Severe metabolic acidosis and disturbances of calcium metabolism induced by acetazolamide in patients on haemodialysis

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SUMMARY

1. To investigate mechanisms of extrarenal buffering in uraemic acidosis, we studied the effects of the carbonic anhydrase inhibitor, acetazolamide, in normal subjects and in patients with end-stage kidney disease on maintenance haemodialysis with virtually no urine output.

2. Acetazolamide (500 mg) was administered daily for 7 days, after pretreatment for 1 month with 1,25-dihydroxyvitamin D (n=12) or placebo (n=12); only placebo was administered to a third group (n=12) of haemodialysis patients. In addition, acetazolamide was administered to normal control subjects (n=12).

3. Treatment with acetazolamide resulted in a more marked metabolic acidosis in haemodialysis patients than in normal control subjects and the effect in haemodialysis patients was attenuated by prior treatment with 1,25-dihydroxyvitamin D.

4. The administration of acetazolamide to haemodialysis patients led to an increase in serum inorganic phosphorus, bone isoenzyme of alkaline phosphatase and parathyroid hormone, and a reduction in serum calcium, whereas acetazolamide had no effect on these variables in normal subjects. In contrast, in the haemodialysis patients previously treated with 1,25-dihydroxyvitamin D, acetazolamide increased serum inorganic phosphorus, bone isoenzyme of alkaline phosphatase, parathyroid hormone and serum calcium.

5. We hypothesize that the metabolic acidosis induced by acetazolamide in haemodialysis patients may result from interference with the mechanisms of extrarenal buffering.

6. As parathyroid hormone, 1,25-dihydroxyvitamin D and carbonic anhydrase are thought to be involved in bone buffering, we suggest that the marked acidosis seen in haemodialysis patients treated with acetazolamide may be due to impaired parathyroid hormone-mediated bone buffering.

Key words: acetazolamide, acid–base balance, acidosis, calcium metabolism, haemodialysis, uraemia, vitamin D

Abbreviations: C-PTH, C-terminal (65–84) portion of parathyroid hormone; M-PTH, mid-region (44–68) of parathyroid hormone; 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone.

INTRODUCTION

Acetazolamide, a non-competitive carbonic anhydrase inhibitor, is widely used in the treatment of glaucoma because it reduces the rate of formation of aqueous humour [1]. Acetazolamide has long been known to cause metabolic acidosis by the inhibition of carbonic anhydrase activity in the proximal tubular epithelium with consequent bicarbonate diuresis [2]. Metabolic acidosis is an almost invariable consequence of the long-term administration of this agent. However, it is reported as self-limiting and mild, with a plasma bicarbonate level rarely below 18 mmol/l [2]. A few cases of symptomatic acidosis have been reported in elderly patients, diabetic patients and in patients with renal failure [3–6]. It is not clear how acidosis develops in these patients. It has been suggested that in some patients with renal failure excessive bicarbonate may be lost by the tubule, and acetazolamide would be expected to inhibit the partial reabsorption of this bicarbonate [7]. Acetazolamide has been advocated as the agent of choice for the prevention of acute glaucoma, which may occur in some patients with end-stage kidney disease during haemodialysis [8, 9]. Although data regarding the effect of haemodialysis on the kinetics of this drug are not conclusive, a dose of 500 mg/day has commonly been considered safe and efficacious.

We undertook a controlled study comparing the effects of acetazolamide or placebo administered to normal subjects and to patients with end-stage kidney disease maintained on haemodialysis as well as the effects of acetazolamide administration to a third group of uraemic patients on haemodialysis after pretreatment for 1...
month with 1,25-dihydroxyvitamin D \( [1,25-(\text{OH})_2\text{D}_3; \text{calcitriol}] \). The specific purposes of the study were: (1) to examine whether acetazolamide, by inhibiting extrarenal carbonic anhydrase, may induce a more severe metabolic acidosis in patients on haemodialysis with virtually no urine output than in subjects with normal renal function; (2) to elucidate the possible role of 1,25-(\text{OH})_2\text{D}_3 and parathyroid hormone (PTH) in the mechanisms by which the inhibition of carbonic anhydrase by acetazolamide impairs the extrarenal buffering of uraemic acidosis in haemodialysis patients.


