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Research Article

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A Micropuncture Study of the Effect of Parathyroid Hormone on Renal Bicarbonate Reabsorption

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A B S T R A C T   Renal micropuncture and clearance experiments were carried out in rats to study the effect of parathyroid hormone (PTH) on renal tubular HCO_3^- reabsorption. The rats were studied during an initial period of parathyroid deficiency (acute thyroidparathyroidectomy, TPTX) and during infusion of large amounts of bovine PTH. Under normal acid-base conditions, PTH administration to TPTX rats caused a significant rise in proximal tubular fluid HCO_3^- concentration ($TF_{HCO_3^-}$), a decrease in fluid reabsorption, and a fall in proximal HCO_3^- reabsorption from 94.0 to 88.2% ($P < 0.01$). In control experiments with mannitol infusion, a comparable reduction in proximal fluid reabsorption occurred without any significant effect on intraluminal HCO_3^- concentration. During acute intravenous HCO_3^- loading, PTH inhibited proximal HCO_3^- reabsorption. However, no change in whole kidney HCO_3^- reabsorption was observed in these experiments or in the animals studied under normal acid-base conditions. The findings are consistent with the view that PTH inhibits proximal tubular HCO_3^- reabsorption with normal or high filtered loads of HCO_3^-, but distal segments of the nephron are able to reabsorb the excess delivered from the proximal tubule. Measurements of urinary ammonium and titratable acid indicate that net acid excretion ($NH_4^+ + TA - HCO_3^-$) increases significantly after PTH administration. These results do not provide support for the view that PTH excess causes metabolic acidosis by reducing renal acid excretion.

INTRODUCTION

A number of studies have suggested that parathyroid hormone (PTH)[1] influences bicarbonate reabsorption

by the renal tubules (1-9). Infusion of purified parathyroid extract into intact dogs under normal acid-base conditions causes a transient rise in urinary bicarbonate excretion (7), and when infused into parathyroidectomized bicarbonate-loaded animals, a sustained reduction in tubular reabsorption of bicarbonate results (7-9). These effects were shown to be independent of changes in renal hemodynamics, extracellular volume, ultrafilterable calcium, and calcitonin (7-9). From these observations, it could be concluded that PTH has little or no significant effect on the bicarbonate reabsorptive mechanism when the filtered load of bicarbonate is normal or low, but that the hormone inhibits reabsorption when the filtered bicarbonate level is abnormally high. Such a conclusion could have important implications with regard to the underlying mechanism of action of PTH on bicarbonate reabsorption. For example, inhibition of renal carbonic anhydrase activity with acetazolamide reduces bicarbonate reabsorption over a wide range of filtered bicarbonate concentrations, even when the filtered load is quite low (10, 11). Furthermore, if PTH inhibits bicarbonate reabsorption only when the filtered bicarbonate concentration is high, it would be unlikely that excess endogenous parathyroid secretion can produce metabolic acidosis, as has been suggested for several different clinical conditions (5, 6, 12).

The present experiments were carried out to gain further information about the effect of PTH on renal tubular reabsorption of bicarbonate. Micropuncture measurements of proximal tubular bicarbonate transfer were made in thyroidparathyroidectomized (TPTX) rats before and after administration of exogenous PTH, under normal acid-base conditions and during acute bicarbonate loading. We found that PTH caused a moderate reduction in proximal bicarbonate reabsorp-

tion in TPTX rats, whether the filtered load of bicarbonate was normal or elevated. Whole kidney bicarbonate reabsorption was unaltered, however, under these experimental conditions. These observations suggest that distal segments of the nephron are not inhibited by PTH, and were able to reabsorb the excess bicarbonate rejected by the proximal tubules. Net acid excretion increased after PTH, as a result of a rise in titratable acid and ammonium excretion. The observations do not provide support for the view that PTH excess causes metabolic acidosis.

METHODS

White male Sprague-Dawley rats weighing 225–335 g were maintained on a regular rat pellet diet and given 5% sucrose drinking solution ad lib. for at least 4 days before the day of the experiment to insure adequate hydration. Food, but not drinking solution, was withheld on the night before the experiment. On the day of the experiment, anesthesia was induced with i.p. Inactin, 10 mg/100 g body weight. The trachea was cannulated, and PE 50 tubing was inserted into a jugular vein for administration of i.v. fluids. A second PE 50 tubing was inserted into the same vein for pulse injection of FD & C green no. 3 dye (Keystone Aniline and Chemical Co., Chicago, Ill.). A carotid artery was cannulated with heparinized PE 50 tubing connected to a Statham strain gauge, model P 23 Dc (Statham Instruments Div., Gould Inc., Oxnard, Calif.), for continuous recording of blood pressure by a Grass polygraph, model 5D (Grass Instrument Co., Quincy, Mass.). In all experiments, total TPTX was carried out as soon as the jugular and carotid vessel surgery had been completed. A volume of Ringer's lactate solution equal to 1% of body weight was given i.v. over a 1–2-min period, followed by one of the i.v. solutions described below.

