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Research Article

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Etiology of Hyperparathyroidism and Bone Disease during Chronic Hemodialysis

III. EVALUATION OF PARATHYROID SUPPRESSIBILITY

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ABSTRACT Parathyroid function was assessed by calcium infusions (4–8 h) in 16 patients with chronic renal insufficiency being treated by long-term hemodialysis. The concentrations of two immunoreactive species of parathyroid hormone in plasma (iPTH-9, mol wt 9500; iPTH-7, mol wt 7000) were estimated by radioimmunoassays utilizing two relatively specific antisera. Control values of the smaller species, iPTH-7, were uniformly high, whereas values of iPTH-9 were normal in 12 of 19 studies. Response of iPTH-7 to calcium infusions was variable, with significant decreases occurring only five times in 27 infusions. Concentrations of iPTH-9, however, decreased during every calcium infusion. In contrast to these acute responses, five of six patients studied during periods of dialysis against both low (< 6 mg/100 ml) and high (7–8 mg/100 ml) calcium concentrations in the dialyzate showed a decrease in values of iPTH-7 during the period of dialysis against the higher calcium concentration. It is concluded that plasma concentrations of iPTH-9 reflect primarily the moment-to-moment secretory status of the parathyroid glands, while concentrations of iPTH-7 reflect more closely chronic parathyroid functional status. It is further concluded that the failure of iPTH-7 to decrease during induced hypercalcemia should not be equated with autonomy of parathyroid gland function.

INTRODUCTION

The serum calcium concentration is acknowledged to be the major factor influencing the secretion of parathyroid hormone (PTH),¹ doing so by negative feedback

control (1, 2). Based on this relationship, the use of calcium infusion as a means of testing parathyroid responsiveness has gained wide acceptance. Until recently, however, PTH secretory responses have been evaluated only indirectly by assessment of changes in urinary excretion or serum concentration of phosphorus (3–6). With the introduction of radioimmunoassay techniques for estimation of serum or plasma concentrations of PTH (iPTH) (7–11), it has become feasible to evaluate parathyroid functional status more directly.

Reiss, Canterbury, and Egdahl (10) reported that, in patients with secondary hyperparathyroidism due to mild to moderate renal insufficiency, calcium infusions resulted in a progressive decrease in serum iPTH, the rate of decrease being inversely related to the degree of renal insufficiency. We have recently performed similar studies in patients being treated by chronic hemodialysis and have observed that the responses were variable, depending in large measure on the immunoreactive species of PTH estimated by the antiserum being used. Two relatively specific antisera were used in these studies to estimate the plasma concentrations of two molecular species of immunoreactive parathyroid hormone: iPTH-7 with a molecular weight of about 7000, and iPTH-9 with a molecular weight of about 9500. Control plasma values of the smaller species, iPTH-7, were uniformly high, whereas values of iPTH-9 were normal in 12 of 19 studies. Responses of iPTH-7 to calcium infusions were variable, significant decreases occurring only five times in 27 infusions. Concentrations of iPTH-9, however, decreased during every calcium infusion, sometimes to undetectable values. Despite lack of acute suppression of iPTH-7 in many patients, all patients showed long-term suppression after serum phosphorus was decreased by oral administration of phosphate-binding agents and after dialysis was instituted against high concentrations of calcium (12).

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¹ *Abbreviations used in this paper:* iPTH, immunoreactive species of parathyroid hormone; PTH, parathyroid hormone.

METHODS

Patient selection. 16 patients were randomly selected for this study from among a group of patients undergoing treatment by long-term hemodialysis for renal insufficiency. Initial studies were performed immediately before or during their training for hemodialysis. In some patients, repeat studies were performed after variable periods of dialysis.

Dialysis. Details of the technique of dialysis have been reported (13, 14). The only variables introduced into the dialysis regimen were the use of oral aluminum hydroxide therapy and changes in the concentration of calcium in the dialyzate. All patients who were dialyzed against calcium concentrations in excess of 6 mg/100 ml received aluminum hydroxide orally in sufficient dosages to maintain the plasma concentration of phosphorus below 6 mg/100 ml.

Calcium infusion. All calcium infusions were performed with calcium gluconate diluted in 500 ml of 0.45% saline. The concentration of calcium was adjusted so that the infusion rate of 60 ml/h would correspond to 4 mg Ca/kg body wt per h. The infusion rate was controlled by either a syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.) or a roller pump (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). At the beginning of the investigation, calcium infusions lasted 4 h; later, calcium infusions were given for 8 h. In some cases, however, calcium infusions were terminated earlier than the projected duration when plasma calcium was found to be more than 15 mg/100 ml or when patients became symptomatic (for example, nauseated). Blood was drawn before, every 2 h during, and 24 h after the calcium infusion. Calcium infusion was performed on the day after a dialysis, with a minimum interval of 18 h between the end of a dialysis and the beginning of calcium infusion.