METHODS

Study protocol

We conducted a randomized clinical trial to investigate the effects of a 7-day course of acetazolamide therapy on some biochemical measures of calcium metabolism (serum concentrations of total and ionized calcium, inorganic phosphorus, PTH and bone isoenzyme of alkaline phosphatase) and acid–base balance (blood pH, bicarbonate concentration and \( \text{PCO}_2 \), serum unmeasured anion concentration and serum acetate), and on the serum level of potassium, in 24 patients with end-stage kidney disease on maintenance haemodialysis and in 12 healthy subjects. The study design is shown in Fig. 1. Twelve haemodialysis patients were given 1,25-(\text{OH})_2\text{D}_3 for 4 weeks before receiving acetazolamide to examine its possible influence on the acid–base and calcium-related abnormalities induced by acetazolamide. Twelve healthy subjects and 12 haemodialysis patients were given placebo and served as control groups. Written informed consent was obtained from the patients and the healthy subjects. Each haemodialysis patient underwent four investigations of the biochemical measures of calcium metabolism and acid–base balance, which were carried out at intervals of 1 week, before receiving acetazolamide. Investigations were repeated on the last day of acetazolamide administration and on the seventh day after it had been discontinued. Each healthy subject underwent four investigations of the biochemical measures of calcium metabolism and acid–base balance, which were carried out every 2 days, before receiving acetazolamide. Investigations were repeated on the last day of acetazolamide administration and on the seventh day after it had been discontinued. The blood samples obtained from the patients on haemodialysis were taken immediately before dialysis. In addition, the serum concentration of acetate was measured 1 and 2 h after the end of the session. In the dialysis patients given 1,25-(\text{OH})_2\text{D}_3 the blood samples were taken approximately 1–2 h after the last dose of 1,25-(\text{OH})_2\text{D}_3. In healthy subjects and in haemodialysis patients receiving acetazolamide the blood specimens were taken 1–2 h after the last dose of acetazolamide.

Patients and subjects

Thirty-six patients with end-stage kidney disease enrolled in a chronic haemodialysis programme and 24 healthy subjects were studied.

Haemodialysis patients. To be eligible for inclusion in the study, haemodialysis patients had to fulfil the following two criteria: (1) relatively normal acid–base parameters and serum inorganic phosphorus concentration, namely predialysis blood levels of pH > 7.34, bicarbonate > 18 mmol/l and inorganic phosphorus < 1.9 mmol/l; (2) absence of residual diuresis. More specifically, all haemodialysis patients had virtually no urine output and showed normal serum concentrations of bone isoenzyme of alkaline phosphatase and total and ionized calcium. Patients were excluded if they were receiving agents known to influence acid–base balance and calcium metabolism, except for phosphate binders.

Thirty-six patients (23 men and 13 women) were included in the study. They ranged in age from 35 to 66 years (mean ± sd 47 ± 9 years). The mean duration of dialysis was 52 months (range 12–86 months). The causes of the underlying renal failure were chronic glomerulonephritis, chronic pyelonephritis and polycystic kidney disease. Patients received 4 h of dialysis three times weekly. Acetate dialysis (dialysate acetate; 39.5 mmol/l) and capillary dialysers (Cordis Dow Corp., Miami, FL, U.S.A.) were used throughout the study. The concentrations of calcium, sodium and glucose in the dialysate were...
Group 1. Twelve patients were allocated to this group. They were given a 1,25-(OH)\textsubscript{2}D\textsubscript{3} for 4 weeks before the administration of acetazolamide. The initial dose was 0.25 \( \mu \)g/day. Subsequent doses were adjusted to maintain the serum concentrations of total and ionized calcium below 2.5 and 1.25 mmol/l, respectively. The mean (±SEM) value of serum 1,25-(OH)\textsubscript{2}D\textsubscript{3} during 1,25-(OH)\textsubscript{2}D\textsubscript{3} supplementation was 86 ± 7.5 pmol/l. Acetazolamide therapy was started when the biochemical parameters of calcium metabolism and acid–base balance were stabilized for at least 2 weeks. Acetazolamide was administered orally in a single dose of 500 mg daily for 7 days. Each patient was given 1,25-(OH)\textsubscript{2}D\textsubscript{3} during acetazolamide therapy and for 1 week after it had been discontinued, as shown in Fig. 1.