The left kidney was exposed through a small lateral abdominal incision, dissected free of perirenal adipose tissue, and placed in a Lucite cup attached to the animal table. The kidney was immobilized by silicone grease (Dow Corning Corp., Midland, Mich.) and cotton packed between it and the kidney cup. Mineral oil warmed to 37–38°C flowed continuously over the kidney. A fiber optic light system was used to illuminate the kidney surface, which was viewed through a stereomicroscope at X150 magnification. The urinary bladder was externalized through a small suprapubic incision, and PE tubing, flanged at the tip, was inserted into the lumen and ligated in a manner which eliminated bladder dead space. Body temperature of the rat was monitored continuously via a rectal thermistor and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) and was maintained at 37–38°C by adjusting the voltage of a 40-W bulb under the animal table. Further details of the methods are given in the individual protocols described below.

Effect of parathyroid extract under hydropenic, normal acid-base conditions. In nine rats, a continuous infusion of Ringer's lactate solution was given i.v. throughout the experiment at 50 µl/min. A 30-µCi prime of [carboxyl-^{14}C]inulin (New England Nuclear, Boston, Mass.) was given and an amount added to the infusion to deliver 30 µCi/h. Tubular fluid collections were begun approximately 30–45 min later, 35–90 min after TPTX. The last surface convolution of proximal convoluted tubules was selected for micropuncture by three criteria: contiguity to a vascular "star"; sequential appearance of i.v. pulse-injected FD & C green dye; disappearance beneath the kidney surface of oil droplets injected
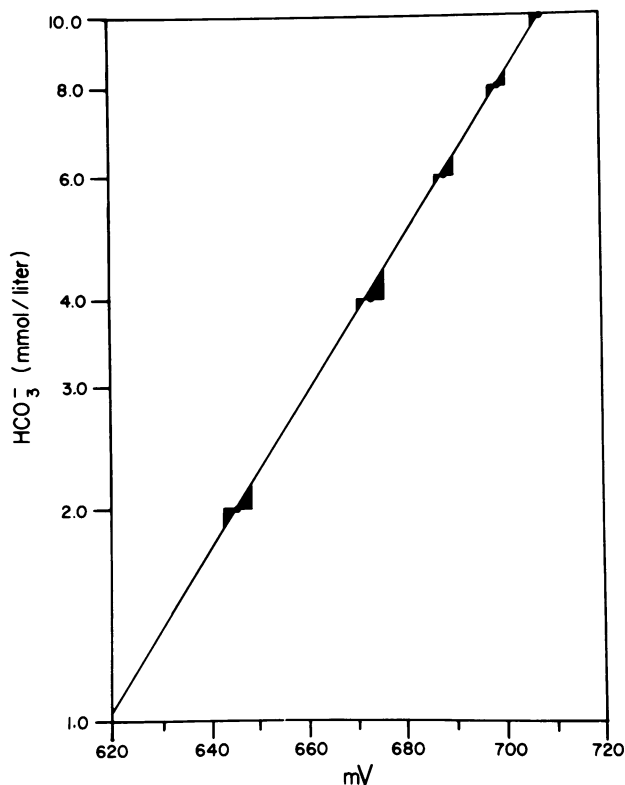

FIGURE 1   Measurements of bicarbonate concentrations in standard solutions with 48 quinhydrone microelectrodes. The horizontal distance to the right and left of each point represents ±1 SE. The reproducibility of the method is ±0.3 mmol/liter.

into the tubular lumen, and failure of the droplets to reappear in other convolutions. Two tubular fluid collections were made from each of the selected end-proximal convolutions. The first was made into a micropipet filled with Sudan black-colored castor oil, by previously described techniques (13, 14). The tubule distal to the collection site was blocked by a large column of the oil, injected before starting the tubular fluid collection. The collection was made at a rate which maintained the oil block in a constant position, by adjusting the level of a mercury bulb connected to the micropipet. The collection was timed with a stopwatch. This first sample was used to measure [^{14}C]inulin concentration and single nephron glomerular filtration rate (SNGFR) (see below). After this collection had been completed, the tubule was repunctured, either slightly proximal to the first hole or through the same hole, with a pipet of larger tip diameter to prevent leakage of tubular fluid around the pipet. This second pipet contained a quinhydrone microelectrode. The method for preparation of the quinhydrone electrodes has been described previously (13–15). The pipet was filled with Sudan black-stained mineral oil that had been continuously bubbled with 5% CO_2. If the oil column from the first tubular fluid collection was no longer visible, additional oil was injected into the lumen. The rate of tubular fluid collection was again adjusted to maintain the oil column in a constant position. This second collection was continued until tubular fluid fully surrounded the microelectrode. The pipet was then withdrawn from the tubule and the tip of the pipet immediately sealed with 3 M KCl-agar.

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After 5 min, the potential of the quinhydrone electrode was measured at 37°C against a standard calomel electrode in 3 M KCl bubbled with 5% CO_2, using a model 25 Radiometer pH meter (London Co., Cleveland, Ohio). A standard curve was established on the day of each experiment with isotonic saline solutions containing 2, 4, 6, 8, or 10 mmol/liter bicarbonate, using individual quinhydrone electrodes. In each case, the measurement was made consistently at 5 min after the pipet tip had been sealed with the KCl-agar. The bicarbonate concentrations in the tubular fluid were determined from the HCO_3^-, vs. millivolt plot of the standard solutions. Preliminary tests of the accuracy and reproducibility of bicarbonate determinations with quinhydrone microelectrodes were carried out, using the standard solutions. The results of measurements with 48 microelectrodes are shown in Fig. 1. They indicate that these microelectrodes can determine HCO_3^- concentrations reliably in this range within ± 0.3 mmol/liter.