Analytical methods. Total plasma calcium was measured by atomic absorption spectroscopy (15), ionized calcium by a flow-through electrode (Orion Research, Inc., Cambridge, Mass.) (16), and phosphorus by the method of Fiske and Subbarow adapted for the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (17).

Radioimmunoassays of PTH. Radioimmunoassay of PTH in plasma samples was done by the method of Arnaud, Tsao, and Littledike (11) with minor modifications. Two separate antisera (GP1M and CH14M) were used. They were selected from a large group of antisera produced against bovine and porcine PTH on the basis of their ability to react selectively with one of two molecular species of human PTH which have molecular weights of about 9500 and 7000. These were isolated in a relatively homogeneous state by gel filtration of desalted media from large-scale cultures of parathyroid adenoma explants (18). They each produced hypercalcemia and increased renal excretion of phosphate in conscious, perfused, parathyroidectomized rats. The 9500 mol wt form (iPTH-9) could not be distinguished immunologically from the major PTH species which is extracted from parathyroid adenomas (18, 19). The 7000 mol wt form (iPTH-7) could not be distinguished immunologically from the predominant form of PTH which circulates in human hyperparathyroid plasma or serum (18).

Mixtures of the two forms of PTH in varying concentration ratios were prepared. Antisera were then studied to determine their relative ability to react selectively with either of the species in the mixtures. The details of these investigations will be published separately; however, it was found that antiserum GP1M reacted almost exclusively with iPTH-7, and CH14M, with iPTH-9. Gel filtration of

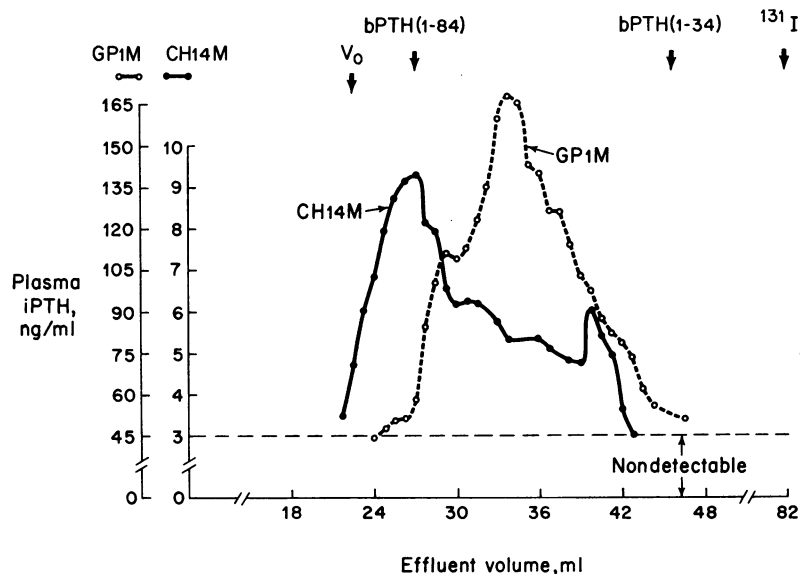


FIGURE 1 Gel filtration of 1 ml of plasma from patient with chronic renal failure. Bio-Gel P-30 column, 1 × 100 cm; 0.2 M ammonium acetate (pH 4.6) and bovine serum albumin (1 mg/100 ml) as eluant; 3 ml/h flow rate; 0.75-ml fractions. Positions of calibration substances are indicated at the top of the figure. Fractions were assayed for immunoreactive PTH with antisera CH14M and GP1M. The interrupted line near the bottom of the figure represents the limit of detection in this assay. bPTH, bovine PTH.

