Study of the Renal Tubular Interactions of Thyrocalcitonin, Cyclic Adenosine 3′,5′-Monophosphate, 25-Hydroxycholecalciferol, and Calcium Ion

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ABSTRACT Acute clearance studies were performed in thyroparathyroidectomized animals to determine the actions and interactions of thyrocalcitonin (TCT), cyclic adenosine 3′5′-monophosphate (cAMP), 25-hydroxycholecalciferol (25HCC), and calcium ion on the reabsorption of phosphate, calcium, sodium, and potassium by the kidney. The infusion of 25HCC in a dosage of 60 U/h to moderately saline-expanded animals (2.5% body weight) induced a fall in the excretion of all of the ions under study after 90–120 min similar to that observed in previous experiments from this laboratory. Mean decrements in fractional excretion were: phosphate, 42.0% ($P < 0.005$); calcium, 25.0% ($P < 0.005$); sodium, 23.4% ($P < 0.001$); and potassium, 14.7% ($P < 0.005$). The superimposition of either porcine or salmon TCT (1–100 MRC U/h for 2 h) resulted in no further alterations in electrolyte excretion. However, the infusion of TCT during steady-state saline expansion, before the administration of 25HCC, obviated the renal transport effects of the vitamin D metabolite. Both in the latter studies, as well as those in which similar doses of TCT were given to hydropenic animals, the hormone itself failed to induce any consistent alteration in electrolyte excretion. Cyclic AMP (50 mg/h) caused an increase in the excretion of phosphate, sodium, and potassium and no change in calcium excretion. Like TCT, the nucleotide blocked the action of 25HCC on the kidney. Raising the mean level of serum ultrafilterable calcium to 3.02±0.25 mEq/liter from 1.62±0.17 mEq/liter likewise prevented enhanced ionic reabsorption due to 25HCC.

INTRODUCTION

It is now well established that two of the factors which are important in controlling phosphate, calcium, and sodium transport in the nephron are parathyroid hormone (PTH)[1] and the status of the extracellular fluid volume (1–9). The actions of PTH on renal transport can be largely, but not entirely reproduced by the administration of cyclic adenosine 3′,5′monophosphate (cAMP) (10–14). Recently, two additional agents which may play significant roles in the regulation of ionic transport in the kidney have been identified. These substances, thyrocalcitonin (TCT) and vitamin D, were originally considered to have effects directed only upon the skeleton and the gastrointestinal tract. However, it has now been demonstrated that, at least in man (15–18) and the rat (19–23), TCT induces an increased excretion of sodium, calcium, and phosphate and that vitamin D_3 and both its 25-hydroxylated and 1,25-dihydroxylated derivatives can enhance the reabsorptive rates of all three ions (24, 25). Additionally, evidence is presently available which suggests that intracellular calcium ion concentration may be a major determinant of the degree to which the biological effects of these substances become manifest (26–29).

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[1] *Abbreviations used in this paper:* ADH, aqueous vasopressin; cAMP, cyclic adenosine 3′,5′-monophosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid; GFR, glomerular filtration rate; 25HCC, 25-hydroxycholecalciferol; PAH, *p*-aminohippurate; PTH, parathyroid hormone; SUF, serum ultrafilterable; TCT, thyrocalcitonin; TPTX, thyroparathyroidectomized.

The present studies were performed with the following aims: (*a*) to evaluate the efficacy of TCT and cAMP as well as alterations in extracellular fluid calcium concentration in initiating or modifying alterations in electrolyte transport in the kidney of the dog; (*b*) to investigate whether the renal effects of one or more of these agents could be shown to act either synergistically or antagonistically to the others and/or to vitamin D; and (*c*) to attempt to elucidate the mechanism(s) by which the actions of TCT, cAMP, and vitamin D on renal reabsorptive processes might be interrelated.

METHODS

Acute clearance studies, each lasting approximately 6–8 h, were performed on female mongrel dogs weighing 16–22 kg which had been thyroparathyroidectomized (TPTX) at least 3 days before their use in an experimental study. Completeness of glandular ablation was confirmed by a postoperative decline in serum calcium concentration of at least 30%, and thyroid hormone was replaced as synthroid, 0.3 mg/day by mouth. In general, tetany was treated with calcium infusion, but occasionally parathyroid extract (Lilly) was required. In these cases, the animals were allowed to stabilize for an additional period of at least 48 h before study. Vasopressin tannate in oil (1 cc, Parke, Davis & Co.) was given 16 h before the experiment, and the dogs were deprived of food and water overnight. To obviate any influence of diurnal variations in electrolyte excretion on the results of these studies, all of the experiments were performed during the same time period, which began at 8:00 a.m. Light anesthesia was induced either with sodium pentothal (25 mg/kg) or sodium pentobarbital (20 mg/kg). A retention catheter was passed into the bladder, and polyethylene cannulas were introduced into a hindlimb vein for the infusion of fluids and into a jugular vein for the withdrawal of blood specimens. An endotracheal tube was passed and the dogs were ventilated with a Harvard respirator. Inulin, *p*-aminohippurate (PAH) and aqueous vasopressin (ADH) were infused in physiological saline solution at a rate of 1 ml/min in quantities sufficient to provide plasma levels of 20–30 mg/100 ml and 1–3 mg/100 ml for the former two substances, and to deliver 30–60 mU/kg per h of the latter. After an equilibration period of 50–60 min, the following experimental maneuvers were performed.

Hydropenic studies. In five dogs, after the collection of two to four control periods, porcine or salmon calcitonin,[2] in dosages of from 1 to 100 MRC U/h was infused for a period of 2½ to 3 h during which time urine collections were continued. The powdered hormone was diluted in normal saline solution (the pH of which was adjusted to 4.0 with HCl) to an appropriate final concentration and was administered with a constant infusion pump in a volume of 0.02–0.04 ml/min. In some of the experiments, a 1–2% solution of gelatin was added to the TCT infusion for purposes of "stabilizing" the material. However, since the addition of this substance had no apparent effect, its use was discontinued.