After two or three end-proximal tubules had been sampled in each rat, 16 U purified bovine PTH (The Wilson Laboratories, Chicago, Ill.) was given i.v. and an amount of hormone was added to the i.v. infusion to deliver 16 U/h continuously for the second period of the experiment. Starting approximately 10 min later, additional end-proximal convolutions were punctured twice by the same techniques used in the first period. These were "fresh" tubules, not the same ones sampled during the first period. Timed urine collections were obtained from the urinary bladder before and after PTH administration.

Effect of continuing hypoparathyroid state under hydropenic, normal acid-base conditions. Six additional rats were prepared by exactly the same techniques described above. After TPTX, they were given a continuous infusion of Ringer's lactate solution at 50 µl/min. Tubular fluid and urine collections were made over a total time period which matched that of the preceding group of animals. However, no PTH was given to these six rats. For analysis of the data in this group, the experiment was arbitrarily divided into two halves, to correspond in time to the two periods of the preceding group of animals.

Effect of mannitol on proximal bicarbonate reabsorption. Since PTH administration is known to reduce fluid reabsorption in the proximal tubule (16–18), the effect on bicarbonate of inhibition of fluid reabsorption by an agent other than PTH was studied. For this purpose, mannitol was infused in seven additional rats. The rats were prepared in the same way described in the preceding sections, including TPTX. Tubular fluid and urine samples were collected in the first period during i.v. Ringer's lactate infusion at 50 µl/min. At the end of this period, 0.7 ml of 2% mannitol in Ringer's solution was given i.v. and 2% mannitol was added to the i.v. infusion, which was continued at 50 µl/min for the remainder of the experiment. 5–10 min after the mannitol had been started, additional tubular fluid and urine samples were collected.

Effect of PTH on bicarbonate reabsorption during acute bicarbonate loading. In nine rats, bicarbonate reabsorption by the proximal tubules was measured during progressive elevation of serum bicarbonate concentration, in an initial hypoparathyroid period and after administration of PTH. The rats were prepared for the experiment as described in the first section, including TPTX. After the surgery was completed, an i.v. infusion of $NaHCO_3$, 750 mmol/liter, was started at 50 µl/min. The intention of the slow infusion of this hypertonic solution was to raise serum bicarbonate concentration progressively without markedly expanding extracellular fluid volume (19–21). Approximately 10 min after the infusion had been started, end-proximal convolutions were selected and

punctured twice for inulin and bicarbonate measurements, as described in the first section. In six of the nine rats, after two or three tubules had been sampled, 16 U PTH was given i.v. as a prime and an amount of hormone added to the infusion to deliver 16 U/h throughout the remainder of the experiment. Additional tubular fluid and urine collections were then obtained. In the other three rats, PTH was not given, but tubular fluid samples were collected over a total period of time that corresponded to the other six experiments.

In all experiments, the volume of tubular fluid collected for [^{14}C]inulin measurement was determined by transferring the sample into a constant-bore capillary tube (0.1-mm internal diameter) (Corning Glass Works, Science Products Div., Corning, N. Y.) and measuring the length of the column under a microscope with an eyepiece micrometer (22). The sample was then washed out of the capillary tube into liquid scintillation counting vials containing 15 ml counting solution: 100 ml Bio-Solv (Beckman Instruments, Inc., Fullerton, Calif.) 42 ml Liquifluor (New England Nuclear, Boston, Mass.) made up to 1,000 ml with scintillation-grade toluene (Fisher Scientific Co., Pittsburgh, Pa.). Radioactive counts were measured with a Nuclear-Chicago liquid scintillation counter Unilux II A (Amersham/Searle Corp., Arlington Heights, Ill.). Portions of plasma, obtained from arterialized blood collected from the cut end of the tail in heparinized capillary tubes at 30-min intervals, were analyzed for [^{14}C]inulin concentration by the same methods. The plasma inulin concentration was corrected for a plasma water content of 94%. The plasma inulin concentration at the midpoint of each tubular fluid collection was calculated from a time plot, and this concentration was used to calculate the tubular fluid/plasma inulin ratio (TF/P_{In}). SNGFR was calculated from the expression:

$$SNGFR = TF/P_{In} \times TFR,$$

where TFR is the tubular fluid flow rate in nanoliters per minute.

Arterial blood samples were collected periodically from the carotid artery catheter for measurement of pH, total CO_2, and phosphorus using methods described previously (14, 15, 23). Serum calcium was measured by the method of Meites (24). Plasma HCO_3^- concentrations at the midpoint of tubular fluid and urine collections were obtained from a time plot, and these values used to calculate tubular fluid/plasma HCO_3^- (TF/$P_{HCO_3^-}$) ratios and filtered load of bicarbonate for single nephrons and the whole kidney. Timed urine collections from the urinary bladder were analyzed for the same constituents, as well as for [^{14}C]inulin. Glomerular filtrate rate (GFR) for the two kidneys was calculated from the expression:

$$GFR = U/P_{In} \times V,$$

where U/P_{In} is the ratio of inulin in the urine vs. plasma, and V is the urine flow rate in milliliters per minute. In all experiments, the excreted fraction of phosphorus (EF$_p$) was used as the criterion for the adequacy of the parathyroidectomy (EF$_p < 1\%$) and for the biologic activity of the administered PTH (EF$_p > 5\%$).

