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### **Research Article**

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# Parathyroid Hormone Secretion in Vivo

## DEMONSTRATION OF A CALCIUM-INDEPENDENT, NONSUPPRESSIBLE COMPONENT OF SECRETION

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**ABSTRACT** The response of normal bovine parathyroid glands to hypercalcemia was assessed in vivo by radioimmunoassay of immunoreactive parathyroid hormone concentrations in parathyroid effluent blood obtained by surgical cannulation of both anesthetized and nonanesthetized calves. Hypercalcemia was induced for periods of 0.3–35 h by intravenous infusion of a solution of calcium chloride. Assessment of immunoreactivity in effluent and peripheral blood included measurements of selected samples by use of a radioimmunoassay specific for a site residing in the biologically active portion of the hormone molecule. In all instances, the concentration of immunoreactive parathyroid hormone in hypercalcemic venous effluent from a superior parathyroid gland exceeded that of the peripheral blood. Failure of hypercalcemia to suppress completely secretion by normal parathyroids indicates that a portion of parathyroid hormone secretion occurs independent of blood calcium concentration. Consequently, continued parathyroid hormone secretion despite hypercalcemia can no longer be regarded as a unique feature of parathyroid neoplasia.

### INTRODUCTION

Several lines of evidence have suggested that hypercalcemia may not completely suppress the secretion of parathyroid hormone (1–4). Nevertheless, certain aspects of the previous experiments leave some doubt regarding the general validity of the conclusion that there is a major calcium-independent component of hormone secretion in vivo. Although in vitro studies have demonstrated release of immunoreactive material from parathyroid

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glands despite a supraphysiologic calcium concentration of the bathing medium (1, 2), the possibility of non-specific leakage of hormone from intracellular stores secondary to reduced viability of those tissues cannot be readily excluded. Transplantation of multiple isologous parathyroid glands in rats was accompanied by a persistent elevation of plasma calcium concentration (3), but the induced hypercalcemia was not severe (plasma calcium concentration approximately 12 mg/100 ml) and hence did not adequately examine the issue of hormone secretion in markedly hypercalcemic states such as severe primary hyperparathyroidism. While persistence of detectable amounts of immunoreactive parathyroid hormone in the peripheral blood of cows during induced hypercalcemia (4) in our own earlier studies was felt to be consistent with continued secretion, the possibility that the basal concentration detected represented merely a lingering component of hormone secreted before suppression was not eliminated. The above considerations together with the relevance of the question of nonsuppressible secretion to parathyroid disorders in man has led us to assess the response of normal bovine parathyroid glands to sustained hypercalcemia in vivo by measurement of hormone concentration in parathyroid effluent blood.

### METHODS

The arteriovenous difference in concentrations of parathyroid hormone across a parathyroid gland was measured in 12 anesthetized and 2 conscious calves during induced alterations of plasma calcium concentration. The animals ranged in age from 2 to 14 wk. Anesthesia was induced and maintained by inhalation of a mixture of halothane and nitrous oxide.

The vein draining the area of the superior parathyroid gland was located and cannulated with either polyethylene or polyvinyl tubing after all major tributaries not originating from the parathyroid gland had been ligated. During the experiments on anesthetized calves, blood was allowed to flow continuously through the venous cannula without restriction and was collected and measured volumetrically during timed intervals. In the experiments to be performed

on conscious calves, the free end of the polyvinyl cannula was exteriorized to the lateral surface of the neck by threading it through a needle which had been inserted through the skin and muscles of the neck to the region of the cannulated vein. Once exteriorized, the free end of the parathyroid effluent cannula was connected to a catheter of the same diameter placed in the jugular vein by a Teflon cuff, slightly larger in diameter. By uncoupling the two catheters at the cuff, recycling of parathyroid effluent blood to the systemic circulation could be intermittently interrupted for collection of parathyroid effluent after recovery from anesthesia. In the experiments to be performed under anesthesia, a cannula was placed in the saphenous artery. In the conscious calves, a small artery in the neck was cannulated for the collection of arterial blood. To inhibit clot formation in the cannulae, sodium heparin (300 U/kg body weight) was administered intravenously immediately before cannulating the blood vessels. For maintenance of anticoagulant effect, one-half of the initial dose of sodium heparin was administered intravenously every hour for the duration of the experiment.

