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STUDIES ON THE RENAL CLEARANCE OF DIFFUSIBLE CALCIUM AND THE ROLE OF THE PARATHYROID GLANDS IN ITS REGULATION§

Calcium is excreted by the kidneys after tubular reabsorption of its diffusible (non-protein-bound) component. There is no evidence to date to suggest that tubular secretion of calcium occurs in the mammalian nephron. In fact, recent studies utilizing the stop-flow technique indicate that calcium is reabsorbed in both the proximal and distal portion of the nephron.[1] The stop-flow studies[1] together with observations on the effect of osmotic diuretics (mannitol, sucrose, NaCl, and $NaHCO_3$) and metabolic inhibitors (phloridzin, dinitrophenol, sodium azide) on calcium excretion, indicate that this ion is reabsorbed by a process of active tubular transport.[2,3,4] The complexing of calcium ion in the extracellular fluids (and therefore in the glomerular filtrate) by organic or inorganic anions such as sulfate,[4] phosphate,[2,3] citrate,[5] or versenate,[2,3] markedly decreases the tubular reabsorption of calcium and may increase calcium excretion from a normal value of 1-5 per cent to 60-80 per cent of the filtered load.[2,3,4] These observations

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suggest that calcium is actively reabsorbed in the ionic form, that complexed calcium is largely excreted, and that factors directly altering the tubular reabsorption of calcium do so by affecting the ionic component.

As 95-99+ per cent of the filtered calcium is normally reabsorbed, a change in reabsorption of only 5 per cent could cause a six-fold alteration in calcium excretion. Furthermore, a 5 per cent increase in the filtered load of calcium caused by an increased glomerular filtration rate or a rise in the proportion of the diffusible fraction without a change in per cent reabsorbed could increase the normal daily excretion of calcium four-to five-fold. These illustrations and the problem of variable alterations in calcium complexes indicate the difficulty of interpreting variations in the renal excretion or clearance of calcium.[2,6] However, available evidence supports the concept that for any given filtered load or degree of complex binding, calcium excretion depends on homeostatic mechanisms which regulate active tubular reabsorption and assist in the usual maintenance of ionic serum calcium within normal limits.

Certain observations suggest that parathyroid secretion may be one of the main homeostatic factors controlling tubular reabsorption of calcium. Low phosphorus diets or diets with a high calcium-to-phosphorus ratio are associated with parathyroid atrophy or hypofunction[7,8,9] and significantly increased calcium excretion.[10,11,12] Prolonged intravenous calcium infusions or the rapid liberation of osseous calcium into the extracellular fluids (bone tumors, hyperthyroidism, immobilization) also produce secondary hypofunction of the parathyroids* and hypercalciuria.

Conversely, high phosphorus or low calcium diets (low calcium-to-phosphorus ratio) and extra-osseous disorders leading to calcium depletion (chronic diarrhea, steatorrheas, and vitamin D deficiency) cause significant decreases in calcium excretion and hyperplasia of the parathyroid glands.[7-9,12-18] The above observations suggest that conditions causing parathyroid overactivity are accompanied by a *decreased* renal clearance of calcium, while those causing hypofunction of the parathyroids are accompanied by an *increased* clearance.

Despite hypocalcemia, the excretion of calcium may be normal or even somewhat elevated in primary hypoparathyroidism, particularly of recent origin (postsurgical). Furthermore, one is struck by the frequency of normal calcium excretion in hyperparathyroidism with hypercalcemia, provided that renal function is intact. In fact, to the authors' knowledge,

* In unpublished observations the authors have observed that patients with non-parathyroid disorders causing hypercalciuria with or without hypercalcemia (bone tumors, hyperthyroidism, etc.) may respond to a calcium loading test[18] with a phosphorus excretion pattern resembling hypoparathyroidism.

primary hyperparathyroidism is the only clinical disorder in which hypercalcemia may occur without increased urinary calcium excretion. It was first postulated in 1952 by Talbot that parathyroid hormone increases the tubular reabsorption of calcium.[14] More recently, the studies of Talmage, Kraintz, and Buchanan[15,16] have conclusively shown that parathyroidectomy *increases* and administration of parathyroid extract *decreases* the renal clearance of calcium in the rat. The present study concerns the effects of parathyroid activity on the renal clearance of diffusible calcium in man and dog.

METHODS AND PROCEDURES

CLINICAL STUDIES

1. *General methodology.* The studies were performed on 24 healthy males between the ages of 24 and 45 years, 3 hypoparathyroid patients (postsurgical), and 5 patients with hyperparathyroidism secondary to parathyroid adenomas. In all experiments in which infusions of calcium were given, 1 gm. of calcium ion, as the chloride or gluconate salt,* was administered over a four-hour period (8:30 a.m. to 12:30 p.m.). Approximately one-half-hour clearance periods were obtained one hour before, during, and two to four hours after discontinuing the calcium infusion. A mild water diuresis (3-5 cc./min.) was continually maintained, and the subjects reclined throughout, standing only to void.

Venous samples of blood for calcium determination were collected anaerobically (under oil) at the mid-point of each clearance period and transferred to Lavietes ultrafiltration units.[17] Anaerobic ultrafiltration was carried out at room temperature for 14-16 hours. Analysis of the ultrafiltrates for T.C.A. precipitable material was uniformly negative. Samples obtained at two-to four-hour intervals during ultrafiltration showed no significant differences in calcium concentration. The pH of the final ultrafiltrate has always been within 0.1 pH unit of the original anaerobically collected plasma. Replicate determinations of diffusible calcium on the same plasma sample set up in separate ultrafiltration units agreed within ± 2 per cent; however, repeated determinations of diffusible calcium on fasting samples from the same individual, under standardized dietary conditions, for four consecutive mornings disclosed a maximum percentage deviation from the mean of 7.5 per cent.

No significant difference between simultaneously drawn arterial and venous samples was detected. Samples of plasma obtained at one-minute intervals for five minutes after

* Calcium chloride was administered in the paired studies with and without parathyroid extract. In all other experiments, calcium gluconate was used. Howard, Wilde, and Malvin[1] have recently suggested that the organic anion, gluconate, may interfere with the tubular reabsorption of calcium; and Chen and Neuman[2] observed that calcium administered as the gluconate was excreted more rapidly than calcium as the chloride. It is possible, therefore, that had calcium chloride been used throughout the present studies, the rate of change and the peak calcium clearances attained might have been somewhat lower. However, we are unable to compare the calcium chloride with the calcium gluconate experiments because they were carried out on different patients undergoing different types of studies. For comparison, paired experiments using the chloride and gluconate salts must be done on the same individual.

applying the tourniquet to the arm indicated that *this* duration of venous congestion did not significantly alter the concentration of calcium in the plasma or ultrafiltrate. All syringes and glassware used were washed with double glass-distilled water; all chemical analyses were done in duplicate. Plasma calcium was corrected to its concentration in plasma water by measuring serum proteins by the copper sulfate-specific gravity method. Calcium was analyzed in all fluids by the method of Yanagisawa[18] as modified by Kingsley and Robnett.[19] Phosphorus was determined by the method of Fiske and Subbarow. The technique for inulin clearance measurement and the analysis of inulin and creatinine are described in previous publications from this laboratory.[20]

Crude defatted beef parathyroid glands and human parathyroid glands obtained from autopsy material* were purified through the stage of ultrafiltration by a modification of the method of Rasmussen.[21] The defatted glands were extracted with sub-boiling hydrochloric acid (0.2 per cent) for five minutes (stage I). This acid extract was rapidly filtered, cooled, and re-extracted with cold acetone. The acetone precipitate was discarded and the supernatant (stage II) was ultrafiltered at 4° through a visking membrane for 24 hours. The ultrafiltrate was stage III.

2. *Experiments:* (a). The effect of Lilly parathyroid extract (PTE)** on the clearance of diffusible calcium during a calcium infusion was determined in paired studies (with and without PTE) on seven normal subjects and two patients with hypoparathyroidism maintained on constant calcium and phosphorus diets. Two-hundred units of the Lilly PTE were administered intramuscularly every two hours, beginning two hours before initiating the experiment. A total of 800--1,000 units was usually given.

(b). Six normal subjects had comparable paired clearance studies during calcium infusions after they had ingested either a high calcium-low phosphorus diet (Ca 3 gm., P 0.35 gm.) or a low calcium-high phosphorus diet (Ca 0.2 gm., P 2-3 gm.) for five days. PTE was not administered to this group. All paired studies were separated by an interval of one week. One hypoparathyroid patient on a high calcium-normal phosphorus diet received only one calcium infusion without a paired study.

(c). The acute effect (three-five hours) of a single intramuscular injection of the beef and human extract on calcium clearance was observed in the three hypoparathyroid patients.

(d). Five normal subjects were maintained on a constant calcium and phosphorus diet (Ca 1 gm., P 1.5 gm.) for six-seven days. After two control days, 200 units of Lilly PTE were given intramuscularly every six hours for two or three days; two recovery days followed. Urine was collected in 12- or 24-hour periods, and bloods were collected at the beginning, mid-point, and end of each period for the determination of calcium clearance.

(e). Four normal subjects on constant calcium and phosphorus intakes (Ca 1 gm., P 1.5 gm.), after two control days received 20-30 gm. of calcium carbonate orally for

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three days, followed by four recovery days. Calcium carbonate ingestion was then resumed for an additional three days accompanied by 800 units of Lilly PTE per day. Appropriate blood and urine collections were obtained to determine the relationship of the calcium concentration in the serum to its urinary excretion.

(f). Five patients with primary hyperparathyroidism were studied before and after adenoma removal for alterations in calcium clearance.

