Effects of Hepatectomy, Nephrectomy, and Nephrectomy/Uremia on the Metabolism of Parathyroid Hormone in the Rat

GINO V. SEGRE, PIERRE D'AMOUR, ANNE HULTMAN, and JOHN T. POTTS, JR.,
Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT Reports from several laboratories, showing extensive hepatic extraction of circulating parathyroid hormone, led us to examine the effect of near-total hepatectomy on the metabolism of the hormone to circulating fragments, and on its clearance from plasma. The rate of disappearance of ^{125}I-labeled and unlabeled bovine parathyroid hormone from plasma, and the appearance, disappearance, and chemical and immunochemical characteristics of circulating fragments were examined by gel filtration and either sequence-specific radioimmunoassays or sequence analysis using the Edman reaction. Results from awake rats subjected to near-total hepatectomy were compared with those found in sham-treated, nephrectomized, and short-term uremic rats (studied 2 d after nephrectomy). When compared with the sham-treated group, all other groups clear ^{125}I-labeled hormone more slowly; after hepatectomy, however, the clearance rate is most strikingly decreased.

After injection of intact hormone, the concentration of carboxy-terminal fragments in the circulation of hepatectomized rats is greatly reduced at all time intervals when compared with that in sham-treated rats. Sequence analysis of plasma samples, collected from rats into which ^{125}I-labeled hormone had been injected, shows that carboxy-terminal fragments having positions 34 and 37 of the intact hormone sequence as their amino-terminal amino acids are abundant in sham-treated, nephrectomized, and nephrectomized/uremic rats, but are undetectable in hepatectomized rats. The data suggest that inasmuch as the liver in vivo generates most of the carboxy-terminal fragments resulting from the metabolism of injected hormone, specific cell types within the liver must be the principal locus of the responsible enzyme(s); thus, studies of the enzymic properties of isolated hepatic cells in vitro most likely will yield information of physiologic relevance to the metabolism of the hormone in the intact animal.

INTRODUCTION

Parathyroid hormone (PTH),[1] an 84-amino-acid, single-chain polypeptide, undergoes proteolysis after secretion that results in the appearance of specific hormonal fragments in plasma (1–9). Metabolism of intact hormone after secretion appears to account for at least part of the heterogeneity of circulating PTH. Recent reports have indicated that hormonal fragments, in addition to being generated after secretion, also are released by the parathyroid glands, particularly during hypercalcemic challenge, and may contribute significantly to the concentration of the multiple immunoreactive forms of the hormone in the circulation (10–15).

Fragments resulting from metabolism of intact bovine PTH (bPTH) injected intravenously into animals are indistinguishable by both physicochemical and immunochemical criteria from those in the circulation of patients; the principal fragments appear to be ~60% as large as the intact hormone and consist of the middle and COOH-terminal portions of the hormonal sequence (1–10, 16–21). Sequential Edman degradation of fragments recovered from plasma after injection of ^{125}I-bPTH in vivo showed that the major sites of proteolysis are between positions 33 and 34 and between 36 and 37 of the intact sequence, yielding fragments that appear to be identical with those found in studies

Dr. Segre is a recipient of Research Career Development Award AM 00070 from the National Institute of Arthritis, Metabolism, and Digestive Diseases. Dr. D'Amour was a Fellow of the Medical Research Council of Canada and is presently at Hôpital St. Luc, Montreal, Canada.

Received for publication 14 February 1980 and in revised form 22 October 1980.

[1] *Abbreviations used in this paper*: MRCU, Medical Research Council Units; PTH, parathyroid hormone; bPTH, bovine parathyroid hormone.

of unlabeled hormone. In addition, hydrolysis of ^{125}I-bPTH occurs at the same peptide bonds in different species (6, 8).

Evidence for the roles of the kidneys, liver, and bone in the metabolism and clearance of intact hormone and in the release and clearance of fragments of the hormone was reviewed recently (22). Metabolism and/or clearance of intact PTH and hormonal fragments are principally by hepatic and renal mechanisms (2–5, 22–26). Abundant evidence points to the role of the kidneys in hormonal metabolism. In 1968, Berson and Yalow (26) showed prolonged survival of immunoreactive PTH in patients with renal failure after therapeutic parathyroidectomy, suggesting that the kidney's role in peripheral metabolism is important.

Subsequently, Singer et al. (23) and Hruska et al. (3) confirmed and extended these observations by demonstrating an arteriovenous difference in immunoreactive PTH concentrations of 20% across the canine kidney. Experiments in rats indicate that intact bPTH, bPTH-(1–34), and immunoreactive COOH-terminal fragments all are cleared by glomerular filtration with subsequent reabsorption, and that the first two peptides also are extracted by peritubular uptake (24). Quantitatively, the kidneys are of paramount importance in removing COOH-terminal fragments from the circulation. Recent studies in man show a marked reduction in circulating COOH-terminal fragments after successful renal transplantation (27), and support these observations in animals indicating that the kidneys—but no other organs, including the liver (5, 28)—remove large amounts of these fragments. In addition, the isolated perfused dog kidney appears to release COOH- and NH_2-terminal fragments into the circulating medium when exposed to intact hormone (29).

Earlier studies by Davis and Talmage (30) pointed to the potential importance of the liver in the inactivation of PTH, and Fang and Tashjian (31), using partially hepatectomized rats, showed the central role of the liver in clearing immunoreactive PTH from plasma. Subsequent studies by Neuman et al. (4, 32) identified the liver as the principal site of PTH deposition, and showed that nephrectomy had only minor effects on the rate of peripheral degradation of the hormone. The intact peptide was the principal form of the hormone found in liver extracts, and although hepatic uptake was relatively independent of the dose of hormone administered, uptake was influenced by the biologic activity of the preparation. Canterbury et al. (2), using the isolated perfused rat liver, were the first to show that hepatic metabolism of intact hormone resulted in the release of fragments into the perfusate. These fragments were similar in size and immunoreactive properties to those found in plasma in vivo, and the NH_2-terminal fragments were found to activate renal adenylate cyclase. Later studies confirmed that the liver is both a source of circulating COOH-terminal fragments (5) and a major site of PTH uptake (4, 5, 25, 28). Hepatic extraction appears to depend on recognition of specific structural features located within the middle portion of the PTH sequence: both ^{125}I-bPTH (5, 28) and synthetic ^{125}I-bPTH-(28–48) (28) are removed efficiently, but neither ^{125}I-bPTH-(1–34) (5, 28) nor endogenously produced ^{125}I-labeled COOH-terminal fragments (28) (recovered by gel filtration of plasma from dogs given ^{125}I-bPTH intravenously) are cleared. Hormonal fragments recovered from liver homogenates after intravenous injection of ^{125}I-bPTH into rats are chemically identical with those found in plasma (25).

