses of 10-60 U/hr was superimposed on a control hydropenic state. When a new steady state had been reached (usually requiring 1½-2½ hr), the intravenous administration of 25HCC, 50-60 U/hr, was begun and continued for an additional 2 hr.

Blood was drawn at the beginning of each steady-state plateau, before each experimental maneuver, and approximately every 30-45 min throughout the studies. Blood for calcium determinations was obtained anaerobically, the serum was separated and was then ultrafiltered under oil by

^a The 25-hydroxycholecalciferol utilized in these studies was generously supplied by Dr. Hector F. DeLuca, Department of Biochemistry, University of Wisconsin.

^b Obtained as a highly purified preparation from Wilson Laboratories, Chicago, Ill.

means of centrifugation through an Amicon Centriflo centrifuge cone (Amicon Corp., Lexington, Mass.). Blood and urine calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Corp., Norwalk, Conn.) and sodium and potassium were analyzed on an internal lithium standard flame photometer (Instrumentation Laboratory Inc., Lexington, Mass.). Analysis of inulin and PAH were performed on plasma and urine according to a modification of the method of Führ, Kaczmarczyk, and Krüttgen (28) and of Brun (29), respectively. Total serum and urine phosphate were analyzed by the method of Fiske and Subbarow (30) adapted to the AutoAnalyzer.

The percentage of filtered sodium, potassium, calcium, and phosphate appearing in the urine was determined from the ratio of the clearance of each ion to that of inulin, multiplied by 100.

Standard statistical methods were employed (31).

RESULTS

Table I summarizes the data for both the control studies (group A) and for those experiments in which either vitamin D₃ (group B) or its 25-hydroxylated metabolite was administered intravenously in graded dosage (groups C, D, and E). In Table II is presented the protocol of a representative experiment. Because the animals in groups D and E behaved similarly, the data from all ten studies have been analyzed together. With the exception of the group B study, no significant change in urine flow rate occurred. GFR and renal plasma flow varied randomly in each of the groups. The infusion of the vehicle (propylene glycol) for periods of 25–125 min in the five control animals in which modest saline expansion was sustained, resulted in no change in absolute phosphate excretion, whereas a mild but significant ($P < 0.02$) increase in the percentage of filtered phosphate excreted ensued. In contrast, when 10,000 U of vitamin D₃ was given, both absolute and percentage phosphate excretion declined significantly; a mean fall of 39% from control levels in percentage excretion was obtained ($P < 0.05$)⁴ with a range of 32–44%. Whereas 10–12 U of 25HCC had no discernible effect on electrolyte transport (group C, Table I), the infusion of 25–120 U of 25HCC (groups D and E, Table I) however, resulted in major depressions in both absolute and percentage excretion rates of phosphate. In nine of the ten studies, absolute phosphate excretion fell and, as was the case in the group B experiments, the percentage of filtered phosphate excreted declined in *every* experiment. Mean fall for all ten studies (groups D and E) was 47% ($P < 0.005$, range: 13–89%). Serum phosphate concentration was essentially unchanged by each of the experimental maneuvers employed, as in the control studies.

Alterations in the renal transport of sodium and cal-

cium in response to the agents listed in Table I generally showed a parallelism. Whereas sustained saline expansion did not produce a statistically significant change in either the absolute or percentage excretion rates of sodium and calcium, the values for each of these measurements did decline significantly in the animals receiving either vitamin D₃ or 25HCC (Table I, groups B, D, and E), as was the case for phosphate. Serum ultrafilterable calcium showed no consistent change in any of the groups studied and the mean arterial blood pressure monitored in a few experiments showed no tendency towards any consistent variation. In preliminary studies performed to establish a dosage of vitamin D₃ which would produce a consistent effect on phosphate excretion, a range of 100–5000 U of this substance was found to be ineffective (data not shown).

The relationship of the changes in phosphate transport to those of calcium and sodium are depicted in Fig. 1 for both the control studies (open symbols) and those experiments in which 25HCC was administered (closed symbols). These data, plotted for each of the ions as changes in the percentage of filtered load excreted into the urine demonstrate the following. First, the control studies can be separated from the 25HCC experiments in that in the former, no change or an increase in percentage phosphate excretion occurred, while in the latter, depressions in phosphate excretion invariably resulted. Secondly, for the most part the changes in sodium and calcium excretion followed, in direction, those for phosphate. And third, in a general way, the greater the change in phosphate excretion the greater the alteration also in the transport of sodium and calcium, regardless of whether one examines the data from the control animals or from the 25HCC studies.