Group 2. Twelve patients were assigned to this group. They were given a placebo solution for 4 weeks before acetazolamide therapy. Acetazolamide was administered orally in a single dose of 500 mg daily for 7 days. Placebo was given during acetazolamide therapy and for 1 week after it had been discontinued. The mean (±SEM) value of serum 1,25-(OH)\textsubscript{2}D\textsubscript{3} was 35 ± 3.5 pmol/l.

Group 3. Twelve patients were allocated to this group. They were given a 6-week course of placebo solution. At the beginning of the fifth week a second placebo, delivered in tablet form, was administered and continued for 7 days.

Healthy subjects. Twenty-four healthy volunteers (16 men and eight women) were included in the study. They ranged in age from 32 to 63 years (mean ±SD 50 ± 13 years). None had evidence of renal, cardiac, pulmonary or metabolic diseases. They were randomly allocated to one of two groups as shown in Fig. 1.

Group 4. Twelve subjects were assigned to this group. After an 8-day control period to obtain the basal values of the biochemical parameters of calcium metabolism and acid–base balance, each subject was given acetazolamide in a single oral dose of 500 mg daily for 7 days.

Group 5. Twelve subjects were allocated to this group. They underwent the same schedule as the subjects assigned to group 4, but they received a placebo, delivered in tablet form, instead of acetazolamide.

Laboratory methods

Measurements of blood pH and \( P_{\text{CO}_2} \) were performed on arterial samples using a digital acid–base analyser (Radiometer, Copenhagen, Denmark). Blood bicarbonate concentration was calculated by the Henderson–Hasselbach equation. Sodium and potassium were measured by flame photometry, chloride by a Chloride Titrator (American Instruments Co., Inc.), total calcium by atomic-absorption spectrometry, ionized calcium by an Orion SS-220 ion selective electrode (Orion Research, Cambridge, MA, U.S.A.) and inorganic phosphorus by the method of Fiske & Subbarow [12]. Alkaline phosphatase isoenzymes were determined by the method of Whitby & Moss [13]. Serum acetate concentration was measured by the gas chromatographic method of Nielsen et al. [14]. Serum level of immunoreactive PTH was measured by using two different antisera. The first assay used an antiserum which reacted with the C-terminal (65–84) portion (C-PTH). In the second assay PTH was measured with a mid-region assay using an antiserum which reacted with the 44–68 region (M-PTH). Serum levels of 1,25-(OH)\textsubscript{2}D\textsubscript{3} were determined by a competitive protein binding assay [15]. The unmeasured anion concentration was calculated as [(Na\textsuperscript{+}) – [Cl\textsuperscript{–}]] + [HCO\textsubscript{3}\textsuperscript{–}].

Statistical analysis

Results are expressed as means±SEM. Paired and unpaired two-tailed Student’s t-test was used to compare group means and to assess the statistical significance of changes. In the analysis of the effects of acetazolamide therapy we compared changes in treated groups with changes in placebo groups. In addition, when appropriate, tests were done by comparing haemodialysis and healthy treated groups, and changes in each group separately. Statistical significance was defined as \( P<0.05 \).

