# Selective Uptake of Intact Parathyroid Hormone by the Liver

## DIFFERENCES BETWEEN HEPATIC AND RENAL UPTAKE

K. MARTIN, K. HRUSKA, A. GREENWALT, S. KLAHR, and E. SLATOPOLSKY

*From the Renal Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110*

ABSTRACT Hepatic and renal extraction of immunoreactive parathyroid hormone (i-PTH) was studied in awake dogs with explanted kidneys and chronic indwelling hepatic vein catheters. After a single injection of bovine PTH 1-84 (b-PTH 1-84), hepatic arteriovenous (A-V) differences for immunoreactive PTH (i-PTH) was 39% at 2 min after injection but decreased to 0% by 25 min, despite high levels of i-PTH in the arterial circulation. Gel filtration of arterial and hepatic venous samples obtained when hepatic A-V differences for i-PTH were demonstrable revealed hepatic uptake of the intact hormone and addition of a smaller COOH-terminal fragment, eluting just after the intact hormone, to the hepatic venous blood. Gel filtration of samples obtained 20–30 min after injection of b-PTH 1-84 (when no hepatic A-V difference for i-PTH was demonstrable) revealed no detectable intact hormone in the circulation. Levels of COOH-terminal fragments of the hormone at the time were identical in arterial and hepatic venous samples. In additional experiments no hepatic A-V difference was observed after the injection of the synthetic bovine PTH 1-34 (syn b-PTH 1-34). By comparison there was a demonstrable A-V difference of 20% across the kidney for both intact PTH and COOH-terminal fragments that persisted until i-PTH disappeared from the circulation. The kidney also demonstrated an A-V difference of 22% after injection of syn b-PTH 1-34. These studies demonstrate selective extraction of intact PTH but not of its fragments by the liver. The kidney, on the other hand, extracted the intact hormone and both COOH and $NH_2$ terminal fragments. The studies demonstrate that the kidney was the only organ of those examined that detectably removed the fragments of PTH from the circulation.

This work was presented in part at the Central Society for Clinical Research, Chicago, Ill., November 1975 and at the American Federation for Clinical Research, Atlantic City, N. J., May 1976.

Dr. Martin was the recipient of a fellowship from the Kidney Foundation of Eastern Missouri, St. Louis, Mo.

*Received for publication 3 March 1976 and in revised form 4 June 1976.*

## INTRODUCTION

Immunoreactive circulating parathyroid hormone (PTH)[1] exists as a heterogenous mixture of the intact hormone and its fragments (1–4). There is some controversy as to whether the parathyroid glands secrete exclusively the intact 84-amino acid polypeptide (5) or whether they also secrete some of the fragments found in the circulation (4, 6). The major target organs of PTH are kidney (7) and bone (8), with other possible sites of action in the liver (9) and the intestine (10). To date, the kidney and the liver have been shown to be able to degrade the hormone (11, 12).

Previous studies from this laboratory demonstrated a 20% extraction of delivered carboxyl (COOH) terminal PTH immunoreactivity by the kidney of awake dogs (13). This arteriovenous (A-V) difference of 20% was maintained as the circulating form of the hormone changed from the intact species predominately to COOH-terminal fragments. Isolated perfused dog kidneys in vitro demonstrated uptake and progressive degradation of intact bovine (b)-PTH 1-84 (14) with the production and subsequent metabolism of smaller molecular weight fragments from both the COOH and amino ($NH_2$) terminal portions of the molecule.

The dog studies in vivo (13) also demonstrated that extrarenal sites of metabolism accounted for approximately 40% of the removal of COOH-terminal immunoreactivity from the circulation after the injection of b-PTH 1-84. Also, approximately 60% of the removal

[1] *Abbreviations used in this paper:* A-V, arteriovenous; ICG, indocyanine green; b-PTH, bovine parathyroid hormone; i-PTH, immunoreactive parathyroid hormone; syn-PTH, synthetic parathyroid hormone.

rate of $NH_2$-terminal immunoreactivity was accounted for by extrarenal sites when bovine synthetic PTH 1-34 (syn b-PTH 1-34) was injected.

The liver is apparently a major extrarenal site for the degradation of PTH. A prolonged disappearance time of injected radiolabeled $^{125}$I-PTH has been demonstrated in partially hepatectomized rats (15). Studies in vitro have demonstrated progressive degradation of the intact hormone and release of hormonal fragments into the perfusion medium by the isolated perfused rat liver (12). The fragments produced included a large COOH-terminal fragment wiith an estimated mol wt of 7,000 that appeared to be secondarily degraded to a COOH-terminal fragment, approximate mol wt 3,500. Also, an $NH_2$-terminal fragment, approximate mol wt 3,500, was detected in the perfusate.

The present studies were undertaken to examine further the role of the liver in the metabolism of PTH in vivo. They demonstrate selective uptake of b-PTH 1-84 by the liver and the absence of detectable hepatic uptake of smaller COOH-terminal fragments or of injected syn b-PTH 1-34. Concomitant studies in the kidney demonstrated uptake of the intact hormone and its fragments.