The data were also analyzed with regard to the time sequence of the alterations in phosphate transport induced by vitamin D₃ and its 25-hydroxylated metabolite. In Fig. 2 the chronologic course of events after the administration of these compounds is compared to the pattern of phosphate transport obtained in the control studies. Maintenance of the modest saline expansion employed in these experiments can be seen to have resulted in a mildly, ever-increasing phosphaturia as the experiments continued. Cholecalciferol produced a fall in phosphate excretion with an onset at about 50–60 min, whereas the action of 25HCC became evident at 30–40 min and peaked at about 90–120 min. Thus, not only was approximately 100–400 times as much of the parent compound required to induce changes in electrolyte transport, but these changes were of smaller magnitude than those induced by the small amounts of the metabolite employed. Furthermore, administration of 25HCC shortened the lag time of response demon-

⁴ Calculated as: $(C_P/C_{In} \times 100 \text{ [control]} - C_P/C_{In} \times 100 \text{ [experimental]}) / (C_P/C_{In} \times 100 \text{ [control]}) \times 100$.

TABLE I
Summary of the Data for Control Studies and after the

Study	V		C _{In}		C _{PAH}		U _{PV}		C _P /C _{In} × 100	
	C‡	E‡	C	E	C	E	C	E	C	E
	ml/min		ml/min		ml/min		μmoles/min		%	
A. Control animals										
D-18	6.2	4.6	140	125	352	339	9.3	15.6	4.5	6.9
D-47	12.4	13.8	52	44	77	97	15.5	14.4	25.1	31.9
D-48	6.7	9.6	27	31	60	54	13.4	17.3	34.7	41.2
D-49	10.8	9.6	78	53	—	—	13.8	11.4	23.0	28.5
D-51	3.5	3.8	30	28	—	—	15.1	14.6	25.1	25.6
Means	7.8	8.3	65	56	163	163	13.4	14.7	22.5	26.8
±SE	1.6	1.8	21	18	93	89	1.1	1.0	4.9	5.6
P	>0.5		>0.1		>0.9		>0.4		<0.025	
B. + 10,000 U cholecalciferol										
D-23	2.1	1.6	69	63	—	—	1.5	0.8	1.8	1.0
D-71	4.5	4.1	64	72	113	124	27.5	25.4	14.7	10.7
D-72	3.6	2.6	42	52	78	77	16.6	12.1	19.2	11.4
D-76	3.6	2.9	45	56	71	78	16.5	13.3	26.1	17.4
D-77	3.5	1.8	67	71	261	187	43.5	27.2	38.7	25.0
Means	3.5	2.6	57	63	131	117	21.1	15.8	20.1	13.1
±SE	0.4	0.5	6	4	44	26	7.0	4.8	6.1	4.0
P	<0.025		>0.1		>0.5		>0.10		<0.05	
C. + 10–12 U 25-hydroxycholecalciferol										
D-15	2.1	2.4	47	45	—	—	13.0	15.7	12.8	16.0
D-18	4.6	3.6	125	133	339	344	15.6	19.2	6.9	7.4
D. + 25 U 25-hydroxycholecalciferol										
D-17	4.1	3.0	51	47	—	—	24.9	11.8	20.1	10.5
D-21	4.4	2.2	43	45	—	—	3.8	1.8	6.5	3.0
D-22	2.8	6.2	40	43	274	209	22.6	1.8	33.5	3.8
D-68	2.4	1.6	40	37	105	124	21.5	9.4	23.4	11.3
E. + 100–120 U 25-hydroxycholecalciferol										
D-26	2.2	1.9	63	105	128	139	43.8	34.0	22.4	10.9
D-35	2.2	2.6	98	104	213	277	12.1	8.3	4.5	3.9
D-64	10.3	10.0	112	111	196	135	40.3	24.4	18.4	12.4
D-55	5.2	1.8	61	52	—	—	14.7	5.1	19.8	7.4
D-37	1.2	1.1	83	81	219	172	19.5	20.8	16.3	9.1
D-69	5.8	4.6	49	41	—	—	19.9	15.5	28.3	20.7
Means§	4.1	3.5	64	67	189	176	22.3	13.3	19.3	9.3
±SE	0.8	0.9	8	9	26	24	3.8	3.3	2.8	1.7
P	>0.3		>0.5		>0.5		<0.005		<0.005	

* Abbreviations: V = urine flow rate; C_{In} = glomerular filtration rate (clearance of inulin); C_{PAH} = effective renal plasma flow (clearance of PAH); U_{PV}, U_{NaV}, U_{CaV} = absolute excretion rates of phosphate, sodium, and calcium, respectively; C_P/C_{In}, C_{Na}/C_{In}, and C_{Ca}/C_{In} = the fraction of filtered phosphate, sodium, or calcium (respectively) appearing in the urine; S_P = (total) serum phosphate concentration; S_{UF_{Ca}} = serum ultrafiltrable calcium concentration. ‡ C,E = means of two to four consecutive urine collection periods during the control phase of the study (C) and at the time of peak effect of the infused vitamin or its metabolite (E). In the case of the group A and C studies, in which no effect was noted, collection periods were selected at times utilized for the group B, D, and E studies (90–120 min after the beginning of the infusion).

§ Means are those for four studies in group D and six studies in group E.