Volume expansion studies. In these studies, animals were expanded with 25–30 ml/kg physiological saline solution containing 4–4.5 mEq/liter calcium over a 20–30 min period, after which the infusion rate was adjusted constantly to match urine flow. As in previous studies from this laboratory (24, 25), this moderate degree of volume expansion (approximately 2.5% body wt) was utilized as the "control" situation because, in hydropenic, TPTX animals, phosphate excretion is so low as to render any further decline difficult or impossible to detect (24). Steady-state expansion was considered to have been achieved when the urinary flow rate of five to seven consecutive 10-min urine collections did not vary by more than ±5%. In nine such experiments, an infusion of 25HCC[3] was then begun at a rate of 0.02–0.04 ml/min so that approximately 60 U of this substance, dissolved in propylene glycol, was delivered per hour. 2 h later, TCT in dosages from 1 to 100 MRC U/h was begun as a continuous infusion for an additional 2 h while 25HCC administration was continued.

In 11 additional experiments, after the accomplishment of steady-state saline diuresis, the sequence of administration of the two agents was reversed. TCT was begun first, in dosages of from 40 to 100 MRC U/h, and then, after 2 h, 25HCC infusion (50–60 U/h) was added while the TCT administration continued.

Another 10 animals were expanded in the same manner as that described above, but the saline solution in these studies contained calcium in a concentration of 15–20 mEq/liter. In five of these studies 25HCC was then infused for 2½–3 h, while in the other five experiments only the vehicle (propylene glycol) was given, at the same rate (0.02 ml/min) as that used for the metabolite, once steady-state expansion had been achieved.

cAMP studies. In seven experiments, dibutyryl cAMP (Schwarz-Mann) was infused intravenously at a rate of approximately 50 mg/h for 1½–2 h (in a total volume of 0.04 ml/min) in hydropenic animals. The intravenous administration of 25HCC (50–60 U/h) was then superimposed on the cAMP infusion for an additional 2 h.

Analytical methods. Blood samples were obtained at the end of the equilibration period, just before the introduction of each experimental maneuver, and at intervals of 30–45 min throughout the studies. Urine was collected every 10–30 min by means of air washout and/or manual bladder compression. Blood and urine samples were analyzed for inulin, PAH, calcium, phosphate, sodium, and potassium by methods previously described (24).

Blood for calcium and phosphate was obtained under mineral oil, the serum was separated anaerobically, and each sample was then centrifuged through a pre-wetted CF-50 centrifuge cone (Amicon Corp., Lexington, Mass.) to which oil had been added, in order to determine the ultrafilterable serum concentration of these ions.

Statistical analyses of control versus experimental data within each experimental group were performed by means of Student's *t* test for paired values. Because of the variation in the mean rates of electrolyte excretion during the control (sustained volume expansion) phase from one group of animals to another, the changes induced by 25HCC alone were compared to those obtained with the metabolite after pretreatment with TCT or after the production of hypercalcemia, by an analysis of covariance (30). Some of the statistical determinations as well as the data analyses were performed on a model PDP-10 computer (Digital Equipment Corp., Maynard, Mass.) located at the Univer-

[2] Kindly supplied by Dr. James Bastian, Armour Pharmaceutical Co., Kankakee, Ill.

[3] Generously provided by Dr. John Babcock, Upjohn Co., Kalamazoo, Mich.

TABLE I

*Single and Combined Effects of Thyrocalcitonin and 25-Hydroxycholecalciferol on Renal Electrolyte Transport and Renal Hemodynamics**

Study	Period, agent, dose	C_{In}	C_{PAH}	FF	U_PV	SUF_P	C_P/C_{In}	$U_{Ca}V$	SUF_{Ca}	C_{Ca}/C_{In}	$U_{Na}V$	C_{Na}/C_{In}	U_KV	C_K/C_{In}
		ml/min	*ml/min*		*μM/min*	*mM/liter*		*μEq/min*	*mEq/liter*		*μEq/min*		*μEq/min*	
A. Superimposition of thyrocalcitonin on the action of 25-hydroxycholecalciferol														
1	SVE	60	—	—	14.7	1.24	0.198	6.9	1.82	0.067	1056	0.125	40.2	0.154
	+25HCC, 60 U/h	52	—	—	5.1	1.35	0.074	3.4	1.81	0.036	475	0.064	11.0	0.065
	+TCT, porcine, 18 U/h	51	—	—	5.3	0.92	0.112	4.5	1.88	0.046	503	0.071	15.7	0.095
2	SVE	112	—	—	40.3	1.94	0.184	36.2	2.29	0.141	2199	0.142	129.0	0.302
	+25HCC, 60 U/h	111	—	—	25.0	1.83	0.124	34.1	2.59	0.119	1864	0.125	89.3	0.226
	+TCT, procine, 1 U/h	114	—	—	21.0	2.18	0.084	37.7	3.15	0.091	1926	0.131	88.9	0.252
3	SVE	112	205	0.55	16.8	1.17	0.132	27.2	2.07	0.118	1371	0.091	104.0	0.268
	+25HCC, 60 U/h	111	186	0.60	12.9	1.15	0.103	27.4	2.00	0.122	1256	0.085	94.0	0.278
	+TCT, porcine, 51 U/h	109	186	0.59	11.8	1.17	0.093	26.4	2.17	0.112	1209	0.086	84.0	0.230
4	SVE	72	235	0.31	29.5	1.95	0.210	6.5	1.42	0.064	619	0.061	79.3	0.249
	+25HCC, 60 U/h	74	203	0.36	24.4	1.99	0.166	2.4	1.38	0.024	230	0.022	65.2	0.226
	+TCT, salmon, 98 U/h	78	185	0.42	19.7	1.86	0.136	2.9	1.76	0.021	262	0.025	50.3	0.172
5	SVE	76	—	—	23.0	1.78	0.170	2.8	1.12	0.033	447	0.041	123.0	0.360
	+25HCC, 60 U/h	90	—	—	0.5	1.74	0.003	2.1	1.27	0.019	352	0.027	90.0	0.239
	+TCT, salmon, 62 U/h	109	—	—	3.6	1.95	0.017	2.0	1.28	0.014	415	0.025	76.6	0.176
6	SVE	43	97	0.44	3.4	2.00	0.041	8.8	1.82	0.114	516	0.086	86.0	0.439
	+25HCC, 60 U/h	40	86	0.47	1.6	2.00	0.021	5.3	2.10	0.065	414	0.076	75.0	0.392
	+TCT, salmon, 98 U/h	65	149	0.44	1.9	1.99	0.015	5.5	2.07	0.041	548	0.063	99.0	0.334
7	SVE	89	—	—	14.1	1.49	0.109	7.0	1.61	0.049	623	0.048	34.9	0.140
	+25HCC, 60 U/h	103	—	—	10.8	1.47	0.073	5.4	1.58	0.034	512	0.036	44.1	0.122
	+TCT, salmon, 58 U/h	81	—	—	16.1	1.53	0.130	5.9	1.60	0.043	444	0.039	57.2	0.186
8	SVE	67	141	0.48	33.5	2.00	0.254	17.8	0.80	0.334	1159	0.136	87.8	0.330
	+25HCC,, 60 U/h	62	141	0.42	20.1	1.95	0.165	17.7	1.08	0.263	1008	0.128	70.0	0.289
	+TCT, salmon, 70 U/h	50	142	0.35	19.4	1.82	0.214	21.4	1.20	0.360	1142	0.183	77.9	0.424
9	SVE	53	—	—	26.7	1.80	0.285	10.3	1.58	0.123	817	0.114	80.8	0.449
	+25HCC, 60 U/h	52	—	—	17.4	1.77	0.191	8.7	1.69	0.098	563	0.081	73.5	0.410
	+TCT, salmon, 45 U/h	63	—	—	21.2	1.79	0.192	12.8	1.84	0.110	684	0.082	65.5	0.295
Means	SVE	76	170	0.45	22.4	1.71	0.176	13.7	1.61	0.116	979	0.094	85.0	0.293
SEM		8	31	0.05	3.8	0.11	0.025	3.7	0.15	0.030	185	0.013	10.8	0.036
	+25HCC	77	154	0.46	13.1	1.69	0.102	11.8	1.72	0.087	742	0.072	68.0	0.250
		9	26	0.05	3.1	0.14	0.022	3.9	0.16	0.026	178	0.013	8.8	0.037
	+TCT	80	166	0.45	13.3	1.69	0.110	13.2	1.88	0.093	793	0.078	68.3	0.240
		8	12	0.05	2.6	0.14	0.023	4.2	0.19	0.036	178	0.017	8.3	0.033
P values:	25HCC vs. SVE	>0.60	>0.10	>0.50	<0.005	>0.50	<0.005	<0.01	>0.05	<0.005	<0.005	<0.001	<0.02	<0.005
	TCT vs. 25HCC	>0.50	>0.50	>0.60	>0.80	>0.90	>0.40	>0.05	<0.05	>0.60	>0.05	>0.30	>0.90	>0.70