## METHODS

*Preparation of dogs.* Studies were performed on female mongrel dogs weighing 12–32 kg. Dogs were fed standard Purina dog chow (Ralston Purina Co., St. Louis, Mo.) and had free access to water. Before study, a catheter was placed in a hepatic vein by a modification of the procedure described by Shoemaker et al. (16). Through a midline abdominal incision, the left common hepatic vein was identified. A guide wire was introduced through a 16-gauge needle, over which a polyethylene catheter was advanced 1–2 cm into the vein and sutured in place. The catheter was brought out through a stab wound in the right flank and filled with heparin. Initial studies were performed 4–10 days after surgery. Patency of the catheter was maintained for 8–10 wk by filling the catheter with heparin every 5–7 days. In addition to the hepatic vein catheter, another group of dogs had the left kidney explanted to a subcutaneous position, as previously described (13). Three dogs had an additional catheter placed in the portal vein via a mesenteric vein.

*Study protocol.* Dogs were studied awake after a 12–14-h fast. The femoral artery was catheterized by the Seldinger technique (17), and a polyethylene catheter was advanced into the aorta to the approximate level of the celiac axis. A foreleg vein catheter was placed for infusion of solutions, and a catheter was placed in the bladder for collection of urine.

Hepatic plasma flow was measured by the constant infusion of indocyanine green (ICG) (Hynson, Westcott & Dunning, Inc., Baltimore, Md.), which also served as a marker for adequacy of hepatic venous sampling to exclude contamination of samples with inferior vena caval blood (18). After a priming dose of 300 μg/kg, ICG was infused in normal saline containing 0.6% bovine serum albumin at 60–80 μg/min with a Harvard syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.). Standards were prepared in dog's plasma and read in a Coleman spectrophotometer (Coleman Instruments Div., Perkin Elmer Corp., Oak Brook, Ill.) at a wavelength of 806 μm.

Glomerular filtration rate was measured by exogenous creatinine clearance. In those dogs with explanted kidneys renal plasma flow was measured by the infusion of para-aminohippurate. Renal extraction of para-aminohippurate also served as a marker for adequacy of renal venous sampling.

After 30–45 min of equilibration, highly purified b-PTH 1–84 or syn b-PTH 1–34 (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) was administered by rapid intravenous injection (< 5 s) through the foreleg catheter. Blood samples were drawn simultaneously from aorta, hepatic vein, renal vein, and portal vein at 1–10-min intervals after injection of hormone. In addition, studies were performed with a constant infusion of b-PTH 1–84 in normal saline containing 1% bovine serum albumin under ice.

*Sources of PTH.* Highly purified b-PTH 1–84 for administration and for the radioimmunoassay standard was obtained from Inolex Corp., Biomedical Div., Glenwood, Ill. (sp act 900–1,500 U/mg in the Munson bioassay). Syn b-PTH 1–34 was obtained from Beckman Instruments, Inc. (sp act 3,700 U/mg in renal adenylate cyclase system). Both b-PTH 1–84 and syn b-PTH 1–34 were reconstituted in 0.2% acetic acid, pH 3.5, and added to hypoparathyroid dog serum for injection.

*Chemical determinations.* Creatinine was measured by the Jaffe reaction as described by Folin (19) and adapted for the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Para-aminohippurate was determined by the method of Harvey and Brothers (20) as adapted for the Technicon Autoanalyzer.

*Radioimmunoassay methods.* Plasma levels of immunoreactive PTH (i-PTH) after injection of b-PTH 1–84 or syn b-PTH 1–34 were determined as described previously (13) with minor modifications. In studies with b-PTH 1–84, i-PTH was determined with antiserum CH9, a predominantly COOH-terminal antiserum, as characterized previously (13). In the present studies, the CH9 antiserum was used in a final dilution of 1 : 75,000. Antiserum CH9N, previously characterized as having specific binding determinants for the amino terminal portion of the PTH molecule (13), was used in the studies with syn b-PTH 1–34 in a final dilution of 1 : 8,000.

Assay additions were made with an automatic pipetting station (Micromedic Systems, Inc., Horsham, Pa. model 24004) with multiple dilutions of each sample in duplicate. b-PTH purified for iodination (Inolex) and syn b-PTH 1–34 (Beckman) were iodinated with iodine-131 by a modification of the Greenwood et al. technique (21) as previously described (13). Iodinated peptides were added to the assay tubes after 3–4 days of initial incubation. After 1–2 days of additional incubation, total counts added were determined; after separation of free from bound counts by charcoal-coated dextran as previously described (13), the supernate was discarded, and the charcoal pellet was counted in a gamma well spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill., model 5360). The counts were coded on a Teletype tape and fed via a Hewlett-Packard tape reader (model 2748B) to a Hewlett-Packard calculator (model 9810A) (Hewlett-Packard Co., Palo Alto, Calif.) programmed to calculate the i-PTH for each assay tube, with a logit transformation of the standard curve. The standard curve and the pattern of immunoreactivity in samples of column eluates were plotted on a Hewlett-

Packard plotter (model 9862A). Samples assayed in this way, i.e., counting charcoal pellet only, correlated closely with results obtained by the standard method of counting charcoal and supernate ($r = 0.98$).