Consecutive paired arterial and venous samples were taken by collecting total blood flow from each cannula concurrently for intervals of 5, 10, or 15 min. Packed cell volume of effluent blood was determined so that plasma flow rate could be calculated. Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  for analysis. Alterations in plasma calcium concentration were induced by a constant infusion of either a solution of calcium chloride or disodium ( $\text{Na}_2$ ) EDTA into the jugular vein. Plasma calcium concentration of arterial samples was determined by automatic fluorometric titration using ethyleneglycol bis (aminoethylether) tetraacetic acid as the calcium chelator (5). Plasma parathyroid hormone concentrations were determined in both arterial and venous samples by radioimmunoassay (6) using antisera obtained by immunizing guinea pigs with bovine parathyroid hormone and employing a highly purified parathyroid hormone preparation (7) for use as a standard and for preparation of  $^{125}\text{I}$ -labeled hormone. In our conventional assay, an antiserum (GP-133) which recognizes antigenic sites located in the 19-34 and 53-84 regions of the amino acid sequence of the hormone molecule (8) was used. Since one of these recognition sites (53-84) is definitely outside the biologically active region of the molecule (9), this assay will detect both intact hormone and biologically inactive carboxyl terminal fragments of the hormone molecule. Therefore, arteriovenous difference of immunoreactive hormone during hypercalcemia was determined in one or two pairs of samples in each experiment on anesthetized calves using another antiserum (GP-1) which had been preincubated with an excess of a peptide fragment (53-84) of parathyroid hormone. Modification of antiserum GP-1 in this fashion restricts antigenic recognition to a portion of the hormone molecule that is requisite for biological activity since the antigenic determinant includes all or some of the 14-30 region of the molecule (8). This "N assay" provides assurance that the immunoreactivity detected at least contains the region of hormone sequence required for biological activity.

Gel filtration chromatography of parathyroid effluent plasma from two calves (C-34 and C36) was carried out on  $1.2 \times 60$ -cm columns of Bio-Gel P-100 (100-200 mesh, Bio-Rad Laboratories, Richmond, Calif.) at  $4^{\circ}\text{C}$  with an eluting buffer of 0.05 M sodium barbital (pH 8.6) containing 2% human plasma. Fraction size was 1.01 ml. To mark the elution pattern of intact parathyroid hormone, the plasma samples were cochromatographed with radiiodinated, highly purified bovine parathyroid hormone. After counting the radioactivity of each fraction in a gamma scin-

tillation spectrophotometer, immunoreactivity was determined in 300- $\mu\text{l}$  portions of each fraction using the N assay.

Secretion rate for the superior parathyroid gland (nanograms per minute) was calculated as the product of the effluent plasma flow rate (milliliters per minute) and the arteriovenous difference of immunoreactivity (nanograms per milliliter) as determined by the GP-133 assay.

## RESULTS

Although the parathyroid venous effluent concentration of immunoreactive parathyroid hormone (GP-133 assay) was reduced by the induction of hypercalcemia (Fig. 1), the hormone concentration of effluent blood remained well above the arterial concentration despite sustained hypercalcemia (i.e., greater than 12 mg/100 ml for up to 3.6 h in duration) in all 12 anesthetized calves (Table I). (Although the data are not shown, secretion rate of the parathyroid gland during normocalcemic periods before and after hypercalcemia was similar suggesting that the reduction in secretion rate associated with hypercalcemia was due to an effect of calcium rather than an impairment of gland function due to venous cannulation.) Likewise, assay of representative paired arterial and venous samples from each of these experiments using modified GP-1 antiserum (N assay) also revealed a greater concentration of immunoreactive material in the