TABLE I—(*Continued*)

B. Addition of 25-hydroxycholecalciferol to sustained infusion of thyrocalcitonin														
1	SVE	97	—	—	24.1	3.22	0.068	3.1	1.77	0.016	356	0.024	—	—
	+TCT, porcine, 42 U/h	76	—	—	19.5	3.06	0.087	2.6	1.73	0.021	317	0.033	—	—
	+25HCC 60 U/h	65	—	—	16.8	3.12	0.084	3.1	1.75	0.027	310	0.036	—	—
2	SVE	52	98	0.53	26.6	2.42	0.213	6.6	1.58	0.056	336	0.044	47.9	0.245
	+TCT, procine, 42 U/h	36	81	0.44	14.5	2.23	0.180	2.8	1.68	0.047	169	0.033	26.5	0.201
	+25HCC 60 U/h	41	103	0.40	16.2	2.23	0.178	2.2	1.55	0.035	152	0.026	38.2	0.250
3	SVE	83	251	0.33	26.0	1.41	0.222	49.7	2.11	0.284	2149	0.205	136.0	0.355
	+TCT, salmon, 98 U/h	72	245	0.29	18.4	1.20	0.214	46.6	2.45	0.264	1986	0.223	153.0	0.431
	+25HCC 60 U/h	85	234	0.36	23.1	1.47	0.184	47.8	2.23	0.252	2079	0.199	131.0	0.405
4	SVE	37	79	0.47	9.0	1.35	0.183	9.7	2.14	0.124	513	0.109	50.4	0.362
	+TCT, salmon, 98 U/h	32	64	0.50	8.8	1.21	0.228	10.0	1.93	0.163	550	0.137	52.6	0.443
	+25HCC 60 U/h	33	69	0.48	8.9	1.18	0.234	10.5	1.94	0.152	507	0.124	44.0	0.428
5	SVE	55	86	0.64	18.1	2.44	0.134	5.9	1.36	0.078	583	0.071	122.0	0.482
	+TCT, salmon, 92 U/h	55	90	0.61	19.2	2.50	0.141	5.6	1.36	0.075	553	0.072	57.6	0.315
	+25HCC 60 U/h	54	133	0.41	23.0	2.43	0.178	6.7	1.32	0.094	601	0.080	62.2	0.322
6	SVE	70	—	—	10.2	3.94	0.117	9.8	1.64	0.087	499	0.050	71.7	0.265
	+TCT, salmon, 70 U/h	71	—	—	17.8	3.71	0.212	8.0	1.56	0.073	507	0.051	58.7	0.235
	+25HCC 60 U/h	60	—	—	23.1	4.48	0.269	5.6	1.80	0.052	347	0.041	40.6	0.189
7	SVE	102	—	—	8.2	1.91	0.042	5.4	1.57	0.034	562	0.039	72.6	0.174
	+TCT, salmon, 49 U/h	108	—	—	18.2	1.86	0.090	4.4	1.58	0.026	629	0.042	98.5	0.219
	+25HCC 62 U/h	100	—	—	7.3	1.91	0.038	4.2	1.38	0.031	540	0.039	49.1	0.135
8	SVE	75	163	0.46	0.9	1.72	0.007	3.9	1.83	0.029	63	0.006	36.0	0.135
	+TCT, salmon, 49 U/h	92	155	0.59	1.3	1.87	0.008	4.8	1.65	0.032	195	0.015	62.3	0.173
	+25HCC 62 U/h	92	132	0.70	4.8	2.09	0.025	2.8	1.71	0.018	234	0.019	66.5	0.198
9	SVE	61	91	0.67	23.1	2.25	0.169	5.9	1.20	0.081	559	0.064	63.5	0.421
	+TCT, salmon, 49 U/h	63	92	0.68	21.3	2.17	0.156	6.0	1.43	0.066	763	0.084	89.7	0.413
	+25HCC, 62 U/h	65	128	0.51	17.9	2.18	0.127	5.3	1.28	0.064	672	0.076	85.5	0.442
10	SVE	56	92	0.61	15.1	1.80	0.154	6.8	1.04	0.123	504	0.068	52.5	0.309
	+TCT, salmon, 49 U/h	47	112	0.42	13.1	1.57	0.177	5.9	1.09	0.115	474	0.072	61.9	0.410
	+25HCC 62 U/h	53	114	0.46	15.0	1.71	0.164	6.3	1.04	0.113	549	0.075	76.7	0.472
11	SVE	68	—	—	9.8	1.87	0.078	4.3	1.17	0.055	209	0.022	42.9	0.211
	+TCT, salmon, 49 U/h	67	—	—	14.0	2.00	0.108	4.4	1.19	0.056	218	0.023	43.0	0.213
	+25HCC 62 U/h	57	—	—	12.1	1.91	0.111	2.6	1.11	0.042	144	0.018	31.3	0.155
Means	SVE	69	123	0.53	15.6	2.21	0.126	10.1	1.58	0.088	576	0.064	69.5	0.296
±SE		±6	24	0.05	2.6	0.24	0.021	4.0	0.11	0.022	165	0.016	10.6	0.035
	+TCT	65	120	0.50	15.0	2.13	0.146	9.2	1.61	0.085	578	0.071	70.4	0.305
		±7	24	0.05	1.8	0.23	0.020	3.8	0.11	0.022	152	0.018	11.3	0.034
	+25HCC	64	130	0.47	15.3	2.25	0.145	8.8	1.56	0.080	558	0.067	62.5	0.300
		±6	19	0.04	1.9	0.27	0.023	4.0	0.11	0.021	162	0.016	9.4	0.041
P values:	+TCT vs. SVE	>0.30	>0.50	>0.50	>0.80	>0.05	>0.05	>0.05	>0.60	>0.50	>0.90	>0.05	>0.90	>0.70
	+25HCC vs. TCT	>0.60	>0.25	>0.50	>0.80	>0.10	>0.90	>0.30	>0.20	>0.10	>0.40	>0.10	>0.20	>0.70