Endogenous canine i-PTH assayed in the above system (b-PTH 1–84 standard) was subtracted as a background from all samples (normal range 0.1–0.45 ng b-PTH eq/ml).

*Gel filtration.* Arterial and hepatic venous plasma samples (1.5 ml) were filtered on 50–90 × 1.5-cm columns of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.), calibrated by the elution position of dextran blue, $^{131}$I-b-PTH 1–84, $^{125}$I-insulin, and $^{131}$I-syn b-PTH 1–34. The column eluant was 0.15 M ammonium acetate with 2.5 mg/ml bovine serum albumin, pH 5.5. Column flow rate was 5–10 ml/h, and 0.5-ml fractions were collected.

i-PTH in the column eluates was determined in two radioimmunoassays, one using antiserum CH9 preincubated with excess syn b-PTH 1–34 (10 ng/tube at 4°C for 24 h with constant agitation) to render it COOH-terminal-specific and the other using CH9N, an $NH_2$-terminal-specific antiserum as previously described (13). To correct for any effect of column buffer on the binding between PTH and the antisera, appropriate controls for nonspecific binding, tracer binding, and standard curves were performed with volumes of column eluant equal to the test sample volumes. The pH of this assay mixture was 8.2 as compared to a pH of 8.6 for standard assay mixtures. The effect of this addition of column buffer on the radioimmunoassays was insignificant.

*Calculations.* Hepatic plasma flow was calculated by dividing the infusion rate of ICG (in micrograms per minute) by the hepatic A-V difference. Renal plasma flow was calculated by the Wolff modification of the Fick principle (22). The percent hepatic and renal extraction of i-PTH was determined by dividing the A-V difference by the arterial i-PTH concentration.

## RESULTS

*Adequacy of hepatic vein sampling.* Table I portrays the results of 12 determinations of hepatic ICG extraction, hepatic plasma flow, and hepatic blood flow in eight unanesthetized dogs. Hepatic extraction of ICG was 15.8±1.1%, similar to values reported previously (18). ICG extraction remained constant throughout the studies, with a coefficient of variation ranging from 2 to 8% within each study. Hepatic venous samples with greater than 5% variation from the mean ICG extraction were uncommon, and they were considered contaminated with vena caval blood. These samples were not used in assays for i-PTH nor in gel filtration studies. The calculated hepatic blood flows by this technique were slightly higher than the values reported with ICG in anesthetized dogs (18), but were comparable to those reported with bromsulphalein in awake dogs (23). In additional studies the hepatic extraction of ICG was measured simultaneously with i-PTH, but clearances of ICG and hepatic plasma flow were not determined. The extraction of ICG in these studies was always constant and equal to values reported in Table I.

TABLE I

*Hepatic Plasma and Blood Flow Determined by the Clearances and Hepatic Extraction of ICG*

| Dog | Weight | $C_{ICG}$ | Extr | HPF | HBF |
|---|---|---|---|---|---|
| | *kg* | *ml/kg/min* | *%* | *ml/kg/min* | *ml/kg/min* |
| 1 | 14.5 | 5.50 | 21 | 26.4 | 38.4 |
| | 14.5 | 5.60 | 21 | 27.0 | 37.0 |
| 2 | 22.9 | 3.57 | 19 | 18.7 | 37.5 |
| | 22.9 | 4.30 | 19 | 24.4 | — |
| 3 | 17.5 | 3.50 | 16 | 21.0 | 36.3 |
| | 16.5 | 1.90 | 14 | 14.8 | 29.6 |
| 4 | 12.5 | 3.46 | 10 | 36.4 | — |
| | 12.5 | 3.60 | 11 | 32.1 | 42.8 |
| 5 | 16.8 | 4.76 | 19 | 25.1 | 37.5 |
| 6 | 15.5 | 2.20 | 14 | 16.2 | 27.9 |
| 7 | 17.3 | 3.40 | 13 | 27.0 | 36.5 |
| 8 | 31.8 | 4.25 | 13 | 32.1 | 45.9 |
| Mean | | 3.86 | 15.8 | 25.1 | 36.9 |
| SEM | | 0.33 | 1.1 | 1.9 | 1.68 |
| *n* | | 12 | 12 | 12 | 10 |

$C_{ICG}$, indocyanine green clearance; Extr, hepatic extraction of ICG; HPF, hepatic plasma flow; HBF, hepatic blood flow.

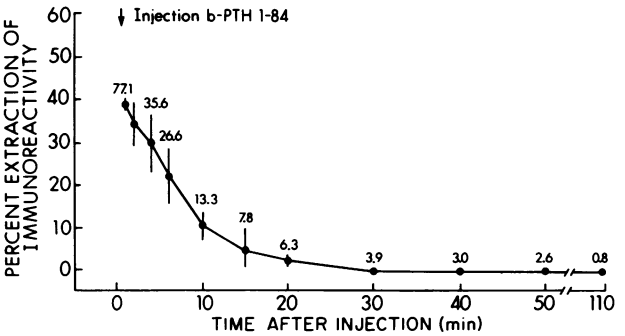


FIGURE 1 Hepatic extraction of i-PTH after a single injection of b-PTH 1–84. Each point represents the mean± SE of the percent extraction seen in seven dogs. The mean arterial i-PTH concentration in nanograms per milliliter at each time of sampling is shown above the standard error bars. Background mean i-PTH was 0.13 ng/ml.