RESULTS

Effects of acetazolamide on acid–base balance

The changes in blood pH, bicarbonate concentration, \( P_{\text{CO}_2} \) and serum unmeasured anion concentration induced by acetazolamide are shown in Fig. 2. In healthy subjects acetazolamide caused a mild metabolic acidosis, presumably by increasing the urinary bicarbonate loss. The mean values of blood pH and bicarbonate concentration before acetazolamide were 7.42 ± 0.006 and 23.2 ± 0.3 mmol/l, respectively, and the urinary excretion of bicarbonate in all subjects was below 3 mmol/24 h. During acetazolamide therapy, blood pH and bicarbonate concentration decreased to 7.36 ± 0.004 mmol/l (\( P<0.0001 \)) and 18.5 ± 0.3 mmol/l (\( P<0.0001 \)), respectively, whereas the urinary excretion of bicarbonate rose to 25 mmol/24 h (\( P<0.0001 \)). In haemodialysis patients acetazolamide caused a more severe metabolic acidosis than in healthy subjects, despite the fact that these patients had virtually no urine output. The changes from basal at the end of acetazolamide treatment for uraemic patients given acetazolamide alone and uraemic patients pretreated with 1,25-(OH)\textsubscript{2}D\textsubscript{3} and then given acetazolamide were summarized in Table 1. Administration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} blunted the decline in blood pH, bicarbonate concentration and \( P_{\text{CO}_2} \). The mean of the individual decreases in blood pH, from pretreatment values to the lowest values during acetazolamide therapy, was 0.07 ± 0.005 in uraemic patients given 1,25-(OH)\textsubscript{2}D\textsubscript{3} and then given acetazolamide. In uraemic patients given acetazolamide alone (\( P=0.0001 \)). The corresponding decline in bicarbonate concentration was 3.7 ± 0.7 mmol/l in the first group and 8.1 ± 0.7 mmol/l in the second group (\( P=0.003 \)). The \( P_{\text{CO}_2} \) level at the end of acetazolamide
Fig. 2. Changes in blood pH, bicarbonate concentration, $P_{CO_2}$ and serum anion gap in haemodialysis patients and healthy subjects after acetazolamide therapy or placebo. Acetazolamide (500 mg/day) was administered from day 1 to day 7. Haemodialysis patients: ○, placebo; ▼, acetazolamide; ▽, acetazolamide and 1,25-(OH)$_2$D$_3$. Healthy subjects: ○, placebo; ●, acetazolamide. Values are means ± SEM. Statistical significance: *$P<$0.03, †$P<$0.005, ‡$P<$0.0001 compared with placebo.

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<tr>
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<th>Group 1 (n=12)</th>
<th>Group 2 (n=12)</th>
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<tr>
<td></td>
<td>Before acetazolamide</td>
<td>After acetazolamide</td>
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<tr>
<td>Blood pH</td>
<td>7.35 ± 0.01</td>
<td>7.27 ± 0.01</td>
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<tr>
<td>Serum bicarbonate (mmol/l)</td>
<td>19.6 ± 0.9</td>
<td>16.8 ± 0.5</td>
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<td>Blood $P_{CO_2}$ (kPa)</td>
<td>5.19 ± 0.1</td>
<td>4.85 ± 0.1</td>
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<td>Serum anion gap (mol/l)</td>
<td>19.3 ± 1.6</td>
<td>22.1 ± 1.5*</td>
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<td>Serum total calcium (mmol/l)</td>
<td>2.31 ± 0.05</td>
<td>2.55 ± 0.05§</td>
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<tr>
<td>Serum ionized calcium (mmol/l)</td>
<td>1.15 ± 0.03</td>
<td>1.39 ± 0.03§</td>
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<tr>
<td>Serum inorganic phosphorus (mmol/l)</td>
<td>1.46 ± 0.07</td>
<td>2.21 ± 0.14†</td>
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<td>Serum sodium (mmol/l)</td>
<td>139.7 ± 0.23</td>
<td>144.3 ± 0.35‡</td>
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<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.85 ± 0.23</td>
<td>5.48 ± 0.21*</td>
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<td>Serum chloride (mmol/l)</td>
<td>101 ± 0.2</td>
<td>105 ± 0.4‡</td>
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<td>Serum acetate (mmol/l) Before dialysis</td>
<td>0.15 ± 0.06</td>
<td>0.18 ± 0.06</td>
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<td>1 h after dialysis</td>
<td>0.32 ± 0.15</td>
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<td>2 h after dialysis</td>
<td>0.19 ± 0.13</td>
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Table 1. Changes in some parameters of acid–base balance and calcium metabolism and serum concentrations of sodium and potassium in uraemic patients given acetazolamide with (group 1) and without (group 2) 1,25-(OH)$_2$D$_3$ pretreatment

Values are shown as means ± SEM. Statistical significance: *$P<$0.05, †$P<$0.01, ‡$P<$0.001, §$P<$0.0001 compared with placebo.

therapy was lower in uraemic patients not given 1,25-(OH)$_2$D$_3$ than in uraemic patients given 1,25-(OH)$_2$D$_3$, [4.26 ± 0.6 vs 4.85 ± 0.1 kPa (31.9 ± 4.5 vs 36.3 ± 0.8 mmHg); $P=0.01$. In haemodialysis patients the mean value of serum unmeasured anion concentration showed a slight increase after acetazolamide administration. The mean of the individual changes was 3 ± 2 mmol/l and there was no significant difference between uraemic
Acetazolamide and haemodialysis

patients given 1,25-(OH)\textsubscript{3}D\textsubscript{3} and uraemic patients given acetazolamide alone. During acetazolamide therapy there was no significant difference in the mean serum concentrations of acetate between these two groups of uraemic patients and the haemodialysis patients given placebo, either before dialysis or 1 and 2 h after the end of the session. Furthermore, in the two groups of haemodialysis patients receiving acetazolamide we found no significant change in the mean serum concentrations of acetate at any time after acetazolamide therapy. Blood pH, bicarbonate concentration, \(P_{\text{CO}_2}\) and serum anion gap, which were altered by acetazolamide administration, progressively returned to pretreatment values within 7 days of stopping the agent.