The present studies principally focus on the role of the liver in the metabolism of PTH. Unlabeled hormone and ^{125}I-bPTH were injected into hepatectomized and sham-operated rats. The chemical nature of the fragments resulting from proteolysis of ^{125}I-bPTH was defined by sequence analysis, and the immunochemical properties of these fragments were assessed by sequence-specific radioimmunoassays. The disappearance of intact hormone and the appearance and disappearance of COOH-terminal fragments were estimated from experiments with both labeled and unlabeled hormonal preparations. Additional control studies, using the same analytic methods, were performed in nephrectomized rats. The results emphasize the quantitative importance of the liver in the clearance of intact hormone, as well as the precise and unique chemical properties of the PTH fragments resulting from hepatic metabolism of the hormone, findings necessary to validate the biological significance and specificity of our studies of PTH metabolism by specific populations of hepatic cells in vitro (see accompanying report).

METHODS

Preparation of PTH and albumin. Intact bPTH and bPTH-(53–84), a product of tryptic digestion of highly purified native hormone after reversible blockade of ε-amino groups of lysine residues, were prepared (33, 34). Biologic activity of bPTH-(1–84) was 3,000 Medical Research Council Units (MRCU)/mg in the in vitro rat cortical adenylate cyclase assay (35) and 2,500 MRCU/mg in the in vivo chick hypercalcemia assay (36). Bovine serum albumin (fraction V) was purchased from Armour Pharmaceutical Co., Phoenix, Ariz. Iodinations were performed with Na^{125}I or Na^{131}I (New England Nuclear, Boston, Mass.) by modifications (37) of the method of Hunter and Greenwood (38). Iodinated bPTH was purified initially by adsorption to QUSO-G-32 (Philadelphia Quartz Co., Valley Forge, Pa.) and then both labeled bPTH and bovine serum albumin were gel filtered as previously described (6).

Aliquots of a single preparation of bPTH (76×10^6 cpm, 200–300 mCi/mg sp act) in 0.5 ml of 0.05 M barbital buffer, 10% plasma (vol/vol), pH 8.6, to which ^{131}I-labeled bovine serum albumin (1.25×10^6 cpm) had been added, were quick-frozen in acetone-dry ice (Airco Industrial Gases, Airco, Inc., Murray Hill, N. J.) and stored at −70°C. Aliquots were used within 10 d, and each aliquot was thawed only once.

Preparation of animals. Four groups of white male rats were used (CD-1, Charles River Breeding Laboratories, Wilmington, Mass.), weighing 263±26 g. Surgery was conducted under ether anesthesia.

Sham-treated rats (group 1) underwent surgery that consisted of implantation of injection and sampling cannulas, incision of the abdominal wall, gentle manipulation of the organs, and closure of the abdominal wound. Rats were subjected to hepatectomy and portacaval shunt (group 2) by the technique of Funovics et al. (39) within the 3 d preceding the experiment. On the morning of the experiment, the lobes of the liver were ligated and excised after reopening of the abdominal incision, and injection and sampling cannulas were implanted. A minimal amount (<500 mg) of tissue adherent to the inferior vena cava was allowed to remain, and the abdominal wound was closed. Animals that bled excessively were excluded. Rats were subjected to bilateral nephrectomy, through flank incisions (group 3), as well as surgery for the implantation of injection and sampling cannulas. Rats were subjected to bilateral nephrectomy through flank incisions 45–48 h before initiation of the experiment (group 4); on the morning of the experiment, injection and sampling cannulas were implanted. Blood urea nitrogen in these uremic animals was 280±30 mg/dl.

In the interval between the initial surgery and the day of the experiment, animals in groups 2 and 4 were maintained on standard rat chow and water ad lib. Experiments were initiated within 3 h of the time that the cannulas were placed.

Protocol for animal studies. Studies with ^{125}I-bPTH were performed in 120 awake rats. Fifteen rats from each of the above groups were used in two separate experiments. In each experiment the labeled bPTH and bovine serum albumin were injected (20–30 s) into the external jugular vein. Blood samples (700 μl) were removed through a cannula in the internal carotid artery at the following intervals after injection of the hormone: 2, 4, 6, 8, 12, 24, 48, and 96 min. The schedule for the bleeding of the 15 animals used in each experiment is shown in Table I. Inasmuch as preliminary studies revealed that animals frequently became hypoglycemic after hepatectomy, a solution of 10% dextrose and normal saline (700 μl) was administered to replace blood removed at each sampling in all groups. Blood was collected in heparinized syringes. In each experiment, samples, obtained from six rats at each interval, were pooled and immediately centrifuged at 3,000 rpm for 15 min at 4°C. The plasma was removed, portioned into two vials, quick-frozen in acetone-dry ice, and stored at −70°C for analysis. Each vial was thawed only once for gel filtration.

Plasma volumes of each group were measured in 12 of the 15 animals. The concentration of ^{131}I-labeled bovine serum albumin at zero time was estimated by extrapolating from the concentration of ^{131}I at the various times sampled in animals 7–15. In animals 1–3, the volume of distribution of the labeled albumin was estimated from the 8-min sample (40). (Earlier experiments showed that samples obtained at this time give the best correlation with estimates found by the extrapolation method.) Plasma volumes (milliliters) in the different group were as follows: group 1, 11.3±0.5; group 2, 9.5±0.5; group 3, 10.4±0.4; group 4, 12.1±0.4. Because the data of Fang and Tashjian (31) indicated that acute removal of 1.5 ml of blood did not alter the disappearance rates of immunoreactive PTH in rats, no attempt was made to match the plasma volumes in these groups.

TABLE I
Protocol for Blood Sampling after Injection of ^{125}I-bPTH

	Time (min)							
Animals	2	4	6	8	12	24	48	96
1–3	x	x	x	x				
4–6	x	x						
7–9			x	x	x	x		
10–12							x	x
13–15					x	x	x	x

The metabolism of unlabeled bPTH (2 μg) was studied after intravenous injection of the hormone into two animals from each of the above groups. Using the same methods, 1-ml blood samples were removed at 12 and 24 min, and the plasma was collected and stored. These samples were not pooled, but were chromatographed separately.