* Each value represents the mean of two to four consecutive collection periods during steady-state moderate saline expansion (SVE), or during the constant infusion of 25-hydroxycholecalciferol (+25HCC) or thyrocalcitonin (+TCT) or both. Abbreviations: C_{In} = glomerular filtration rate (clearance of inulin); C_{PAH} = effective renal plasma flow; FF = filtration fraction; U_PV, $U_{Ca}V$, $U_{Na}V$, U_KV = absolute excretion rates of phosphate, calcium, sodium, and potassium, respectively; C_P/C_{In}, C_{Ca}/C_{In}, C_{Na}/C_{In}, C_K/C_{In} = fractional excretion rates of phosphate, calcium, sodium and potassium, respectively; SUF_P, SUF_{Ca} = serum ultrafilterable concentrations of phosphate and calcium.
For results of statistical analysis by covariance, see text.

TABLE II

*Effects of Thyrocalcitonin Infusion in Hydropenic Animals**

Study phase	C_{In}	C_{PAH}	FF	U_PV	C_P/C_{In}	SUF_P	$U_{Ca}V$	C_{Ca}/C_{In}	SUF_{Ca}	$U_{Na}V$	C_{Na}/C_{In}	U_KV	C_K/C_{In}
	ml/min	*ml/min*		*μM/min*		*mM/liter*	*μEq/min*		*mEq/liter*	*μEq/min*		*μEq/min*	
H	67	149	0.49	18.6	0.132	2.26	2.3	0.025	1.50	142	0.016	42.1	0.182
	±9	±39	±0.11	±5.3	±0.039	±0.25	±0.9	±0.013	±0.20	±58	±0.007	±10.4	±0.053
+TCT	64	123	0.47	14.9	0.114	2.15	1.9	0.027	1.37	131	0.016	43.0	0.197
	±6	±18	±0.08	±4.6	±0.038	±0.24	±0.5	±0.011	±0.19	±37	±0.005	±10.3	±0.046
P‡	>0.70	>0.60	>0.70	>0.25	>0.25	>0.40	>0.50	>0.80	<0.05	>0.80	>0.50	>0.80	>0.80
						n = 5							

n = number of studies; Other abbreviations as in Table I.

* Data given are the means (±SEM) for five experiments. In each study, the means of two to four consecutive collection periods during control, hydropenic (H) conditions were compared to those obtained following 2½–3 h of thyrocalcitonin (TCT) infusion.

‡ Computed utilizing Student's t test for nonindependent variables.

sity of Pennsylvania Medical School Computer Facility, by means of a remote teletype unit.

RESULTS

Combination studies: 25HCC and TCT. In Table I, part A, are given the results of those studies in which the infusion of TCT was superimposed upon the renal effects of 25HCC. It has previously been determined in this laboratory that the administration of propylene glycol alone, during sustained volume expansion of this degree leads either to no change (25) or a slight rise (24) in the excretion of phosphate, sodium, and calcium as the expanded state is maintained. Similar to the findings in the former studies (24), the administration of 25HCC to the saline-expanded animals resulted in an invariable fall in the absolute and fractional excretion rates for phosphate, sodium, and calcium in each of the nine studies. In addition, potassium excretion also declined. The absolute excretion rates of phosphate (U_PV), sodium ($U_{Na}V$), calcium ($U_{Ca}V$), and potassium (U_KV) fell by 41.5% ($P < 0.005$), 24.2% ($P < 0.005$), 13.9% ($P < 0.01$), and 20.0% ($P < 0.02$), respectively. There was a mean reduction in fractional phosphate excretion of 42% from control levels ($P < 0.005$), and the decrements for sodium, potassium, and calcium were 23.4% ($P < 0.001$), 14.7% ($P < 0.005$), and 13.9% ($P < 0.01$), respectively. Furthermore, these decreases in electrolyte excretion were not accompanied by any significant variation in GFR (C_{In}), effective renal plasma flow (C_{PAH}), or filtration fraction (FF), nor were there any consistent changes in serum ultrafilterable (SUF) calcium or phosphate. When porcine (three experiments) or salmon (six studies) TCT in dosages from 1 to 100 MRC U/h was added to the infusion of 25HCC, the absolute and fractional excretion rates of the ions under study, as well as the measurements of renal hemodynamics and the value for SUF phosphate, remained stable. There was a mild increase in the SUF calcium value which was statistically significant at the 0.05 level.