*Infusion of Cholecalciferol and 25-Hydroxycholecalciferol**

SP		U _{Na} V		C _{Na} /C _{Tn} × 100		U _{Ca} V		C _{Ca} /C _{Tn} × 100		SUF _{Ca}	
C	E	C	E	C	E	C	E	C	E	C	E
<i>mmoles/liter</i>		<i>μEq/min</i>		<i>%</i>		<i>μEq/min</i>		<i>%</i>		<i>mEq/liter</i>	
1.49	1.82	1419	1100	7.2	6.4	15.3	10.6	8.2	7.3	1.32	1.17
1.18	1.02	1814	1927	26.4	33.1	28.9	37.4	25.7	35.1	2.12	2.42
1.48	1.19	1006	1406	25.4	29.8	31.4	43.4	33.7	41.4	3.53	3.43
0.78	0.75	1675	1494	16.2	22.2	37.7	34.3	17.5	26.9	2.77	2.64
2.01	2.03	719	672	17.9	18.0	6.9	8.3	17.2	18.6	1.34	1.58
1.39	1.36	1327	1398	18.6	21.9	24.0	26.8	20.4	25.9	2.22	2.25
0.20	0.24	205	209	3.5	4.7	5.6	7.2	4.3	6.0	0.43	0.40
>0.8		>0.9		>0.05		>0.40		>0.05		>0.7	
1.20	1.26	469	295	5.3	3.5	13.9	7.6	5.8	3.8	3.50	3.27
2.93	3.30	999	804	11.4	8.0	21.2	19.6	38.8	20.4	0.86	1.33
2.08	2.02	780	603	13.0	8.0	16.9	14.0	21.4	10.9	1.91	2.48
1.40	1.37	750	626	11.8	8.1	11.4	9.2	14.7	8.3	1.73	1.95
1.69	1.52	913	460	10.7	5.0	12.5	7.7	10.6	5.6	1.76	1.91
1.86	1.89	782	558	10.4	6.5	15.2	11.6	18.3	9.8	1.95	2.19
0.31	0.37	90	85	1.3	1.0	1.8	2.3	5.7	2.9	0.43	0.33
>0.7		<0.02		<0.005		<0.02		<0.05		>0.1	
2.23	2.17	72	111	1.1	1.8	1.2	1.3	0.6	0.6	4.28	4.98
1.82	1.95	1100	770	6.4	4.3	10.6	5.6	7.3	5.1	1.17	0.82
2.43	2.44	592	256	8.3	3.8	18.9	9.2	7.7	4.3	2.41	2.32
1.36	1.32	863	430	15.2	7.3	22.6	12.3	17.4	8.7	1.52	1.61
1.70	1.11	348	515	7.0	9.4	13.1	14.6	11.1	9.9	1.49	1.70
2.34	2.24	670	431	11.8	8.3	10.6	7.3	12.7	10.9	2.12	1.84
3.09	2.99	569	477	7.8	4.8	13.4	11.1	5.4	3.0	1.89	1.74
2.76	2.02	553	604	4.2	4.2	10.8	11.0	4.6	4.7	2.34	2.23
1.94	1.83	2199	1864	15.9	13.9	36.2	34.1	14.1	11.9	2.29	2.51
1.24	1.35	1056	475	12.5	6.4	6.9	3.4	6.7	3.6	1.82	1.81
1.44	2.82	351	298	3.8	2.9	6.3	5.8	3.1	2.8	2.40	2.77
1.45	1.66	1295	1056	18.9	17.3	16.6	16.4	29.7	27.6	1.15	1.29
1.98	1.98	849	640	10.5	7.8	15.5	12.5	11.3	8.7	1.94	1.98
0.20	0.20	177	152	1.6	1.5	2.8	2.7	2.5	2.4	0.14	0.14
>0.9		<0.02		<0.02		<0.05		<0.01		>0.6	

TABLE II
*Representative Experiment: Effect of 25-Hydroxycholecalciferol and Parathyroid
 Hormone on Renal Electrolyte Transport**