Gel filtration of plasma samples. For each of the two experiments with radioiodinated hormone, duplicate aliquots of pooled plasma from each interval were gel filtered on columns (1.2 × 100 cm) of Bio-Gel P-100, 100–200 mesh (Bio-Rad Laboratories, Richmond, Calif.) at 4°C with an eluting buffer of 0.1 M ammonium acetate with 1% (vol/vol) out-dated blood-bank plasma, pH 5.0. The procedure used to chromatograph samples from experiments using unlabeled hormone was identical except that the eluting buffer consisted of 0.05 M barbital with 10% plasma, pH 8.6 (the same buffer that is used in the radioimmunoassay). Fraction size was 0.9 ml. Tracer amounts of ^{131}I-bPTH and Na^{131}I in amounts that were insufficient to interfere with the subsequent radioimmunoassay were added to each sample before chromatography to calibrate the column. The ^{125}I and ^{131}I in each fraction were counted in a dual-channel spectrometer (Packard Instruments Co., Inc., Downers Grove, Ill.) with appropriate correction of ^{125}I counts for ^{131}I. Recovery of ^{125}I and of immunoreactive PTH was determined for all samples, and ranged from 84 to 93% and 77 to 95% for samples from studies using ^{125}I-labeled and unlabeled hormone, respectively.

Quantitation and statistical analysis. The area under the peaks of radioiodinated intact hormone and fragments in each chromatogram was calculated by planimetry, and the amount of intact hormone and labeled fragments at each time point was determined as follows: ^{125}I eluting in the region of intact hormone (or fragments) after chromatography of 1-ml samples times recovery of ^{125}I from the column times the average plasma volume of the group of rats. The injected hormone dose was determined by estimating the percentage of the labeled preparation that eluted in the position of intact hormone (a portion of the preparation is excluded by the gel, and a small amount of free iodide is also apparent) and multiplying by the total dose of ^{125}I injected into the rats.[2] The

[2] When radioiodinated PTH is chromatographed in nondenaturing solvents containing carrier protein, a portion of the radioactivity is excluded from the gel, particularly if plasma is used to protect the tracer. Dissociation of this noncovalent interaction by protein denaturants in the column effluent showed that nearly all of this material now elutes in the position of intact hormone (8, 44). In their elegant studies, Barrett et al. (44) showed how these differences in analytic techniques (chromatography with denaturing and nondenaturing solvents) influence the results of studies of PTH metabolism. The nature of the interaction of ^{125}I-bPTH with large molecular weight proteins is not known. It occurs with preparations labeled with chloramine T and by the electrolytic method (unpublished observations by G. V. Segre), but only to a very limited extent with unlabeled hormone. Our decision to calculate the data shown in Fig. 1 and Table II on gel filtration

percentage of injected dose was estimated by dividing the former number by the latter.

The resultant data from each of the four groups were subjected to a balanced two-way analysis of variance (replicate chromatograms for samples from each interval in the first and second experiments vs. the time interval). No significant differences were found between the two experiments, and no interaction effects were observed. Accordingly, the four values determined for samples from each time interval were pooled to obtain a mean and SEM.

The decay of ^{125}I-bPTH was calculated by fitting the data from each group to a sum of two exponentials of the form $A_1e^{-k_1t} + A_2e^{-k_2t}$ using program SAAM-27 (41).

Edman degradations. Gel filtration fractions from the region of the chromatogram containing the COOH-terminal radioiodinated fragments were pooled and lyophilized and then dissolved in heptafluorobutyric acid. Automated degradations were performed in a model 890 Sequencer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) using a single-coupling, double-cleavage program (42). All procedures for converting the anilinothiazolinone derivatives of the amino acids and for identifying the radioiodinated derivatives were previously reported (6, 8). All samples were degraded for at least 25 cycles. The results are expressed after correction for a repetitive yield of 92% for each cycle of degradation, a figure derived by averaging the results from six degradations of myoglobin.

Radioimmunoassays. Radioimmunoassays that measure specific regions of the PTH sequences were performed, using GP-1 and nonequilibrium conditions (18, 37). GP-1 is an antiserum that contains antibody populations that recognize two major antigenic determinants in the bPTH molecule: one includes some or all of the 14–27 region, and the other includes some or all of the 53–84 region (18, 37). By preincubating the antiserum with an excess of either bPTH-(1–34) or bPTH-(53–84), antigenic recognition is restricted to either the COOH- or the NH_2-terminal portions of the sequence. The same preparation of highly purified bPTH used in the animal studies and for labeling was also used as the standard.

RESULTS

Metabolism of ^{125}I-bPTH. The disappearance of ^{125}I-bPTH from the plasma in all groups follows higher than first-order kinetics, is fastest in the sham-operated group, and appears to be delayed most strikingly in the hepatectomized group (Fig. 1). Fitting the data to the sum of two exponentials gave a satisfactory fit in all groups (Table II). The fast component in the sham-treated group ($t_{1/2}$ = 1.7 min) is statistically different from those found in the other groups. The half-life of the fast component in the hepatectomized rats is 3.3 min, which was not significantly different from that found in groups 3 and 4. Calculation of the area under the first component also shows significant differences only in the sham-operated rats when compared with the rats in the other three groups (Table II).

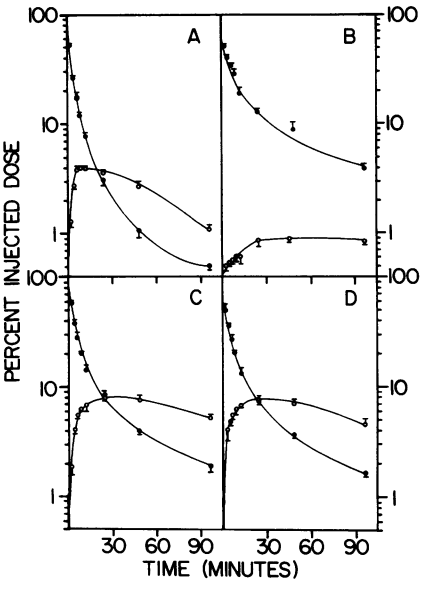

FIGURE 1 The disappearance of ^{125}I-bPTH (●) and the appearance and disappearance of radioiodinated fragments (○) in (A) sham-treated rats; (B) hepatectomized rats; (C) nephrectomized rats; and (D) nephrectomized/uremic rats. Data are expressed as mean±SEM (n=4). For details of the calculations, see text.