Per cent reabsorption of bicarbonate by the proximal tubule was calculated as

$$[1 - (TF/P_{HCO_3^-} \times P/TF_{In})] \times 100.$$

Absolute rate of bicarbonate reabsorption was calculated for each tubule by the following:

$$T_{HCO_3^-} = SNGFR \times P_{HCO_3^-}/nl \ [\% \ HCO_3^- \ reab.].$$

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TABLE I

Plasma Acid-Base and Calcium Values

	First period				Second period			
	pH	Pco₂	HCO₃⁻	Ca⁺⁺	pH	Pco₂	HCO₃⁻	Ca⁺⁺
		mm Hg	*mmol/liter*	*mg/100 ml*		*mm Hg*	*mmol/liter*	*mg/100 ml*
Group I (9) TPTX-PTH	7.39	33.3	19.5	7.8	7.45 0.04	28.1 2.6	18.8 1.6	8.7* 0.3
Group II (6) TPTX-TPTX	7.38	34.0	18.2	7.8	7.44 0.05	26.7 2.1	18.2 2.7	7.6 0.1
Group III (7) TPTX-Mannitol	7.33	41.7	21.3	7.4	7.39* 0.02	35.9 3.4	21.6 1.4	8.0 0.4

Numbers in parentheses are the number of animals.
Data expressed as mean±SED, calculated by paired t test.
* $P < 0.01$, second period compared with first period.

The data were "normalized" for differences in SNGFR and expressed as

$$[T_{HCO_3^-}/SNGFR] \times 100.$$

Absolute bicarbonate reabsorption for the whole kidney was calculated by

$$R_{HCO_3^-} = [GFR \times P_{HCO_3^-}/ml] - U_{HCO_3^-}V.$$

As in the case of the single nephron data, the whole kidney data were "normalized" for differences in GFR:

$$[R_{HCO_3^-}/GFR] \times 100.$$

In individual rats, all tubular fluid data for the first period were averaged and compared with the average values from the same animal obtained during the second period. Statistical analysis was by paired Student's t test, and are expressed as mean ± standard error of difference (SED).

Effect of PTH on urinary ammonium and titratable acid excretion. In four rats, urinary excretion of ammonium and titratable acid was measured during an initial hypoparathyroid period, and during administration of PTH. In this group, food was not withheld on the night before the experiment. The rats were prepared surgically by the same techniques described for the first group of animals except that abdominal surgery and isolation of one kidney for micropuncture was omitted. The urinary bladder was exposed and catheterized as described above. Starting approximately 60 min after

TPTX, two timed urine collections were obtained under oi in preweighed tubes. PTH was then administered i.v., as in the preceding experiments, and two additional timed urine collections were obtained. Each urine sample was analyzed for pH, CO_2 content, ammonium (25), and titratable acid (26). The values in individual animals were averaged before and after PTH administration and analyzed statistically by paired t test.

RESULTS

Table I shows the plasma acid-base and calcium values for the three groups of hydropenic rats. As can be seen, there were no significant changes in plasma HCO_3^- concentrations between the first and second periods of the experiments. In all three groups, P_{CO_2} fell and pH rose during the second half of the experiment, but this achieved statistical significance only in the mannitol group. The reason for the tendency toward hyperventilation is probably decreasing levels of anesthesia. Serum calcium concentration increased significantly during PTH administration ($P < 0.01$) but remained constant in the other two groups of animals.

Table II shows the urinary electrolyte and GFR

TABLE II

Urinary Electrolyte Excretion and GFR Values

	First period						Second period					
	V	$U_{N_4^+}V$	pH	$U_{HCO_3}^-V$	EFp	GFR	V	$U_{N_4^+}V$	pH	$U_{HCO_3}^-V$	EFp	GFR
	μl/min/kg	*μeq/min/kg*		*mmol/min/kg*	*%*	*ml/min/kg*	*μl/min/kg*	*μeq/min/kg*		*mmol/min/kg*	*%*	*ml/min/kg*
Group I (9)	0.019	1.41	6.31	0.03	0.28	8.59	0.045* 0.005	2.37 0.67	6.16 0.20	0.11 0.05	8.84* 2.23	8.46 0.75
Group II (6)	0.022	2.12	6.32	0.03	0.15	9.46	0.022 0.007	1.28 0.74	6.21 0.09	0.02 0.02	0.16 0.02	7.70 0.71
Group III (7)	0.024	3.66	6.37	0.07	0.40	9.34	0.086* 0.013	7.57 1.82	6.13 0.02	0.06 0.02	0.58 0.36	9.03 0.41

Numbers in parentheses are the number of animals.
Data expressed as mean±SED, calculated by paired t test.
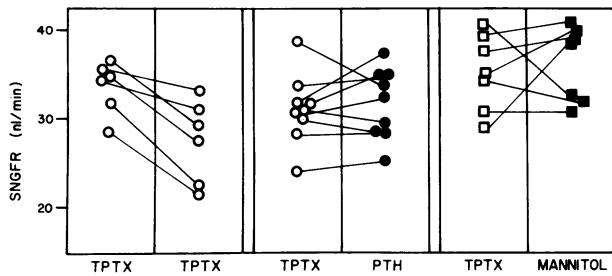* $P < 0.01$, second period compared with first period.