TABLE 1. THE EFFECT OF DIET AND PARATHYROID EXTRACT ON THE ENDOGENOUS CLEARANCE OF DIFFUSIBLE CALCIUM*

Patient	$\frac{C_{CA}}{C_{I_n}}$ X 100†	*Serum calcium (mg.%)*	$\frac{C_{CA}}{C_{I_n}}$ X 100	*Serum calcium (mg.%)*
	High calcium-Low phosphorus		*Low calcium-High phosphorus*	
Av.	2.0	9.0	0.3	9.7
Bu.	3.6	8.9	1.8	8.4
Ro.	5.2	9.0	1.0	9.0
Ke.	5.0	9.2	1.0	8.2
Ga.	3.3	10.0	0.5	8.7
Mo.	2.8	10.0	0.2	9.0
	Without PTE		*With PTE*	
Ho.	2.5	9.4	1.6	9.0
Co.	5.8	9.2	2.8	9.8
Ma.	0.7	8.2	0.2	8.6
Fo.	2.8	9.1	1.5	9.1
Sh.	4.7	9.8	2.0	10.0
Na.	1.8	9.3	1.1	11.8‡
Me.	8.0	9.2	1.8	9.0
Hypoparathyroid Patients				
Bu.	21.	6.5	0.8	6.8
Ma.	1.6	7.1	0.7	8.5

* The mean of two control periods prior to the infusion of calcium.

† $\frac{\text{Calcium clearance}}{\text{Inulin clearance}}$ X 100.

‡ This subject received PTE for two days prior to as well as on the experimental day.

ANIMAL EXPERIMENTS

Six parathyroidectomized and two sham-operated dogs were studied.

(a). The immediate effect of parathyroidectomy on serum calcium levels and calcium clearances were followed in six animals.

(b). In four parathyroidectomized dogs, the acute effects of intramuscular injection of beef and human parathyroid extract on calcium clearance were studied.

In all experiments the "clearance" of calcium was calculated as the ratio of the observed clearance to that of the simultaneously measured inulin or creatinine $\left(\frac{C_{Ca}}{C_{In}} \times 100\right)$. This represents the per cent of the filtered load excreted or the calcium cleared per 100 cc. of glomerular filtrate in cc. per minute. This calculation standardized

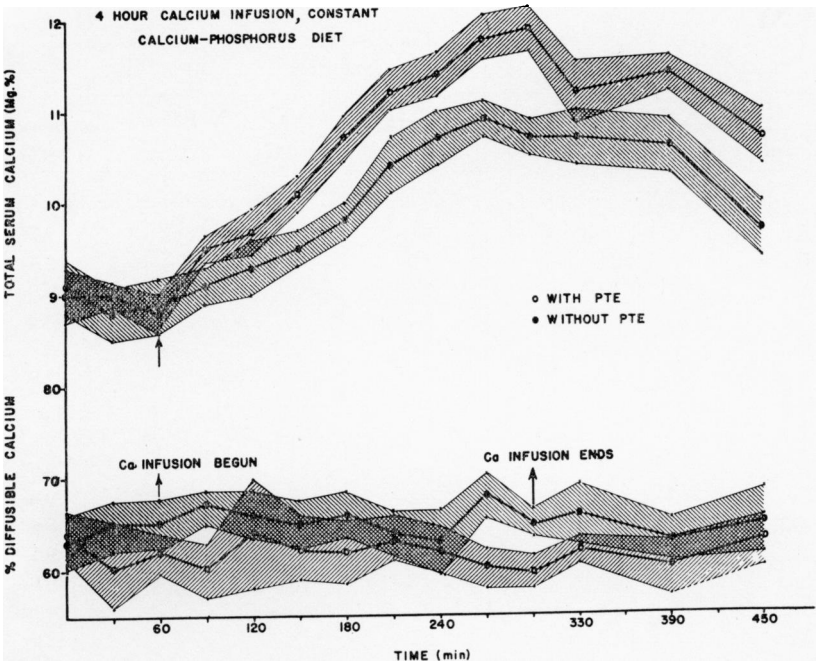

FIG. 1. The effect of a constant infusion of calcium on mean total and diffusible (%) calcium of the serum. The shaded areas represent standard errors of the mean values.

the clearances for variations in size of the individuals and corrected for changes in filtered load not due to changes in plasma concentration of diffusible calcium.

The effect of all the studies mentioned above on the metabolism and excretion of phosphorus will be published separately.[23]

RESULTS

THE EFFECT OF DIET AND LILLY PTE ON THE ENDOGENOUS CLEARANCE OF DIFFUSIBLE CALCIUM

Table 1. Endogenous calcium clearances were obtained during the control periods prior to the infusion of calcium. The mean clearance on the high calcium-low phosphorus diets was 3.7, while on the low calcium-high phosphorus diets it was 0.8 (Group b, page 5). The mean clearance on the

1 Gm. calcium-1.5 Gm. phosphorus diet without PTE was 3.8; with PTE it was 1.6 (Group a, page 5). The differences in clearances caused by diet and PTE are significant at the 1 and 5 per cent levels, respectively, without a statistically significant difference in serum values.

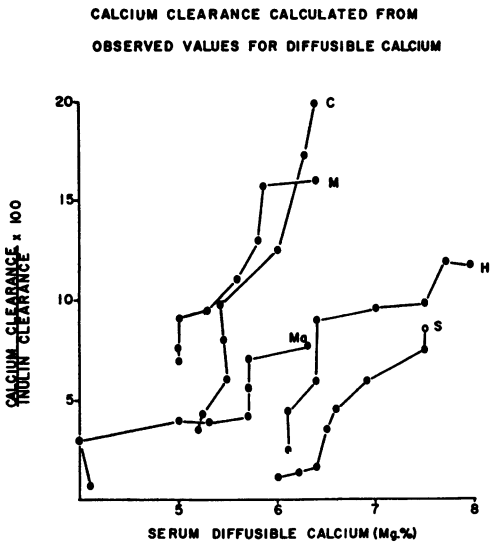

FIG. 2. The renal clearance of calcium calculated from the observed values of diffusible calcium in the ultrafiltrate. Each curve represents a separate subject.

Following his paired study, it was determined that patient Me. (Table 1) probably had idiopathic hypercalciuria. This accounted for his high initial clearance value. The number of subjects studied does not allow us to calculate a reliable mean endogenous calcium clearance for a heterogenous population. This is evident from the overlap of the clearances of the subjects on the high calcium and normal calcium diets. The clearances of two hypoparathyroid patients were within the range observed in the control subjects on normal calcium and phosphorus diets despite distinctly reduced levels of serum calcium. These patients had not been receiving vitamin D.

THE EFFECT OF CALCIUM INFUSIONS ON THE RENAL CLEARANCE AND SERUM PARTITION OF CALCIUM

Figure 1 illustrates the change in total and per cent diffusible calcium in the serum before, during, and after the infusion (Group a, page). The subjects on a normal calcium and phosphorus diet showed an approximate increment in the total serum calcium of 2 mg. per cent during the infusion with a gradual fall toward normal in the subsequent 2½ hours. The mean value for the per cent diffusible calcium (64.0, S.E.±2.5 per cent did not change significantly during this period. This value (64.0) was extremely close to the mean of all subjects observed in the present study (65.1 per cent). The increment in serum calcium when PTE was simultaneously administered was approximately 3 mg. per cent. Again no significant change in per cent diffusible calcium occurred. Although not shown, the pattern

of change in serum calcium was not significantly altered when the subject switched from the high calcium-low phosphorus to the low calcium-high phosphorus diets.

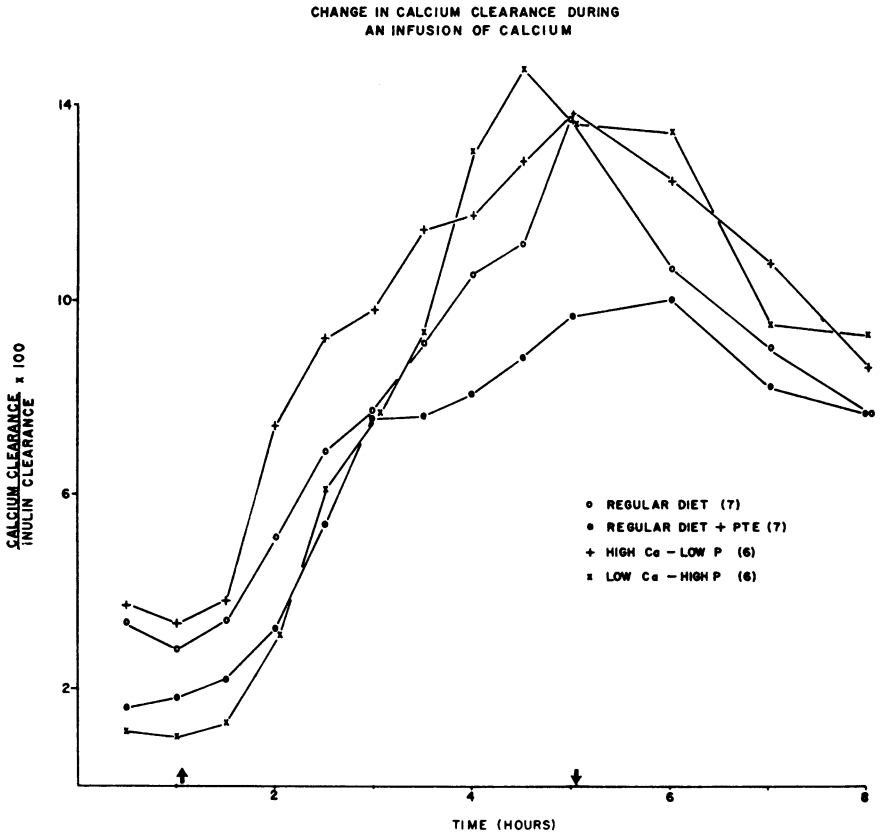

FIG. 3. The diffusible calcium clearance before, during, and after the infusion of calcium plotted against time. Each curve represents the mean of the values obtained from each individual in his respective experimental group. The numbers in parentheses after the group identifications represent the number of subjects. The arrows indicate the beginning and end of the infusion.