In the second set of experiments, the order of administration of the two agents was reversed (Table I,

TABLE III

Bioassay of Thyrocalcitonin Employed in the Infusion Studies[4]

Specimen	Concentration	Mean final serum (±SD) calcium level	Estimated potency	Calculated potency
Aliquot No. 1		8.99±0.17	40 MRC U/ml	28 MRC U/ml
Standard		8.58±0.17		
Aliquot No. 2		8.17±0.32	30 MRC U/ml	31.5 MRC U/ml
Standard	2.6 mU/100 g body wt	9.19±0.16		
Standard	8.0 mU/100 g body wt	8.22±0.20		
Aliquot No. 3		8.25±0.37	60 MRC U/ml	58.4 MRC U/ml
Standard	2.6 mU/100 g body wt	9.19±0.16		
Standard	8.0 mU/100 g body wt	8.22±0.16		

TABLE IV

*Renal Transport Effects of Cyclic Adenosine 3',5'-Monophosphate Infusion and of Superimposed 25-Hydroxycholecalciferol**

Study	Period	V	C_{In}	U_PV	SUF_P	C_P/C_{In}	$U_{Ca}V$	SUF_{Ca}	C_{Ca}/C_{In}	$U_{Na}V$	C_{Na}/C_{In}	U_KV	C_K/C_{In}
		ml/min	*ml/min*	*μM/min*	*mM/liter*		*μEq/min*	*mEq/liter*		*μEq/min*		*μEq/min*	
1	C	0.3	53	1.6	2.69	0.011	0.8	1.10	0.014	57	0.007	37.3	0.152
	+cAMP	1.8	50	28.1	1.97	0.295	5.0	1.05	0.103	439	0.065	67.8	0.366
	+25HCC	2.0	51	32.6	1.36	0.472	4.9	1.18	0.081	465	0.063	48.0	0.261
2	C	0.6	68	9.7	1.84	0.078	2.1	1.58	0.020	183	0.018	61.2	0.214
	+cAMP	2.8	75	18.9	1.50	0.230	6.5	1.40	0.062	588	0.053	136.0	0.537
	+25HCC	1.3	61	24.2	1.34	0.297	4.2	1.43	0.049	354	0.040	67.4	0.352
3	C	0.2	33	5.2	2.54	0.062	0.3	1.50	0.006	22	0.004	18.0	0.129
	+cAMP	0.3	30	17.2	1.74	0.329	0.4	1.54	0.009	92	0.020	27.9	0.258
	+25HCC	0.3	40	24.3	1.50	0.413	0.4	1.58	0.007	93	0.016	10.9	0.075
4	C	0.3	63	5.9	2.00	0.047	0.2	1.31	0.002	49	0.005	13.5	0.050
	+cAMP	0.7	66	23.1	1.70	0.206	0.2	1.47	0.002	176	0.018	56.2	0.216
	+25HCC	0.9	63	33.8	1.44	0.372	0.3	1.40	0.003	205	0.022	37.0	0.145
5	C	0.1	48	3.7	2.12	0.037	0.1	1.08	0.002	37	0.006	9.0	0.047
	+cAMP	0.4	75	26.2	1.79	0.195	0.2	1.21	0.002	126	0.013	36.6	0.121
	+25HCC	0.4	61	37.2	1.40	0.439	0.2	1.17	0.003	134	0.016	37.1	0.164
6	C	0.5	128	53.7	2.12	0.197	0.7	1.36	0.004	115	0.006	22.4	0.075
	+cAMP	1.1	152	74.6	1.82	0.273	3.1	1.36	0.015	394	0.019	33.6	0.091
	+25HCC	1.1	118	67.3	1.26	0.458	1.9	1.40	0.017	343	0.022	29.2	0.084
7	C	0.1	65	6.8	2.50	0.036	1.9	0.80	0.005	4	0.0004	3.3	0.013
	+cAMP	0.2	50	30.8	2.26	0.273	2.0	0.75	0.006	15	0.0021	20.8	0.102
	+25HCC	0.2	71	43.0	1.94	0.345	2.0	0.95	0.008	36	0.0032	25.1	0.092
Means	C	0.3	65	12.4	2.26	0.067	0.9	1.25	0.008	67	0.007	23.5	0.097
±SE		±0.1	12	7.0	0.12	0.023	0.3	0.10	0.003	24	0.002	7.5	0.027
	+cAMP	1.0	71	31.3	1.83	0.257	2.5	1.25	0.028	261	0.027	54.1	0.242
		±0.4	15	7.5	0.09	0.018	0.9	0.10	0.015	80	0.009	15.0	0.062
	+25HCC	0.9	66	37.5	1.46	0.399	2.0	1.30	0.024	233	0.026	36.4	0.168
		±0.2	9	5.6	0.09	0.024	0.7	0.08	0.011	60	0.007	6.8	0.039
P values:	cAMP vs. Control	<0.05	>0.30	<0.001	<0.005	<0.001	>0.05	>0.80	>0.10	<0.02	<0.05	<0.02	<0.01
	25HCC vs. cAMP	>0.50	>0.50	<0.05	<0.005	<0.005	>0.10	>0.20	>0.10	>0.40	>0.90	>0.10	>0.05

* Each value represents the mean of two to four consecutive collection periods obtained during the control, hydropenic phase of each experiment (C), after 2 h of cyclic AMP infusion (+cAMP) at 50 mg/h, and after an additional 1½–2 h of cyclic AMP with superimposed 25-hydroxycholecalciferol (+25HCC) at 60 U/h. V = urine flow rate. Other abbreviations as in Table I.

part B). Superimposed upon steady-state saline diuresis, TCT failed to induce any consistent variation either in absolute or in fractional excretion rates for any of the ions in the 11 studies. As in the previous experiments, GFR, C_{PAH}, and FF, as well as the SUF calcium concentration were not altered by the hormone. However, as opposed to its effects when administered first, when the 25HCC was added in those animals which were undergoing a constant infusion of TCT, there occurred no reductions in electrolyte excretion as are usually associated with 25HCC administration (Table I, part A). This lack of effect of 25HCC, furthermore, could not be attributed to changes in renal hemodynamics or to alterations in the serum values of calcium or phosphate. Thus, although unable to exert any direct effects on electrolyte excretion itself, TCT did prevent the action of 25HCC from becoming apparent.

