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Research Article

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Thyrocalcitonin, EGTA, and Urinary Electrolyte Excretion *

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Summary. The infusion of thyrocalcitonin (TCT) into thyroparathyroidectomized rats, given either no exogenous parathyroid hormone or a constant infusion of this hormone, leads to a transient phosphaturia and a decreased excretion of urinary magnesium, calcium, and hydroxyproline without a change in glomerular filtration rate. The changes in phosphate excretion may be due to a direct effect of the hormone upon renal tubular function or they may be a consequence of the fall in plasma calcium brought about by the action of TCT upon bone. In support of this latter alternative is the fact that the infusion of sodium ethylenebis-oxyethylenenitrilotetraäcetic acid (EGTA, a specific chelator of calcium) also leads to phosphaturia presumably as a consequence of hypocalcemia. However, EGTA infusion leads to enhanced urinary hydroxyproline excretion and sustained phosphaturia. These latter observations are interpreted to mean that alterations in the local ionic environment of osteolytic cells lead to changes in their activity and constitute a local regulatory system whose activity is modulated by the hormones, thyrocalcitonin and parathyroid hormone.

Introduction

Since the identification (1–4) and purification (5) of thyrocalcitonin, a number of studies have been undertaken in an effort to define its site of action. Most of these have indicated that a major site of action is upon bone (3, 4, 6–8), either to enhance mineral deposition or to suppress bone resorption. Either effect could presumably account for the fact that this hormone lowers the concentration of both calcium and phosphate in the plasma (3). However, none of these studies has ruled out the possibility that there are other sites of action within the organism.

The purpose of this report is to describe effects of TCT upon the renal excretion of electrolytes in thyroparathyroidectomized rats. The results indicate that thyrocalcitonin alters the excretion of several electrolytes, but it is not clear whether these changes represent a direct effect of TCT upon renal function or are mediated by hormonally induced changes in plasma electrolytes.

Methods

Male Holtzman rats, weighing 90 to 100 g, were given 100 U of vitamin D_2 by oral intubation and then maintained on a stock diet containing adequate quantities of this vitamin. When they reached 120 to 140 g, they were thyroparathyroidectomized by surgery. Immediately afterward, the perfusion of a standard solution, with or without parathyroid hormone (PTH), was begun at a rate of 3.0 to 3.5 ml per hour. The preparation of the animal, method of obtaining blood samples, analytic procedures, and perfusion technique were carried out as previously described (9). All animals were perfused with a solution containing 4% dextrose, 5 mM calcium, 5 mM magnesium, 20 mM sodium, and 2.5 mM potassium, all as their chloride salts. Parathyroid hormone (1 μg per

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3 ml) was added in some cases. The perfusion was sustained for 17 to 20 hours before urine collections were begun. Collections were made during a control period of several hours before TCT was added to the perfusate (40 μg per 3 ml) and infused for a period of 4 hours, after which the control infusion was continued for several more hours. Urine samples were collected at half-hour intervals. Blood samples were obtained during the control period, at the midpoint of the TCT perfusion, and at the end of the study. Other animals received no TCT but were perfused with a solution in which sodium chloride was replaced by sodium EGTA. The EGTA was perfused at a rate of 60 μmoles per hour. Determination of the plasma calcium in animals receiving EGTA was done by an ethylenediaminetetraäcetic acid (EDTA) titration method (10). Urinary creatinine excretion was followed as a measure of glomerular filtration rate. Creatinine was measured by the method of Bonsnes and Taussky (11). In some instances inulin excretion was measured in animals maintained on a constant infusion of inulin (9, 12). Urinary hydroxyproline was measured by the method of Prockop and Udenfriend (13).

Results

As discussed in a previous paper (9), the rates of urine flow were from 0.05 to 0.15 ml per hour less than the measured rates of perfusate infusion in all animals. However, there was no significant fluid retention. The animals usually weighed 3 to 4 g less after a 50-hour experiment than before surgery. We concluded that the discrepancy between urine flow and infusion rate was due to insensible water loss, difficulties in maintaining constant rates of infusion, and possibly slight evaporation from the urine samples as they stood in the fraction collector.