FIGURE 2 SNGFR in TPTX rats. Multiple measurements in each period have been averaged in individual animals, and the average values are shown. The lines connect data from individual animals. Three groups of rats are shown.

FIGURE 4 Proximal tubular fluid bicarbonate concentration $TF_{HCO_3^-}$ in TPTX rats. The data are from the same three groups of rats shown in Figs. 2 and 3. Multiple measurements in each period have been averaged in individual animals, and the average values are shown.

data for the same three groups of animals. In all animals, EF_P was <1% during TPTX periods, indicating successful total parathyroidectomy. In group I, administration of PTH caused a significant increase in urine flow rate (V) and phosphorus excretion. There was no significant effect on urinary pH or bicarbonate excretion, however. GFR remained constant throughout the experiment. During prolonged TPTX (group II), all measured electrolytes and GFR remained unchanged throughout the experiment. In the group given mannitol, urine flow increased significantly, whereas electrolyte excretion and GFR showed no statistically significant changes.

The data on SNGFR measurements in the same three groups of rats are shown in Fig. 2. All measurements in individual animals were averaged for each period, and the average values are shown. As can be seen, SNGFR fell moderately in the animals that were studied over a prolonged period of parathyroid deficiency, from 33.4 to 28.3 nl/min ±1.5 SED (P <0.02). In the other two groups, in which either PTH or man-
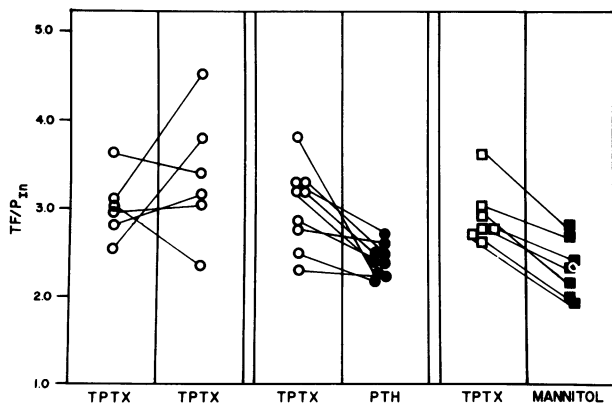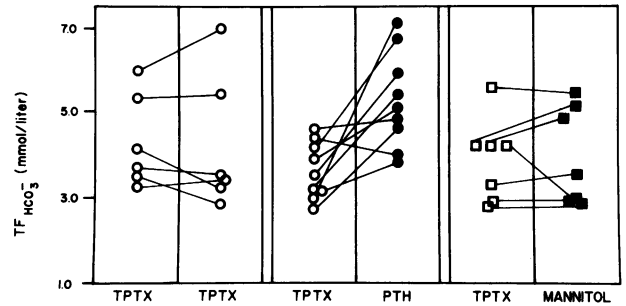

FIGURE 3 Proximal tubular fluid/plasma inulin ratios TF/P_{In} in TPTX rats. Multiple measurements in each period have been averaged in individual animals, and the average values are shown. The lines connect data from individual animals. The data are from the same groups of rats and tubules as shown in Fig. 2.

nitol was administered in the second period of the experiment, there was no significant change in SNGFR (P > 0.5). The reason for the decline in SNGFR with prolonged parathyroid deficiency is not clear. However, we noted that blood pressure frequently declined gradually in this group of animals, and this could have been responsible for the fall in surface SNGFR, as well as the small decrease in GFR (Table II). Experiments were terminated if the mean blood pressure fell below 110 mm Hg.

Fig. 3 shows TF/P_{In} measured in the last accessible proximal convolutions, for the same three groups of rats. As in Fig. 1, the values for each period in each animal have been averaged, and the averages for individual animals are presented. In the six rats studied during prolonged parathyroid deficiency (left panel), TF/P_{In} either remained constant or rose as the experiment progressed. The mean value of 3.36 in the second period was not significantly different from the mean value of the first period, 2.98 (P > 0.3). In contrast, in eight of the nine rats given PTH, TF/P_{In} fell significantly from 3.04 to 2.46±0.15 SED (P < 0.01). This indicates that a reduction in fractional reabsorption of fluid occurred with the administration of PTH. Since SNGFR was relatively constant in this group of animals, absolute proximal fluid absorption also fell as a result of PTH administration. These observations are in agreement with those of others that PTH reduces fluid reabsorption in the proximal tubule of the dog (16, 17) and rat (18). In the seven rats given mannitol, TF/P_{In} fell from 2.92 to 2.37±0.07 SED (P < 0.001). Proximal fluid reabsorption was thus reduced in both fractional and absolute amounts.