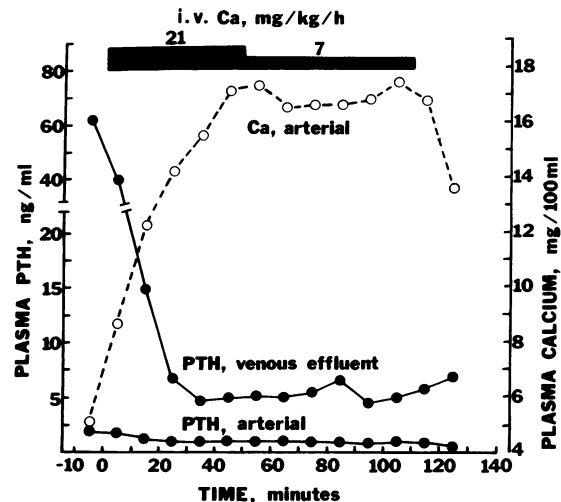


FIGURE 1 Incomplete suppression of parathyroid secretion in a calf during hypercalcemia. Parathyroid effluent blood was collected during general anesthesia by surgical cannulation of the vein draining a superior parathyroid gland. Arterial and venous blood samples were collected concurrently by continuous flow for 10-min intervals. Immunoreactive parathyroid hormone concentration was measured using antiserum GP-133. The initial hypocalcemia was induced by intravenous infusion of  $\text{Na}_2\text{EDTA}$ . The rapid increase in arterial concentration of calcium was accompanied by a sharp decline in parathyroid secretion. (Blood flow through the gland remained relatively constant while the hormone concentration declined). The persistence of a significant arteriovenous difference in concentration of immunoreactivity despite hypercalcemia demonstrates an inability of calcium to completely suppress secretion.

TABLE I  
*Persistent Arteriovenous Difference in Parathyroid Hormone (PTH) Concentration across a Superior Parathyroid Gland during Experimentally Induced Hypercalcemia in 12 Anesthetized Calves*

Calf	No. of samples	Duration of hypercalcemia h	Plasma calcium* mg/100 ml	Plasma PTH				PTH secretion rate** ng/min
				GP-133‡		GP-1 (N-Assay)§		
				Venous	Arterial	Venous	Arterial	
C-5	9	2.0	12.9–15.5	5.9±0.4	0.1±0.01	10.4	UD''	5.2
C-7	11	1.8	12.6–19.2	0.9±0.06	0.2±0.04	1.5	UD	1.3
C-13	11	1.8	14.3–17.5	2.7±0.1	0.4±0.03	3.3	1.2	4.5
C-15	16	3.6	12.0–20.0	1.2±0.09	0.3±0.02	1.1	UD	3.4
C-20	5	0.8	12.1–14.9	3.3±0.06	0.7±0.02	1.7	—¶	4.3
C-21	4	0.3	12.1–13.1	3.4±0.1	0.5±0.06	2.7	UD	3.1
C-27	6	1.0	13.2–16.1	8.4±0.5	0.4±0.01	5.5	UD	4.9
C-30	6	1.0	12.0–15.3	2.9±0.4	0.6±0.06	2.5	UD	7.4
C-31	14	2.6	12.3–16.4	1.4±0.1	0.6±0.02	1.8	UD	1.0
C-33	12	1.8	12.5–16.3	4.4±0.2	0.3±0.01	4.2	UD	10.1
C-34	8	1.3	12.1–15.7	17.8±0.4	0.5±0.02	13.2	UD	8.1
C-36	6	1.5	12.6–17.4	41.0±4.0	0.4±0.02	>20.0	UD	8.0

\* Range of arterial plasma calcium concentration of samples collecting during calcium infusion.