1 ml of plasma from one of the patients in this study was performed to examine the various species of immunoreactive PTH detected by these antisera and to determine possible areas of overlap (Fig. 1). Gel filtration was carried out with a Bio-Gel P-30 column, 1×100 cm, 0.2 M ammonium acetate (pH 4.6) and bovine serum albumin (1 mg/ml) as eluant, 20 cm hydrostatic pressure (siphon), 3 ml/h flow rate, and 0.75-ml fractions. The column was calibrated with blue dextran, ^{125}I -labeled and unlabeled bovine PTH (amino acids 1-84), ^{125}I -labeled and unlabeled synthetic bovine PTH (amino acids 1-34), and ^{125}I -I⁻; locations of these calibration substances are shown at the top of the figure. No more than 50 μl of fractions were added to immune mixtures (total volume, 500 μl); this quantity of 0.2 M ammonium acetate did not influence the immune reaction between ^{125}I -labeled bovine PTH and either antiserum. Fractions were assayed for immunoreactive PTH by using antisera CH14M (anti-bovine) and GP1M (anti-porcine). Reference standard for the CH14M antiserum was a 20% pure preparation of human PTH obtained from parathyroid adenomas and probably representative of the 9500 mol wt species of the hormone (19). Reference standard for GP1M was a crude desalted preparation of medium from large-scale culture of parathyroid adenomas and is probably 2-5% human PTH (18). These two reference standards were selected because of the essentially identical immunodilution curves obtained with these standards and with the immunoreactive substances eluting in comparable position on gel filtration, permitting quantitative comparisons to be made. As illustrated in the figure, only two major peaks of immunoreactivity were detected, one by each of the antisera. The major immunoreactivity detected by antiserum CH14M eluted in a position corresponding to that of bovine PTH 1-84 (mol wt 9500); a small secondary peak of immunoreactivity corresponded to a molecular weight of about 4000. The major immunoreactivity detected by antiserum GP1M eluted in a position corresponding to a molecular weight of about 7000. The nonideal leading and trailing slopes of these major peaks may represent minor contributions from other species or fragments of PTH, although we favor the view that they more likely indicate variability due to the large plasma protein load required for satisfactory detection of immunoreactivity by antiserum CH14M. As indicated in Fig. 1, the nanogram equivalents of impure human PTH detected by antiserum CH14M represent minute amounts of hormone, since only 50 μl of each fraction could be added to the incubation mixture. This gel filtration pattern indicates that each of the antisera employed in the present study is relatively specific for a single major species of immunoreactive PTH in the plasma of patients with chronic renal failure. Additional fragments which may be present are either poorly recognized by these antisera or contribute to observed immunoreactivity in only a minor way.

Standard hyperparathyroid plasma was used in both radioimmunoassays, as previously described (11), and values for iPTH are expressed in microliter equivalents of this serum per milliliter (μl eq/ml). We recognize that this is a compromise since the ideal standard would contain the same concentration ratio of the two molecular species of PTH under consideration as was present in each unknown plasma. However, we had little choice, since we did not know this ratio in each plasma. We nevertheless have confidence in the values for iPTH obtained since all plasmas were studied in at least three concentrations, and immunodilution curves constructed from the data thus obtained were parallel (i.e.,

no significant difference in slope) to those obtained with standard hyperparathyroid plasma.

The normal range for iPTH-7 is from undetectable (<10 μl eq/ml) to 45 μl eq/ml with values measurable in greater than 95% of normal subjects. The normal range for iPTH-9 is from undetectable (<30 μl eq/ml) to 250 μl eq/ml, with values measurable in greater than 90% of normal subjects. It should be recognized that the apparent discrepancy in the normal ranges for iPTH using these two antisera relates primarily to the assay standard which was used. This hyperparathyroid plasma probably contains as much as four to five times more iPTH-7 than iPTH-9 (unpublished data from separate gel filtration studies of this plasma). Therefore, the assay done with CH14M, which recognizes primarily iPTH-9, requires considerably more of the standard plasma to produce an inhibition of antibody binding of ^{125}I -labeled bovine PTH than is required in the assay done using GP1M.

RESULTS

Results are summarized in Tables I and II.

Plasma calcium and phosphorus. Plasma calcium concentration increased progressively during all 27 calcium infusions, with a mean maximal increase of 5.2 mg/100 ml (range, 3.2-7.5 mg/100 ml). Plasma calcium decreased after infusion, but in no case did it return to the preinfusion concentration during the 24-h study. Plasma concentration of ionized calcium generally changed in parallel with that of total calcium. Plasma concentration of phosphorus often decreased early in the infusion but in most cases had increased by the end of infusion (mean change, +0.9 mg/100 ml; range, -2.7-+4.4). In contrast to plasma calcium, however, plasma phosphorus was often lower at 24 h than it was before infusion. In a few instances, calcium infusion was performed when plasma phosphorus was higher than anticipated, but no adverse reactions were observed in these cases. The means and standard deviations of plasma total calcium, ionized calcium, and phosphorus are shown in Fig. 2 as a function of time for the 15 8-h infusions for which complete data are available.