In Fig. 4 are plotted the mean free-flow end-proximal tubular fluid HCO_3^- ($TF_{HCO_3^-}$) concentrations for the same three groups of rats, measured with quinhydrone microelectrodes. In the six animals studied over a period of prolonged parathyroid deficiency, $TF_{HCO_3^-}$ at the end of the proximal convoluted tubule ranged from 3.2
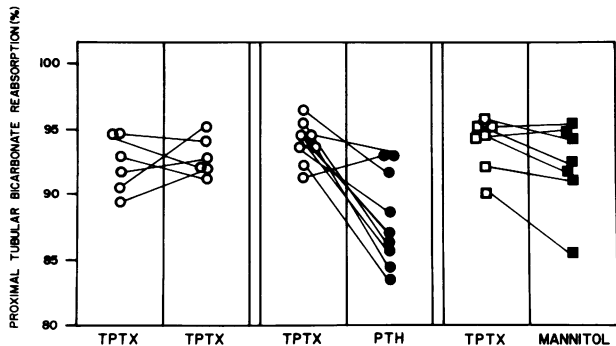
FIGURE 5 Proximal tubular HCO_3^- reabsorption in TPTX rats. The data are from the same three groups of rats shown in Figs. 2–4. Multiple measurements in each period have been averaged in individual animals, and the average values are shown.

to 6.0 mmol/liter in the first period (mean 4.3). There was no significant change throughout the period of observation, the mean value being 4.2 mmol/liter in the second period ($P > 0.5$). In sharp contrast, in the rats given PTH, $TF_{HCO_3^-}$ rose from 3.6 to 5.1 mmol/liter ±0.4 SED ($P < 0.02$). The seven animals given mannitol showed no significant change in $TF_{HCO_3^-}$, the mean being 3.9 mmol/liter before mannitol and 4.0 mmol/liter after mannitol ±0.2 SED ($P > 0.5$).

Fig. 5 shows the calculated end-proximal fractional HCO_3^- reabsorption for the same three groups of rats. No significant change in percent HCO_3^- reabsorption occurred in the rats observed during prolonged parathyroid deficiency, 90.5 vs. 91.5% ±0.9 SED ($P>0.3$). PTH administration caused a significant reduction in percent HCO_3^- reabsorption in eight of the nine rats, the mean value falling from 94.0 to 88.2% ±1.2 SED ($P < 0.01$). Mannitol infusion caused a small decrease in fractional HCO_3^- reabsorption from 94 to 92.3% ±0.5 SED ($P < 0.05$).

The results of the acute i.v. bicarbonate loading experiments are shown in Figs. 6 and 7. Fig. 6 plots whole kidney HCO_3^- reabsorption, on the ordinate, against the corresponding plasma HCO_3^- concentration, during parathyroid deficiency (O) and during PTH administration (●). In the six rats that received PTH, whole kidney GFR was 13.7 ml/min per kg before hormone administration and 12.5 ml/min per kg ±1.3 SED after hormone administration ($P > 0.3$). In the other three rats, which remained parathyroid deficient throughout the experiment, there was also no tendency for GFR to change during the course of the bicarbonate infusion. It is clear that under the two hormonal conditions, renal HCO_3^- reabsorption rose progressively, with no evidence for a tubular maximal. Purkerson and co-workers (19) observed in normal rats a similar linear increase in tubular HCO_3^- reabsorption if extracellular volume expansion is avoided. As shown
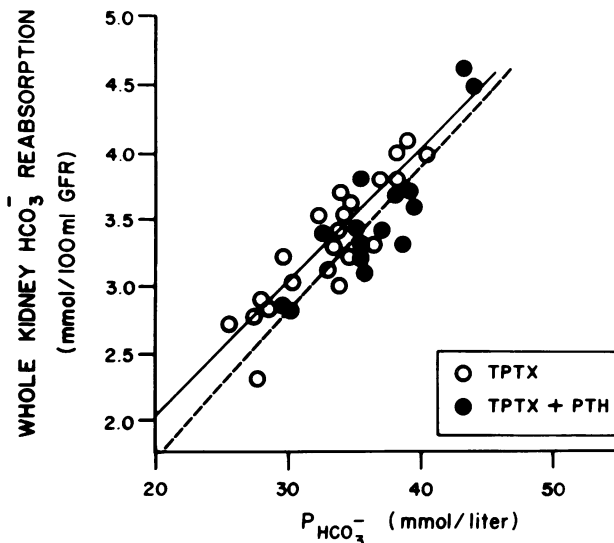

FIGURE 6 Whole kidney HCO_3^- reabsorption during hypertonic $NaHCO_3$ infusion in TPTX rats. The data have been "normalized" for differences in GFR. The two lines are the calculated linear regression lines for TPTX (——) and TPTX + PTH (– – –).

in Fig. 6, no effect on whole kidney HCO_3^- reabsorption was detectable during acute administration of large amounts of PTH. The linear regression line for the hypoparathyroid data is $y=0.10x+0.03$ ($P<0.001$) and for the PTH data is $y=0.11x-0.53$ ($P<0.001$). The two lines are not statistically different.

The measurements of proximal tubular HCO_3^- reabsorption during acute i.v. bicarbonate loading are
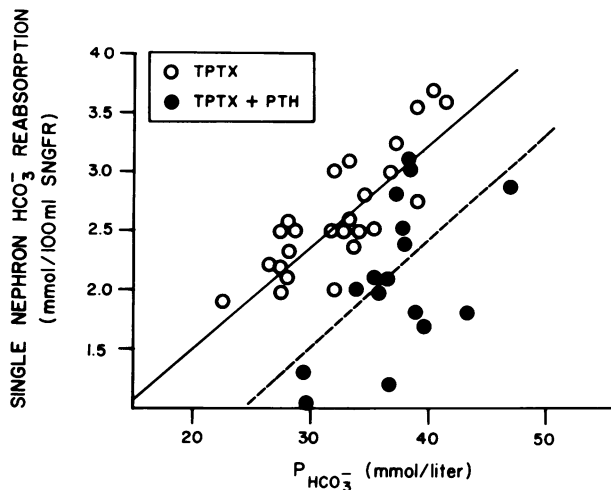

FIGURE 7 Proximal tubular HCO_3^- reabsorption during hypertonic $NaHCO_3^-$ infusion in TPTX rats. The data are from the same animals shown in Fig. 6. The data have been "normalized" for differences in SNGFR. The two lines are the calculated linear regression lines for TPTX (——) and TPTX + PTH (– – –).