Time	Specimen	V ml/min	C _{in} ml/min	C _{PAH} ml/min	U _{N_aV} μEq/min	C _{N_a} /C _{in} X 100 %	U _{PV} μmoles/ min	C _P /C _{in} X 100 %	U _{CaV} μEq/min	C _{Ca} /C _{in} X 100 %	SUF _{Ca} mEq/liter	MABP mm Hg	Hct %	
0	Begin sustaining infusion of inulin, PAH, and ADH in physiological saline solution at 1 ml/min.													
2	Inject inulin and PAH priming doses.													
54-72	Expand with physiologic saline solution (containing 4.0 mEq/liter Ca) at 25 ml/min.													
72-212	Readjust infusion rate to match urine flow.													
212-225	U ₁	2.2	98	235	504	3.8	9.4	4.9	12.2	5.2	2.39		35	
225-237	U ₂	2.4	100	194	610	4.5	10.2	5.2	11.5	4.8	2.40		24	
237-250	U ₃	2.2	95	211	544	4.2	12.6	5.2	8.6	3.9	2.32		24	
250-265	U ₄	2.3	97	219	551	4.2	11.4	4.8	10.0	4.5	2.29	120	22	
270	Begin infusion of 25 HCC, 5 U/ml at 0.191 ml/min.													
265-280	U ₅	2.0	80	196	476	4.5	9.2	4.9	8.8	5.1	2.16		22	
280-296	U ₆	2.8	108	266	667	4.7	11.2	4.7	11.7	5.4	2.00		24	
296-310	U ₇	2.1	91	257	493	4.2	8.5	4.4	10.0	5.5	2.00		23	
310-326	U ₈	2.8	110	278	654	4.4	9.4	4.2	12.1	5.2	2.11		23	
326-340	U ₉	2.4	98	276	554	4.0	7.1	3.7	9.9	4.4	2.30	140	23	
340-355	U ₁₀	1.9	95	246	438	3.2	4.2	2.2	8.8	3.8	2.43		23	
355-371	U ₁₁	1.8	96	233	404	3.1	6.2	3.0	6.5	2.8	2.42	140	23	
380	Begin infusion of parathyroid hormone, at 0.97 U/min.													
371-385	U ₁₂	1.8	84	203	410	3.8	13.1	6.8	5.6	2.7	2.48		25	
385-403	U ₁₃	2.3	75	204	510	5.6	22.4	12.6	8.2	4.3	2.54		25	
403-420	U ₁₄	2.9	87	240	639	6.0	28.9	13.4	9.4	4.4	2.47	150	25	
420-434	U ₁₅	3.1	93	222	677	5.9	27.2	11.5	9.6	4.4	2.35		26	
434-454	U ₁₆	2.9	89	236	650	5.9	22.9	9.9	9.4	4.6	2.30		26	

Dog No. 739, female, 17.0 kg, TPTX.
 Abbreviations: MABP = mean arterial blood pressure; Hct = hematocrit; other abbreviations as in Table I.

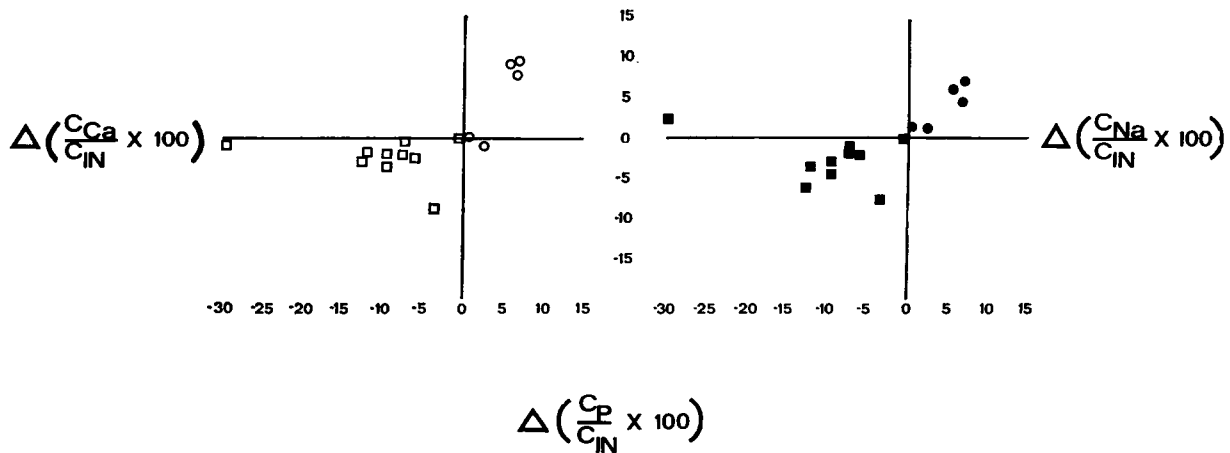


FIGURE 1 Alterations from base line levels in percentage phosphate excretion for control studies (circles) and those in which 25HCC was administered (squares) compared with the simultaneously determined changes in percentage sodium excretion (right panel) and calcium excretion (left panel). Each point represents the difference between the means for two to four consecutive collection periods obtained by subtracting the control values from those in the experimental phase of each study (see text).

strated for the pharmacologic doses of vitamin D utilized.

Combination experiments. The effect of 25HCC on the well known phosphaturic action of PTH was evaluated by superimposing the administration of each of these agents on the other. In Fig. 3 are shown the experimental observations obtained in six studies in which infusion of PTH in doses ranging from 15 to 60 U/hr was added to the continuous intravenous administration of 50–60 U of 25HCC per hr. The values represent the means of two to three consecutive collection periods during the peak effect of each phase of the experiment. PTH can be seen to have reversed the antiphosphaturia induced by 25HCC when given in dosages of 30–60 U per hr, whereas further depression of phosphate excretion induced by the metabolite occurred at the lowest dose level (15 U/hr) of PTH employed.