The slow component of the intact-hormone disappearance curves also is significantly faster in the sham-treated rats ($t_{1/2}$ = 13.0 min) than in the rats in the other three groups. Inspection of the data and of the equation fit to the data suggested that groups 3 and 4 could not be distinguished from each other, but that the results in group 2, the hepatectomized animals, were different from those in groups 3 and 4. Attempts to fit the data from groups 2, 3, and 4 to a single equation confirmed this hypothesis: although the data from groups 3 and

TABLE II

Analysis of the Disappearance of ^{125}I-bPTH from the Plasma of Rats in Groups 1–4 Using Computer Program SAAM-27

Group	Treatment	Fast component		Slow component	
		$t_{1/2}$	Area	$t_{1/2}$	Area
		min		*min*	
1	Sham-operated	1.7	215	13.0	244
2	Hepatectomized	3.3	300	44.4	1218
3	Nephrectomized	2.6	306	33.6	583
4	Nephrectomized/uremic	2.7	298	33.8	537

$t_{1/2}$ = 0.693/K; area = $\overline{A_i/K_i}$ for each component.

without denaturing solvents is based on our earlier observations in which an excellent quantitative correlation was found between the results obtained with labeled and unlabeled PTH when both were analyzed by chromatography with nondenaturing solvents (1). From these studies we deduced that most, if not all, of the ^{125}I-bPTH excluded by the column also was bound to proteins in the circulation, and was thereby cleared from plasma and reacted with specific tissue sites much more slowly than did unlabeled or ^{125}I-labeled hormone that was not bound to these proteins.

4 could be fitted to a single equation satisfactorily, the data from the hepatectomized group were impossible to fit. Thus, the disappearance of ^{125}I-bPTH from the plasma of hepatectomized animals is significantly slower than it is in all other groups.

In the sham-treated animals, radioiodinated fragments are present after 2 min, reach a maximum concentration between 8 and 12 min, and decrease thereafter. The apparent half-life of the disappearance of these fragments is ~39 min. Most likely this is an overestimate, inasmuch as the generation of fragments, not being instantaneous, probably occurs during at least the initial portion of the experiment. At 12 min, when the plasma concentration of radioiodinated fragments is at its maximum, the amount of these fragments is 4.0±0.2% of the ^{125}I-bPTH that was injected. This contrasts sharply with the results from chromatography of plasma samples from the hepatectomized rats. In this group, the maximum plasma concentration of labeled fragments is not achieved before 24 min, and at 24 min, the amount of ^{125}I-labeled COOH-terminal fragments in the circulation constitutes only 0.9±0.06% of the injected dose of labeled intact hormone. The concentration of these fragments decreases only slightly between 48 and 96 min. The late-eluting radioiodinated fragments in the plasma of the hepatectomized rats are not resolved as distinctly upon chromatography as are those in the other three groups, but give a pattern similar to that observed when unlabeled hormone was injected into the hepatectomized rats (Fig. 3B).

In nephrectomized and nephrectomized/uremic rats, the time course of the appearance and disappearance of radiolabeled COOH-terminal fragments and the plasma concentration of these fragments are indistinguishable. Circulating radioiodinated fragments reach a maximum concentration later (24 min), and the amount of these fragments in plasma at this time is twofold greater (8.0±0.4 and 7.8±0.5% of the injected dose of hormone in nephrectomized and nephrectomized/uremic rats, respectively) than in the sham-treated rats. The decay rate of the ^{125}I-labeled fragments in groups 3 and 4 is decreased; the apparent $t_{1/2}$ = 110–120 min in both groups. This reduced clearance probably accounts for both the higher peak concentration of circulating fragments and the later time at which the maximum concentration occurred.

To establish the chemical identity of the circulating ^{125}I-labeled COOH-terminal fragments, paired plasma samples were obtained at 8, 12, and 24 min after injection of ^{125}I-bPTH into each of the four groups and also at 48 min after injection of the hormone into hepatectomized rats. After chromatography of each sample, fractions containing the radiolabeled fragment peak from each profile were pooled and degraded separately in the Sequenator. Analysis of the samples from the sham-treated, nephrectomized, and nephrectomized/uremic rat groups gave results that are qualitatively indistinguishable from one another and from data we obtained previously in anesthetized rats (6). In all samples, the greatest release of iodotyrosyl radioactivity occurs at cycle 10, representing cleavage between positions 33 and 34 of the intact sequence. The second greatest release of radioactivity occurs at cycle 7, which is consistent with proteolysis between positions 36 and 37. In all samples, there is a significant release of iodotyrosyl radioactivity after one cycle, representing hydrolysis between positions 42 and 43, that increases in a time-dependent fashion from 8 to 24 min. Fig. 2 illustrates the data from degradation of the 12-min samples.

Significantly increased release of iodotyrosyl radioactivity at 7 and 10 cycles of degradation is not observed upon analysis of any of the eight samples obtained from the hepatectomized rats. The pattern of radioactivity released is more variable than that seen in the samples from the other three groups, an observation that may be related to the relatively small amount of radioiodinated fragments available for analysis. In general, release of radioactivity above background occurs only at cycles 1 and 6 (Fig. 2B). However, many of the samples from the hepatectomized rats show no discernible pattern when degraded: the small amount of iodotyrosyl radioactivity released is distributed randomly throughout the cycles of degradation.

Metabolism of unlabeled bPTH. The immunoreactivity in the plasma from two animals from each group was examined after intravenous injection of intact unlabeled PTH. Samples obtained 12 and 24 min after injection were chromatographed, and each fraction was assayed using both NH_2- and COOH-terminal-specific antisera. Analysis of each of the two samples obtained at one time in each group shows that they are essentially identical. In the sham-operated rats, the concentration of immunoreactive COOH-terminal fragments is great-

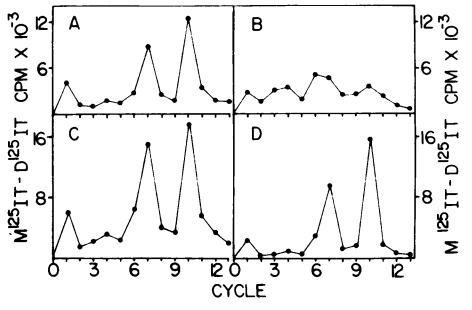

FIGURE 2 Specific iodotyrosyl radioactivity released at each cycle of Edman degradation of fraction pooled from the portion of the gel profile of the 12-min samples that contain ^{125}I-labeled fragments in (A) sham-treated rats; (B) hepatectomized rats; (C) nephrectomized rats; and (D) nephrectomized/uremic rats. M^{125}IT-D^{125}IT, monoiodotyrosyl-diiodotyrosyl radioactivity.