TABLE III

TABLE III
Urinary Ammonium and Titratable Acid Excretion

First period (TPTX)				Second period (TPTX + PTH)			
$U_{NH_4^+}V$	$U_{TA}V$	pH	$U_{HCO_3^-}V$	$U_{NH_4^+}V$	$U_{TA}V$	pH	$U_{HCO_3^-}V$
$\mu eq/min/kg$	$\mu eq/min/kg$		$\mu mol/min/kg$	$\mu eq/min/kg$	$\mu eq/min/kg$		$\mu mol/min/kg$
0.99	0.12	6.37	0.04	3.98*	1.56*	5.95*	0.06
				0.74	0.25	0.07	0.03

Data expressed as mean±SED, calculated by paired t test.
* $P < 0.05$, second period compared with first period.

plotted in Fig. 7. As in the case of the whole kidney data, the single nephron HCO_3^- reabsorption values have been "normalized" for differences in SNGFR. In the six rats that received PTH, SNGFR was 44.9 nl/min before hormone administration and 42.7 nl/min ±2.5 SED after hormone administration ($P > 0.5$). Similarly, no significant changes in SNGFR occurred in the three rats that remained parathyroid deficient throughout the experiment. The mean TF/P_{In} ratio was 2.83 before PTH administration and fell to 2.44 ±0.10 SED after PTH administration ($P < 0.01$). As shown in Fig. 7, with i.v. HCO_3^- loading, proximal HCO_3^- reabsorption rose linearly, with no evidence of a maximum rate. In sharp contrast to the whole kidney data, however, PTH administration significantly reduced proximal tubular HCO_3^- reabsorption. The linear regression line for the hypoparathyroid data is $y = 0.08x$ −0.23 ($P < 0.001$) and for the period during PTH administration is $y = 0.09x - 1.25$ ($P < 0.05$). The slopes of the two regression lines are not different from one another, but the intercept of the TPTX + PTH values is significantly lower than the TPTX values ($P < 0.01$). Thus, under conditions of acute bicarbonate loading, PTH reduced HCO_3^- reabsorption in the proximal convoluted tubule, but not by the whole kidney.

The effect of PTH administration on urinary ammonium and titratable acid excretion was studied in four rats. These animals were not fasted overnight, and had no abdominal surgery other than a suprapubic incision to externalize the urinary bladder. Plasma HCO_3^- concentration was between 22.0 and 25.5 mmol/ liter during the clearance measurements. The results are shown in Table III. As can be seen, titratable acid excretion increased markedly after PTH administration ($P < 0.02$), presumably because of the increase in phosphate in the urine. Ammonium excretion also increased significantly, and this was accompanied by a fall in urine pH from 6.37 to 5.95±0.07 SED ($P = 0.01$). There was no significant change in bicarbonate excretion. Net acid excretion ($NH_4^+ + TA$ − HCO_3^-) increased from 1.07 $\mu eq/min$ per kg to 5.48 $\mu eq/min$ per kg after PTH administration.

DISCUSSION

The results of the present study demonstrate that acute administration of bovine PTH to TPTX nonexpanded rats produces a small but significant reduction in HCO_3^- reabsorption by the proximal tubule. This was demonstrable in animals with serum HCO_3^- concentrations ranging from 18 to 40 mmol/liter. The reduction in reabsorption was the result of a combination of a decrease in fluid absorption (TF/P_{In}) and a rise in the concentration of HCO_3^- in the proximal tubular fluid ($TF_{HCO_3^-}$). The rise $TF_{HCO_3^-}$ suggests that the mechanism of HCO_3^- inhibition was not simply retention of water in the tubular lumen, since inhibition of proximal fluid absorption by mannitol did not result in any significant change in $TF_{HCO_3^-}$. Several other possibilities can be considered. PTH might have inhibited active H^+ secretion via some effect on intracellular pH or carbonic anhydrase. If H^+ secretion is dependent upon active sodium transport, the hormone might have reduced H^+ secretion by inhibiting Na^+ transport. Finally, PTH might have increased the back diffusion of HCO_3^-, as well as sodium, from plasma into the lumen by increasing the permeability of the intercellular tight junctions. The observations do not distinguish among these various possibilities.

The mechanism of the effect of mannitol on HCO_3^- reabsorption is probably complex. The osmotic effect in the lumen would be expected to cause a fall in $TF_{HCO_3^-}$, as well as other electrolytes. On the other hand, mannitol could exert a solvent drag effect across the tubular epithelium which might return HCO_3^- to the tubular lumen from the intercellular spaces, where it is presumably present in high concentrations. This would tend to counteract the dilutional effect of the mannitol on luminal concentrations. Our findings are in fact consistent with such a combination of effects, since no change in $TF_{HCO_3^-}$ was found in spite of a significant decrease in fluid absorption. The small decrease in proximal HCO_3^- reabsorption with mannitol was directly proportional to the decrease in water reabsorption. No significant changes in either TF/P_{In} or $TF_{HCO_3^-}$ occurred in those experiments in which neither PTH

nor mannitol was given. This tends to exclude possible uncontrolled effects on HCO_3^- reabsorption, such as volume expansion and systemic acid-base changes occurring during the course of the experiments.