As noted previously (9), there were always sodium retention and potassium loss during the period of control infusion. In thyroparathyroidectomized animals not receiving a constant infusion of PTH, calcium was retained at a rate of 11 ± 3 μmoles per hour, and in those receiving PTH, 6 ± 2 μmoles per hour. The animals not receiving PTH were in magnesium balance, and those receiving PTH were in negative balance, excretion exceeding intake by 2 to 4 μmoles per hour.

As shown in Table I, the infusion of either TCT or EGTA led to little change in creatinine excretion. Because of the difficulties in obtaining large plasma samples, plasma creatinine was not measured, but was assumed to be constant and the rate of creatinine excretion was assumed to be a measure of glomerular filtration rate. To test this supposition, we determined the effect of TCT upon inulin excretion in three animals receiving constant infusion of inulin. The rates of inulin excretion were 0.66 ± 0.02 μg per minute during the control period and 0.63 ± 0.08 during the infusion of thyrocalcitonin.

TABLE I

*The effect of TCT and EGTA upon urinary creatinine excretion**

Experimental period	Creatinine excretion†
	μmole/minute
Control	0.032 ± 0.002
TCT	0.031 ± 0.004
Post-TCT	0.028 ± 0.003
Control	0.034 ± 0.003
EGTA	0.032 ± 0.004
Post-EGTA	0.029 ± 0.004

* TCT = thyrocalcitonin; EGTA = ethylenebis-oxyethylenenitrilotetraäcetic acid.

† Based upon four animals in each group. TCT was given at a rate of 40 μg per hour and EGTA at a rate of 60 μmoles per hour. All animals were maintained on a constant infusion of parathyroid hormone, 1 μg per hour.

The effect of TCT infusion upon the rate of excretion of urinary electrolytes is shown in Figure 1. The results are the mean rates of excretion of phosphate, calcium, magnesium, and sodium before, during, and after TCT infusion into four thyroparathyroidectomized rats receiving no parathyroid hormone (left) and four animals maintained on a constant infusion of parathyroid hormone, 1 μg per hour (right). Potassium excretion was also measured, but since there was no consistent change in the rates of its excretion, the data were not plotted. Also shown in Figure 1 are the plasma calcium and phosphate values before, during, and after TCT infusion into the two groups of animals. A statistical evaluation of the urinary data is recorded in Table II and of the plasma data in Table III.

The results indicate that TCT infusion leads to a rise in phosphate excretion and a fall in both calcium and magnesium excretion in thyroparathyroidectomized rats. These changes were considerably greater in animals maintained on a constant infusion of parathyroid hormone and were accompanied by a significant sodium diuresis in this group. TCT infusion led to a significant fall in both plasma phosphate and plasma calcium

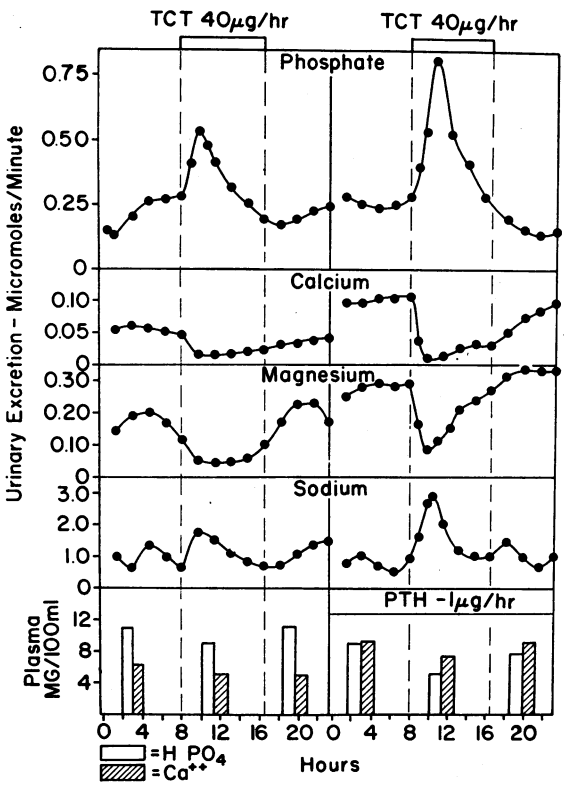

FIG. 1. THE EFFECT OF THYROCALCITONIN (TCT) INFUSION UPON URINARY AND PLASMA ELECTROLYTES IN THYROPARATHYROIDECTOMIZED RATS (LEFT) AND SIMILAR ANIMALS MAINTAINED ON A CONSTANT INFUSION OF PARATHYROID HORMONE (PTH) (RIGHT). The values represent the means of the values obtained from four animals in each set.