Parathyroid hormone. Plasma iPTH-7 was above normal in all patients before infusion (174 μl eq/ml or greater in all but one patient). The logarithmic mean of preinfusion values was 785 μl eq/ml. The values decreased by a mean of 10% by the end of infusion (range, -34%-+56%). A decrease of greater than 24% (three times the coefficient of variation) from control values occurred only five times during the 27 infusions and not at all during the five 4-h infusions. In 14 cases, iPTH-7 was lower at 24 h than at the end of the infusion; in five cases, values at 24 h had rebounded toward or above control values. The mean and standard deviation for 15 8-h infusions are plotted as a function of time in Fig. 2. By regression analysis (least squares) of plasma iPTH-7 values during individual calcium infusions, a significant ($P < 0.05$) negative slope was obtained in 6 of the

TABLE I
Effect of Calcium Infusion on Plasma Calcium and Phosphorus

Patient	Previous dialyses		Length of infusion	Plasma Ca			Plasma Ca ⁺⁺			Plasma P		
	Ca	Duration		Before infusion	End of infusion	24 h after	Before infusion	End of infusion	24 h after	Before infusion	End of infusion	24 h after
	mg/ 100 ml	mo		hr	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml
1	5	6	6	7.8	11.2	9.3	2.6	3.5	3.5	11.2	8.5	9.0
	8	1	6	9.4	13.3	10.3	—	—	—	5.7	4.9	3.7
2	5	23	4	9.2	12.9	10.1	3.7	5.1	3.8	8.5	7.9	7.7
	8	2	4	10.0	13.9	11.4	—	—	—	6.0	6.2	5.7
3	8	1	8	9.1	12.8	10.3	—	—	—	2.8	3.6	2.4
	8	2½	8	9.1	12.3	10.2	—	—	—	4.9	4.3	3.6
4	5	1	8	7.0	12.7	11.0	2.7	5.3	3.8	2.1	3.0	4.1
	6	1½	6	7.7	11.8	8.8	—	—	—	6.8	6.5	6.0
5	7.5	½	8	8.7	13.3	—	2.8	5.0	—	4.8	5.3	—
	7.5	6	8	9.1	15.7	12.6	3.4	6.4	5.9	4.2	4.4	4.1
6	5	1	8	8.5	14.4	11.0	3.3	6.3	4.4	5.8	6.4	4.6
	8	1	7	9.8	16.0	12.3	—	—	—	4.3	5.8	3.8
7	5	1	8	9.0	16.0	12.9	3.1	7.3	5.4	3.6	5.4	5.6
	8	3	6	9.4	14.5	12.5	—	—	—	4.4	8.8	6.8
8	6	15	4	7.9	12.0	—	—	—	—	5.4	7.6	—
	6	24	8	9.2	16.3	14.0	4.1	8.6	7.5	3.4	5.1	5.5
9	5	7	8	9.0	14.6	11.3	3.9	6.1	4.0	9.3	12.2	10.9
	8	2	4	11.3	15.4	14.2	—	—	—	4.7	5.7	5.3
10	7.5	½	8	9.0	13.8	11.7	4.5	7.2	5.6	3.2	4.3	4.0
	5	2½	8	9.7	15.7	12.0	3.4	6.8	4.4	3.6	5.8	4.8
11	5.5	1½	4	11.5	17.7	—	—	—	—	5.3	6.3	—
	5.5	7	8	10.0	14.9	12.2	4.8	6.7	5.2	6.6	7.5	5.4
12	7	1	8	8.9	15.6	10.3	3.9	6.9	—	5.4	6.3	4.3
13	5	25	8	8.5	16.0	11.6	3.4	6.3	4.4	10.7	10.4	9.4
14	6	5	8	7.9	13.5	10.2	3.3	6.3	4.3	7.9	10.3	9.6
15	5	1½	8	8.8	13.4	10.9	3.4	6.4	4.1	2.5	2.8	2.3
16	5	½	8	8.7	15.5	12.5	3.5	6.1	6.0	4.3	6.0	6.4

27 infusions (linear: no. 12; quadratic: nos. 7, 8, 10, 15, and 16). When the entire group of infusions, or just the 15 8-h infusions for which complete data were available, were subjected to regression analysis (with or without logarithmic transformation of the iPTH values to decrease heteroschedasticity), however, no significant deviation of the slope from zero was found. Similarly, comparison of iPTH-7 values (log) at each time point with the control values by Dunnett's test (20) showed that at no time were the values, as a group, significantly different from control.