**Effect of acetazolamide on serum concentration of potassium**

Acetazolamide induced opposite changes in serum potassium concentration in healthy subjects and in haemodialysis patients. In healthy subjects the carbonic anhydrase inhibitor induced a decrease in serum potassium concentration and a rise in its urinary loss. The mean values of serum potassium level and its fractional urinary excretion before acetazolamide were 4.2 ± 0.03 mmol/l and 8.6 ± 0.2%, respectively. These values changed to 3.7 ± 0.05 mmol/l (\(P=0.002\)) and 12.4 ± 0.04% (\(P=0.003\)) after acetazolamide therapy. Serum potassium concentration and fractional urinary excretion of potassium progressively returned to pretreatment values within 7 days of stopping the agent. In haemodialysis patients given acetazolamide the mean serum potassium level showed a significant rise when compared with that of uraemic patients given placebo (5.4 ± 0.14 vs 4.8 ± 0.1 mmol/l, \(P=0.002\)). Such a rise was greater in uraemic patients given acetazolamide alone than in uraemic patients pretreated with 1,25-(OH)\textsubscript{3}D\textsubscript{3}. The means of the individual increases were 0.6 ± 0.06 mmol/l and 0.46 ± 0.09 mmol/l, respectively. The serum potassium concentration, raised by acetazolamide administration, progressively returned to pretreatment values within 7 days of stopping the agent.

**Effect of acetazolamide on calcium metabolism**

The mean serum levels of inorganic phosphorus, PTH and bone isoenzyme of alkaline phosphatase before starting acetazolamide therapy were slightly lower in uraemic patients given 1,25-(OH)\textsubscript{3}D\textsubscript{3} than in uraemic patients not given 1,25-(OH)\textsubscript{3}D\textsubscript{3}. However, only the difference in serum inorganic phosphorus was statistically significant (\(P<0.05\)). The effects of acetazolamide on serum concentrations of total calcium, inorganic phosphorus, bone isoenzyme of alkaline phosphatase and PTH are shown in Fig. 3. The serum levels of total and ionized calcium showed opposite changes in the two treated groups of haemodialysis patients. Uraemic patients given only acetazolamide showed a fall in serum concentrations of total and ionized calcium. The means of the individual decreases were 0.21 ± 0.02 mmol/l and 0.19 ± 0.02 mmol/l. Conversely, in uraemic patients given 1,25-(OH)\textsubscript{3}D\textsubscript{3}, acetazolamide led to a rise, rather than a fall, in serum concentration of total and ionized calcium. The means of the individual increases were 0.39 ± 0.04 mmol/l and 0.22 ± 0.03 mmol/l, respectively. The serum concentration of inorganic phosphorus showed a significant rise in both treated groups of haemodialysis patients. However, in uraemic patients given 1,25-(OH)\textsubscript{3}D\textsubscript{3} the mean of the individual rises was slightly greater than that of uraemic patients given only acetazolamide (0.73 ± 0.9 vs 0.46 ± 0.4 mmol/l, \(P=0.02\)). The mean serum concentrations of C-PTH, M-PTH and bone isoenzyme of alkaline phosphatase rose in both uraemic groups given acetazolamide. The serum levels of total and ionized calcium, inorganic phosphorus, PTH and bone isoenzyme of alkaline phosphatase, altered by acetazolamide administration, progressively returned to pretreatment values within 7 days of stopping the agent. Conversely, acetazolamide did not induce any significant change in these biochemical parameters of calcium metabolism in healthy subjects.