(Figure 1 and Table III). The changes in plasma phosphate were more marked in the group receiving a constant infusion of PTH. In this group the plasma calcium and phosphate returned to control values after TCT infusion had been stopped. However, in the group not given parathyroid hormone, plasma calcium remained low after cessation of TCT infusion even though plasma phosphate returned to control values (Table III). This persistently low plasma calcium was accompanied by a persistent reduction in rate of urinary excretion (Figure 1).

A most important feature of the TCT–induced phosphaturia was the fact that it was not sustained. The rates of urinary phosphate excretion returned to control values even during the continued infusion of TCT (Figure 1). Similarly, the rate of magnesium excretion, in the animals given a constant infusion of PTH (Figure 1, right), returned to nearly the control rate during the later hours of TCT infusion.

The rates of urinary hydroxyproline excretion were measured before and during TCT infusion in three animals maintained on a constant infusion of parathyroid hormone. The rate of excretion was 0.42 ± 0.04 μg per minute during the control period, and this fell to 0.15 ± 0.05 μg per minute during the first hour of TCT infusion. A similar fall in hydroxyproline excretion has been reported by Kohler and Pechet (14).

Two interpretations of the above data seem

TABLE II

*The effect of TCT upon the excretion of urinary electrolytes in thyroparathyroidectomized rats with and without a constant infusion of PTH**

		Rate of urinary excretion†							
		Without PTH				With PTH			
	Hours	HPO_4^-	Ca^{++}	Mg^{++}	Na^+	HPO_4^-	Ca^{++}	Mg^{++}	Na^+
		μmoles/minute							
Control	0–4	0.19 ± 0.09	0.058 ± 0.007	0.17 ± 0.07	0.8 ± 0.5	0.26 ± 0.06	0.08 ± 0.03	0.28 ± 0.07	1.0 ± 0.2
	4–8	0.26 ± 0.06	0.054 ± 0.004	0.16 ± 0.05	1.0 ± 0.6	0.24 ± 0.05	0.11 ± 0.04	0.29 ± 0.07	0.7 ± 0.3
TCT	8–12	0.41 ± 0.09	0.020 ± 0.005	0.06 ± 0.03	1.4 ± 0.7	0.73 ± 0.14	0.02 ± 0.02	0.11 ± 0.05	2.1 ± 0.4
p‡		<0.01	<0.002	<0.01		<0.001	<0.001	<0.001	<0.001
	12–16	0.26 ± 0.09	0.002 ± 0.007	0.06 ± 0.3	1.9 ± 0.4	0.39 ± 0.08	0.03 ± 0.03	0.23 ± 0.06	1.2 ± 0.3
p			<0.002	<0.01		<0.02	<0.01		
Post-TCT	16–20	0.18 ± 0.11	0.0027 ± 0.006	0.18 ± 0.06	0.8 ± 0.3	0.18 ± 0.05	0.05 ± 0.02	0.31 ± 0.05	1.1 ± 0.4
p			<0.002				<0.1		
	20–24	0.22 ± 0.07	0.0039 ± 0.008	0.22 ± 0.5	1.2 ± 0.4	0.13 ± 0.04	0.08 ± 0.03	0.33 ± 0.04	0.9 ± 0.2
p			<0.1						

* PTH = parathyroid hormone.
† Four rats in each group.
‡ p values compared to first control period.