Reversal of the sequence of administration of these two agents resulted in the data shown in Fig. 4. In three experiments, the infusion of 25HCC at 50–60 U/hr reversed the phosphaturia due to PTH when the hormone was given at rates of 10–30 U/hr; however at higher dosage levels of PTH (50–60 U/hr) the phosphaturic action of the hormone continued unperturbed despite the addition of 25HCC. Determinations of GFR and renal plasma flow in these combination experiments revealed that the alterations in phosphate excretion induced by PTH and 25HCC could not be accounted for on the basis of variations in renal hemodynamics.

DISCUSSION

The studies herein reported were performed with the objective of evaluating any potential effect of vitamin D on the kidney in the absence of uncontrolled factors which could make interpretation of the experimental observations difficult or impossible. To this end, the following steps were taken. First, all of the experiments were undertaken in animals in which complete ablation of the thyroid and parathyroid glands had been accomplished and the animal had been allowed to achieve a state of stable hypoparathyroidism. Hypothyroidism was averted by replacement therapy. Secondly, because in the parathyroidectomized animal, phosphate excretion is frequently so low as to defy accurate measurement, steady-state volume expansion, a well known phosphaturic maneuver (18, 21, 27), was employed as the control experimental situation. Furthermore, we selected as our expanding volume an amount of saline (25 ml/kg body weight) which has been demonstrated to inhibit electrolyte transport mainly in the proximal tubule, without major effects on the distal nephron (18, 27). Third, in order to obviate any effect of alterations in serum calcium concentration per se on phosphate transport in the kidney (16, 22), calcium was included in the infusion solution. Fourth, since alterations in renal hemodynamics can, of themselves, induce changes in electrolyte handling in the nephron (18–22), we have included for consideration only those animals in which substantial variations in GFR and renal plasma flow

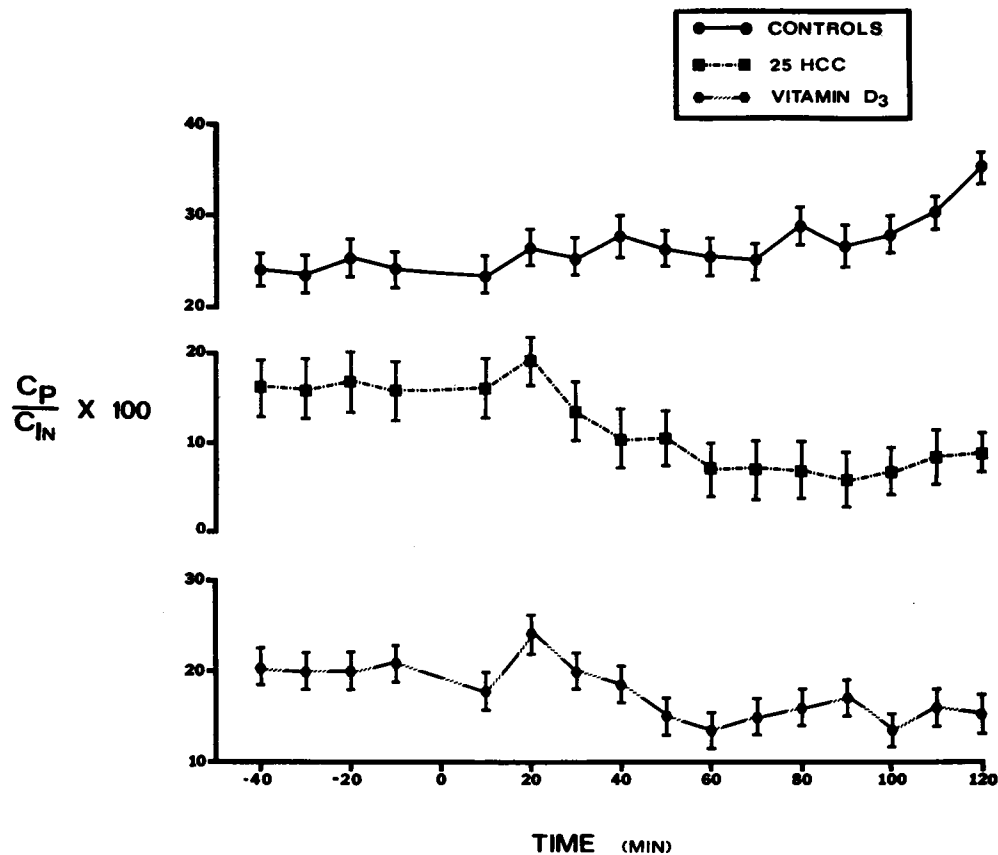


FIGURE 2 Time course of the effects of sustained saline expansion (controls, top panel), 25–120 U of 25-hydroxycholecalciferol (middle panel), and 10,000 U of cholecalciferol (bottom panel) on the percentage of filtered phosphate excreted. Each point represents the mean of all animals studied in each group at the times indicated.

did not occur. Fifth, all of the studies reported are *acute* clearance observations, since chronic investigations have the disadvantage that actions of the vitamin on gut and bone which would interfere with the interpretation of the results cannot be excluded once time has been allowed for these other homeostatic mechanisms to come into play. Finally, to eliminate any possible influence of fluctuating levels of endogenous antidiuretic hormone on electrolyte transport in the distal nephron (32), exogenous vasopressin was continuously provided throughout the study.