The period-to-period variation in per cent diffusible calcium, shown for group a in Figure 1, was great enough in each subject to account for the irregularity in the clearance curves illustrated in Figure 2. These curves were typical of all subjects studied. This degree of variability in per cent diffusible calcium seemed greater than could be accounted for by errors in obtaining the samples, the ultrafiltration, or the chemical analysis. How-

ever, this random variation in diffusible calcium probably represents an undisclosed technical variable rather than a true physiological change in calcium-binding to plasma proteins. Therefore, in each individual experiment, calcium clearance has been calculated using a mean figure for the per cent diffusible calcium. The latter was obtained for *each experiment* by dividing the sum of the determined per cent diffusible calcium from each mid-point blood by the number of samples collected. The use of mean values

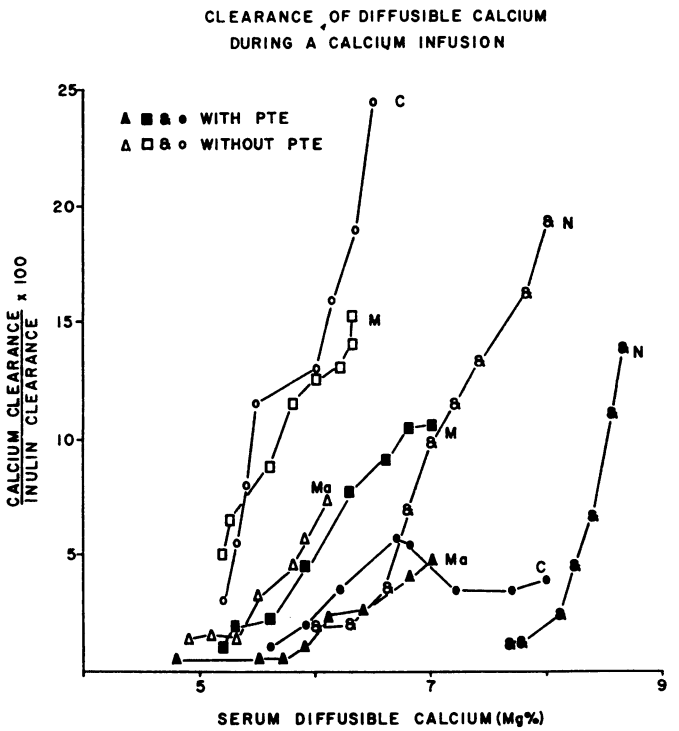

FIG. 4. The effect of parathyroid extract (Lilly) on the diffusible calcium clearance during a calcium infusion. The curves represent the paired experiments on four subjects.

for per cent diffusible calcium did not alter the nature of the results obtained.

In all subjects, the four-hour infusion of calcium caused a progressive rise in calcium clearance. Mean values for the normal subjects at the end of the infusion ranged from 10 to 15 units. The clearances began to decrease soon after terminating the infusion (Fig. 3). This pattern could be correlated with the simultaneous changes in serum calcium or filtered load of this ion (Fig. 1). The decrease in the clearance of calcium that accompanied

TABLE 2. COMPARISON OF CALCIUM CLEARANCE AT COMPARABLE CONCENTRATIONS OF DIFFUSIBLE CALCIUM DURING THE INCREASE AND DECREASE IN SERUM CALCIUM

Patient	*Serum diffusible calcium (mg.%)*	*CCa* ratio with increasing serum calcium*	*CCa* ratio with decreasing serum calcium*
Ho.	6.3	9.2	8.5
	6.7	9.4	9.1
	7.1	14.9	11.2
Ma.	4.9	3.0	6.3
	7.2	7.2	4.0
Fo.	6.5	4.8	4.3
	6.9	7.1	7.1
	7.3	15.6	8.5
	7.4	15.0	12.4
Sm.	7.0	9.0	6.6
	7.6	7.9	6.4
Co.	5.6	11.3	11.9
	6.1	12.7	12.2
	6.3	18.3	14.0
Me.	5.5	13.5	11.2
	5.8	15.7	13.1
Av.	6.3	5.7	11.3
	6.4	9.8	13.5
Bu.	6.6	9.0	9.0
	7.6	12.0	12.1
Ro.	7.2	12.3	11.8
	7.3	9.9	17.2
Ke.	5.8	12.8	14.8
	7.0	17.0	13.6
	7.8	19.8	17.2
Ga.	10.5	11.0	13.1
	11.3	13.3	12.9
Mo.	7.8	4.5	4.7
	8.1	5.3	5.7
	8.6	6.4	6.1
	8.8	6.4	6.3

* $\frac{\text{Calcium clearance}}{\text{Inulin clearance}} \times 100.$

the falling filtered load differs from the pattern observed following a single large intravenous injection of calcium. In the latter circumstance in both humans [28] and dogs,[2] the maximal clearance is observed one-two hours *after* the peak blood level is attained. This difference may be due to an artifact created by urinary dead space and the extremely rapid changes in serum calcium that accompany a single injection; or it represents a physiological "lag" in the rise in calcium clearance masked by the continuous infusion of calcium used in the present studies. The absence of even a relative increase in calcium clearance after discontinuing the infusion is evident from the data presented in Table 2. For any given concentration of diffusible calcium, the clearance is either the same or somewhat higher on the ascending limb of the clearance curve.

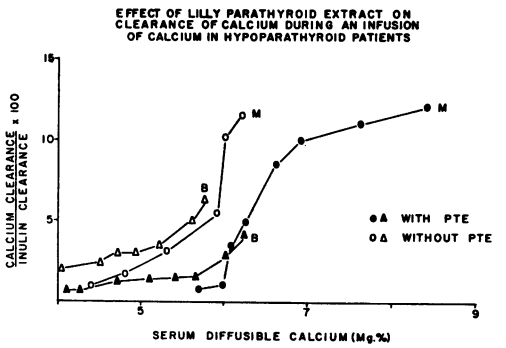

FIG. 5. The effect of parathyroid extract (Lilly) on the diffusible calcium clearance during a calcium infusion in hypoparathyroid patients. The curves represent the paired experiments on two patients.

Variations in the degree of water diuresis during the calcium infusion did not affect the rate of calcium excretion. This was also noted by Chen and Neuman.[2]

1. *The effect of PTE on calcium clearance.* In all subsequent figures only the calcium clearances during the calcium infusion have been plotted. PTE caused a significant reduction in calcium clearance *for any given level of diffusible calcium* (*or filtered load*) in four of the seven normal subjects in this group (Fig. 4) and in two hypoparathyroid patients (Fig. 5). It is apparent that the general pattern of the curves was similar for each individual but differed markedly from subject to subject. In three of the paired studies in this group PTE did not cause a significant reduction in calcium clearance (Fig. 6).*

One hypoparathyroid patient (La.) not receiving PTE in a paired study has been included. Her endogenous and peak calcium clearance (11 and 30.5) were the highest observed in any subject (Fig. 7). She ingested a

* This was not due to inactive extract, at least with regard to phosphorus excretion, for in these three studies PTE caused a marked increase in the clearance of phosphorus.[23]

TABLE 3. TUBULAR REABSORPTION OF CALCIUM (MG./MIN) AS FILTERED LOAD INCREASED

Patient	*Control*	*1 Hour*	*2 Hours*	*3 Hours*	*4 Hours*	*Control*	*1 Hour*	*2 Hours*	*3 Hours*	*4 Hours*
	High Phosphorus—Low calcium diet					*High calcium—Low phosphorus diet*				
Mo.	12.9	12.7	12.8	15.4	15.9	12.8	16.0	16.4	26.3	26.6
Av.	7.4	7.5	7.2	7.4	7.8	8.1	6.1	7.5	8.8	10.5
Bu.	6.4	4.9	7.4	7.1	7.9	7.7	7.4	9.5	9.5	10.3
Ro.	4.7	5.7	6.1	5.4	6.4	5.4	4.3	5.3	4.4	7.6
Ga.	3.6	3.9	4.7	4.9	5.1	4.5	5.9	5.2	6.2	6.4
Ke.	5.0	6.1	6.8	8.1	8.4	7.4	7.3	8.1	9.5	10.0
	Standard diet without PTE					*Standard diet with PTE*				
Ho.	6.8	8.9	9.2	9.0	9.4	7.5	5.1	6.9	9.7	8.4
Co.	4.3	4.0	3.8	3.8	4.4	6.6	6.0	6.6	7.4	9.5
Ma.	7.1	6.9	7.6	7.7	8.7	6.4	7.4	8.2	8.7	9.8
Me.	6.0	6.3	6.4	7.5	7.1	..	6.6	7.2	7.8	8.9
Sh.	7.4	14.0	15.8	15.5	16.6	7.0	9.9	11.7	12.8	13.2
Fo.	7.5	8.9	9.7	9.1	9.8	7.2	7.6	8.8	9.6	9.6
Na.	7.3	9.7	11.6	14.3	..	..	..	..	..	..
	Hypoparathyroid patients without PTE					*Hypoparathyroid patients with PTE*				
Bu.	7.4	6.8	7.4	7.8	8.2	3.0	6.7	8.5	8.8	9.8
Ma.	4.7	5.0	5.9	6.0	7.3	7.0	7.8	8.3	9.5	9.8
La.	3.5	4.3	5.1	4.6	5.7	..	..	..	..	..

high calcium (at least 3 Gm.)-normal phosphorus diet with a vitamin D intake not exceeding 10- 15,000 U./day.