*Studies with single injection b-PTH 1-84.* After single injections of b-PTH 1-84 (5 μg/kg) (assayed with antiserum CH9) the mean percent extraction of immunoreactivity by the liver was 38.7±1.2% in seven dogs 2 min after injection, and fell progressively to 0% after 15–25 min (Fig. 1). This occurred despite a lack of change in hepatic blood flow as measured by extraction of ICG and despite persisting high levels of immunoreactive PTH in the circulation that did not return to base-line values until 150–180 min after injection. In five additional dogs in which renal extraction was determined simultaneously with hepatic extraction (Fig. 2), the pattern of hepatic extraction was similar to that seen in Fig. 1. However, renal extraction of i-PTH was constant at 20±0.68% and remained stable until i-PTH disappeared from the circulation.

The possibility that the cessation of extraction of PTH by the liver might represent a saturation phenome-

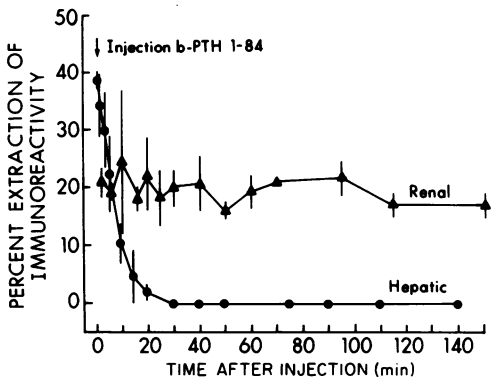


FIGURE 2 Hepatic (●) and renal (▲) extraction of i-PTH after a single injection of b-PTH 1–84. Each point represents the mean±SE of the percent extraction in five dogs.

non at the site of PTH uptake was investigated by reinjecting b-PTH 1-84 after extraction had completely ceased after an initial injection. The results of such studies in three dogs are shown in Fig. 3. The initial pattern of hepatic b-PTH extraction was similar to that already described in the previous studies. After the injection of b-PTH 1-84, there was an initial high percent extraction (38%), which fell to zero after approximately 20 min. Reinjection of equal amounts of b-PTH 1-84 at 50–60 min when plasma i-PTH had fallen to levels of 2.6±0.4 ng/ml resulted again in a high percent extraction (34%), which fell to zero within 25 min as before. When the second injection was given earlier (at 20 min) a similar pattern was reproduced. Thus, the cessation of hepatic extraction after the initial injection of b-PTH 1-84, despite the persistence of relatively high concentrations of immunoreactivity in

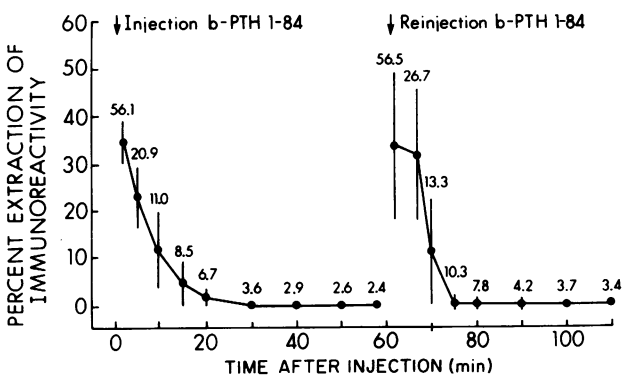


FIGURE 3 Hepatic extraction of i-PTH after a single injection of b-PTH 1–84 and after a repeat injection of b-PTH 1–84, 50–60 min (shown as 60 min) after the initial injection. Each point represents the mean extraction seen in three dogs. Mean arterial i-PTH concentration in nanograms per milliliter at each time of sampling is shown above the standard error bars. Background mean i-PTH was 0.11 ng/ml.

the circulation, cannot be explained solely by saturation of a site that extracts the hormone, since readministration of b-PTH 1-84 led to reappearance of extraction, which again decreased and rapidly disappeared.

*Gel filtration studies.* To examine if hepatic extraction of immunoreactivity after the injection of b-PTH 1-84 was related to extraction of a particular species of i-PTH, gel filtration of simultaneously obtained arterial and hepatic venous samples was performed on columns of Bio-Gel P-10. Control gel filtration studies of the purified b-PTH 1-84 used for injection into the dogs revealed a single homogenous peak of immunoreactivity eluting with the b-PTH 1-84 marker. Fig. 4 portrays the elution pattern for COOH-terminal i-PTH in arterial and hepatic venous samples obtained from a dog 5 min after injection of b-PTH 1-84. Considerable degradation of the injected intact hormone was already present, as shown by the elution pattern of immunoreactivity in the arterial sample obtained from the aorta. Two peaks of immunoreactivity that appear in the effluent after the $^{131}$I-b-PTH 1-84 marker are evident. This pattern is similar to that previously reported for COOH-terminal immunoreactivity in pools of arterial serum or plasma obtained 0–15 min after injection of b-PTH 1-84 (13). The elution pattern of hepatic venous COOH-terminal i-PTH obtained simultaneously with the arterial sample demonstrates a markedly smaller peak of immunoreactivity in the elution position of the $^{131}$I-b-PTH 1-84 marker and a larger peak of immunoreactivity eluting after the $^{131}$I-b-PTH 1-84 marker.