In the present series of experiments, the intravenous infusion of 10,000 U of vitamin D₃ resulted in a mean depression of phosphate excretion of 39% from control levels, with a concomitant decrease in the excretion of sodium and calcium. This dosage of the vitamin was arrived at by an evaluation of the effects of a wide range of doses (100–10,000 U) on phosphate excretion. Only at the 10,000 U level was there a consistent and reproducible effect of cholecalciferol within the 2–3 hr period of observation employed. The administration of

25–120 U of 25HCC likewise produced an invariable fall in the percentage of filtered phosphate which appeared in the urine (mean decrease of 47%), and also induced a small but significant decline in the percentage excretion rates of sodium and calcium (Table I and Fig. 1). These actions of both the parent vitamin and its active metabolite could be shown *not* to be attributable either to alterations in renal hemodynamics or to changes in the concentration of serum ultrafiltrable calcium since these (latter) parameters showed no consistent variation whereas phosphate excretion fell in each study. Thus, the action of cholecalciferol and that of its 25-hydroxylated metabolite on electrolyte transport would appear to be the result of a direct *tubular* effect. Furthermore, not only was as little as 25 U of 25HCC effective in enhancing the reabsorption of phosphate, but it was capable of antagonizing the phosphaturic action of volume expansion in so doing.

There is presently available a large body of data obtained both from clearance and micropuncture investigations which strongly suggests that phosphate is

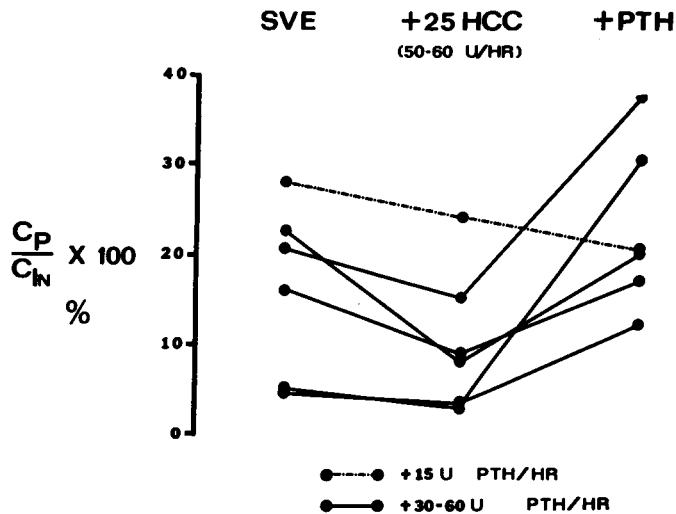


FIGURE 3 Effect on the per cent of filtered phosphate excreted of infusing graded doses of parathyroid hormone to animals receiving 25-hydroxycholecalciferol (25-HCC) at a rate of 50-60 U/hr, superimposed upon steady-state volume expansion (SVE). Each point represents the mean of two to three consecutive clearance periods. The observations shown for the first experimental maneuver were obtained after the infusion of 5HCC for 1½-2 hr, after which the infusion was continued, and the second maneuver (continuous intravenous administration of PTH) was begun. The values for the combined effects of 25HCC and PTH were obtained after the PTH had been infused for approximately 2 hr.

handled in the kidney by the processes of filtration at the glomerulus and then tubular reabsorption (18, 27, 33-39). Conclusive evidence for net phosphate secretion has not been forthcoming. Furthermore, the majority of micropuncture investigations in the rat (36, 38, 39), as well as those in the dog (18, 27), have demonstrated that phosphate reabsorption has either been completed, or else is virtually complete, by the time the tubular fluid leaves the proximal convoluted tubule. Additionally, a major proportion of filtered sodium and calcium (of the order of 70-80%) is reabsorbed in the proximal nephron (19, 40-43). Therefore, in the mammalian kidney, the proximal nephron serves as a major site for both sodium and calcium reabsorption and represents probably the sole locus of phosphate transport. Rarely, if ever, do agents which alter the transport of one ion have no effect on the reabsorption of others. Thus, for example, saline expansion can be shown to inhibit not only sodium transport but that of phosphate (18, 21, 27) and calcium (19, 20, 44, 45) as well. In addition, there are data available which indicate that when maneuvers are performed which *enhance* rather than *inhibit* fractional reabsorption, the transport of these three ions is affected similarly (18, 20, 21, 46). In this regard, clearance studies have demonstrated that aortic clamping

(for example) reduces the excretion of sodium, phosphate, and calcium (20, 21). Therefore, the action of vitamin D and its biologically active metabolite on all three of these ions is not unexpected, and indeed provides further evidence for a relationship between the tubular transport of sodium on the one hand and that of calcium and phosphate on the other. The greater magnitude of the effect of the metabolite on phosphate than on calcium and sodium reabsorption obtained in these clearance experiments is also not unexpected, since sodium and calcium transport occur throughout the entire nephron whereas phosphate reabsorption is limited to the proximal convolution. These observations also emphasize the fact that the proximal tubule is a rather important site of the action of vitamin D.