‡ Immunoreactive plasma PTH concentration determined using antiserum GP-133 which has antigenic recognition sites in two regions of the PTH molecule (19–34 and 53–84). Values are mean±SE for either all arterial or venous samples collected during hypercalcemia.

§ Immunoreactive plasma PTH concentration determined using antiserum GP-1 that had been preincubated with an excess of PTH fragment, 53–84. This restricts the antigenic recognition site to the amino terminal region of the molecule which is essential for biological activity. Values are results of assay of one or two pairs of arterial and venous samples collected during the hypercalcemic phase of each experiment.

'' Undetectable. The assay was able to detect a plasma immunoreactive PTH concentration of 0.95 ng/ml.

¶ Sample not assayed.

\*\* Secretion rate of the single superior parathyroid gland based on measurement of immunoreactivity using the GP-133 assay.

venous effluent blood during hypercalcemia (Table I). With only one exception, immunoreactive hormone was undetectable in arterial blood using the N assay. All samples tested for N assay reactivity were run in a single assay, and the minimum detectable plasma hormone concentration was 0.95 ng/ml.

The differences in hormone concentration of venous effluent blood observed between calves (Table I) appears to be due mainly to differences in the proportion of nonparathyroid venous blood contained in the samples. The variation between calves is less when results are expressed as secretion rate (Table I) since it is the product of effluent flow rate and the difference in arteriovenous concentration. In other words, when the parathyroid venous blood was diluted by blood from venous tributaries of nonparathyroid origin, the concentration gradient was reduced but the flow rate was increased. Thus, secretion rate varied less than effluent concentration.

The results of gel filtration chromatography of parathyroid effluent plasma (Fig. 2) indicate that the immunoreactivity measured in these samples by the N assay has an elution pattern that coincides with that of radiolabeled intact parathyroid hormone. No hormonal

fragments having antigenic sites recognizable by this assay were detectable in these samples.

In the experiments on conscious calves (C-104 and C-113), hypercalcemia was maintained for a much longer duration. Although in calf C-104, technical problems necessitated several interruptions of the intravenous infusion of calcium, plasma calcium was continuously maintained above 12 mg/100 ml for a period of 11 h. During this period of hypercalcemia, the concentration of immunoreactivity (GP-133 assay) in parathyroid effluent plasma continuously exceeded that of peripheral plasma (Fig. 3A). In calf C-113, hypercalcemia was maintained for 35 h (Fig. 3B). With the exception of the initial 4 h of infusion, secretion rate of the gland was assessed at hourly intervals. The gland continued to secrete immunoreactive hormone (GP-133 assay) despite hypercalcemia throughout the period of assessment. After termination of the calcium infusion, the return to normocalcemia during an intravenous infusion of Na<sub>2</sub>EDTA was accompanied by a rapid (within 30 min) increase in secretion rate (Fig. 3B).

## DISCUSSION

The results of the studies in anesthetized calves offer a direct demonstration that continuous secretion of hor-