est in the samples obtained at 12 min, whereas in the other three groups, the concentration of these fragments is greatest in samples obtained after 24 min. Accordingly, results of analyses of 24-min samples from one animal in each group are shown in Fig. 3. The concentration of immunoreactivity eluting in the position of intact hormone is approximately fivefold higher in the hepatectomized rats and 2.5-fold higher in both the nephrectomized and nephrectomized/uremic rats than in the sham-treated rats. Essentially equal concentrations of NH_2- and COOH-terminal immunoreactivity are found in the peak coeluting with ^{131}I-bPTH. COOH-terminal, but not NH_2-terminal, immunoreactivity elutes later than does intact hormone. In all groups except the hepatectomized rats, a discrete peak of COOH-terminal immunoreactivity is seen, eluting at a volume identical with that of the radioiodinated fragments in studies with ^{125}I-bPTH. Chromatography of plasma from hepatectomized rats shows detectable concentrations of COOH-terminal immunoreactivity in many fractions after intact hormone, but these low concentrations do not appear to define a discrete immunoreactive peak (Fig. 3B). These data, as well as the other results obtained in studies of unlabeled hormone, are consistent with those obtained with ^{125}I-bPTH.

DISCUSSION

The current studies extend our knowledge concerning the peripheral metabolism of intact PTH to hormonal fragments and the clearance of intact hormone and its fragments from the circulation by liver and kidney. Although many laboratories, employing multiple ap-

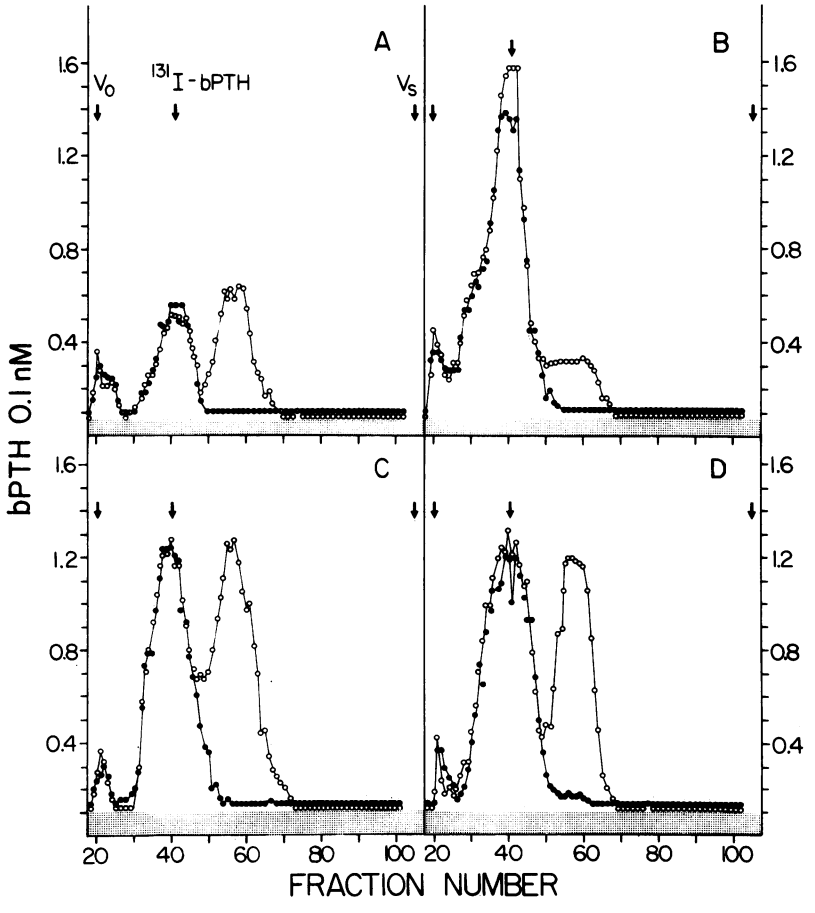

FIGURE 3 Immunoreactivity in fractions after gel filtration of plasma obtained 24 min after intravenous injection of unlabeled bovine PTH into (A) sham-treated rats; (B) hepatectomized rats; (C) nephrectomized rats; and (D) nephrectomized/uremic rats. Specific NH_2-terminal (●) and COOH-terminal (○) immunoreactivity was measured by radioimmunoassay antiserum GP-1 preincubated with excess bPTH-(53–84) and bPTH-(1–34), respectively. The shaded area represents the detection limits of the radioimmunoassays. The elution position of fractions within the void volume (V_0) and the elution positions of intact bovine PTH and ^{131}I are indicated by the arrows.

proaches, have contributed to our current understanding of the heterogeneity of circulating immunoreactive PTH and the peripheral metabolism of the polypeptide, many issues remain unresolved. It is important, therefore, to discuss not only the results of our present investigations, but also both the utility and limitations of the methods employed.

First, our experimental design, using injected hormone, does not permit us to address the issue of whether secretion of fragments by the parathyroid glands contributes significantly to the concentration of the multiple hormonal forms in plasma (10–15). Second, it is central to some but not all of our conclusions that the metabolism of ^{125}I-bPTH is sufficiently similar to that of the unlabeled hormone to permit analyses of the radioiodinated hormone to be considered valid reflections of the fate of the unlabeled preparations. Limitations that potentially result from the use of ^{125}I-bPTH, therefore, must be considered. The results of Neuman et al. (32) and Teitelbaum et al. (43) indicated that hepatic uptake decreases and renal uptake increases when PTH of low biologic potency is injected into rats. However, Teitelbaum et al. (43) found no differences when the clearance from plasma of biologically active, electrolytically iodinated bPTH was compared with that of chloramine T-labeled hormone in rats. Alternatively, Martin et al. (24) suggested that renal peritubular uptake of PTH requires that the hormone be biologically active. In both our earlier and current studies, neither qualitative nor significant quantitative differences were seen when we compared the results from experiments with radioiodinated and unlabeled, biologically active hormone. In the intact dog, the metabolism of both preparations appeared to follow the same kinetics, as reflected by serial measurements in plasma (1, 8), and in the rat, the tissue distribution of the two hormonal preparations was qualitatively similar (25). We recognize that proteolysis of PTH, particularly as detected by sequence analysis of the metabolites of ^{125}I-bPTH, cannot be assumed to be identical with that resulting from the metabolism of unlabeled hormone. However, the coincidence in the rates of metabolism of the two preparations and in their localization in tissues establishes a very high probability that both hormonal preparations are metabolized by the same enzyme(s). Accordingly, the use of the Edman technique to define the chemical specificity of these proteolytic events seems warranted, especially as exploration of the enzyme(s) involved in PTH metabolism proceeds to the cellular and subcellular level. Monitoring of the products of PTH metabolism by sequence analysis will serve to validate the relevance of observations made in vitro to those occurring in vivo.