Mention must also be made of the relative potencies of cholecalciferol and 25-hydroxycholecalciferol in inducing the alterations in electrolyte transport described, and of the differences noted in the time course of events resulting from the administration of these two substances. Comparisons of the actions of vitamin D₃ and 25HCC in bone and gut test systems reveal a characteristic pattern: first, less of the active metabolite is required to induce the same (or greater) effect than

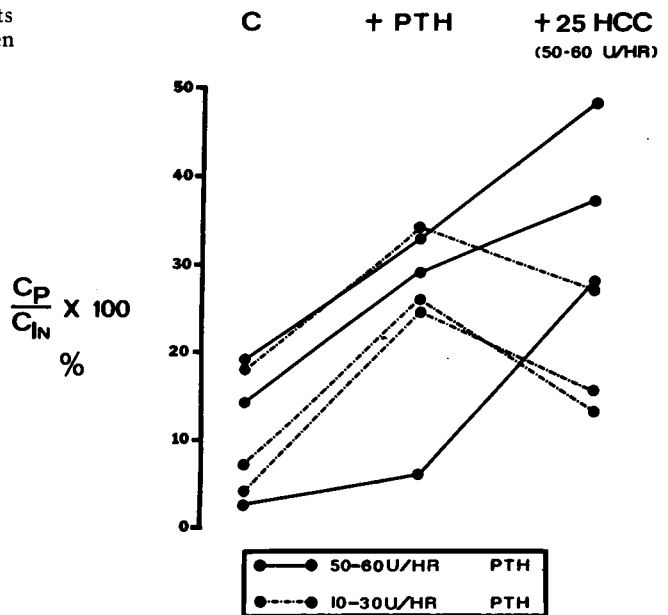


FIGURE 4 The changes induced in the percentage of filtered phosphate excreted by superimposing the infusion of 25-hydroxycholecalciferol (25HCC) at 50-60 U/hr on graded doses of parathyroid hormone (PTH), compared to control observations (C). Each point represents the mean of two to three consecutive collection periods. Experimental observations were obtained after approximately 1½-2 hr of PTH infusion. PTH was then continued and the determinations repeated when the 25HCC had been infused continuously for a period of 1½-2½ hr.

that produced by the parent compound, and secondly, 25HCC seems to shorten the time required for the biological effect to become manifest (47). Since the hydroxylation step occurs in the liver and is product inhibited (48), at least a portion of this reduction in the characteristic lag time of action of vitamin D may be a reflection of the time required for 25-hydroxylation and release of this substance into the circulation in sufficient amounts to induce biological effects. In the experimental results presented in this communication, 25HCC produced an effect on renal electrolyte transport greater than that induced by 400 times as much vitamin D₃, shortened the time required for the onset of this action, and produced a more sustained effect. These observations suggest, but do not prove, that the effect of vitamin D on the nephron may well proceed by similar biochemical and physiological mechanisms to those operative in mediating its biological actions on the skeleton and gastrointestinal tract (47).

In this regard, it seems pertinent to include in this discussion the recently reported experimental evidence which indicates that 25HCC is further converted to a more polar compound (49-51) subsequently identified as the 1,25 dihydroxylated (1,25DHCC) form of the parent vitamin (52, 53). This process occurs primarily, if not entirely, in the kidney (54, 55), and 1,25DHCC has been shown to enhance the absorption of calcium from the gut contents (56-58). Furthermore, while the metabolism of cholecalciferol both to 25HCC and 1,25-DHCC is blocked by the prior administration of actinomycin D, the biological effect of the dihydroxylated vitamin on gut calcium transport is not prevented by this inhibitor of protein synthesis, suggesting that the action of this compound does not involve DNA transcription (59). Although the action of 1,25DHCC on the kidney has not been studied, the experimental observations just cited suggest at least two plausible explanations for the delay which we have demonstrated in the manifestation of the effects of 25HCC on renal electrolyte transport. This time lag could be the result either of the time required for the conversion of 25HCC to one or more additional more polar metabolites which represent the "tissue active" forms of the vitamin in the kidney, or might be related to the necessity for new protein synthesis to take place, or both.

Additional evidence in support of the view that the vitamin does have a direct renal action and that this action is the enhancement of phosphate transport is provided by the data obtained in those experiments in which the acute effects of 25HCC and PTH were superimposed upon each other. By manipulating the dosages of the two agents, we were able to show that a distinct antagonism exists between them, which *must* be the

case if the effect of the vitamin is real and has physiological significance.