TABLE III

Change in plasma calcium and phosphate during infusion of TCT or EGTA into thyroparathyroidectomized animals maintained with a constant infusion of electrolytes with or without PTH

		Without PTH		With PTH			With PTH	
		HPO_4^-	Ca^{++}	HPO_4^-	Ca^{++}		HPO_4^-	Ca^{++}
		mg/100 ml		*mg/100 ml*			*mg/100 ml*	
Control		10.8 ± 0.4*	6.2 ± 0.2	9.4 ± 0.5	8.7 ± 0.4	Control	10.6 ± 0.7	8.7 ± 0.4
TCT		8.6 ± 0.5	4.9 ± 0.3	6.3 ± 0.6	7.1 ± 0.4	EGTA	8.6 ± 0.6	5.0 ± 0.4
	p	<0.01	<0.01	<0.001	<0.01		<0.01	<0.01
Post-TCT		10.9 ± 0.05	4.7 ± 0.3	8.8 ± 0.4	8.4 ± 0.3	Post-EGTA	10.0 ± 0.6	8.0 ± 0.5

* Standard error. Four rats in each group.

possible. Either TCT has a direct effect upon renal tubular function; or the fall in plasma calcium, which this hormone produces by its action upon bone, leads to an alteration in renal tubular function, resulting in phosphaturia.

The first of these alternatives can best be tested by the direct infusion of TCT into the renal artery. However, this was not possible with the present experiments in small animals. The other possibility could be tested by determining the effect of a fall in plasma calcium, produced by some other means, upon urinary electrolyte excretion. The agent chosen to induce hypocalcemia was EGTA, which is a highly specific chelator of calcium. The effect of EGTA infusion upon the excretion of phosphate, calcium, and hydroxyproline in the urine, and the concentration of calcium and phosphate in the plasma, of four thyroparathyroidectomized animals maintained on a constant infusion of PTH is shown in Figure 2. EGTA infusion led to a fall in plasma calcium, a fall in plasma phosphate, a rise in urinary phosphate excretion, and a significant increase in rate of urinary hydroxyproline excretion. In contrast to the situation with TCT (Figure 1), EGTA-induced phosphaturia was sustained throughout the period of infusion and continued for a time after cessation of EGTA infusion. The changes in both plasma calcium and phosphate, induced by EGTA, were highly significant (Table III), and both returned to control values after EGTA infusion had been terminated.

To test whether EGTA might be acting by some mechanism other than that of chelating calcium, we infused this substance into three animals as its calcium rather than its sodium salt. The rate of Ca–EGTA infusion was identical to that used in the above experiments. There was no change in rate of phosphate excretion in any of the animals infused with Ca–EGTA. The mean rate of phosphate excretion was 0.21 ± 0.8

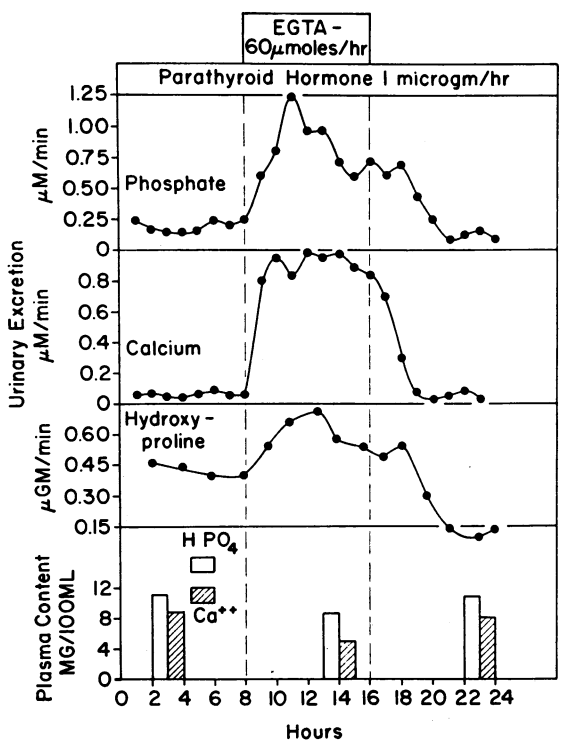

FIG. 2. THE EFFECT OF EGTA INFUSION UPON URINARY PHOSPHATE, TOTAL CALCIUM, AND HYDROXYPROLINE, AND UPON PLASMA CALCIUM AND PHOSPHATE IN THYROPARATHYROIDECTOMIZED RATS MAINTAINED ON A CONSTANT INFUSION OF PTH. The values represent the means of the values from four separate animals. The differences in rates of excretion of calcium, phosphate, and hydroxyproline were all significantly greater during the period of EGTA infusion as contrasted to both the control and postinfusion periods ($p < 0.001$). EGTA = ethylenebisoxyethylenenitrilotetraacetic acid.