Fig. 5 shows the elution pattern for COOH-terminal immunoreactivity in arterial and hepatic venous sam-

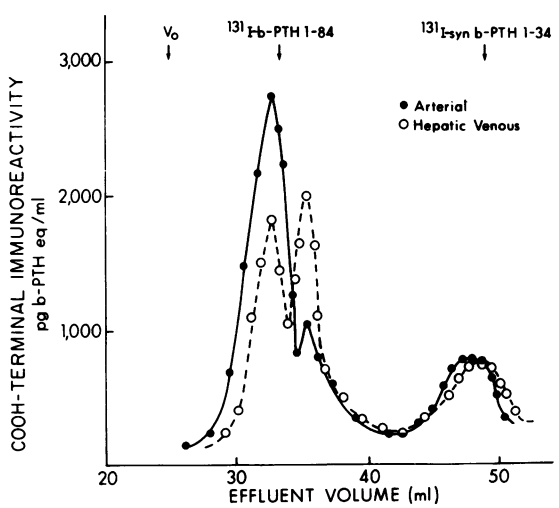


FIGURE 4 Elution pattern of COOH-terminal i-PTH in arterial (●) and hepatic venous (○) plasma obtained from a dog 5 min after injection of b-PTH 1–84. COOH-terminal i-PTH was determined with the CH9 antiserum preincubated with excess syn b-PTH 1–34. Gel filtration was performed on a 1.5 × 50 cm column of Bio-Gel P-10.

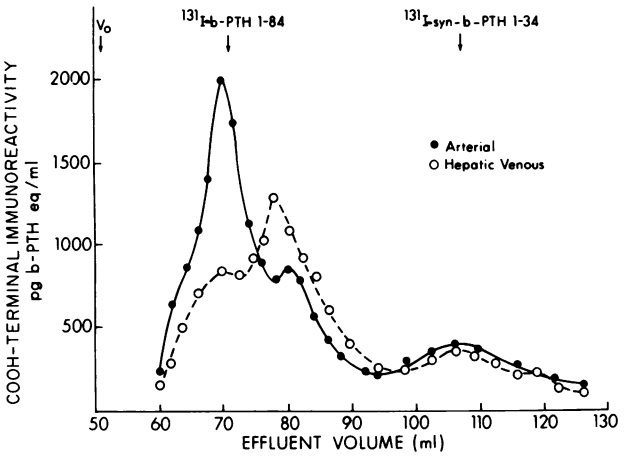


FIGURE 5 Elution pattern of COOH-terminal i-PTH in arterial (●) and hepatic venous (○) plasma pools from three dogs. The samples were taken 6 min after injection of b-PTH 1–84. COOH-terminal i-PTH was determined with the CH9 antiserum preincubated with excess syn b-PTH 1–34. Gel filtration was performed on a 1.5 × 90 cm column of Bio-Gel P-10.

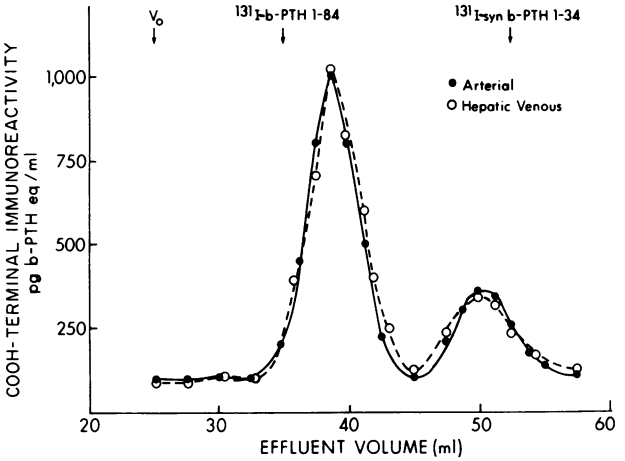


FIGURE 6 Elution pattern of COOH-terminal i-PTH in arterial (●) and hepatic venous (○) plasma obtained from a dog 20 min after injection of b-PTH 1–84. COOH-terminal i-PTH was determined as in Figs. 5 and 6. Gel filtration was performed on a 1.5 × 50 cm column of Bio-Gel P-10.

ples in plasma pools obtained from three dogs 6 min after injection of b-PTH 1-84. Again the elution pattern of i-PTH in arterial plasma revealed a large peak eluting with the $^{131}$I-b-PTH marker representing intact hormone, a smaller peak representing a COOH-terminal fragment (apparent mol wt 6,000–7,000) and a small amount of COOH-terminal immunoreactivity eluting with the syn b-PTH 1-34 marker (apparent mol wt 3,500). Again in hepatic venous plasma, the peak representing intact hormone was markedly reduced, and the amount of the COOH-terminal peak (apparent mol wt 7,000) was increased as compared to arterial samples. The elution patterns of $NH_2$-terminal immunoreactivity (not illustrated) revealed peaks consistent with intact hormone and a smaller, late-eluting fragment appearing with the syn b-PTH 1-34 marker. Again, there was evidence for a large hepatic uptake of $NH_2$-terminal immunoreactivity in the elution position of the intact hormone marker but not difference between the arterial and venous elution patterns for the late-eluting fragment.