Plasma iPTH-9 was assayed during only 19 infusions because of limited availability of plasma. Control values were normal (< 250 μ l eq/ml) in 12 cases and only slightly increased (260 μ l eq/ml) in 1. During all infusions in which the control value was measurable, the concentration of iPTH-9 had decreased within 2 h, in three cases to undetectable values. For the entire group of infusions, or just the 14 8-h infusions, a significant negative (quadratic) regression ($P < 0.001$)

was observed, with or without logarithmic transformation of the data. For the calculation of the regression and the mean values shown in Fig. 2, undetectable values were taken to be 25 μ l eq/ml (detection limit = 30 μ l eq/ml). The mean decrease from control was 44% at 2 h and remained at essentially the same value for the duration of infusion. Values at 24 h generally rebounded toward control values but reached or exceeded control in only three cases.

Six patients received a calcium infusion during periods of low (5 mg/100 ml) and high (7.5–8 mg/100 ml) dialyzate calcium concentrations (not during actual dialysis). Control values of iPTH-7 were lower in all patients but one during use of the higher calcium concentration, with a mean decrease of 52% (range, 0 to –83%). Despite the difference in preinfusion values, however, there was no consistent difference in response to calcium infusion between the two levels of dialyzate calcium. Of special interest is patient 6, whose plasma iPTH-7 decreased into the normal range 24 h after

TABLE II
Effect of Calcium Infusion on Plasma iPTH*

Patient	Plasma iPTH											
	iPTH-7 (antiserum GP1M)						iPTH-9 (antiserum CH14M)					
	Control	2 h	4 h	6 h	8 h	24 h	Control	2 h	4 h	6 h	8 h	24 h
	<i>μl eq/ml</i>						<i>μl eq/ml</i>					
1	8800	6800	6700	7000	—	5300	400	100	58	60	—	300
	1500	1500	1300	1600	—	1200	120	45	48	50	—	48
2	1100	1100	1100	—	—	940						
	1100	690	950	—	—	800						
3	1100	930	930	630	840	600	480	110	160	110	160	200
	1200	1000	880	790	970	920	750	100	150	110	140	250
4	1000	910	840	810	890	790	370	160	98	98	130	90
	590	490	390	460	—	470	250	170	84	68	92	94
5	870	810	1100	820	810	—	62	40	ND†	ND	ND	—
	460	450	430	360	390	390	55	36	31	ND	—	32
6	760	420	570	760	950	690						
	61	49	60	70	—	39						
7	730	610	470	480	420	440	110	ND	ND	ND	ND	ND
	290	240	280	280	—	240	47	ND	ND	ND	ND	ND
8	640	770	760	—	—	—						
	540	450	440	420	390	350	100	85	85	92	138	84
9	550	480	570	450	390	450	100	90	86	140	85	120
	270	240	260	—	—	360	ND	ND	ND	ND	ND	75
10	510	320	200	300	110	560	120	ND	130	60	—	120
	890	610	730	600	720	710	110	88	75	60	72	120
11	260	320	330	—	—	—						
	610	620	590	490	520	—						
12	4400	4200	3800	4100	4100	3000	260	112	128	89	77	89
13	2900	3000	2000	1700	2400	2000	205	140	119	111	75	85
14	1900	3300	3200	2000	1300	2500	350	240	220	202	180	230
15	1400	1200	1200	1100	1100	1300	334	180	104	278	155	176
16	180	160	160	150	150	160						

* See Table I for previous dialyses and duration of infusion.

† ND, not detectable.

calcium infusion during high calcium dialysis. Plasma iPTH-9 was lower during the high calcium dialyzate period in three of the four patients in whom it was measured.

DISCUSSION

Berson and Yalow (21) presented the first evidence suggesting that more than one molecular species of PTH may circulate in human plasma. This concept has been confirmed subsequently in several laboratories (19, 22, 23), although there has been controversy about the relative significance of each of the circulating forms of the hormone (23). Canterbury and Reiss (24) and Habener, Powell, Murray, Mayer, and Potts (25), in recent reports of gel filtration studies of human plasma samples, have suggested that a substantial fraction of parathyroid hormone in peripheral plasma has a molecular weight of about 7000 while that secreted by the parathyroid gland has a molecular weight of 9500.