TABLE IV

The source of urinary phosphate during TCT and EGTA infusion in thyroparathyroidectomized rats

No.	Treatment	Excess urinary phosphate	Loss of extracellular fluid phosphate
		μmoles	*μmoles*
4	TCT, 40 μg/hour	23 ± 4	20 ± 4
4	TCT, 40 μg/hour	46 ± 7	42 ± 5
4	EGTA, 60 μmoles/hour; PTH, 1 μg/hour	228 ± 42*	25 ± 4

*Significantly greater ($p < 0.001$) than loss from extracellular fluid.

μmole per minute during the control period, and 0.17 ± 0.6 μmole per minute during the period of Ca–EGTA infusion. Thus it seemed clear that EGTA, when infused as its sodium salt. was exerting its effects upon renal phosphate excretion because of its ability to lower plasma ionized calcium.

Because of the sustained elevation of phosphate excretion during EGTA infusion as compared to that in animals given TCT, it became of interest to calculate the source of urinary phosphate under the different experimental conditions. Two values were calculated: first, the excess of urinary phosphate, that is, the amount of phosphate excreted during the period of TCT or EGTA infusion in excess of that expected from the control rates of excretion; and second, the amount of phosphate disappearing from the extracellular fluids (ECF), based upon the assumption that the animals were in fluid balance (see above) and that the ECF composed 20% of the body weight. The values are shown in Table IV. When TCT was infused, the loss of phosphate from the ECF was of the same order of magnitude as that appearing in the urine. However, when EGTA was infused, urinary phosphate excretion far exceeded the loss of phosphate from the ECF.

Discussion

The present results indicate that thyrocalcitonin infusion leads to phosphaturia under conditions where there is no change in the rate of parathyroid hormone secretion or in glomerular filtration rate. However, our results do not establish whether this is a direct effect of TCT upon the renal tubule or is brought about as a consequence of the action of TCT upon bone. However, the studies with EGTA and Ca–EGTA infusions indicate that a fall in plasma calcium induced by EGTA can lead to an increased rate of urinary phosphate excretion (Figure 2). A similar result has been reported by Lavender and Pullman (15) and by Estep and co-workers (16). They have found that the infusion of calcium directly into the renal artery of the dog leads to phosphate retention, and the infusion of EDTA, a chelating agent similar to EGTA, leads to phosphate diuresis. In the present experiments the results obtained with Ca–EGTA infusion rule out the likelihood that this effect is exerted via the chelation of other cations. This being the case, it could be argued that the sequence of events after TCT infusion (Figure 1) is as follows: *1*) An inhibition of bone resorption leads to a fall in plasma calcium and in urinary calcium and hyproxyproline; *2*) the fall in plasma calcium leads to an increase in phosphate excretion; and *3*) this phosphaturia is not sustained because TCT prevents the mobilization of phosphate from bone.

An important difference between the response of the rat to TCT as compared to EGTA is the lack of a sustained phosphaturia with EGTA. The most likely explanation for this difference is that TCT prevents the mobilization of further phosphate from bone. Hence, when urinary phosphate excretion increases, plasma phosphate falls, and in spite of altered tubular phosphate transport, the decrease in filtered load leads to a decline in phosphate excretion. This interpretation is borne out by the fact (Table IV) that the amount of phosphate appearing in the urine was the same order of magnitude as that which disappeared from the ECF. This interpretation is also supported by the fact (14) that TCT depresses the rate of hydroxyproline excretion, which, under this circumstance, is probably a measure of the rate of bone resorption (14, 17).