The alterations induced in electrolyte excretion by 25HCC alone (Table I, part A) were compared to those produced by the metabolite after pretreatment of the animals with TCT (Table I, part B) by an analysis of covariance. Alterations in absolute as well as fractional excretion rates for phosphate, sodium, and calcium obtained with 25HCC differed significantly from those observed after TCT administration, as follows: U_PV, $P < 0.025$; C_P/C_{In}, $P < 0.01$; $U_{Ca}V$, $P < 0.025$; $C_{Ca}C_{In}$, $P < 0.05$. While the changes in fractional potassium excretion with, versus without TCT, were also significant ($P < 0.05$), those for U_KV failed to reach the 95% confidence level, most likely because of the wide variation in base line values for the individual studies (Table I, parts A and B).

Hydropenia: TCT studies. In order to rule out the possibility that prior volume expansion had obscured any potential effects of TCT on renal-electrolyte transport, the infusion of this agent was performed in five hydropenic animals. Mean values for the parameters under study during control and experimental periods

TABLE V

*Effects on Renal Function of Sustained Increase in Extracellular Fluid Calcium with and without the Superimposition of 25-Hydroxycholecalciferol**

Study	Period	C_{In}	C_{PAH}	FF	U_{PV}	SUF_P	C_P/C_{In}	$U_{Ca}V$	SUF_{Ca}	C_{Ca}/C_{In}	$U_{Na}V$	C_{Na}/C_{In}	$U_{K}V$	C_K/C_{In}
		ml/min	*ml/min*		*μM/min*	*mM/liter*		*μEq/min*	*mEq/liter*		*μEq/min*		*μEq/min*	
A. Sustained hypercalcemic volume expansion with superimposed propylene glycol infusion														
1	SVE	39	104	0.38	14.0	2.50	0.145	10.5	3.10	0.086	261	0.046	63.8	0.316
	+PG	40	101	0.40	16.0	2.53	0.240	17.9	3.74	0.121	427	0.075	67.3	0.340
2	SVE	37	—	—	23.8	2.73	0.240	5.4	2.46	0.059	303	0.059	55.3	0.340
	+PG	29	—	—	17.9	2.80	0.219	3.3	2.27	0.050	203	0.052	54.3	0.424
3	SVE	63	166	0.38	29.7	1.93	0.245	49.4	3.53	0.223	1104	0.137	76.0	0.391
	+PG	50	128	0.39	21.4	1.46	0.300	44.4	4.13	0.218	762	0.115	62.2	0.387
4	SVE	67	127	0.53	40.8	2.23	0.269	135.9	5.08	0.399	2251	0.260	154.0	0.886
	+PG	64	110	0.58	34.6	1.65	0.381	135.6	5.49	0.450	2128	0.278	154.5	1.383
5	SVE	81	199	0.41	6.6	2.20	0.187	28.3	2.61	0.134	1044	0.099	74.3	0.325
	+PG	63	163	0.39	8.0	2.10	0.197	22.7	2.90	0.124	626	0.078	50.5	0.282
Means	SVE	57	149	0.43	23.0	2.30	0.217	45.9	3.40	0.180	993	0.120	84.7	0.452
±SE		±9	±21	±0.04	±6.0	±0.14	±0.022	±23.8	±0.47	±0.061	±361	±0.038	±17.7	±0.109
	+PG	49	126	0.44	19.6	2.10	0.267	44.8	3.70	0.193	829	0.120	77.8	0.563
		±7	±14	±0.05	±4.4	±0.25	±0.033	±23.6	±0.55	±0.070	±338	±0.041	±19.4	±0.206
P		>0.05	>0.05	>0.30	>0.10	>0.10	>0.10	>0.60	>0.05	>0.30	>0.10	>0.90	>0.25	>0.30
B. Sustained hypercalcemic volume expansion with superimposed 25-hydroxycholecalciferol infusion														
1	SVE	33	62	0.53	9.4	2.61	0.110	7.6	3.04	0.076	154	0.034	52.8	0.342
	+25HCC	26	55	0.47	5.8	2.62	0.085	4.6	3.38	0.052	86	0.024	61.2	0.432
2	SVE	36	88	0.41	27.4	2.31	0.330	17.9	2.59	0.192	551	0.111	51.3	0.396
	+25HCC	27	95	0.29	21.3	2.38	0.329	13.4	2.50	0.197	383	0.103	44.5	0.442
3	SVE	106	321	0.39	36.9	2.37	0.148	18.7	2.92	0.061	595	0.042	95.0	0.220
	+25HCC	111	271	0.41	29.7	1.81	0.148	18.5	2.46	0.068	564	0.037	99.5	0.220
4	SVE	71	176	0.40	30.2	1.34	0.324	86.6	2.68	0.462	1352	0.143	114.0	0.578
	+25HCC	51	103	0.50	28.3	1.69	0.333	88.8	3.49	0.501	1411	0.216	140.0	1.070
5	SVE	26	86	0.30	20.5	1.76	0.117	28.3	4.22	0.263	466	0.128	66.5	0.690
	+25HCC	17	86	0.20	19.9	1.67	0.119	19.3	2.88	0.392	328	0.138	53.2	0.840
6	SVE	71	—	—	37.3	2.30	0.230	56.3	2.66	0.301	1803	0.175	94.7	0.326
	+25HCC	61	—	—	28.4	1.79	0.261	93.8	3.22	0.479	2070	0.247	99.3	0.480
Mean	SVE	57	147	0.41	26.9	2.12	0.210	35.9	3.02	0.226	820	0.106	79.1	0.425
±SE		±13	±48	±0.04	±4.3	±0.19	±0.041	±12.2	±0.25	±0.062	±254	±0.023	±10.6	±0.071
	+25HCC	49	122	0.37	22.1	1.99	0.211	39.7	2.99	0.282	806	0.128	83.0	0.581
		±14	±38	±0.06	±3.7	±0.17	±0.044	±16.6	±0.18	±0.083	±314	±0.037	±14.9	±0.128
P		>0.05	>0.10	>0.40	<0.02	>0.40	>0.90	>0.60	>0.90	>0.10	>0.80	>0.20	>0.50	>0.05

For results of statistical analysis by covariance, see text.

* Each value is the mean of two to four consecutive collection periods obtained during sustained hypercalcemic volume expansion (SVE) of modest degree, or after 2½–3 h of propylene glycol (PG) or 25-hydroxycholecalciferol (25HCC) infusion. Abbreviations as in Table I.

are given in Table II. Despite the absence of saline expansion, TCT can be seen not to have altered fractional ionic excretion or renal hemodynamics. Neither did absolute excretion rates demonstrate any significant change. However, TCT did reduce the level of SUF calcium in these hydropenic animals from 1.50±0.20 to 1.37±0.19 mEq/liter ($P < 0.05$).