The present study was possible because of a combination of circumstances in our laboratory. First, two immunoreactive species of PTH were purified from media of cultured parathyroid adenomas in sufficient amounts for study (18). Second, two antisera (11) (from among a large number examined) were found which exhibited such specific affinity for each of the two identified iPTH forms that, in mixtures of the two forms, for practical purposes each antiserum recognized only one. The gel filtration patterns of plasma from one of the patients in this study and from patients with various forms of hyperparathyroidism confirm this specificity. Further confirmation of this interpretation is provided by the observation that the immunoreactive species recognized by CH14M sometimes became undetectable during calcium infusion, while that recognized by GP1M was still greatly elevated. We interpret this to indicate that antiserum

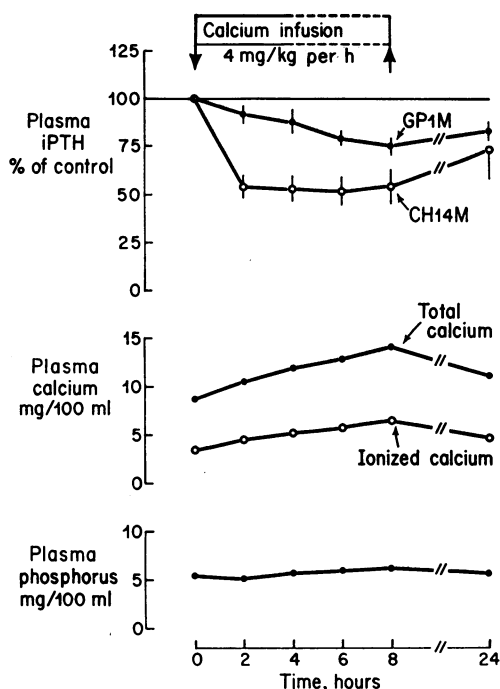


FIGURE 2 Plasma total and ionized calcium, phosphorus, and iPTH, as means \pm SE, before, during, and after 8-h calcium infusion. Values for iPTH-7 (antiserum GP1M) and iPTH-9 (antiserum CH14M) are expressed as percentages of control values. For total calcium, ionized calcium, and phosphorus, SE was less than height of symbols showing means.

CH14M does not recognize the species recognized by antiserum GP1M. As a result, we conclude that the immunoreactivity detected by antiserum CH14M in the region corresponding to a molecular weight of 7000 represents either the trailing edge of the 9500 mol wt species or one or more smaller molecular weight species which disappear from the circulation with half-times similar to that of the 9500 mol wt species.

Results of the present study demonstrate that each of the forms of iPTH estimated by the two antisera reflects a different aspect of parathyroid function. The observation that values of iPTH-7 were increased above normal in all of the patients is consonant with previous reports that secondary hyperparathyroidism begins to develop early in chronic renal insufficiency (26, 27). Furthermore, these values consistently decreased during periods of hemodialysis against high dialyzate concentrations of calcium, in keeping with our previous report that the incidence of roentgenographic bone disease (primarily osteitis fibrosa) was lower in patients so treated (13). In a recent case report, we demonstrated roentgenographic healing of osteitis fibrosa concomitantly with decreasing concentrations of iPTH-7 during 6 mo of he-

modialysis against dialyzate calcium concentrations of 8 mg/100 ml, coupled with decrease of plasma phosphorus (28), further confirming this relationship. These data suggest that iPTH-7 probably reflects closely the chronic state of parathyroid function. We believe that this sort of observation supplies further evidence that PTH-7 may be biologically active, although the data confirm only that it reflects biologic activity.

Since the presumptive cause of secondary hyperparathyroidism is hypocalcemia, perhaps as a result of phosphorus retention (27), one would expect that induced hypercalcemia should inhibit secretion of PTH and decrease its plasma concentration. The failure of iPTH-7 to decrease significantly in many of these patients during acute calcium infusions is probably a manifestation of either a long circulating half-time of iPTH-7 or an inability to degrade or excrete this moiety in the presence of renal insufficiency, or both. A similar observation was made by Berson and Yalow (21) in that they found the disappearance of iPTH from the plasma after parathyroidectomy to be significantly slower in uremic patients than in those with normal renal function. They also reported a difference in the disappearance of circulating iPTH as assessed by two separate antisera (29) in one patient with renal failure.

Despite this lack of acute responsiveness of iPTH-7, however, it was clearly decreased by prolonged hemodialysis against high concentrations of dialyzate calcium (coupled with a decrease of plasma phosphorus by use of oral aluminum hydroxide). It is appropriate to conclude, therefore, that secretion of PTH was suppressible by the intermittent induction of minimal hypercalcemia (< 11 mg/100 ml) but that the long circulating half-time of iPTH-7 precluded our detecting significant acute changes.

In contrast to our observations suggesting that iPTH-7 reflects chronic parathyroid functional status, values of iPTH-9 were within or near the normal range in the majority of these patients with chronic renal failure. Roentgenographic evidence of osteitis fibrosa was present in all but a few of the cases studied, and therefore we assume that all patients had secondary hyperparathyroidism of clinical significance. This lack of correspondence between iPTH-9 and clinical status indicates that iPTH-9 does not reflect the chronic state of parathyroid function in chronic renal failure. In response to acute calcium infusions, however, iPTH-9 decreased in every instance, sometimes to undetectable values. We conclude from this observation that iPTH-9 has a much shorter circulating half-time than does iPTH-7 and that it reflects the rate of secretion of PTH. It is probable that, once in the plasma, iPTH-9 is cleaved to iPTH-7 (18), leaving the latter as the major circulating form of PTH (19).