Previous studies of the effect of vitamin D on renal electrolyte transport have produced conflicting results. To a large extent, the controversy surrounding these experimental observations is the result of the fact that there exist multiple defects in experimental design which render meaningful interpretation of the data difficult. The landmark experiments of Harrison and Harrison (7), which originally suggested the possibility of a direct renal action of vitamin D, have been criticized on the basis that they were performed in intact animals so that the effect of varying PTH levels on urinary phosphate excretion related to vitamin D-induced elevations in serum calcium cannot be separated from a direct renal action of the vitamin. Similar objections can be leveled at studies in rachitic children, such as those by Klein and Gow (12) in which vitamin D in rather large doses seemed to improve phosphate reabsorption. An additional problem which complicates the interpretation of previous studies is the effect which changes in renal hemodynamics introduce. Thus, marked elevations in serum calcium have been shown to produce reductions in GFR and renal blood flow (22) which can lead to a depression of the urinary excretions of calcium, sodium, and phosphate. This fact must be taken into consideration in evaluating the studies of Gran, who noted an enhanced calcium reabsorption in rachitic puppies after vitamin D administration for several days (14) and in the interpretation of the chronic studies performed in the rachitic dog by Ney, Kelly, and Barrter (15). Investigations performed in TPTX animals are subject to yet another criticism; that is, the existence of a base line phosphate excretion before administration of the vitamin which is very low will preclude the identification of an antiphosphaturic effect of vitamin D. Such a situation exists in the acute studies of Ney et al. in the dog (15) and those of Crawford, Gribetz, and Talbot in the rat (13). In both of these investigations the administration of vitamin D to very recently PTX animals with tubular phosphate reabsorption of better than 98% resulted in no detectable alteration in phosphate excretion. Finally, the chronic administration of large doses of vitamin D may produce a mild hypercalcemia which has been shown to inhibit the tubular reabsorption of both sodium and phosphate (60). This observation could explain the phosphaturia which developed in the chronic studies of Crawford et al. (13).

When these methodological problems are taken into consideration, there remains experimental evidence which would appear to support our results. Thus, Clark and Rivera-Cordero found that the urinary phosphate

actually decreased both in intact and parathyroidectomized rats given 2500 U of vitamin D daily for 12 days, while serum calcium rose only from 3.55 to 4.06 mEq/liter—levels not expected to cause a diminished GFR—although such data are not given (17). In addition, Gekle, Ströder, and Rostock found an enhancement of phosphate reabsorption by vitamin D in the first 3 hr after the onset of its administration (39). This occurred both in normal and rachitic rats and the effect was found to be independent of changes in GFR and serum calcium concentration.

With the exception of those studies in which only 25 U of 25HCC was utilized, the dosage of vitamin D and its metabolite employed in previous studies as well as those herein reported no doubt are in the pharmacologic range. Thus, despite having shown a direct tubular effect of vitamin D on renal phosphate reabsorption there remains the question of the importance of this phenomenon physiologically. In this regard, there are available in the clinical literature several studies which provide indirect evidence in favor of this postulate (8-12). Harrison and Harrison were originally prompted to open the question of a renal action of vitamin D because of their observation that it was not possible for them to account for the hypophosphatemia encountered in some of the rachitic infants under study in their metabolic unit merely on the basis of gut loss (7). It was their observation that the low phosphate level of such patients was the result of the paradoxical loss of phosphate in the urine, and that this phosphaturia could be corrected by the administration of vitamin D (61). However, secondary hyperparathyroidism is well known to be a frequent, if not invariable, concomitant of vitamin D deficiency rickets (62-64). Therefore, theoretically the phosphaturia might represent the hypersecretion of PTH, and the reduction in the phosphaturia after vitamin D administration could be attributed to suppression of PTH elaboration by an elevation in serum calcium concentration resulting from enhancement by vitamin D of gut absorption of calcium. To counter the latter argument, one would have to demonstrate an improvement in renal phosphate reabsorption before any change in serum PTH levels, or, lacking this assay, at least before any alteration could be noted in the serum calcium level. Now that reliable PTH assays have become available, what is lacking are cases of vitamin D deficiency rickets, which has apparently almost disappeared from the American medical scene. However, there are several cases available in the literature in which serum phosphate rose (and, frequently, in which urine phosphate declined) after vitamin D administration, while no change, or *even a fall* in serum calcium concentration was occurring (8-11).

To the best of our knowledge, the observations reported here represent the first clear cut evidence for enhancement of phosphate (as well as sodium and calcium) reabsorption in the proximal tubule of the intact organism by any humoral or chemical agent. The implications of these data for the study of disease states such as hypophosphatemic rickets are obvious. Investigations aimed at evaluating the physiological significance of this phenomenon in both the experimental and human situations are now in progress.

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