When compared with sham-treated rats, the disappearance rate of injected intact bPTH is decreased in all three other groups, but particularly in the hepatectomized rats. The fast and slow components of PTH disappearance in hepatectomized rats are 2.0 and 3.5 times longer than those in the sham-operated controls. The delay that we found in the hepatectomized rats is greater than that reported by Fang and Tashjian (31), probably because placement of a portacaval shunt enabled us to conduct studies in rats with <5%, rather than 30–40%, of their original liver mass. In both nephrectomized and nephrectomized/uremic animals, the disappearance rates of intact hormone are indistinguishable from each other, and the fast and slow components are approximately 1.5 and 2.6 times longer than they are in the sham-treated rats.

Striking differences were seen in the relative concentrations, kinetics, and chemical nature of the circulating COOH-terminal fragments in the plasma samples from each of the four groups. Plasma from hepatectomized rats contains only very low concentrations of ^{125}I-labeled or immunoreactive COOH-terminal fragments, whereas plasma from nephrectomized and nephrectomized/uremic animals contains COOH-terminal fragments that reach maximum concentrations that are twofold higher and occur later (24 rather than 12 min) than those in sham-treated rats. Although the present studies were not designed to examine the disappearance of COOH-terminal fragments per se, it is clear that with respect to the rate of removal of these fragments in sham-treated rats, the clearance rate in nephrectomized and nephrectomized/uremic rats is reduced significantly.

These results are in agreement with the data of others, and confirm the importance of renal clearance of both intact PTH and COOH-terminal fragments (3, 23–27, 29). We also found that uremia of short duration does not produce effects on the metabolism of either intact hormone or the COOH-terminal fragments, other than those that can be accounted for by nephrectomy. Our data, however, do not address the issue of metabolic alterations due to more chronic uremia, a state that more closely resembles the clinical situation and was reported to reduce the extrarenal clearance of synthetic PTH-(1–34) in dogs (3).

In the present study, sequence analysis of plasma samples obtained after injection of ^{125}I-bPTH into sham-treated, nephrectomized, and nephrectomized/uremic rats shows that the circulating ^{125}I-labeled fragments from each group are structurally identical with each other and with those found after injection of hormone into anesthetized, intact rats (6). In all groups, the dominant circulating COOH-terminal fragments result from hydrolysis of the intact hormone between positions 33 and 34 and between 36 and 37. Edman degradation of the ^{125}I-labeled COOH-terminal fragments in the plasma of hepatectomized rats, however, failed to reveal significant concentrations of fragments having positions 34 and 37 of the intact hormonal sequence as their NH_2-terminal amino acids. Only very

limited amounts of circulating COOH-terminal fragments derived from metabolism of radioiodinated hormone appear to arise from organs other than the liver; sequence analysis of plasma samples from hepatectomized rats shows that they are heterogeneous, consisting of a relatively large number of different fragments rather than a few specific ones. We cannot exclude the possibility that hepatectomy, by an unknown mechanism, increases the removal rate of all COOH-terminal fragments, and increases preferentially the clearance of fragments with positions 34 and 37 of the intact sequence as their NH_2-terminal amino acids. However, it appears far more likely that after hepatectomy, the generation of all COOH-terminal fragments derived from injected, intact hormone is markedly reduced, especially the generation of fragments that result from cleavage of intact hormone between positions 33 and 34 and between 36 and 37.

It is unclear why we found no significant accumulation of COOH-terminal fragments in the plasma of hepatectomized animals, whereas others have presented impressive evidence concerning renal generation of PTH fragments and peritubular metabolism of the hormone (5, 24, 29). Several explanations seem possible. Martin et al. (24) suggested that only biologically active hormone is extracted by peritubular sites. Extrahepatic sources of these PTH fragments may distinguish between biologically active, unlabeled bPTH and ^{125}I-bPTH of low biologic potency. Perhaps radioiodinated bPTH is more readily accepted by hepatic enzymes or binding sites than by peritubular renal sites, thereby accounting for the absence of detectable radioiodinated fragments in the plasma of hepatectomized rats having positions 34 and 37 of the intact sequence as their NH_2-terminal amino acids. On the other hand, much lower concentrations of COOH-terminal fragments were detected in plasma by COOH-terminal specific assays in hepatectomized rats than in the other groups of animals, even when biologically active hormone was used (Fig. 3). This suggests that the liver plays a quantitatively more important role than the kidneys in the generation of circulating fragments. The absence from the plasma of hepatectomized rats of the circulating COOH-terminal fragments of ^{125}I-bPTH that are dominant in the circulation of the other groups of animals may still prove, however, to be related to specific properties of the radioiodinated hormone. Perhaps renal proteolysis of the hormone is associated with release of the fragments back into the circulation that is less than that after hepatic proteolysis of the hormone. In this regard, it is interesting to note that although 20% of the immunoreactive PTH (as measured by a COOH-terminal radioimmunoassay) was removed in a single pass by the kidney of intact dogs in the studies of Martin et al. (5), extraction was constant early after injection of PTH, when intact hormone is the major circulating form, and also at later times, when intact hormone had disappeared from the circulation, and mostly COOH-terminal fragments remained. If renal metabolism of intact hormone resulted in subsequent release of appreciable concentrations of COOH-terminal fragments back into the circulation, arteriovenous differences in COOH-terminal immunoreactivity during the early periods of these studies might have been smaller than they were during the later phases.