One of the questions raised by the normal values of iPTH-9 in most of our patients is how secondary hyperparathyroidism can be present and manifest in the presence of a normal secretory rate. The data are compatible with the following explanation. If we accept the proposition that secretion of PTH begins to increase early in the development of renal insufficiency (26, 27), we can understand the increase in number and size of parathyroid cells (parathyroid hyperplasia) as a response to the chronic demand for more PTH to maintain normal plasma concentration of calcium. During these early stages of renal insufficiency, disposal of iPTH-9 and iPTH-7 is probably normal. As renal insufficiency progresses, however, disposal of iPTH-7 becomes impaired although cleavage of iPTH-9 to iPTH-7 remains intact. Thus, it is probable that, at any given rate of PTH secretion, there is a larger amount of circulating iPTH-7 in patients with renal impairment than in patients with normal renal function. We assume that, in a normal subject, an increased concentration of circulating iPTH-7 characteristic of renal failure would lead promptly to an increase in plasma concentration of calcium and a compensatory decrease in secretion rate of PTH. Since the plasma concentration of iPTH-9 in our patients is normal or increased in the presence of a greatly increased concentration of iPTH-7, we conclude that there is a continuing demand for secretion of PTH. Whether this demand is due to relative or absolute hypocalcemia, or to some other factor, cannot be concluded from our data. The observation that iPTH-9 responds promptly to induced hypercalcemia, however, suggests that some semblance of normal feedback control is still present.

Addendum. Continuing examination of the specificity of the two antisera described in this manuscript has been performed on many gel filtration studies of sera from both normal subjects and patients with a variety of diseases. Studies of sera in which the 9000-9500 mol wt species of PTH was not so completely overwhelmed by the 4000-7000 mol wt species have disclosed that antiserum GP1M reacts with all of the major circulating species of immunoreactive PTH, while antiserum CH14M reacts with primarily the 9000-9500 mol wt species and a smaller fragment in the 2000-2500 mol wt range. Thus, the value for immunoreactive PTH obtained with antiserum GP1M may be considered as representing the summation of all immunoreactive species circulating. Antiserum CH14M, on the other hand, seems to be reasonably specific for the 9000-9500 mol wt species, since the smallest fragment of 2000-2500 mol wt represents but a very small fraction of the total immunoreactivity present. This may explain why antiserum GP1M is more consistently able to differentiate states of increased parathyroid activity, while antiserum CH14M is relatively insensitive in this regard.

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REFERENCES

1. Sherwood, L. M., G. P. Mayer, C. F. Ramberg, Jr., D. S. Kronfeld, G. D. Aurbach, and J. T. Potts, Jr. 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. *Endocrinology*. **83**: 1043.
2. Arnaud, C. D., T. Littledike, and H. S. Tsao. 1970. Calcium homeostasis and the simultaneous measurement of calcitonin and parathyroid hormone in the pig. In *Proceedings of the Second International Symposium on Calcitonin*, London, 1969. S. Taylor and G. Foster, editors. William Heinemann, Ltd., London. 95.
3. Howard, J. E., T. R. Hopkins, and T. B. Connor. 1952. The use of intravenous calcium as a measure of activity of the parathyroid glands. *Trans. Assoc. Am. Physicians Phila.* **65**: 351.
4. Kyle, L. H., J. J. Canary, D. H. Mintz, and A. de Leon. 1962. Inhibitory effects of induced hypercalcemia on secretion of parathyroid hormone. *J. Clin. Endocrinol. Metab.* **22**: 52.
5. Nordin, B. E. C., and R. Fraser. 1954. The effect of intravenous calcium on phosphate excretion. *Clin. Sci. (Oxf.)*. **13**: 477.
6. Goldsmith, R. S., A. D. Mason, Jr., W. H. Hall, and J. F. Powers. 1962. A rapid calcium infusion test for hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **22**: 741.
7. Berson, S. A., R. S. Yalow, G. D. Aurbach, and J. T. Potts, Jr. 1963. Immunoassay of bovine and human parathyroid hormone. *Proc. Natl. Acad. Sci. U. S. A.* **49**: 613.
8. Berson, S. A., and R. S. Yalow. 1966. Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia, and bronchogenic carcinoma. *Science (Wash. D. C.)*. **154**: 907.
9. Reiss, E., and J. M. Canterbury. 1968. A radioimmunoassay for parathyroid hormone in man. *Proc. Soc. Exp. Biol. Med.* **128**: 501.
10. Reiss, E., J. M. Canterbury, and R. H. Egdahl. 1968. Experience with a radioimmunoassay of parathyroid hormone in human sera. *Trans. Assoc. Am. Physicians Phila.* **81**: 104.
11. Arnaud, C. D., H. S. Tsao, and T. Littledike. 1971. Radioimmunoassay of human parathyroid hormone in serum. *J. Clin. Invest.* **50**: 21.
12. Goldsmith, R. S., J. Furszyfer, W. J. Johnson, A. E. Fournier, and C. D. Arnaud. 1971. Control of secondary hyperparathyroidism during long-term hemodialysis. *Am. J. Med.* **50**: 692.
13. Fournier, A. E., W. J. Johnson, D. R. Taves, J. W. Beabout, C. D. Arnaud, and R. S. Goldsmith. 1971. Etiology of hyperparathyroidism and bone disease during chronic hemodialysis. I. Association of bone disease with potentially etiologic factors. *J. Clin. Invest.* **50**: 592.
14. Fournier, A. E., C. D. Arnaud, W. J. Johnson, W. F.