2. *The effect of high calcium-low phosphorus and high phosphorus-low calcium diets on calcium clearance.* Earlier studies indicate that these two diets are capable of producing hypofunction and hyperfunction, respectively, of the parathyroid glands.[1,8,9] The marked difference in endogenous calcium

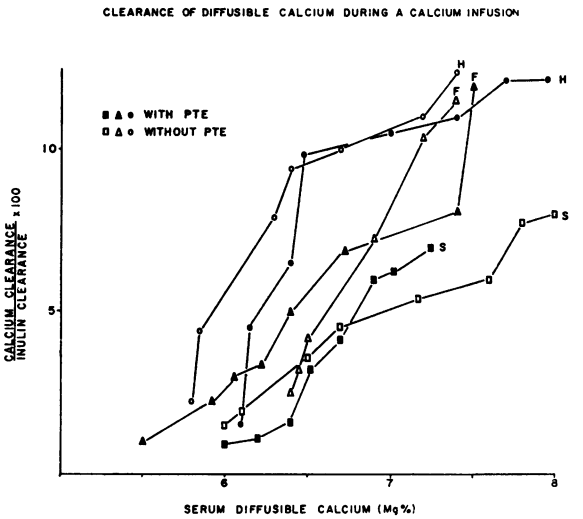

FIG. 6. Three paired experiments during a calcium infusion illustrating the failure of parathyroid extract (Lilly) to depress the clearances.

clearance caused by these two diets had been referred to in an earlier section (Table 1). Raising the serum calcium level by the infusion rapidly eliminated the difference between subjects on a high calcium and low calcium diet (Fig. 8). The difference in the initial slope of the curves on the two diets accounts for the pattern of the mean clearance curves plotted against time in Figure 3.

THE EFFECT OF BEEF AND HUMAN PARATHYROID EXTRACT ON THE ENDOGENOUS CLEARANCE OF CALCIUM IN HYPOTHYROID PATIENTS AFTER SINGLE OR MULTIPLE INJECTIONS (GROUP C)

Table 4 (1) *Patient Bu.* (24-year-old male). This patient had a total parathyroidectomy for papillary adenocarcinoma. Severe tetany developed two days postoperatively and the clearance of calcium was 2.1, an obviously high figure for a total serum calcium of 6.5 mg.%. A course of AT-10 increased the concentration of calcium in his serum to 8.1 mg.%, and at this

TABLE 4. THE EFFECT OF SINGLE AND MULTIPLE INJECTIONS OF BEEF AND HUMAN PTE ON THE ENDOGENOUS CLEARANCE OF CALCIUM IN HYPOPARATHYROID PATIENTS

Patient	Period	Duration	Total Serum Calcium (mg. %)	Diffusible Calcium (mg. %)	$\frac{C_{C_a}}{C_{I_n}}$ X100*	$\frac{C_P}{C_{I_n}}$ X100**
1. *Bu.*						
Hypoparathyroidism following total thyroidectomy						
3/25/57—Tetany 2 days postoperative		24 hrs.	6.5	4.1†	2.1‡	
4/3/57—7 days after starting A.T.-10		24 hrs.	8.1	5.1†	3.8‡	
4/27/57—24 days after A.T.-10 discontinued		24 hrs.	5.6	3.5†	2.2‡	
4/29/57—after 2 days of Lilly PTE (800 U./day)		24 hrs.	8.5	5.4†	0.6‡	
5/6/57—6 days after PTE discontinued		24 hrs.	7.1	4.5†	2.5‡	
2. *Bu.*						
Following clearance periods morning of Day 1,	Day 1	30 min.	7.8	4.7	4.3	
200 U. Lilly PTE I.M. every 6 hrs. through	" "	30 min.	7.6	4.5	3.9	
clearance periods of Day 2.	" "	30 min.	7.5	4.5	3.5	0.5
	Day 2	30 min.	8.6	5.0	2.4	
	" "	30 min.	8.6	4.8	1.9	
	" "	30 min.	8.7	5.1	2.1	7.0
3. *Ma.*						
High Ca-low P diet. No Vit. D.	Day 1	2 hrs.	7.0	4.6	2.7	
After clearance period on day 1, 4 injections	Day 2	2 hrs.	8.4	5.7	0.9	
Beef PTE, stage 1, I.M. every 6 hrs.						
4. *Ma.*						
2 injections human PTE, stage III, I.M.		30 min.	6.4	4.3	0.7	
		30 min.	6.8	4.6	0.9	1.5
	PTE→	60 min.	6.2	4.2	1.1	
		60 min.	6.5	4.4	0.4	
	PTE→	60 min.	7.0	4.7	0.2	36.0
		60 min.	6.9	4.6	0.3	
		60 min.	6.9	4.6	0.3	17.5
5. *Ma.*						
1 injection human PTE, stage I, I.M.		20 min.	6.9	4.5	0.6	1.6
		20 min.	7.2	4.7	0.6	
		30 min.				4.2
	PTE→	60 min.	6.9	4.5	0.5	14.0
		60 min.	7.0	4.6	0.3	25.6
		60 min.	6.8	4.4	0.3	16.8
		60 min.	6.8	4.4	0.3	8.9

6. *Ma.*						
1 injection beef PTE, stage II, I.M.		30 min.	6.3	4.1	1.2	0.4
		30 min.	6.3	4.1	0.8	0.2
	PTE→	30 min. (90§)	7.0	4.6	0.3	12.8
		30 min. (150§)	7.0	4.6	0.3	32.5
		30 min. (300§)	6.8	4.4	0.3	41.5
7. *Ma.*						
1 injection beef PTE, stage III, I.M.		30 min.	6.5	4.2	0.6	3.7
		30 min.	6.1	4.0	0.7	1.9
	PTE→	30 min. (120§)	6.5	4.2	0.6	35.6
		30 min. (180§)	6.5	4.2	0.3	
		30 min. (210§)	6.5	4.2	0.3	38.2
		30 min. (300§)	6.4	4.2	0.6	20.4
8. *La.*						
High Ca.—normal P diet. No Vit. D.		60 min.	7.1	4.6	8.4	
1 injection human PTE, stage I, I.M.		60 min.	6.7	4.4	8.1	6.5
	PTE→	60 min.	6.8	4.4	8.5	
		60 min.	6.8	4.5	3.9	
		60 min.	6.8	4.4	3.2	38.0
9. *La.*						
1 injection beef PTE, stage III, I.M.		60 min.	8.4	5.5	10.2	6.8
		30 min.	8.6	5.6	9.0	8.5
		60 min.	8.6	5.6	7.6	12.6
	PTE→	60 min.	9.1	5.9	2.3	34.1
		60 min.	8.6	5.6	1.2	72.5
		60 min.	8.2	5.3	1.2	58.0
		60 min.	8.2	5.3	1.1	49.0
		60 min.	8.1	5.3	2.0	43.0

* $\frac{\text{Calcium clearance}}{\text{Inulin clearance}} \times 100.$

** $\frac{\text{Phosphorus clearance}}{\text{Inulin clearance}} \times 100.$

† These diffusible calcium concentrations were calculated from the mean per cent diffusible value of all the ultrafiltrates subsequently determined on this patient.

‡ Calculated from 24-hour calcium and creatinine excretion $\left(\frac{C_{C_a} \times 100}{C_{C_r}}\right)$.

§ Time after injection (min.).

level his calcium clearance was 3.8. Twenty-four days after discontinuing the AT-10 (dihydrotachysterol), the calcium clearance at a serum value of 5.6 mg.% was 2.2. After two days of Lilly PTE (800 U./day, I.M.) the clearance had fallen to 0.6 despite a rise in serum calcium to 8.5 mg.%. Withdrawal of PTE was followed by a rapid fall in serum calcium (7.1 mg.%) and a significant *rise* in calcium clearance.[4,8] The patient was maintained on a high calcium—low phosphorus diet *without vitamin D* for approximately three weeks and the experiment tabulated in Table 4 (2) was done. Again, Lilly PTE caused a significant decrease in calcium clearance despite an increase in serum calcium.

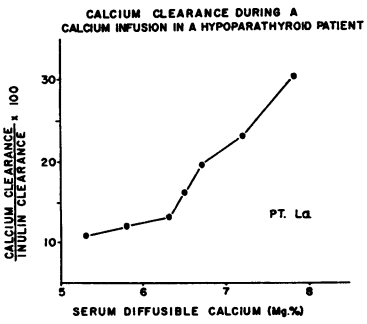

FIG. 7. The effect of a calcium infusion on the diffusible calcium clearance in a hypoparathyroid patient.

Table 4 (3) *Patient Ma.* (45-year-old female). This patient with chronic postsurgical hypoparathyroidism received an I.M. injection every six hours of beef PTE prepared in our laboratory (stage I) after having obtained the two-hour control clearance on the morning of day 1. Comparable samples were collected on the morning of day 2. Serum calcium increased 1.4 mg.%, while calcium clearance decreased from 2.7 to 0.9.

The subsequent acute experiments on patients Ma. and La. (50-year-old female, postsurgical hypoparathyroidism) have been tabulated in Table 4 (4-9) and illustrated in Figures 9 and 10. A single acute intramuscular injection of beef or human PTE caused a moderate-to-marked decrease in calcium clearance in each experiment. The lowest clearance values were obtained in approximately three hours. Phosphorous clearances have been included in Table 4 for comparison. The concentration of calcium in the serum did not rise significantly during these acute experiments. Purification of beef or human extract through the stage of ultrafiltration (stage III)[21] did not cause a loss of either the phosphate-diuretic or calcium-antidiuretic "properties."