The precise role of the kidneys in the generation of circulating fragments from intact hormone remains under study, but our data are at least consistent with earlier reports suggesting a greater quantitative role for the liver than the kidneys in the metabolism of intact PTH, with subsequent release of PTH fragments into the circulation (5, 25). The liver in vivo appears to have a predominant role in the metabolism of injected intact hormone and the subsequent release into the circulation of COOH-terminal fragments, particularly fragments having positions 34 and 37 of the intact hormone as their NH_2-terminal amino acids. Our data also confirm the results of others, showing the importance of hepatic clearance of intact hormone (2, 5, 25, 28, 31) and the major role of the kidneys in the extraction of hormone, especially COOH-terminal fragments (3, 23–27, 29).

Our data regarding the quantitative importance of the liver in the metabolism of PTH in vivo, particularly with respect to its role in the generation and release of specific COOH-terminal fragments into the circulation, are also critical for evaluation of the studies in the accompanying report in which metabolism of PTH by isolated liver cells in vitro is assessed. In addition, these results influence our interpretations of earlier attempts to examine the biologic significance of the peripheral metabolism of the hormone by competitive blocking of hormonal cleavage by use of an alternative substrate, bPTH-(28–48). These studies were only partly successful, owing in part to the high capacity of the cleavage mechanism and the limited solubility of bPTH-(28–48) in aqueous solutions (45). The finding that the liver is the principal organ containing the enzymes responsible for cleavage of PTH suggests that future efforts to design inhibitors having optimal efficacy in intact animals may well derive from studies of these compounds with isolated hepatic cells in vitro.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Robert M. Neer's expert assistance in statistical analysis.

This work was supported in part by grants AM11794 and 04501 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and by contract NAS 9-11011 from the National Aeronautics and Space Administration.

REFERENCES

1. Segre, G. V., H. D. Niall, J. F. Habener, and J. T. Potts, Jr. 1974. Metabolism of parathyroid hormone: physiological and clinical significance. *Am. J. Med.* **56:** 774–784.
2. Canterbury, J. M., L. A. Bricker, G. S. Levey, R. L. Kozlovskis, E. Ruiz, J. E. Zull, and E. Reiss. 1975. Metabolism of bovine parathyroid hormone: immunochemical and biological characteristics of fragments generated by liver perfusion. *J. Clin. Invest.* **55:** 1245–1253.
3. Hruska, K. A., R. Kopelman, W. E. Rutherford, S. Klahr, and E. Slatopolsky. 1975. Metabolism of immunoreactive parathyroid hormone in the dog: the role of the kidney and the effects of chronic renal disease. *J. Clin. Invest.* **56:** 39–48.
4. Neuman, W. F., M. W. Neuman, P. J. Sammon, W. Simon, and K. Lane. 1975. The metabolism of labeled parathyroid hormone. III. Studies in rats. *Calcif. Tissue Res.* **18:** 251–261.
5. Martin, K., K. A. Hruska, A. Greenwalt, S. Klahr, and E. Slatopolsky. 1976. Selective uptake of intact parathyroid hormone by the liver: differences between hepatic and renal uptake. *J. Clin. Invest.* **58:** 781–788.
6. Segre, G. V., P. D'Amour, and J. T. Potts, Jr. 1976. Metabolism of radioiodinated bovine parathyroid hormone in the rat. *Endocrinology.* **99:** 1645–1652.
7. Habener, J. F., G. P. Mayer, P. C. Dee, and J. T. Potts, Jr. 1976. Metabolism of amino- and carboxyl-sequence immunoreactive parathyroid hormone in the bovine: evidence for peripheral cleavage of hormone. *Metab. Clin. Exp.* **25:** 385–395.
8. Segre, G. V., H. D. Niall, R. T. Sauer, and J. T. Potts, Jr. 1977. Edman degradation of radioiodinated parathyroid hormone: application to sequence analysis and hormone metabolism *in vivo*. *Biochemistry.* **16:** 2417–2427.
9. Segre, G. V. 1979. Heterogeneity and metabolism of parathyroid hormone. *In* Endocrinology. L. J. DeGroot, G. F. Cahill, Jr., W. D. Odell, L. Martini, J. T. Potts, Jr., D. H. Nelson, E. Steinberger, and A. I. Winegard, editors. Grune & Stratton, Inc., New York. 613–619.
10. Silverman, R., and R. S. Yalow. 1973. Heterogeneity of parathyroid hormone: clinical and physiologic implications. *J. Clin. Invest.* **52:** 1958–1971.
11. Flueck, J. A., F. P. Di Bella, A. J. Edis, J. M. Kehrwald, and C. D. Arnaud. 1977. Immunoheterogeneity of parathyroid hormone in venous effluent serum from hyperfunctioning parathyroid glands. *J. Clin. Invest.* **60:** 1367–1375.
12. Hanley, D. A., K. Takatsuki, J. M. Sultan, J. M. Schneider, and L. M. Sherwood. 1978. Direct release of parathyroid hormone fragments from functioning bovine parathyroid glands *in vitro*. *J. Clin. Invest.* **62:** 1247–1254.
13. Mayer, G. P., J. A. Keaton, J. G. Hurst, and J. F. Habener. 1979. Effects of plasma calcium concentration on the relative proportion of hormones and carboxyl fragments in parathyroid venous blood. *Endocrinology.* **104:** 1778–1784.
14. Arnaud, C. D., and F. P. Di Bella. 1979. Parathyroid hormone assay: general features. *In* Endocrinology, L. J. DeGroot, G. F. Cahill, Jr., W. D. Odell, L. Martini, J. T. Potts, Jr., D. H. Nelson, E. Steinberger and A. I. Winegrad editors. Grune & Stratton, Inc., New York. 713–716.
15. Morrissey, J. J., F. L. Smardo, D. V. Cohn, and J. W. Hamilton. 1979. Secretion of parathormone and parathormone fragments by dispersed porcine parathyroid cells. Program/Abstracts of the 61st Annual Meeting of the Endocrine Society, Anaheim, Calif. (Abstr. 39)
16. Habener, J. F., D. Powell, T. M. Murray, G. P. Mayer, and J. T. Potts, Jr. 1971. Parathyroid hormone: secretion and metabolism *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* **68:** 2986–2991.
17. Canterbury, J. M., and E. Reiss. 1972. Multiple immunoreactive molecular forms of parathyroid hormone in human serum. *Proc. Soc. Exp. Biol. Med.* **140:** 1393–1398.
18. Segre, G. V., J. F. Habener, D. Powell, G. W. Tregear, and J. T. Potts, Jr. 1972. Parathyroid hormone in human plasma: immunochemical characterization and biological implications. *J. Clin. Invest.* **51:** 3163–3172.
19. Goldsmith, R. S., J. Furszyfer, W. J. Johnson, A. E. Fournier, G. W. Sizemore, and C. D. Arnaud. 1973. Etiology of hyperparathyroidism and bone disease during chronic hemodialysis. III. Evaluation of parathyroid suppressibility. *J. Clin. Invest.* **52:** 173–180.
20. Fischer, J. A., U. Binswanger, and F. M. Dietrich. 1974. Human parathyroid hormone: immunological characterization of antibodies against a glandular extract and the synthetic amino-terminal fragments 1-22 and 1-34 and their use in the determination of immunoreactive hormone in human sera. *J. Clin. Invest.* **54:** 1382–1394.
21. Arnaud, C. D., R. S. Goldsmith, P. J. Bodier, G. W. Sizemore, J. A. Larsen, and S. B. Oldham. 1974. Influence of immunoheterogeneity of circulating parathyroid hormone on results on radioimmunoassays of serum in man. *Am. J. Med.* **56:** 785–793.
22. Martin, K. J., K. A. Hruska, J. J. Freitag, S. Klahr, and E. Slatopolsky. 1979. The peripheral metabolism of parathyroid hormone. *N. Engl. J. Med.* **301:** 1092–1098.
23. Singer, F. R., G. V. Segre, J. F. Habener, and J. T. Potts, Jr. 1975. Peripheral metabolism of bovine parathyroid hormone in the dog. *Metab. Clin. Exp.* **24:** 139–144.
24. Martin, K., K. A. Hruska, J. Lewis, C. Anderson, and E. Slatopolsky. 1977. The renal handling of parathyroid hormone: role of peritubular uptake and glomerular filtration. *J. Clin. Invest.* **58:** 781–788.
25. D'Amour, P., G. V. Segre, S. I. Roth, and J. T. Potts, Jr. 1979. Analysis of parathyroid hormone and its fragments in rat tissues: chemical identification and microscopical localization. *J. Clin. Invest.* **63:** 89–98.
26. Berson, S. A., and R. S. Yalow. 1968. Immunochemical heterogeneity of parathyroid hormone in plasma. *J. Clin. Endocrinol. Metab.* **28:** 1037–1047.
27. Freitag, J., K. J. Martin, K. A. Hruska, C. Anderson, M. Conrades, J. Ladenson, S. Klahr, and E. Slatopolsky. 1978. Impaired parathyroid hormone metabolism in patients with chronic renal failure. *N. Engl. J. Med.* **298:** 29–32.
28. D'Amour, P., P. M. Huet, G. V. Segre, and M. Rosenblatt. 1979. Structural requirement for uptake and cleavage of iodinated bovine parathyroid hormone by the dog liver *in vivo*. Program/Abstracts of the 61st Annual Meeting of the Endocrine Society, Anaheim, Calif. (Abstr. 172)
29. Hruska, K. A., K. Martin, P. Mennes, A. Greenwalt, C. Anderson, S. Klahr, and E. Slatopolsky. 1977. Degradation of parathyroid hormone and fragment production by the isolated perfused dog kidney: the effect of glomerular filtration rate and perfusate Ca^{++} concentration. *J. Clin. Invest.* **60:** 808–814.
30. Davis, R., and R. V. Talmage. 1960. Evidence for liver inactivation of parathyroid hormone. *Endocrinology.* **66:** 312–314.
31. Fang, V. S., and A. H. Tashjian, Jr. 1972. Studies on the role of the liver in the metabolism of parathyroid hormone. I. Effects of partial hepatectomy and incubation of the hormone with tissue homogenates. *Endocrinology.* **90:** 1177–1184.