- Taylor, and R. S. Goldsmith. 1971. Etiology of hyperparathyroidism and bone disease during chronic hemodialysis. II. Factors affecting serum immunoreactive parathyroid hormone. *J. Clin. Invest.* 50: 599.
15. Slavin, W. 1968. Atomic Absorption Spectroscopy. John Wiley & Sons, Inc., New York.
16. Moore, E. W. 1970. Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. *J. Clin. Invest.* 49: 318.
17. Fiske, C. H., and Y. Subbarow. 1925. Colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375.
18. Arnaud, C. D., G. W. Sizemore, S. B. Oldham, J. A. Fischer, H. S. Tsao, and E. T. Littledike. 1971. Human parathyroid hormone: glandular and secreted molecular species. *Am. J. Med.* 50: 630.
19. Arnaud, C. D., H. S. Tsao, and S. B. Oldham. 1970. Native human parathyroid hormone: an immunochemical investigation. *Proc. Natl. Acad. Sci. U. S. A.* 67: 415.
20. Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50: 1096.
21. Berson, S. A., and R. S. Yalow. 1968. Immunochemical heterogeneity of parathyroid hormone in plasma. *J. Clin. Endocrinol. Metab.* 28: 1037.
22. Sherwood, L. M., J. S. Rodman, and W. B. Lundberg, Jr. 1970. Evidence for a precursor to circulating parathyroid hormone. *Proc. Natl. Acad. Sci. U. S. A.* 67: 1631.
23. Potts, J. T., Jr., H. Keutmann, H. D. Niall, J. Habener, G. Tregear, L. J. Deftos, J. L. H. O'Riordan, and G. D. Aurbach. 1972. Chemistry of the parathyroid hormones: physiological and clinical implications. *Excerpta Med. Int. Congr. Ser.* 243: 159.
24. Canterbury, J. M., and E. Reiss. 1971. Fractionation of circulating parathyroid hormone (PTH) in man. *J. Lab. Clin. Med.* 78: 814. (Abstr.)
25. Habener, J. F., D. Powell, T. M. Murray, G. P. Mayer, and J. T. Potts, Jr. 1971. Parathyroid hormone: secretion and metabolism *in vivo* (human/bovine/gel filtration/radioimmunoassay/venous catheterization). *Proc. Natl. Acad. Sci. U. S. A.* 68: 2986.
26. Reiss, E., and J. M. Canterbury. 1971. Genesis of hyperparathyroidism. *Am. J. Med.* 50: 679.
27. Slatopolsky, E., S. Caglar, J. P. Pennell, D. D. Taggart, J. M. Canterbury, E. Reiss, and N. S. Bricker. 1971. On the pathogenesis of hyperparathyroidism in chronic experimental renal insufficiency in the dog. *J. Clin. Invest.* 50: 492.
28. Vosik, W. M., C. F. Anderson, W. P. Steffee, W. J. Johnson, C. D. Arnaud, and R. S. Goldsmith. 1972. Successful medical management of osteitis fibrosa due to "tertiary" hyperparathyroidism. *Mayo Clin. Proc.* 47: 110.
29. Berson, S. A., and R. S. Yalow. 1971. Clinical applications of radioimmunoassay of plasma parathyroid hormone. *Am. J. Med.* 50: 623.