Fig. 6 shows the elution pattern of COOH-terminal immunoreactivity in arterial and hepatic venous plasma 20 min after injection of b-PTH 1-84 when no extraction was demonstrable by radioimmunoassay. At this time the intact hormone peak was no longer present, and the predominant peaks were a COOH-terminal fragment (apparent mol wt 7,000) in both artery and hepatic vein samples and also a smaller COOH-terminal peak (apparent mol wt. 3,500). There was no significant difference between the elution patterns from arterial and hepatic venous samples.

*Studies with syn b-PTH 1-34.* To evaluate further the absence of hepatic uptake of the late eluting $NH_2$-terminal fragment seen in the gel filtration studies, experiments were performed with syn b-PTH 1-34. The values for hepatic and renal extraction of immunoreactivity after a single injection of syn b-PTH 1-34 (p $\mu$g/kg) in five dogs are shown in Fig. 7. No extraction of this fragment by the liver was demonstrable at any time, whereas the kidney extracted this fragment throughout its disappearance from the circulation. The mean renal extraction of i-PTH after syn b-PTH 1-34 injections was 22±1.2%. In additional studies there was no aorto-portal venous A-V difference after injection of syn b-PTH 1-34.

*Studies with constant infusion b-PTH 1-84.* Constant infusions of b-PTH 1-84 were performed in three

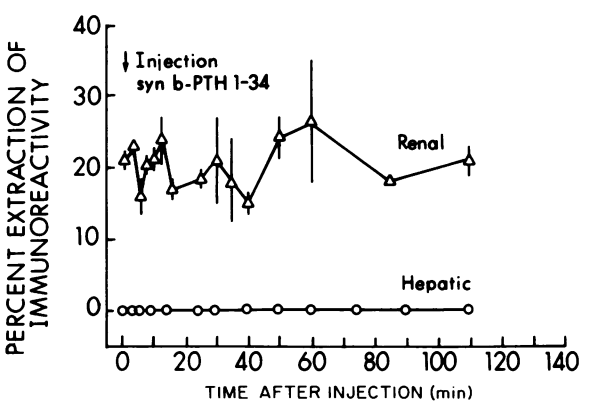


FIGURE 7 Hepatic (○) and renal (△) extraction of $NH_2$-terminal i-PTH after a single injection of syn b-PTH 1–34. Each point represents the mean±SE of the percent extraction seen in five dogs in which simultaneous renal and hepatic venous plasma samples were obtained.

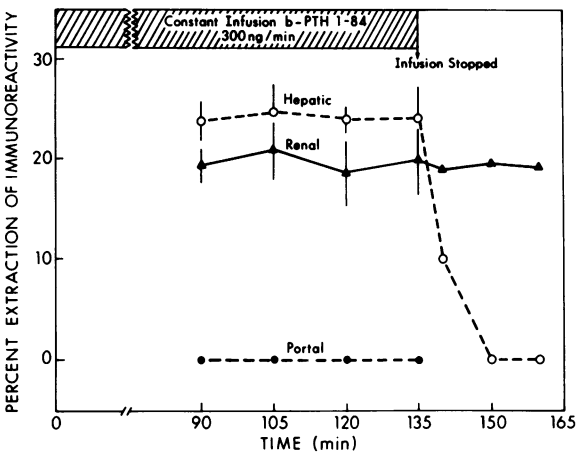


FIGURE 8 Hepatic (○), renal (▲), and portal (●) extraction of i-PTH during constant infusion of b-PTH 1–84. Each point represents mean extraction±SE in three dogs. When infusion was stopped, sampling was continued in one animal.

animals with explanted kidneys in addition to catheters in hepatic and portal veins. Thus, simultaneous extraction of i-PTH by the liver, kidney, and portal systems could be evaluated and compared in the same animals. The results of these studies are shown in Fig. 8. No aorto-portal venous difference of COOH-terminal i-PTH was observed, whereas both the liver and kidney demonstrated constant extraction of 24.1±0.2% and 20.0± 0.7%, respectively. In one animal in whom sampling was continued after the infusion was stopped, the difference between hepatic and renal extraction was apparent and similar to that shown in Fig. 2. Thus, under conditions of constant delivery of intact b-PTH 1-84, the liver demonstrates constant extraction that rapidly disappears upon termination of the infusion.

## DISCUSSION

Degradation of PTH has been demonstrated in studies in vitro using saline extracts of porcine liver (24) and the isolated perfused rat liver (12). The latter studies demonstrated production of PTH fragments by the liver when it was perfused with either tritiated or unlabeled b-PTH 1-84. The fragments generated during liver perfusion were similar to those seen in the fractionation of human hyperparathyroid serum (2). A previous study in vivo of the role of the liver in PTH metabolism documented slower disappearance of injected $^{125}$I-b-PTH after partial hepatectomy in the rat (15).