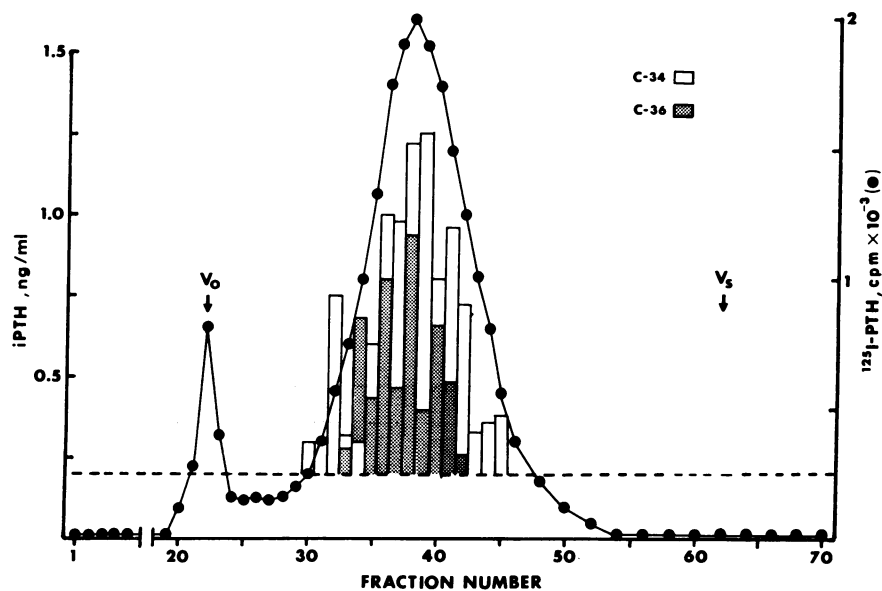


FIGURE 2 Gel-filtration chromatography of hypercalcemic parathyroid effluent plasma. The large peak of radioactivity between the void and salt volumes designates the elution pattern of radioiodinated highly purified bovine parathyroid hormone. The open and hatched bars designate the concentration of immunoreactivity (N assay) in fractions collected during filtration of calf parathyroid effluent plasma containing a high concentration of immunoreactivity (Table I). The two samples were cochromatographed with labeled parathyroid hormone in separate runs. The detection limit of the N assay in these experiments is indicated by the horizontal dashed line. The only detectable immunoreactivity eluted coincident with the radiolabeled marker; no immunoreactivity was detected in fractions eluting either earlier or later than the radioiodinated parathyroid hormone.

mone by parathyroid glands *in vivo*, despite pronounced hypercalcemia, is a general phenomenon. Concentrations of immunoreactivity that were readily detectable by our radioimmunoassays were found in parathyroid effluent blood during hypercalcemia in all 12 animals studied; the persistent arteriovenous differences reflected continued secretion of hormone despite pronounced hypercalcemia. This evidence indicates that a portion of the secretory output of the parathyroid gland is independent of blood calcium concentration and confirms earlier interpretations derived from results of *in vitro* experiments (1) and *in vivo* experiments (3, 4) in which parathyroid secretory activity was assessed indirectly.

The experiment dealing with two additional conscious calves provide important confirmation about several features of the results. First of all, the continuous secretion of immunoreactive parathyroid hormone despite hypercalcemia in fully conscious calves eliminates any artifactual effect of anesthesia. Secondly, the longer duration of these experiments (up to 35 h) and the full responsiveness of the gland to induced hypocalcemia after many hours, diminishes the likelihood that secretion during hypercalcemia represents merely a transient wash out of stored hormone as well as any concern that the gland was injured by the perfusion technique. Based on measurement of parathyroid gland content of hormone (200

ng/mg of wet tissue) (11), the mean superior parathyroid gland secretion rate in hypercalcemic anesthetized calves (5.1 ng/min), and an average gland weight of approximately 30 mg in calves of this size (unpublished observations), we expect that stored hormone (6,000 ng) might, on the average, sustain secretion at the hypercalcemic rate for approximately 20 h. Since we do not know the quantity of stored hormone in C-113, we cannot make a similar calculation for this experiment. Nevertheless, the extension of this experiment far beyond the anticipated time for depletion of gland stores increases the likelihood that newly synthesized hormone contributed to secretion during the period of hypercalcemia. Evidence for continued biosynthesis is further supported by the observation that gland stores were far from depleted during prolonged hypercalcemia in C-113. After termination of the intravenous calcium infusion, administration of Na<sub>2</sub>EDTA brought about a rapid return to normocalcemia and a concurrent pronounced increase in parathyroid secretion rate (Fig. 3B).