Because of the failure of TCT to demonstrate biological activity in the volume expansion experiments, three aliquots of the material utilized in these studies as prepared for administration in our laboratory were returned to the manufacturer for analysis at various times throughout the course of this investigation. The TCT was assayed for its ability to induce hypocalcemia in rats according to a standardized assay procedure (31).[4] The final mean serum calcium levels obtained (five rats per group) compared to a standard hormone preparation were as shown in Table III.

Combination studies: cAMP and 25HCC. The infusion of dibutryl cAMP at a rate of 50 mg/h was performed in 7 hydropenic animals (Table IV). The nucleo-

[4] Kindly performed for us by Dr. James Bastian, Head, Department of Pharmacology, Armour Pharmaceutical Co., Kankakee, Ill.

tide produced an increase in both the absolute and fractional excretion rates of phosphate, sodium, and potassium. The numerical rise in absolute and fractional calcium excretion did not reach statistical significance. Reflective of the marked phosphaturia invariably induced, SUF phosphate declined from 2.26±0.12 to 1.83 ±0.09 ($P < 0.005$). GFR was unchanged, while urine flow rate tripled. When the administration of 25HCC (50–60 U/h) was added to the continued infusion of dibutryl cAMP, the usual effect of the metabolite on electrolyte excretion was obliterated. In fact, mean absolute and fractional phosphate excretion continued to rise (from 31.3±7.5 to 37.5±5.6 μM/min, $P < 0.05$, and from 0.257±0.018 to 0.399±0.024, $P < 0.005$, respectively) under the influence of the cyclic nucleotide, whereas the excretion of sodium, calcium, and potassium showed no further change with the superimposition of the 25HCC infusion. Neither did there occur any further consistent alteration in the SUF concentrations of calcium or phosphorus, nor in GFR or urine flow rate.

Effects of calcium concentration on 25HCC action. In Table V, part B, are given the experimental observations obtained when the infusion of 25HCC was performed in animals prepared in exactly the same manner as those detailed in the studies presented in Table I, part A, except that the concentration of SUF calcium in these moderately expanded animals was first raised. A value of 3.02±0.25 mEq/liter was obtained compared to that of 1.62±0.17 mEq/liter determined in the former studies (Table I, part A). This elevation of SUF calcium to hypercalcemic levels resulted in the failure of 25HCC to induce reductions in electrolyte excretion with the exception of a mild decline in U_PV from 27.0± 4.4 to 22.1±3.7 μM/min ($P < 0.02$). However, C_P/C_{In} showed no essential change (0.210±0.041 → 0.211± 0.004, $P > 0.90$), suggesting that the fall in U_PV was the result of the simultaneous numerical declines in GFR and SUF phosphate which were themselves not statistically significant. The level of SUF calcium in these studies was not significantly different from that of 3.40± 0.47 mEq/liter noted in those experiments in which the vehicle alone was administered (Table V, part A). In the latter studies, there was likewise no significant alteration in renal hemodynamics, and neither U_PV nor any other parameter of electrolyte excretion demonstrated any consistent variation.

As was the case in the TCT-pretreated group (Table I), statistically significant differences were obtained by analysis of covariance for each of the fractional and absolute excretion rates, with a single exception, when the changes produced by 25HCC in hypocalcemic animals (Table I, part A) were compared to those induced by the metabolite in hypercalcemic dogs (Table V, part B). In this group of studies, $U_{Ca}V$ did not reach statistical significance, most likely because of the varying base line levels of calciuria resulting from the infusion of large amounts of calcium. Results of the covariant analyses were: U_PV, $P < 0.05$; C_P/C_{In}, $P < 0.01$; $U_{Na}V$, $P < 0.05$; C_{Na}/C_{In}, $P < 0.025$; $U_{Ca}V$, $P > 0.10$; C_{Ca}/C_{In}, $P < 0.05$; U_KV, $P < 0.025$; C_K/C_{In}, $P < 0.025$.

The 25HCC utilized in these studies, which was obtained as a single lot, was suspended in propylene glycol and refrigerated. Its continued biological activity was verified by the fact that the studies were performed in random order; in particular, the final experiment in this series was that listed as number 9 in Table I, part A, in which the metabolite produced its expected action on electrolyte transport.

DISCUSSION

The experiments described in this communication were prompted by the findings obtained in this laboratory during a previous study of the renal actions of vitamin D and PTH (24, 25). When base line phosphate excretion in TPTX animals was elevated by means of mild volume expansion, it was possible to demonstrate that the acute effect of vitamin D and its metabolites on the kidney is the enhancement of phosphate, sodium, and calcium reabsorption (24, 25). Furthermore, except when given in low dosage, PTH, administered before the infusion of 25HCC, could be shown to prevent these effects of the metabolite on phosphate transport from becoming manifest (24). It therefore seemed pertinent to investigate the interactions of other agents which are important in controlling renal phosphate (as well as calcium and sodium) transport with regard to their capacity to influence the renal effects of vitamin D. Accordingly, the relationships between TCT, cAMP, 25HCC, and serum calcium concentration on ionic excretion were explored, a mechanism which might explain their individual and combined effects was proposed, and an attempt was made to evaluate this proposal. Major emphasis has been placed on the study of alterations in the excretion of the *phosphate* ion, not only because of the predominant effect of PTH on its renal reabsorption, but also because, unlike sodium and calcium, the bulk of its tubular transfer appears to be accomplished in the *proximal* nephron (32–34).

The determination as to whether TCT does or does not possess a renal tubular action seems to depend not only upon the conditions of the experiment, but also on the species studied. Kenny and Heiskell first reported that the subcutaneous injection of a crude extract of thyroid gland induced a phosphaturia and subsequent hypophosphatemia, as well as hypocalcemia without calciuresis, in the intact rat (21). Subsequently, Robinson, Martin and MacIntyre, utilizing a somewhat more purified hormone preparation in PTX rats, showed an acute increase in phosphate excretion pre-

sumably not related to GFR changes (since urinary creatinine excretion did not change) (20). Rasmussen and his colleagues demonstrated that TCT led to a non-sustained phosphaturia in TPTX rats, which effect could be augmented by the simultaneous constant infusion of PTH. In their experiments, the urinary excretion of sodium was also increased, but the excretion rates of magnesium, calcium, and hydroxyproline were decreased by TCT. Furthermore, the effect of the hormone on phosphate excretion could be reproduced by reducing plasma calcium with EGTA to the same degree as that resulting from TCT, itself. They therefore suggested that at least part of the renal effect of TCT on phosphate excretion could be due to hypocalcemia, per se (19). In man, several studies have now demonstrated calciuria and phosphaturia in addition to natriuresis in normal subjects as well as hypoparathyroid patients to various dosages of porcine, salmon, and synthetic human calcitonin (16–20).