In the present studies, a reliable means of repeatedly obtaining hepatic venous blood from awake, resting dogs was utilized, thus avoiding the possible effects of anesthetic agents on hepatic metabolism and PTH degradation. The decrease and disappearance of hepatic i-PTH A-V differences after a single injection of b-PTH 1-84 was reproducible under several different experimental conditions, including seven dogs in which only hepatic extraction was studied (Fig. 1), five dogs with a left explanted kidney, in which simultaneous hepatic and renal venous samples were obtained (Fig. 2), three dogs given two separate injections of b-PTH 1-84 (Fig. 3), and one animal after cessation of constant infusion (Fig. 8). The disappearance of the initial hepatic A-V difference for i-PTH by 15–25 min after injection apparently was not accounted for by a saturation phenomenon, since a reinjection of b-PTH 1-84 at 60 min (Fig. 3) or at 20 min (not shown), resulted in extraction of immunoreactivity by the liver again, in spite of persisting high levels of immunoreactivity remaining in the circulation after the initial injection.

Further investigation of the nature of the immunoreactivity in the circulation by gel filtration studies revealed that in the first several minutes after injection of b-PTH 1-84 when a hepatic A-V difference for i-PTH was detectable there was a large difference between arterial and venous samples in the size of the peak of immunoractivity eluting with the intact hormone marker. This suggests a hepatic uptake mechanism for intact PTH 1-84 with a large capacity, and indicates that the liver is probably one of the major sites of uptake of the intact hormone (PTH 1-84). The gel filtration studies of samples obtained less than 20 min after injection also document the release of a COOH-terminal fragment of i-PTH into the hepatic venous circulation. This fragment elutes just after the intact hormone marker, similar to the previously described COOH-terminal fragment that makes up the greater portion of circulating i-PTH (2), and is thought to represent the initial cleavage product of intact hormone (25). However, at time intervals greater than 20–25 min after injection of b-PTH the major forms of immunoreactivity in the circulation were COOH-terminal fragments eluting after the intact hormone marker. At these times, the elution patterns of i-PTH in arterial and hepatic venous samples were identical. This suggests either a much smaller hepatic uptake rate, undetectable by the method used or the absence of significant hepatic uptake of COOH-terminal i-PTH fragments.

Determinations of $NH_2$-terminal immunoreactivity in arterial and hepatic venous samples subjected to gel filtration also revealed hepatic uptake of i-PTH eluting in the area of the intact hormone but showed no difference between arterial and hepatic venous samples in the peak of $NH_2$-terminal immunoreactivity eluting with the syn b-PTH 1-34 marker. No hepatic uptake of the $NH_2$-terminal fragment of PTH was detectable in a group of five normal dogs with both a hepatic vein catheter and

an explanted kidney given a single injection of the synthetic $NH_2$-terminal fragment, syn b-PTH 1-34. In these studies, as was reported previously (11), this peptide was rapidly cleared from the circulation. The renal extraction of 22% was stable throughout its disappearance from the circulation, and renal clearance accounted for approximately 40% of the total disappearance rate of $NH_2$-terminal i-PTH. Absence of hepatic uptake of $NH_2$-terminal i-PTH was a surprising finding that probably cannot be accounted for by differences between the synthetic bovine and the native canine $NH_2$-terminal tetratricontapeptide, since there was a rapid uptake of the bovine synthetic peptide by the kidney and other unknown sites. Studies done with portal vein catheters in place indicate that neither the intestine or the spleen account for the extrarenal metabolism of syn b-PTH 1-34. Nor do these organs demonstrate detectable uptake of COOH-terminal immunoreactivity after b-PTH 1-84 injections.

The above patterns of hepatic PTH uptake contrast markedly with those by the kidney reported here and in previous studies (13). The kidney demonstrated uptake of COOH-terminal immunoreactivity with an A-V difference of 20% early after injection of PTH 1-84 when intact hormone is the major circulating species and at later times when the intact hormone had disappeared from the circulation and largely COOH-terminal fragments remained. The kidney also avidly extracts injected syn b-PTH 1-34.

The data thus far suggest a selective uptake of intact PTH by the liver. If this is so, then one would expect to find continued and constant extraction of i-PTH by the liver under conditions of constant delivery of intact PTH. The studies in Fig. 8 during constant infusion of b-PTH 1-84 confirm this interpretation and provide additional data to exclude the possibility that hepatic extraction was only occurring at the high plasma levels of immunoreactivity seen after single injection. The plasma levels during constant infusion ($1.6 \pm 0.2$ ng/ml) of b-PTH 1-84 are considerably lower than the level of i-PTH in the plasma when hepatic extraction ceases after single injection.

The physiological role of the differences between hepatic and renal uptake of i-PTH in the overall metabolism of the hormone cannot be ascertained from the type of experiments reported in the present studies. The renal uptake of the COOH-terminal fragments of PTH, shown to be biologically inactive (26), and the failure to demonstrate hepatic uptake of these fragments, suggest that the kidney may have an uptake mechanism linked to degradation of these peptides. Uptake of COOH-terminal fragments may be separable from uptake linked to the biological effects of PTH. Conversely, the liver appears to possess a mechanism specific for the uptake of the intact hormone and to have no role in the catabolism of PTH fragments. Uptake of PTH fragments limited to the kidney could explain the marked accumulation and predominance of COOH-terminal fragments seen in the circulation of patients with chronic renal failure.