The physiological relevance of a nonsuppressible component of parathyroid secretion with sustained hypercalcemia depends upon whether such secretion consists of biologically active hormone. The critical experiment concerning the character of hormone secreted in calves dur-

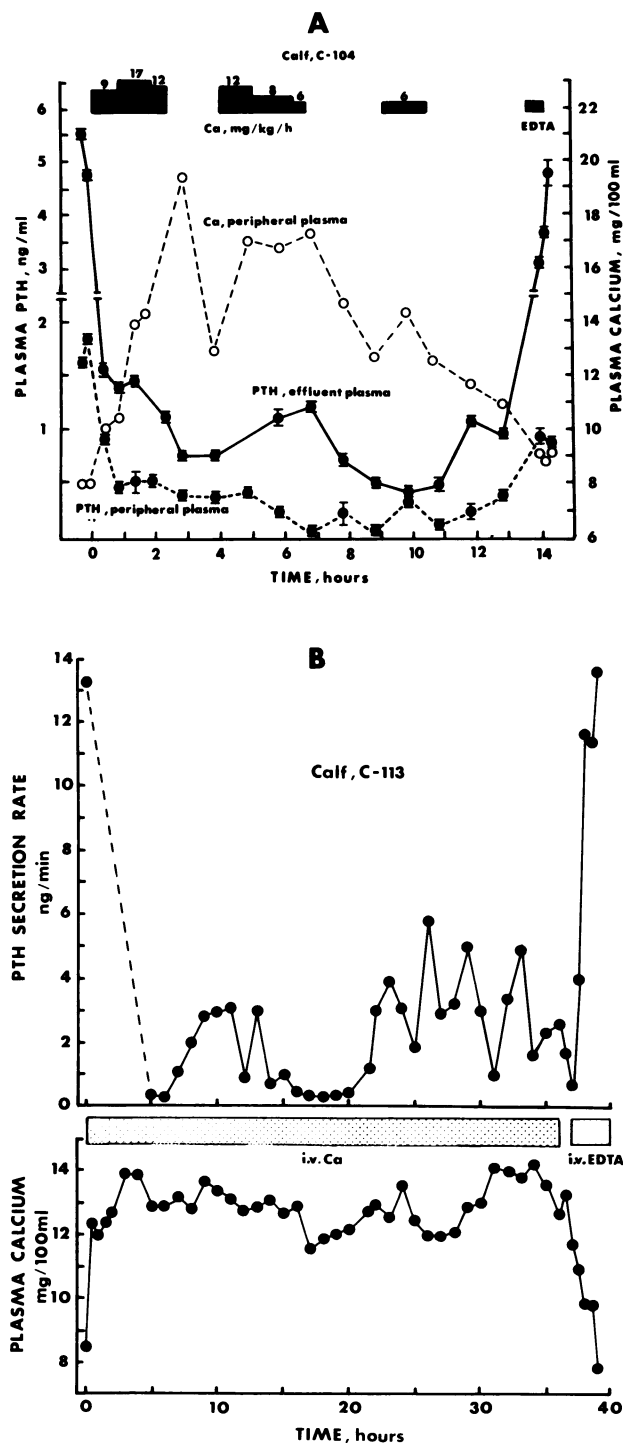


FIGURE 3 Secretion of parathyroid hormone in conscious hypercalcemic calves. (A) Although technical problems necessitated two interruptions of the intravenous calcium infusion, hypercalcemia (above 12 mg/100 ml) was maintained for 11 h. During this period, the concentration of immunoreactivity (GP-133 assay) in the effluent plasma continuously exceeded that of the peripheral plasma. The