CALCIUM CLEARANCE IN NORMAL SUBJECTS DURING AND AFTER THE PROLONGED ADMINISTRATION OF LILLY PTE

The authors have observed that a 12- to 24-hour period of temporary functional hypoparathyroidism (as measured by changing phosporus clearances) follows the iatrogenic "hyperparathyroidism" produced by multiple

injections of Lilly PTE for two days.* It seemed of interest to note the effect of this procedure on the renal clearance of calcium. This was done on five normal subjects. The results are tabulated in Table 5 and Figure 11. In all five subjects, the clearance of calcium rose significantly in the 12- to 24-hour period of functional hypoparathyroidism following withdrawal of the PTE (stage III). Despite the increase in serum during the injection of the extract, only one subject (J.S.) demonstrated a rise in calcium clearance during period II.

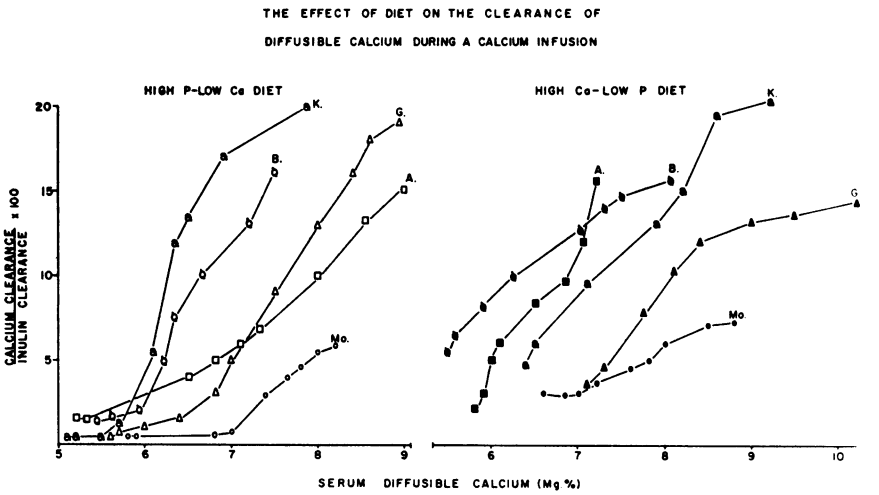

FIG. 8. The effect of altered calcium and phosphorus content of the diet on the diffusible calcium clearance during a calcium infusion. The curves represent the paired experiments on five subjects.

THE EFFECT OF CALCIUM CARBONATE INGESTION WITH AND WITHOUT LILLY PTE ON SERUM CALCIUM AND CALCIUM EXCRETION

These studies were initially done as part of an investigation into the etiology of the milk-alkali (Burnett's) syndrome and have been reported elsewhere in abstract form.[24] The values shown in Figure 12 represent the means of four subjects. The design of the experiment is outlined in the methods and procedure section. Calcium carbonate alone caused a marked increase in urinary calcium and decrease in urinary phosphorus excretion, the latter due primarily to the formation of non-absorbable calcium phos-

*This technique of producing functional hypoparathyroidism was first suggested to the senior author by Dr. Sidney H. Ingbar, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Massachusetts.

TABLE 5. CALCIUM CLEARANCE IN NORMAL SUBJECTS ON CONSTANT NORMAL CALCIUM AND PHOSPHORUS DIETS BEFORE, DURING, AND AFTER THE CHRONIC ADMINISTRATION OF LILLY *PTE*

Patient	*Period*	*Duration*	*Total Serum Calcium (mg. %)*	*Diffusible Calcium (mg. %)*	$\frac{C_{Ca}}{C_{Cr}} \times 100$*	$\frac{C_P}{C_{Cr}} \times 100$†
1. J.S. 6/2/58	Pre-PTE	24 hrs.	10.5	6.8	2.1	21.0
	PTE‡	12 hrs.	11.6	7.5	3.8	—
		12 hrs.	11.6	7.2	5.8	30.3
	Post-PTE	12 hrs.	10.2	6.6	8.7	10.0
		12 hrs.	9.7	6.3	9.7	10.7
		24 hrs.	10.0	6.5	5.1	—
2. H.K. 1/22/58	Pre-PTE	24 hrs.	9.4	6.2	3.0	—
		24 hrs.	9.4	6.2	3.0	7.0
	PTE‡	24 hrs.	10.6	7.1	1.9	14.0
		24 hrs.	11.4	7.6	2.9	32.0
	Post-PTE	12 hrs.	10.9	7.3	5.2	5.0
		12 hrs.	10.9	7.3	4.5	3.0
		24 hrs.	10.9	7.3	3.2	—
3. R.C. 11/10/57	Pre-PTE	24 hrs.	9.2	6.2	0.6	—
		24 hrs.	9.2	6.2	1.0	18.0
	PTE‡	24 hrs.	9.5	7.0	0.7	—
		24 hrs.	10.3	7.2	0.9	32.0
	Post-PTE	12 hrs.	9.7	7.0	2.6	—
		12 hrs.	9.8	7.0	3.1	—
		12 hrs.	9.5	6.7	4.5	7.0
		12 hrs.	9.5	6.7	2.1	—
4. P.B. 4/2/58	Pre-PTE	24 hrs.	8.8	5.6	—	—
		24 hrs.	9.4	5.9	2.9	12.0
	PTE‡	24 hrs.	10.2	6.2	2.5	—
		24 hrs.	10.4	6.9	0.4	25.0
	Post-PTE	12 hrs.	8.2	5.2	4.0	6.0
		12 hrs.	9.9	5.8	3.0	—
5. W.J. 5/3/58	Pre-PTE	24 hrs.	11.2	6.7	3.0	—
		24 hrs.	11.3	7.0	3.0	11.5
	PTE§	24 hrs.	11.2	7.0	2.7	—
		24 hrs.	11.9	7.8	1.7	—
		24 hrs.	12.2	8.0	2.1	22.0
	Post-PTE	12 hrs.	11.2	7.0	6.0	5.0
		12 hrs.	10.7	6.6	3.8	—
		12 hrs.	10.7	6.6	4.0	7.0

* $\frac{\text{Calcium clearance}}{\text{Creatinine clearance}} \times 100.$

† $\frac{\text{Phosphorus clearance}}{\text{Creatinine clearance}} \times 100.$

‡ 800 U. Lilly PTE I.M./day for 2 days.

§ 800 U. Lilly PTE I.M./day for 3 days.

TABLE 6. THE EFFECT OF PARATHYROIDECTOMY ON THE RENAL CLEARANCE OF CALCIUM AND PHOSPHORUS IN HYPERPARATHYROID PATIENTS

Patient	*Period*	*Duration*	*Total Serum Calcium (mg. %)*	*Diffusible Calcium (mg. %)*	$\frac{C_{C_a} \times 100}{C_{C_r}}$*	$\frac{C_P \times 100}{C_{C_r}}$†
Ri.	Preop.	30 min.	13.2	9.2	7.6	17.0
		30 min.	13.2	9.2	7.9	16.0
	Immediate postop.	480 min.	11.4	7.9	3.5	14.2
		480 min.	10.4	7.2	1.0	0.6
		480 min.	9.2	—	—	—
		480 min.	9.1	6.3	0.2	0.2
Au.	Preop.	40 min.	13.4	8.7	4.0	56.0
		40 min.	13.4	8.7	4.3	57.0
Ca.	Preop.	20 min.	14.3	9.3	9.0	67.0
		20 min.	14.5	9.4	9.3	68.0
		50 min.	14.4	9.4	9.5	65.0
	Immediate postop.	370 min.	13.0	8.4	7.4	65.0
	4 days postop.	40 min.	8.6	5.6	4.6	14.0
		40 min.	8.4	5.4	4.5	22.0
		40 min.	8.8	5.6	4.3	15.0
Ba.	Preop.	60 min.	12.0	7.8	6.9	32.0
		60 min.	11.8	7.6	6.9	25.5
		60 min.	11.8	7.6	6.3	23.0
	Postop. 24 hrs.	12 hrs.	9.9	5.7	5.5	1.5
	Postop. 40 hrs.	12 hrs.	7.9	4.7	4.4	0.1
	Postop. 48 hrs.	24 hrs.	9.8	6.1	3.8	0.3
	Postop. 12 days	24 hrs.	9.7	5.4	1.0	18.1
	Postop. 2 mo.	30 min.	9.0	6.0	2.4	20.0
	Postop. 2 mo.	30 min.	9.0	5.9	2.4	20.0
	Postop. 2 mo. (During calcium infusion)	30 min.	11.6	7.6	6.7	23.0
	Postop. 6 mo.	24 hrs.	9.6	5.6	0.4	24.0
	Postop. 6 mo.	24 hrs.	9.6	5.6	0.5	22.0
Bg.	Preop.	24 hrs.	11.4	7.4	4.2	26.0
		24 hrs.	11.9	7.7	3.0	26.6
	Postop. 7 hrs.	11 hrs.	10.1	6.7	4.9	19.7
	Postop. 12 hrs.	4 hrs.	9.9	6.4	5.6	13.8
	Postop. 15 hrs.	3 hrs.	9.7	6.4	5.0	11.6
	Postop. 24 hrs.	6 hrs.	9.3	6.0	2.4	1.9

* $\frac{\text{Calcium clearance}}{\text{Creatinine clearance}} \times 100.$

† $\frac{\text{Phosphorus clearance}}{\text{Creatinine clearance}} \times 100.$

phate in the gastrointestinal tract. Serum calcium increased slightly. When the ingestion of calcium carbonate was combined with PTE administration, the mean serum calcium increased significantly, while calcium excretion was essentially unchanged as compared with calcium carbonate ingestion alone. All four subjects behaved in the same way. The probable inhibition of the parathyroid secretion by the calcium carbonate ingestion was replaced by temporary "hyperparathyroidism" when the extract was simultaneously administered. The comparative changes in serum and urinary calcium reflect these alterations in parathyroid activity.