## ACKNOWLEDGMENTS

This investigation was supported by National Institutes of Health Grants AM09976 and AM05248.

## REFERENCES

1. Arnaud, C. D., R. S. Goldsmith, P. J. Bordier, and G. W. Sizemore. 1974. Influence of immunoheterogeneity of circulating parathyroid hormone on results of radioimmunoassays of serum in man. *Am. J. Med.* **56**: 785–793.
2. 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.
3. Habener, J. F., G. V. Segre, D. Powell, T. M. Murray, and J. T. Potts, Jr. 1972. Immunoreactive parathyroid hormone in circulation of man. *Nat. New Biol.* **238**: 152–154.
4. Silverman, R., and R. S. Yalow. 1973. Heterogeneity of parathyroid hormone. Clinical and physiologic implications. *J. Clin. Invest.* **52**: 1958–1971.
5. 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.
6. Flueck, J. A., A. J. Edis, J. M. McMahon, and C. D. Arnaud. 1975. Human parathyroid tumors secrete COOH-terminal fragments of parathyroid hormone (PTH) in vivo. *Clin. Res.* **23**: 498A. (Abstr.)
7. Rasmussen, H. 1974. Parathyroid hormone, calcitonin and the calciferols. *In* Textbook of Endocrinology. R. H. Williams, editor. W. B. Saunders Company, Philadelphia. 5th edition. 660–773.
8. Rasmussen, H., and P. Bordier. 1974. The Physiological and Cellular Basis of Metabolic Bone Disease. The Williams & Wilkins Company, Baltimore, Md. 142–205.
9. Moxley, M. A., N. H. Bell, S. R. Wagle, D. O. Allen, and J. Ashmore. 1974. Parathyroid hormone stimulation of glucose and urea production in isolated liver cells. *Am. J. Physiol.* **227**: 1058–1061.
10. Birge, S. J., S. C. Switzer, and D. R. Leonard. 1974. Influence of sodium and parathyroid hormone on calcium release from intestinal mucosal cells. *J. Clin. Invest.* **54**: 702–709.
11. Fujita, T., H. Orimo, M. Ohata, M. Yoshikawa, and M. Maruyama. 1970. Enzymatic inactivation of parathyroid hormone by rat kidney homogenate. *Endocrinology*. **86**: 42–49.
12. Canterbury, J. M., L. A. Bricker, G. S. Levey, P. L. Kozlovskis, E. Ruiz, J. E. Zull, and E. Reiss. 1975. Metabolism of bovine parathyroid hormone. Immunological and biological characteristics of fragments generated by liver perfusion. *J. Clin. Invest.* **55**: 1245–1253.
13. 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.

14. Hruska, K. A., A. Greenwalt, P. Mennes, T. Bascom, and E. Slatopolsky. 1974. Metabolism of parathyroid hormone (PTH) by the isolated perfused kidney. *Clin. Res.* **22**: 650A. (Abstr.)
15. 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.
16. Shoemaker, W. C., W. F. Walker, T. B. Van Itallie, and F. D. Moore. 1959. A method for simultaneous catheterization of the major hepatic vessels in a chronic canine preparation. *Am. J. Physiol.* **196**: 311–314.
17. Seldinger, S. I. 1953. Catheter replacement of the needle in percutaneous arteriography. A new technique. *Acta Radiol.* **39**: 368–376.
18. Ketterer, S. G., B. D. Wiegand, and E. Rapaport. 1960. Hepatic uptake and biliary excretion of Indocyanine green and its use in estimation of hepatic blood flow in dogs. *Am. J. Physiol.* **199**: 481–484.
19. Hawk, P. B., B. L. Oser, and W. H. Summerson. 1947. Practical Physiological Chemistry. The Blakiston Co., Div. of McGraw-Hill Book Company, New York. 12th edition. 506.
20. Harvey, R. B., and A. J. Brothers. 1962. Renal extraction of para-amino-hippurate and creatinine measured by continuous in vivo sampling of arterial and renal-vein blood. *Ann. N. Y. Acad. Sci.* **102**: 46–54.
21. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of $^{131}$I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114–123.
22. Wolff, A. W. 1950. The urinary function of the kidney. Grune & Stratton, Inc., Academic Press, Inc., New York. 65.
23. Shoemaker, W. C. 1960. Measurement of hepatic blood flow in the unanesthetized dog by a modified bromosulphalein method. *J. Appl. Physiol.* **15**: 473–478.
24. Fischer, J. A., S. B. Oldham, G. W. Sizemore, and C. D. Arnaud. 1972. Calcium-regulated parathyroid hormone peptidase. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 2341–2345.
25. Segre, G. V., H. D. Niall, J. F. Habener, and J. T. Potts, Jr. 1974. Metabolism of parathyroid hormone. Physiologic and clinical significance. *Am. J. Med.* **56**: 774–784.
26. Canterbury, J. M., G. S. Levey, and E. Reiss. 1973. Activation of renal cortical adenylate cyclase by circulating immunoreactive parathyroid hormone fragments. *J. Clin. Invest.* **52**: 524–527.