32. Neuman, W. F., M. W. Neuman, K. Lane, L. L. Miller, and P. J. Sammon. 1975. The metabolism of labeled parathyroid hormone. V. Collected biological studies. *Calcif. Tissue Res.* **18:** 271–286.
33. Keutmann, H. T., G. D. Aurbach, B. F. Dawson, H. D. Niall, L. J. Deftos, and J. T. Potts, Jr. 1971. Isolation and characterization of the bovine parathyroid isohormones. *Biochemistry.* **10:** 2779–2787.
34. Sauer, R. T., H. D. Niall, M. L. Hogan, H. T. Keutmann, J. L. H. O'Riordan, and J. T. Potts, Jr. 1974. The amino acid sequence of porcine parathyroid hormone. *Biochemistry.* **13:** 1994–1999.
35. Marcus, R., and G. D. Aurbach. 1969. Bioassay of parathyroid hormone *in vitro* with a stable preparation of adenyl cyclase from rat kidney. *Endocrinology.* **85:** 801–810.
36. Parsons, J. A., B. Reit, and C. J. Robinson. 1973. A bioassay for parathyroid hormone using chicks. *Endocrinology.* **92:** 454–462.
37. Segre, G. V., G. W. Tregear, and J. T. Potts, Jr. 1975. Development and application of sequence-specific radioimmunoassays for analysis of the metabolism of parathyroid hormone. *Methods Enzymol.* **37:** 38–66.
38. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature (Lond.).* **194:** 495–496.
39. Funovics, J. M., M. G. Cummings, L. Shuman, J. M. James, and J. E. Fischer. 1975. An improved nonsuture method for portacaval anastomosis in the rat. *Surgery (St. Louis).* **77:** 661–664.
40. Pitts, R. F. 1974. Physiology of the Kidney and Body Fluids. Year Book Medical Publishers, Inc., Chicago, Ill. 11–35.
41. Berman, M., and M. Weiss. 1978. Systems Analysis and Modeling, Operating Manual, Version 27. Laboratory of Theoretical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
42. Edman, P., and E. Begg. 1967. A protein sequenator. *Eur. J. Biochem.* **1:** 80–91.
43. Teitelbaum, A. P., N. Schneider, and W. F. Neuman. 1979. On the relation between peripheral cleavage of parathyroid hormone and its biological activity in kidney. *Metab. Bone Dis. Rel. Res.* **2:** 25–31.
44. Barrett, P. Q., A. P. Teitelbaum, and W. F. Neuman. 1978. The heterogeneity of radioiodinated parathyroid hormone in rat plasma. *Metab. Bone Dis. Rel. Res.* **1:** 263–267.
45. Rosenblatt, M., G. V. Segre, and J. T. Potts, Jr. 1977. Synthesis of a fragment of parathyroid hormone, bPTH-(28–48): an inhibitor of hormone cleavage *in vivo*. *Biochemistry.* **16:** 2811–2816.