THE CLEARANCE OF DIFFUSIBLE CALCIUM IN CLINICAL HYPERPARATHYROIDISM BEFORE AND AFTER REMOVAL OF THE ADENOMA

Only five patients (Group f) with normal renal function were studied (Table 6). Because of the variability of the data, each patient will be discussed separately.

Patient Ri. (48-year-old female). The calcium clearance of approximately 7.8 at a serum calcium of 13.2 mg.% (diff. Ca: 9.2) is below the normal range (Figs. 4, 6, 8) *when compared with the normal at comparable blood levels.* At a serum phosphorus of 2.0, the phosphorus clearance of 13.6 is definitely elevated.[22] Within 24 hours following removal of the adenoma, the serum calcium had fallen to 9.1 mg.% (diff. Ca: 6.3). The clearance of calcium was not sustained and decreased to the low value of 0.2. This should be contrasted with the increased clearance in the normal subjects following withdrawal of the PTE (Fig. 11).

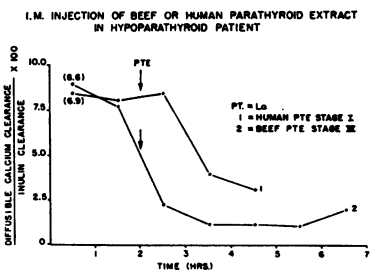

FIG. 9. The effect of a single intramuscular injection of human or beef parathyroid extract on the diffusible calcium clearance in a hypoparathyroid patient. Each curve represents a single experiment. The numbers in parenthesis refer to total serum calcium in mg.%.

Patient Au. (26-year-old male). This patient had only preoperative studies done. The calcium clearance of 4.2 at a serum calcium of 13.4 mg.% (diff. Ca: 8.7) was distinctly low.

Patient Ca. (35-year-old male). This patient had a preoperative clearance of calcium of 9.3. This could be considered a low normal value for a serum calcium of 14.4 mg.% (diff. Ca: 9.4). Four days postoperatively the serum calcium had decreased to 8.5 mg.%, and the phosphorus clearance from 65 to approximately 15. At this point the clearance of calcium was

4.5, considerably higher than would normally be observed at this calcium concentration.

Patient Ba. (55-year-old male). The calcium clearance was approximately 6.7 at a serum calcium concentration of 11.8 mg.% (diff. Ca: 7.6). This clearance was low when compared to those of the normal subjects at similar serum values (Figs. 4, 6, and 8). Forty hours postoperatively serum calcium had decreased to 7.9 mg.% (diff. Ca. 4.7), phosphorus clearance to 0.1, and calcium clearance to 4.4. These phosphorus and calcium clearances are consistent with a period of physiological hypoparathyroidism.

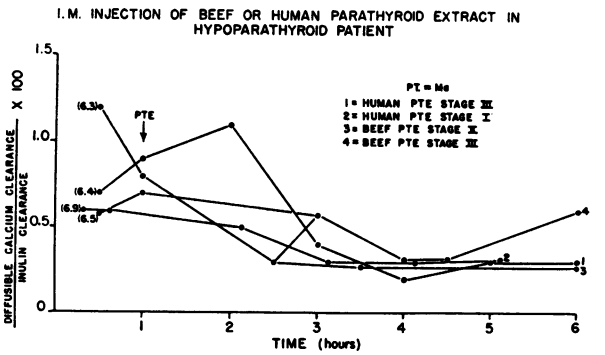

FIG. 10. The effect of a single intramuscular injection of human or beef parathyroid extract on the diffusible calcium clearance in a hypoparathyroid patient. Each curve represents a single experiment The numbers in parenthesis refer to total serum calcium in mg.%.

Two months after surgery a calcium infusion was given to elevate the serum diffusible calcium to levels observed before operation. At a concentration of diffusible calcium of 7.6 mg.%, the clearance was 6.7. This value was comparable to that noted preoperatively. Four months later the clearance of calcium was very low (0.5), and the phosphorus clearance was almost as high as the preoperative value. We were unable to do further studies on this patient to investigate the possibility of recurrent hyperparathyroidism.

Patient Bg. (54-year-old male). This patient demonstrated distinctly low clearances (4.2 and 3.0) referable to his serum calcium of 11.4 mg.% (diff. Ca: 7.4) and 11.9 mg.% (diff. Ca: 7.7) prior to operation. Twelve to 15 hours after removal of the adenoma, his serum calcium had decreased to 9.7 mg.% (diff. Ca: 6.4). Despite this, the clearance of calcium *rose to* 5.0. In the period of 18-24 hours postoperatively, serum calcium was 9.3 mg.%, calcium clearance had decreased to 2.4, and phosphorus clearance was at its lowest level, 1.9.

THE EFFECT OF PARATHYROIDECTOMY AND PARATHYROID EXTRACT ADMINISTRATION ON THE CLEARANCE OF CALCIUM IN THE DOG

The calcium clearances illustrated in Figures 13 and 14 have been calculated from the concentration of total calcium in the serum. Subsequent ultrafiltration of the serum from these animals indicated that the clearance of diffusible calcium would be approximately 30-35 per cent higher. During the five-hour period following parathyroidectomy, the concentration of

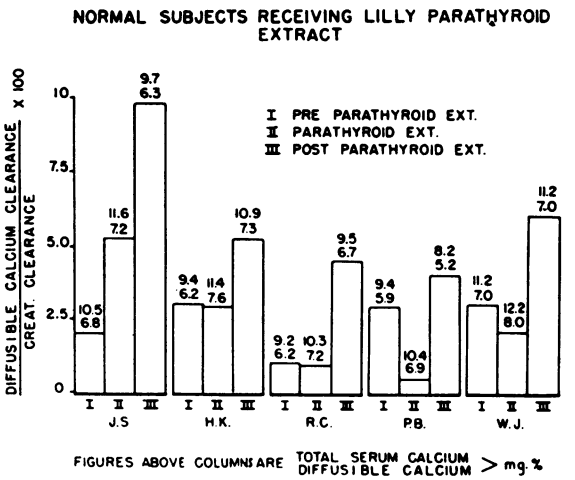

FIG. 11. The endogenous clearance of diffusible calcium, before, during, and after the administration of parathyroid extract (Lilly) for two or three days. Five subjects are represented. The figures above the columns are total serum calcium over the diffusible calcium in mg.%.

calcium in the serum decreased gradually from 9 to 8.5 mg.% (Fig. 13). Despite this fall in filtered load the calcium clearance rose sharply and remained above the control levels for the next five hours. No significant changes in serum calcium or phosphorus or their clearances occurred in two sham-operated dogs over the five-hour period after surgery.

Various stages of beef and human PTE were injected intramuscularly into four stable parathyroidectomized dogs maintained on high calcium diets without vitamin D. Calcium clearances were followed over a period of five-six hours after a single injection. The results are illustrated in Figure 14. Calcium clearance fell to a minimum approximately three hours after the injection. The greatest decreases were observed in the two animals with the highest initial clearances. These two dogs, despite their low serum calcium (7.3 and 4.0 mg.%), had initial clearances comparable to the six

dogs prior to parathyroidectomy (Fig. 13). The results of these dog experiments confirm those of Talmage, Kraintz, and Buchanan in the rat[15,16] and Buchanan, Kraintz, and Talmage in the mouse.[25] Martin, Mikkelson, and Jones, although not measuring clearances, have recently noted that parathyroidectomy in the dog causes a rise in calcium excretion.[26]

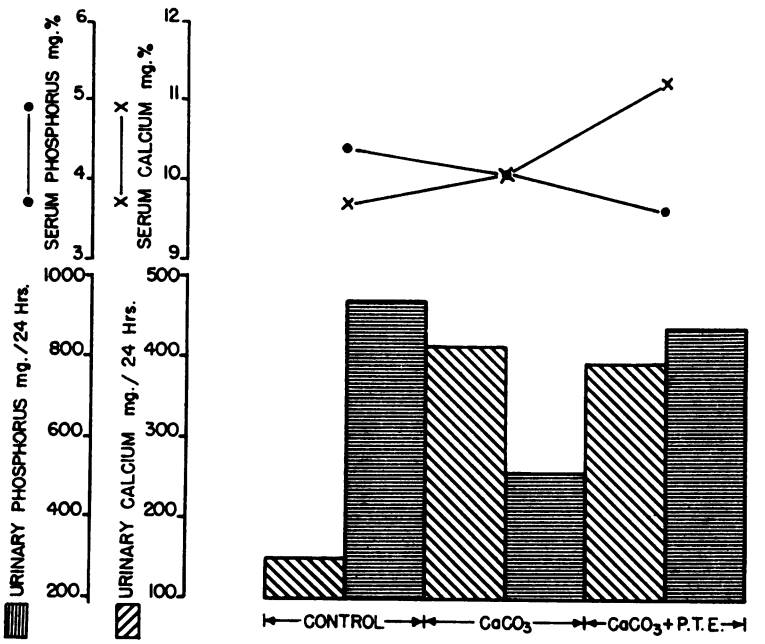

FIG. 12. The effect of calcium carbonate ingestion on the excretion of calcium and phosphorus and the serum concentration of these ions when the carbonate is administered with and without parathyroid extract (Lilly). The values represent the mean of four subjects.