ing persistent hypercalcemia would be direct assay of biological activity of secreted hormone. However, existing bioassays are not sufficiently sensitive to measure hormone concentration in the hypercalcemic parathyroid gland effluent collected in these experiments. However, we have obtained impressive, albeit indirect, evidence that biologically active hormone is secreted despite severe hypercalcemia. Since biological activity of the parathyroid hormone molecule resides in a crucial sequence of only 27 amino acids located at its amino terminal end (9), radioimmunoassays using antisera whose antigenic determinates are located within the amino terminal area of the molecule are helpful in providing assurance that hormone, apparently identical in molecular size to the glandular hormone used as standard, is indeed biologically active. Secretion of intact hormone, presumed to be biologically active, by parathyroids of hypercalcemic, hyperparathyroid patients has already been demonstrated using such criteria (8, 10, 12-14). Use of modified GP-1 antiserum (N assay) in these studies has led to a demonstration that the immunoreactive material secreted into the parathyroid gland effluent contained at least a substantial portion of the sequence of amino acids essential for biological activity (Table I). Furthermore, analysis of parathyroid effluent plasma from two calves by gel-column chromatography (Fig. 2) revealed that the N assay immunoreactivity present in these samples was comprised of a molecular species indistinguishable in size from intact parathyroid hormone (84 amino acids). Furthermore, in view of the evidence suggesting that the N assay immunoreactivity is intact hormone, the close agreement between results of analyses of parathyroid effluent by the N assay and GP-133 assay in 12 calves (Table I) is consistent with the direct evidence from gel filtration that the major portion of effluent immunoreactivity is intact parathyroid hormone. Thus, the foregoing observations strongly suggest that biologically active intact parathyroid hormone is secreted by normal glands during hypercalcemia.

Secretion of parathyroid hormone during hypercalcemia has been considered previously to be a feature unique to primary hyperparathyroidism, i.e., parathyroid neoplasia or hyperplasia. The demonstration of this

solid symbols with bars indicate the mean  $\pm$  SE of quadruplicate determinations on each sample. (B) Despite hypercalcemia of 35 h duration, the superior parathyroid gland continued to secrete immunoreactive hormone. With the exception of a period at the beginning and near the midpoint of the intravenous calcium infusion, the secretion rate was within the range observed in the experiments of shorter duration performed on the anesthetized calves. The periods of reduced secretion rate were associated with partial occlusion of the effluent vessel. The return to normocalcemia (9-11 mg/100 ml), hastened by the intravenous infusion of  $\text{Na}_2\text{EDTA}$  near the end of the experiment, was accompanied by a rapid increase in secretion rate.

phenomenon in normal parathyroid glands may be relevant to the syndromes of primary hyperparathyroidism in man. In view of the response of parathyroid adenomas in man to experimentally induced changes in plasma calcium concentration (15), autonomy of secretion is no longer a satisfactory explanation for the excessive secretory output of hormone associated with this condition. Since plasma parathyroid hormone concentration is inappropriately excessive in the presence of elevated blood calcium, it is clear that some abnormality, albeit more subtle than totally autonomous secretion, must exist in the control of parathyroid hormone output in primary hyperparathyroidism. Our experiments demonstrate that neoplastic changes in parathyroid cells are not a prerequisite to secretion during hypercalcemia. It remains to be determined whether such continued secretion of parathyroid hormone is also characteristic of normal parathyroid glands during hypercalcemia more chronic in duration; i.e., weeks or months as opposed to 35 h. Our present findings provide a stimulus to develop a means, such as induction of vitamin D intoxication, for the production of chronic hypercalcemia in calves so that the issue of persistent secretion of hormone during weeks to months of hypercalcemia may be critically tested. If continued secretion of hormone were to be found characteristic of the parathyroids despite weeks or months of hypercalcemia, such results would strengthen the speculation that excessive hormone secretion, despite associated hypercalcemia, found in patients with parathyroid adenomas may represent, at least in part, an exaggeration of the nonsuppressible component of normal secretion brought on by an increase of glandular mass.

The present findings suggest a need to modify our previous understanding of the homeostatic regulation of parathyroid hormone secretion. Although the control of hormone secretion by blood calcium features a pronounced decrease of secretion rate in association with the induction of hypercalcemia (16), suppression of secretion is incomplete, and at least under acute conditions, a degree of hormone secretion persists which is independent of blood calcium concentration. The physiological significance of this persistent but reduced rate of hormone secretion is at present unknown.

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