One possible theoretical explanation for the rise in $TF_{HCO_3^-}$ concentration after PTH administration is generation of HCO_3^- in the tubular lumen due to a rise in luminal phosphate concentration. Inhibition of phosphate reabsorption by PTH might result in generation of intraluminal HCO_3^- by the reaction

$$H_2CO_3 + HPO_4^= \rightarrow HCO_3^- + H_2PO_4^-.$$
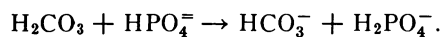
To quantitate the amount of HCO_3^- to be expected from this reaction, we measured total CO_2 and pH of four CO_2-equilibrated solutions, each containing NaCl 150 meq/liter and $NaHCO_3$ 5 meq/liter, but with 2, 4, 6, or 8 mmol/liter of Na_2HPO_4. We found that approximately 0.4 mmol HCO_3^- is generated per millimole phosphate. In the rats given PTH, $TF_{HCO_3^-}$ increased approximately 1.5 mmol/liter. Tubular fluid phosphate concentration would have had to increase by about 4 mmol/liter after PTH to account for this rise in HCO_3^-. In a study by Kuntziger et al. (27), administration of cyclic AMP to parathyroidectomized rats produced only a 0.2 mmol rise in tubular fluid phosphate concentration, as measured by electron microprobe analysis. Thus, it seems unlikely that the effect of PTH on proximal HCO_3^- can be accounted for entirely by intraluminal generation of HCO_3^- via the above reaction.

In spite of the inhibition of proximal HCO_3^- reabsorption by PTH, no significant increase in urinary HCO_3^- excretion occurred in the hydropenic animals, although there were definite increases in urine flow, sodium, and phosphate excretion. We assume, therefore, that all of the excess HCO_3^- delivered out of the proximal tubule was reabsorbed by more distal segments of the nephron. It has been shown that the capacity of the loop of Henle and distal tubule for HCO_3^- reabsorption is normally highly unsaturated in the rat, and that the rate of HCO_3^- reabsorption in these distal segments increases markedly when there is spillover of HCO_3^- from more proximal nephron sites (28, 29). In fact, a comparison of the maximum rates of H^+ secretion and Na^+ reabsorption in the rat distal tubule shows that there is a considerably greater capacity for H^+ secretion than Na^+ reabsorption (29). The moderate increases in sodium excretion without any increase in the urine HCO_3^- flow rate observed after PTH might be attributed, therefore, to proximal fluid inhibition and unequal capacities for Na^+ and H^+ transport in the distal nephron. Our observations do not exclude, however, a distal inhibitory effect of PTH on sodium reabsorption.

Several clearance studies on the effect of PTH on bicarbonate excretion have been reported in dogs (4, 7–9). The results differ in some respects from the present observations in rats. In intact hydropenic dogs studied under normal acid-base conditions, PTH infusion causes a modest rise in urinary pH and HCO_3^- flow rate (4, 7), although the effect is only transient (7). In TPTX dogs acutely loaded with bicarbonate, PTH reduces whole kidney HCO_3^- reabsorption from an elevated level toward more normal levels, but not to abnormally low values (7–9). Thus, in the dog, an inhibitory effect of acute administration of PTH on HCO_3^- reabsorption can be observed for the whole kidney. In contrast, in our rats the effect of PTH could not be detected by clearance measurements, either under normal acid-base conditions or acute HCO_3^- loading. The reason for this difference between rat and dog is not clear, but might be due to a greater capacity of the rat distal nephron to reabsorb HCO_3^-.

A number of authors have suggested that hyperparathyroidism (primary or secondary) might be capable of reducing renal HCO_3^- reabsorption to abnormally low rates, and thereby produce metabolic acidosis (5, 6, 12). However, the results of the present study, as well as studies in dogs (7–9), do not provide support for this view. The results of clearance experiments in dogs indicate that after TPTX, tubular bicarbonate reabsorptive capacity is enhanced and that acute PTH administration reduces reabsorption only to a normal level but not to lower than normal values (7, 8). In the present study, not only did PTH fail to increase bicarbonate excretion in the urine, it actually increased net acid excretion (Table III) by producing a rise in titratable acid and ammonium excretion. The rise in titratable acid can be accounted for by the large increase in phosphate excretion after PTH. The cause of the acute increase in ammonium excretion is not clear, but could be related to the significant fall in urinary pH which occurred after PTH administration. In none of the reported studies has PTH actually lowered plasma HCO_3^- concentration to acidotic levels. Therefore, the evidence thus far does not support the view that acute changes in PTH levels play a direct role in external acid-base balance. However, it is possible that chronic hyperparathyroidism might contribute to metabolic acidosis by an indirect mechanism. Gold and co-workers (30) demonstrated that chronic phosphate depletion, produced by a phosphate-deficient diet over several months, can cause renal bicarbonate wasting in dogs. They suggested that this might be the cause of metabolic acidosis in chronic hyperparathyroidism. Their animals were probably in a hypoparathyroid state (30), however, and since no studies were carried out in chronic hyperparathyroid animals, further experiments are needed to test this hypothesis.

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