DISCUSSION

The excretion of calcium is a function of the filtered load of ionic and complexed calcium and the homeostatic factors regulating active tubular reabsorption. The results of the present investigation indicate that for any given level of dietary calcium and phosphorus or parathyroid activity, the greater the filtered load of calcium the greater was its renal clearance despite an absolute increase in the amount reabsorbed per minute (Figs. 3-6 and Table 3). Chen and Neuman[2] and Williamson and Freeman[27] made similar observations in the calcium-loaded dog. Jahan and Pitts[28] administered Lilly PTE to two normal dogs. As hypercalcemia developed and

the filtered load of calcium increased, the tubular reabsorption of calcium (mg./min.) greatly exceeded the rate observed when the animals were normocalcemic. These authors *correctly* attributed the increased reabsorption to the increased filtered load. It is not possible to conclude from their data, as has been done,[25] that parathyroid hormone caused an increased tubular reabsorption of calcium.

Assuming a normal rate of glomerular filtration and a constant per cent diffusible calcium, Talbot calculated the absolute rate of tubular reabsorption in normal subjects and patients with hypo- and hyperparathyroidism.[24] He used the term "T_m" to refer to absolute and not maximal rate of reabsorption. "Calcium T_m is higher in patients with primary hyperparathyroidism (8.0 Gm. per m^2 per day) than in normal subjects (5.0 Gm. per m^2 per day) or patients with primary hypoparathyroidism (2.0 Gm. per m^2 per day)." From these calculations he concluded: "Doses of parathyroid extract act to boost the lowered serum calcium concentration toward normal, in part, by increasing the efficiency of calcium reabsorption by the renal tubules." Although his conclusion is probably correct, it could not be made by comparing tubular reabsorption at such widely differing filtered loads of calcium. His calculations simply showed that the absolute rate of calcium reabsorption (mg./min) was directly related to the concomitant serum calcium level and, therefore, to the filtered load. It is quite apparent that one cannot interpret the renal clearance of calcium in disease or altered physiological states without the realisation that an increased filtered load per se increases calcium clearance. Recently, Canary and Kyle[29] concluded that parathyroid hormone did not affect the tubular reabsorption of calcium because they found that the *per cent of the filtered load reabsorbed* in hyperparathyroidism was the same as in normal subjects. In interpreting their data, they did not take into consideration their observations that the filtered load of calcium in their hyperparathyroid patients was 10.3±0.8 mg./minute while in their normal subjects it was only 7.1±0.4 mg./minute. Actually, their data indicated that for a filtered load of 10.3 the patients had

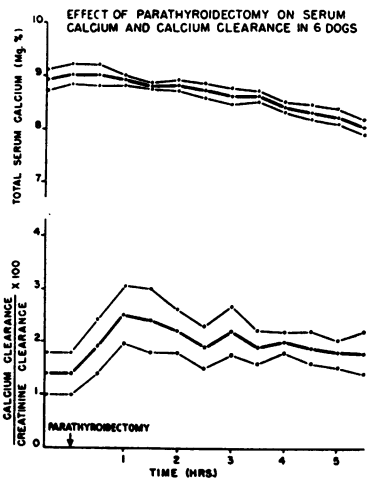

FIG. 13. The effect of parathyroidectomy on the serum calcium and calcium clearance in six dogs. The curves represent the mean values and the standard deviation from the mean.

a *low* clearance of calcium. This was substantiated by the mean clearance of 3.6±0.3 ml./minute.

The results of the present investigation strongly suggest that for any given filtered load of calcium, regardless of the physiological state of calcium metabolism, the greater the level of parathyroid activity of circulating parathyroid hormone(s), the lower will be the renal clearance of diffusible calcium. These observations support the conclusions of Talmage and his associates[15,16,25] that the secretion of the parathyroid glands contributes homeostatically to the regulation of the tubular reabsorption of

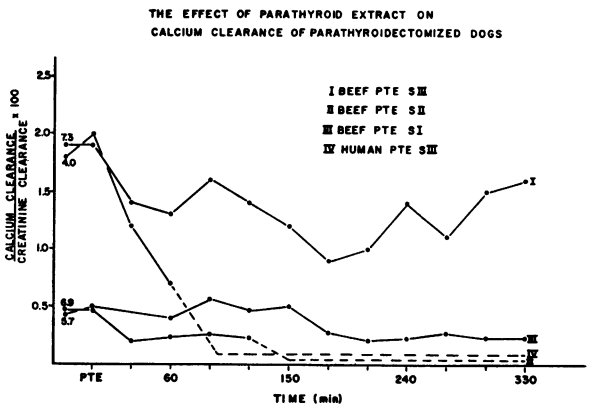

FIG. 14. The effect of beef and human parathyroid extract on the renal clearance of calcium in hypoparathyroid dogs. The dotted termination of two of the curves represents the estimated clearance values. The concentration of calcium in the urine of these samples was too low for accurate determination. The numbers adjacent to the beginning of each curve represent the total serum calcium concentration.

calcium. The summarized results contributing to this conclusion are as follows:

1. The endogenous clearance of calcium was lowered in normal and hypoparathyroid individuals by the administration of various types of human and beef PTE (Table 1, Table 4 (1-9), and Figs. 9 and 10).

2. Normal subjects had significantly higher calcium clearances on high calcium-low phosphorus diets ("physiological parathyroid hypofunction") than when on low calcium-high phosphorus diets ("physiological parathyroid hyperfunction") (Table 1).

3. The endogenous clearances of calcium were normal in two hypoparathyroid patients, despite hypocalcemia (Table 1, Bu. and Ma.), and the highest endogenous and post-calcium infusion clearances observed were found in a hypoparathyroid patient (Fig. 7).

4.The endogenous clearance of calcium increased acutely following PTE administration during the period of physiological hypoparathyroidism in normal subjects (Fig. 11), following accidental removal of the parathyroids (Table 4 (1)), and after removal of a parathyroid adenoma (Table 6, patient Bg.).

5. The administration of Lilly PTE during calcium carbonate ingestion was not associated with an increase in calcium excretion even though serum calcium increased appreciably (Fig. 12).

6. The renal clearance of calcium was relatively low in patients with hyperparathyroidism (Table 6).

7. Parathyroidectomy in the dog resulted in an acute rise in the renal clearance of calcium despite a fall in the filtered load of calcium (Fig. 13).

8. Single injections of various preparations of beef and human PTE in the dog caused a decrease in the clearance of calcium (Fig. 14).

9. Acute administration of Lilly PTE in normal and hypoparathyroid subjects during a calcium infusion lowered the clearance of calcium for any given increment in the filtered load (Figs. 3-5).*

Certain aspects of the above summary require further comment. Although diets with a high Ca/P and a low Ca/P ratio can decrease and increase parathyroid activity,[7-9] respectively, it is not at all certain that the increase and decrease in calcium clearance accompanying these diets[10-12] (Table 1) are due to altered parathyroid activity per se. A slightly greater rise in serum calcium (filtered load) on the high calcium diet might also be a factor in increasing the clearance. Furthermore, phosphorus depletion causes a loss of osseous calcium and increased calcium excretion.[9-10] Whether this process is initiated at the skeletal or renal tubular levels is unknown. However, infusion of calcium into the subjects on the low calcium-high phosphorus diet (a procedure that immediately decreases parathyroid activity) inhibited the homeostatic reduction in the endogenous clearance of calcium (Fig. 8). In one recently completed experiment we have noted that the acute administration of Lilly PTE to a subject who had been on a high calcium-low phosphorus diet lowered his endogenous clearance to the level observed on the low calcium-high phosphorus diet.

The three patients with hypoparathyroidism studied in this investigation were chosen because two (Ma. and Bu.) had not been on vitamin D, and the dose (10,000 to 15,000 U.) taken by the third (La.) was thought to be

* The reason why the administration of PTE in three of the nine subjects (Fig. 6) did not lower the calcium clearance is not readily apparent. A possible explanation may be that the sensitive tubular mechanism affected by parathyroid hormone can be masked by the rapid rise in the filtered load of calcium.

below the therapeutic range necessary to treat hypoparathyroidism. Their endogenous clearances of calcium were normal-to-high. Most patients, however, with chronic untreated hypoparathyroidism (particularly the idiopathic type) have very low levels of calcium excretion. The low levels of filtered calcium certainly contribute to the hypocalciuria. It is possible that after many years of hypoparathyroidism they develop some additional homeostatic mechanism to enhance the reabsorption of calcium. Goldman and Bassett[80] infused calcium into three patients with untreated idiopathic hypoparathyroidism. Assuming a diffusible calcium of 65 per cent, the clearances in these three patients were markedly elevated at hypocalcemic and normocalcemic levels (10 cc., 9 cc., and 11 cc./min. at serum calciums

TABLE 7. THE RATE OF CALCIUM EXCRETION DURING A CALCIUM INFUSION EXTRAPOLATED TO A 24-HOUR LEVEL

Serum calcium during infusion	*No. of Experiments*	*Urine calcium (mg. per 24 hours)*		
		Mean	*Low*	*High*
10.0-10.9	6	1772	1087	2642
11.0-11.9	9	1901	863	2327
12.0-12.9	5	2126	700	3015
13.0-13.9	5	2168	1711	3190
14.0-14.9	3	1607	1056	2258

of 7.2, 7.9, and 10.8 mg.%, respectively). It is apparent that very high clearances of calcium can be observed in hypoparathyroid patients not receiving vitamin D. It has recently been suggested that the hypercalciuria seen in vitamin D-treated cases of hypoparathyroidism is due to a direct effect of vitamin D on the tubular reabsorption of calcium.[81] It seems more reasonable to conclude that vitamin D acting at the osseous level increases serum calcium, and, in the absence of the parathyroid glands, the clearance rises to abnormally high levels. In preliminary experiments we have been unable to detect an effect of vitamin D on the renal clearance of diffusible calcium in normal subjects on diets of varying calcium and phosphorus content.[82]

In some of our patients following removal of a parathyroid adenoma, the renal clearance of calcium did not increase during a period when acute temporary hypoparathyroidism was suspected (Table 6). This contrasted sharply with our normal subjects (Fig. 11). It seems probable that the marked depletion of skeletal calcium that is characteristic of these patients may, by an undisclosed mechanism, enhance the tubular reabsorption of calcium *independent of the level of parathyroid activity.* It is of interest

that when the rate of calcium excretion in our normal subjects receiving calcium infusions is extrapolated at different blood levels to a 24-hour excretion, the amount is considerably in excess of that usually encountered in clinical hypercalcemia (Table 7). This suggests that in chronic hypercalcemic states, usually with osseous depletion of calcium, the tubular reabsorption may be somewhat greater for a given serum calcium level than following an acute infusion of calcium. However, the clearance of calcium is usually lower for any degree of hypercalcemia in hyperparathyroidism than in other hypercalcemic disorders.[88]*

The exact mechanism(s) by which the secretion of the parathyroids decreases the renal clearance of calcium is unknown. It is conceivable that the renal effect is not due to the direct action of the hormone at the renal tubular level but actually is secondary to its acute effects on osseous metabolism. However, in both dogs and humans we observed a fall in calcium clearance without a noticeable skeletal effect (a rise in serum calcium) (Table 4 (1-9)). Unfortunately, the results of infusing parathyroid extract into one renal artery of the dog have to date proved too erratic to interpret.