Of particular note are the changes in phosphate and hydroxyproline excretion produced by EGTA infusion (Figure 2 and Table IV). The excretion of both increases significantly ($p < 0.001$) after EGTA infusion, and the amount of phosphate appearing in the urine is nearly ten times the amount disappearing from the extracellular fluids. Similarly, total calcium excretion is con-

siderably greater than that disappearing from the ECF. These changes indicate that the infusion of EGTA into a thyroparathyroidectomized rat maintained on a constant infusion of parathyroid hormone leads to the mobilization of bone, both mineral and matrix.

It has been known for some time that the infusion of chelating agents into parathyroidectomized animals leads to a mobilization of calcium from bone (18). McLean and Urist (19), and more recently Bronner and Aubert (20), have proposed models to account for this phenomenon. Both models suggest that the equilibrium between blood and bone, in the absence of parathyroid hormone, is maintained by a different type of control mechanism from that which operates when the parathyroid glands are present. Furthermore, neither the previous experiments nor the previous models considered the fate of phosphate or bone matrix, and both assumed that in the absence of parathyroid hormone the calcium that was mobilized came from the so-called exchangeable pool of bone mineral. The present evidence from EGTA infusions indicates that a lowering of plasma calcium leads to a complex series of changes both in the renal tubule and in the bone. In the former, the fall in plasma calcium leads to phosphaturia, and in the latter the fall in plasma calcium or phosphate or both leads to a mobilization of calcium, phosphate, and hydroxyproline, i.e., presumably to an increase in bone resorption with destruction of matrix, rather than, as previously thought, a shift of mineral from the exchangeable bone mineral. The present proposal is more in keeping with the original concept of Albright and Reifenstein (21) that an increase in renal phosphate excretion leads to a fall in plasma phosphate, which leads in turn to an increase in bone resorption. This theory was discarded by many when Barnicot (22) and others showed that parathyroid hormone has a direct effect upon bone resorption. The model of bone resorption that we propose on the basis of past data and the present experiments incorporates the Albright theory, but differs in a fundamental way from those proposed by McLean and Urist (19) and by Bronner and Aubert (20). The rate of bone resorption depends upon two elements: *1*) a local feedback system in which the ionic environment controls the activity of the osteolytic cells; and *2*) systemic modifiers, PTH, TCT, and vitamin D predominantly, which alter the setting or plasma electrolyte concentration about which the local system operates. Thus the system is formally analogous to multienzyme systems in which the end product, in this case an ion rather than a chemical product, regulates the activity of the entire system by negative feedback control.

At present, it is not possible to decide whether the initial fall in plasma calcium or the subsequent fall in plasma phosphate (due to the phosphaturia) is the important local feedback signal. These alternatives are being investigated. The scant evidence available suggests that phosphate may be the important control chemical (23, 24). If this is so, it will provide a rational basis for the therapeutic effectiveness of inorganic phosphate in osteolytic disorders (25).

It is also apparent from the present data that the local ionic environment controls the renal tubular transport of phosphate (Figure 2). A fall in calcium concentration leads to a rise in urinary phosphate excretion, and probably to a rise in intracellular phosphate concentration. A better understanding of these local control mechanisms, at the level of both bone and renal tubule, is obviously important to our eventual understanding of the mode of action of TCT and PTH.

Robinson, Martin, and MacIntyre (26) have recently reported that TCT infusion leads to phosphaturia in parathyroidectomized rats. They concluded that this change in phosphate excretion is brought about by a direct effect of TCT upon the renal tubule. However, their conclusion was based on indirect evidence, and their data do not rule out the alternative explanation suggested by the present study.

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References

1. Hirsch, P. F., G. F. Gauthier, and P. L. Munson. Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the

response to parathyroidectomy in rats. Endocrinology 1963, **73**, 244.