Our own studies with TCT in the dog demonstrated no effect of this hormone on renal electrolyte transport. In the combined studies of TCT and 25HCC action, TCT administration was superimposed upon steady-state volume expansion. Since the latter is a maneuver which has been demonstrated repeatedly to result in a phosphaturia as well as a natriuresis (2–6), it is possible that the action of TCT on the kidney could have been masked. For that reason, TCT infusion was performed also in hydropenic dogs, in which experiments the same result was obtained. These data agree with the findings of others who have given TCT to TPTX dogs either intravenously (35, 36) or directly into the renal artery (36, 37). TCT did result in a decline in serum calcium concentration in the dogs studied by Pak, Ruskin, and Casper (35) and in the experiments of Clark and Kenny (36), whereas we could document a reduction in SUF calcium only in our hydropenic animals (cf. Tables II and I, part B). This state of events is most likely explained by the fact that in the volume expansion studies, a substantial amount of calcium was administered in the infusion mixture, which was not the case in the hydropenic experiments. Despite its lack of effect on urinary electrolyte excretion, TCT did, in fact, invariably block the usual antiphosphaturic, antinatriuretic, and anticalciuric action of 25HCC (Table I). A somewhat analogous situation has recently been reported by Olson, DeLuca, and Potts (38), who studied calcium absorption in isolated vascularly perfused rat intestine. Whereas the infusion of 10 mU of purified porcine TCT into the arterial perfusate produced an immediate fall in intestinal calcium absorption in rats fed vitamin D, it had no effect on the intestines of vitamin D-deficient rats (38).

Details of the cellular processes by which the vitamin promotes an increased uptake of calcium, phosphate, sodium, and potassium by the renal tubular cell are, at present, poorly understood. In the gut, it has been suggested that a binding protein may effect the translocation of calcium, and that vitamin D induces the formation of this protein (39, 40). Indeed, a similar material has been isolated in the kidney (41). However, the fact that the tubular transfer of so many ions is affected by the metabolite suggests a more general action of the vitamin, perhaps related to alterations in membrane permeability, as has been suggested for intestinal calcium and phosphate transport (42). An additional possibility is that vitamin D exerts an effect on specific ATPases located in or near the basal aspect of the cell (43).

The mechanism by which PTH acts on the kidney is thought to involve activation of the adenyl cyclase system in cells of the renal cortex (2, 10–14, 44–47). In a previous series of studies, PTH was administered in a range of doses both before and after the provision of a standard amount (50–60 U/h) of 25HCC (24). Since cAMP infusion can mimic the effects of PTH, at least as regards proximal tubular function (2, 48), and since a major portion of the action by 25HCC on renal transport is thought to occur proximally as well (24), studies were performed in which 25HCC infusion was superimposed upon cyclic AMP administration. In each case, intravenous cAMP induced a rise in the excretion of sodium, potassium, calcium, and phosphate and, as had been demonstrated with TCT (Table I), succeeded in preventing the action of 25HCC on the transport of these ions from becoming manifest (Table IV).

The similar effects of TCT, cAMP, and PTH (24) in blocking the action of 25HCC on renal tubular function could be explained on the basis that each of these agents affects electrolyte transport by a separate mechanism, and that their respective abilities to prevent enhanced reabsorption due to the metabolite are entirely unrelated. Alternatively, since 25HCC does not appear to be the "tissue active" form of vitamin D in the kidney (24, 25), it is possible that these agents prevent the conversion of 25HCC to that derivative of the vitamin which acts directly on the renal tubular cell. While experiments to test this postulate have not been performed, nevertheless there is available recent evidence which indicates that while TCT does, in fact, inhibit the conversion of 25HCC to 1,25DHCC, on the other hand, PTH and cAMP *enhance* this reaction (49, 50). However, if 1,25DHCC must be further metabolized before the kidney-active derivative is formed (25), this hypothesis would have to be reevaluated.

A third contingency, which has the virtue of providing a unifying concept, is that intracellular calcium concentration is a major factor in the regulation of renal electrolyte transport. This thesis is prompted by the results of in vitro studies designed to examine the cellular events effected by the actions of PTH, TCT, and

cAMP. Data obtained by Borle in his studies of calcium flux rates in kidney cells grown in tissue culture (29, 51–53), as well as experimental observations from Rasmussen's laboratory in which isolated renal tubules were utilized as the test system (26, 27, 54, 55) have provided indirect evidence for the view that all three of these agents increase intracellular calcium content. We reasoned, therefore, that an increment in calcium concentration within the cell could have been the common factor responsible for preventing the vitamin D metabolite from enhancing calcium and phosphate (as well as sodium and potassium) uptake by the renal tubule. Since increases in extracellular calcium have been shown to result in elevations in the intracellular content of this ion (52, 56, 57), we decided to test this hypothesis by evaluating whether merely increasing extracellular fluid calcium before administering the metabolite would have the same effect as did both of the hormonal substances and the cyclic nucleotide. In fact, this indeed proved to be the case (see Table V, part B) in that an approximately twofold increase in SUF calcium concentration abolished the usual effect of 25HCC on electrolyte excretion, whereas similar calcium elevations had no effect on transport, in and of themselves (Table V, part A).

Therefore, to the extent that it is possible to extrapolate from the in vitro data cited above (29, 51–55), our results are entirely compatible with the view that alterations in intracellular calcium concentration may play an important role in the regulation of renal transport, although in no sense can these observations be considered to constitute conclusive proof of this thesis. Rather, these data should serve to provide a working hypothesis, which will require further rigorous testing. The mechanism(s) by which alterations in intracellular calcium concentration might change renal electrolyte transport is unclear. The suggestion has been made that the ionic milieu of the cell may alter intermediary metabolism and thus affect the energy systems which fuel ionic pumping (27). It seems likely that a complex series of interrelated biochemical and physicochemical reactions will ultimately be recognized as responsible for the interactions of humoral and ionic substances on renal tubular transport, an example of which interplay has been described in this report.

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