SUMMARY

1. The effect of calcium infusions on the renal clearance of diffusible calcium has been investigated in normal and hypoparathyroid subjects.

2. An increase in the filtered load of calcium increased the renal clearance of diffusible calcium in all subjects.

3. For any given filtered load of calcium, the greater the level of parathyroid activity, the lower the clearance of calcium.

4. Experimental and clinical states of hyperparathyroidism were associated with a relative decrease in calcium clearance.

5. Experimental and clinical states of hypoparathyroidism were associated with a relative increase in the clearance of calcium.

6. Acute administration of parathyroid extract to hypoparathyroid humans and dogs decreased the renal clearance of calcium.

7. The secretion of the parathyroid glands contributes to the homeostatic regulation of the tubular reabsorption of calcium.

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* In a review of 20 recent articles on primary hyperparathyroidism selected at random, 46 cases without renal insufficiency were encountered. Seventeen of these had normal levels of calcium excretion (<200 mg./24 hours). The serum calciums in these 17 ranged from 10.5-18.0 mg.%. To conserve space these references have not been included in the bibliography but may be obtained from the authors on request.

ADDENDUM

Since completion of the manuscript, we have noted the article by Crawford, Loughridge, Milne, and Scribner[32] on organic acid excretion after calcium gluconate infusions. These investigators found that 68-80 per cent of the infused gluconate was excreted as the unaltered anion in the urine. Although they concluded that the gluconate was excreted predominantly as sodium and potassium salts, their findings emphasize the need to compare the effects of chloride and gluconate salts of calcium on the renal clearance of calcium in paired studies.

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REFERENCES

1. Howard, P. J., Wilde, W. S., and Malvin, R. L.: Localization of renal calcium transport; effect of calcium loads and of gluconate anion on water, sodium and potassium. *Amer. J. Physiol.,* 1959, *197,* 337.
2. Chen, P. S., Jr. and Neuman, W. F.: Renal excretion of calcium by the dog. *Amer. J. Physiol.,* 1955, *180,* 623.
3. Chen, P. S., Jr. and Neuman, W. F.: Renal reabsorption of calcium through its inhibition by various chemical agents. *Amer. J. Physiol.,* 1955, *180,* 632.
4. Walser, M. and Browder, A. A.: Ion association. III. The effect of sulfate infusion on calcium excretion. *J. clin. Invest.,* 1959, *38,* 1404.
5. Freeman, S. and Chang, T. S.: Role of the kidney and of citric acid in the production of a transient hypercalcemia following nephrectomy. *Amer. J. Physiol.,* 1950, *160,* 335.
6. Howard, J. E.: *Normal calcium and phosphorus transport and body fluid homeostasis in metabolic interrelations.* Fifth Josiah Macy, Jr. Conference. New York, Josiah Macy, Jr. Foundation, 1954.
7. Bauman, E. J. and Sprinson, D. B.: Hyperparathyroidism produced by diet. *Amer. J. Physiol.,* 1939, *125,* 741.
8. Engfeldt, B., Hjertquist, S. O., and Strandh, J. R. E.: The parathyroid function in longterm dietary experiments. *Acta endocrinol.,* 1954, *15,* 119.
9. Stoerk, H. C. and Carnes, W. H.: Relation of dietary Ca: P ratio to serum Ca and to parathyroid volume. *J. Nutrit.,* 1945, *29,* 43.
10. Copp, H. D.: *Studies on the plasma clearance values of calcium in metabolic interrelations.* Fifth Josiah Macy, Jr. Conference. New York, Josiah Macy, Jr. Foundation, 1954.
11. Goss, H. and Schmidt, C. L. A.: Calcium and phosphorus metabolism in rats during pregnancy and lactation and the influence of the reaction of diet thereon. *J. biol. Chem.,* 1930, *86,* 417.
12. Nicholaysen, R., Eeg-Larsen, N., and Malm, O. J.: Physiology of calcium metabolism. *Physiol. Rev.,* 1953, *33,* 424.
13. Howard, J. E., Hopkins, T. R., and Connor, T. B.: On certain physiologic responses to intravenous injection of calcium salts into normal hyperparathyroid and hypoparathyroid persons. *J. clin. Endocrinol.,* 1953, *13,* 1.
14. Talbot, N. B., Sobel, E. H., McArthur, J. W., and Crawford, J. D.: *Functional endocrinology.* Cambridge, Mass., Harvard University Press, 1952.
15. Talmage, R. V. and Kraintz, F. W.: Progressive changes in renal phosphate and calcium excretion in rats following parathyroidectomy or parathyroid administration. *Proc. Soc. exp. Biol. (N. Y.),* 1954, *87,* 263.

16. Talmage, R. V., Kraintz, F. W., and Buchanan, G. D.: Effect of parathyroid extract and phosphate salts on renal calcium and phosphate excretion after parathyroidectomy. *Proc. Soc. exp. Biol. (N. Y.),* 1955, *88,* 600.
17. Lavietes, P. H.: Anaerobic ultrafiltration. *J. biol. Chem.,* 1937, *120,* 267.
18. Yanagisawa, F.: New colorimetric determination of calcium and magnesium. *J. Biochem.,* 1955, *42,* 3.
19. Kingsley, G. R. and Robnett, A.: New dye method for direct photometric determination of calcium. *Amer. J. clin. Path.,* 1957, *27,* 223.
20. Kleeman, C. R., Maxwell, M. H., and Rockney, R. E.: Mechanisms of impaired water excretion in adrenal and pituitary insufficiency. I. The role of altered glomerular filtration rate and solute excretion. *J. clin. Invest.,* 1958, *37,* 1799.
21. Rasmussen, H. and Westall, R. G.: The partial purification of parathyroid hormone by means of ultrafiltration and displacement chromatography. *Biochem. J.,* 1957, *67,* 658.
22. Bernstein, D., Kleeman, C. R., Rockney, R. E., Dowling, J. T., and Maxwell, M. H.: Unpublished observations.
23. Birkenhager, W. H., Hellendoorn, H. B. A., and Gerbrandy, J.: Effects of intravenous injections of calcium levulinate on calcium and phosphate metabolism. *Clin. Sci.,* 1959, *18,* 45.
24. Kleeman, C. R., Maxwell, M. H., Grossman, M. I., and Rockney, R. E.: Hypercalcemia secondary to calcium carbonate ingestion in duodenal ulcer patients. A forme fruste of Burnett's syndrome? *Clin. Res.,* 1958, *6,* 98.
25. Buchanan, G. D., Kraintz, F. W., and Talmage, R. V.: Renal excretion of calcium and phosphate in the mouse as influenced by the parathyroids. *Proc. Soc. exp. Biol. (N. Y.),* 1959, *101,* 306.
26. Martin, H. E., Mikkelsen, W. P., and Jones, R.: The effect of parathyroidectomy in the dog on serum and urine magnesium levels. *Clin. Res.,* 1959, *7,* 108.
27. Williamson, B. J. and Freeman, S.: Effect of acute changes in acid base balance on renal calcium excretion in dogs. *Amer. J. Physiol.,* 1957, *191,* 384.
28. Jahan, I. and Pitts, R. F.: Effect of parathyroid on renal tubular reabsorption of phosphate and calcium. *J. clin. Path.,* 1959, *12,* 524.
29. Canary, J. J. and Kyle, L. H.: The hypercalciuria of hyperthyroidism in man. *J. clin. Invest.,* 1959, *38,* 994.
30. Goldman, R. and Bassett, S. H.: Effect of intravenous calcium gluconate upon the excretion of calcium and phosphorus in patients with idiopathic hypoparathyroidism. *J. clin. Endocrinol.,* 1954, *14,* 278.
31. Litvak, J., Moldawer, M. P., Forbes, A. P., and Henneman, P. H.: Hypocalcemic hypocalciuria during vitamin D and dihydrotachysterol therapy of hypoparathyroidism. *J. clin. Endocrinol.,* 1958, *18,* 246.
32. Crawford, M. A., Loughridge, L., Milne, M. D., and Scribner, B. H.: Organic acid excretion after calcium gluconate infusions. *J. clin. Path.,* 1959, *12,* 524.
33. Bernstein, D., Kleeman, C. R., Cutler, R. E., Dowling, J. T., and Maxwell, M. H.: Unpublished observations.