2. Foster, G. V., A. Baghdiantz, M. A. Kumar, E. Slack, H. A. Soliman, and I. MacIntyre. Thyroid origin of calcitonin. Nature (Lond.) 1964, **202**, 1303.
3. Hirsch, P. F., E. F. Voelkel, and P. L. Munson. Thyrocalcitonin: hypocalcemic hypophosphatemic principle of the thyroid gland. Science 1964, **146**, 412.
4. Talmage, R. V., J. Neuenschwander, and L. Kraintz. Evidence for the existence of thyrocalcitonin in the rat. Endocrinology 1965, **76**, 103.
5. Tenenhouse, A., C. Arnaud, and H. Rasmussen. The isolation and characterization of thyrocalcitonin. Proc. nat. Acad. Sci. (Wash.) 1965, **53**, 818.
6. Baghdiantz, A., G. V. Foster, A. Edwards, M. A. Kumar, E. Slack, H. A. Soliman, and I. MacIntyre. Extraction and purification of calcitonin. Nature (Lond.) 1964, **203**, 1027.
7. Friedman, J., and L. G. Raisz. Thyrocalcitonin: inhibitor of bone resorption in tissue culture. Science 1965, **150**, 1465.
8. Anast, C., C. D. Arnaud, H. Rasmussen, and A. Tenenhouse. Thyrocalcitonin and the response to parathyroid hormone. J. clin. Invest. 1967, **46**, 57.
9. Arnaud, C., H. Rasmussen, and C. Anast. Further studies on the interrelationship between parathyroid hormone and vitamin D. J. clin. Invest. 1966, **45**, 1955.
10. Technical bulletin no. 6071C. Palo Alto, Spinco Division, Beckman Instruments.
11. Bonsnes, R. W., and H. H. Taussky. On the colorimetric determination of creatinine by the Jaffe reaction. J. biol. Chem. 1945, **158**, 581.
12. Roe, J. H., J. H. Epstein, and N. P. Goldstein. A photometric method for the determination of inulin in plasma and urine. J. biol. Chem. 1949, **178**, 839.
13. Prockop, D. J., and S. Udenfriend. A specific method for the analysis of hydroxyproline in tissues and urine. Analyt. Biochem. 1960, **1**, 228.
14. Kohler, H. F., and M. M. Pechet. The inhibition of bone resorption by thyrocalcitonin (abstract). J. clin. Invest. 1966, **45**, 1033.
15. Lavender, A. R., and T. N. Pullman. Changes in inorganic phosphate excretion induced by renal arterial infusion of calcium. Amer. J. Physiol. 1963, **205**, 1025.
16. Estep, H. L., C. T. Gardner, Jr., J. P. Taylor, A. Minott, and H. St. George Tucker, Jr. Phosphate excretion patterns following intravenous injections of ethylenediaminetetraacetate (EDTA). J. clin. Endocr. 1965, **25**, 1385.
17. Harris, E. D., Jr., and A. Sjoerdsma. Effect of parathyroid extract on collagen metabolism. J. clin. Endor. 1966, **26**, 358.
18. Copp, D. H., H. Moghadam, E. D. Mensen, and G. D. McPherson. The parathyroids and calcium homeostasis *in* The Parathyroids, R. O. Greep and R. V. Talmage, Eds. Springfield, Ill., Charles C Thomas, 1961.
19. McLean, F. C., and M. W. Urist. Bone. An Introduction to the Physiology of Skeletal Tissue, 2nd ed. Chicago, University of Chicago Press, 1961.
20. Bronner, F., and J. P. Aubert. Bone metabolism and regulation of the blood calcium level in rats. Amer. J. Physiol. 1965, **209**, 887.
21. Albright, F., and E. C. Reifenstein, Jr. The Parathyroid and Metabolic Bone Disease: Selected Studies. Baltimore, Williams & Wilkins, 1948.
22. Barnicot, N. A. The local action of the parathyroid and other tissues on bone in intracerebral grafts. J. Anat. (Lond.) 1948, **82**, 233.
23. Raisz, L. G. Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. J. clin. Invest. 1965, **44**, 103.
24. Arnaud, C. D., A. M. Tenenhouse, and H. Rasmussen. Parathyroid hormone. Ann. Rev. Physiol. In press.
25. Dent C. E. Same problems of hyperparathyroidism. Brit. med. J. 1956, **2**, 1419 and 1495.
26. Robinson, C. J., T. J. Martin, and I. MacIntyre. Phosphaturic effect of thyrocalcitonin. Lancet 1966, **2**, 83.