**DISCUSSION**

**Effects of acetazolamide on acid-base balance**

Acetazolamide increases the urinary excretion of bicarbonate and fixed cation, mostly sodium. As a result the concentration of bicarbonate in the extracellular fluid decreases and metabolic acidosis develops. Furthermore, acetazolamide produces a marked increase in potassium excretion, attributable to enhanced secretion in the distal nephron with a consequent decrease in serum potassium concentration [1, 2]. The present study confirms the above sequence of events in healthy subjects who developed a mild metabolic acidosis with plasma bicarbonate concentration rarely below 18 mmol/l after acetazolamide administration. Treatment with acetazolamide resulted in a more severe acidosis in haemodialysis patients than in healthy subjects, despite the fact that these patients could not lose bicarbonate by the formation of bicarbonate-rich urine because they had virtually no urine output. Acetazolamide would not be expected to generate much metabolic acidosis in the absence of a bicarbonate diuresis, yet if severe metabolic acidosis did occur the big question is 'why'? The slight increase in the serum anion gap observed in haemodialysis patients could be attributable to the accumulation of an undetermined anion, perhaps acetazolamide itself. There was no correlation between the rise in serum unmeasured anion concentration and the degree of metabolic acidosis after acetazolamide administration. Furthermore, the relatively small amplitude of such an increase excludes the possibility that the severe metabolic acidosis reflects merely the accumulation of an undetermined anion. We suggest that the marked metabolic acidosis induced by acetazolamide in haemodialysis patients may result from interference with the mechanisms of extrarenal buffering. The persistence of an approximately normal hydrogen ion
balance in certain patients with end-stage kidney disease is well-established. Extrarenal active mechanisms for eliminating hydrogen ions from their exchangeable pool have been proposed to counterbalance the failing renal excretory mechanism. When renal mechanisms for acid excretion fail, acid necessarily is retained because daily production continues. Despite continued acid retention the plasma bicarbonate level eventually stabilizes, and bone carbonates become an increasingly important buffer substance [16-18]. In fact, in patients with untreated uraemic acidosis, bone bicarbonate stores are significantly decreased [19, 20]. Availability of bone buffers depends on the action of PTH, mediated by carbonic anhydrase and 1,25-(OH)$_2$D$_3$ [21, 22]. More specifically, PTH increases the availability of carbonic acid by activating the carbonic anhydrase in the periosseous syncytium [22]. Our findings suggest that the marked metabolic acidosis seen in the haemodialysis patients treated with acetazolamide may be due to impaired PTH-mediated bone buffering. It is conceivable that in these patients the inhibition of the carbonic anhydrase activity reduces the availability of carbonic acid in the periosseous syncytium, and consequently the amount of bicarbonate pushed into the systemic circulation by the pump activity of the osteocytes. As in uraemic patients, the kidney is no longer able to maintain the hydrogen ion balance, this sequence of events may lead to the observed reduction in the concentration of bicarbonate in the extracellular fluid with worsening of the metabolic acidosis. Alternatively, the worsening of metabolic acidosis might be explained, at least in part, by an effect of acetazolamide on acetate metabolism. Acetazolamide might somehow reduce the generation of bicarbonate from acetate metabolism. However, the lack of significant changes in the serum concentration of acetate, either before dialysis or 1 and 2 h after the end of the session, excludes the possibility that acetazolamide may slow hepatic metabolism of acetate in the dialysate to bicarbonate. The worsening of metabolic acidosis secondary to the reduced extrarenal generation of bicarbonate induced by acetazolamide emphasizes the importance of the extrarenal buffer mechanisms in maintaining the hydrogen ion balance in end-stage kidney disease. Recently, Gennari [23] has proposed that patients undergoing haemodialysis are in net zero acid balance and therefore would not utilize bone buffers. The fact that the administration of 1,25-(OH)$_2$D$_3$ in our haemodialysis patients, before acetazolamide was given, did not increase plasma bicarbonate concentration above baseline may be
buffers are relatively spared and pretreatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3} does not influence significantly the plasma bicarbonate concentration. However, in these patients the administration of acetazolamide, by inhibiting carbonic anhydrase, may result in a disequilibrium pH in the periosseous fluid. Under these experimental circumstances the fall in the relative rate of bone buffer release could impair the capacity to maintain plasma bicarbonate concentration. Interestingly, the fall in blood pH and bicarbonate concentration was attenuated by prior treatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3}. We may speculate that in haemodialysis patients with low serum 1,25-(OH)\textsubscript{2}D\textsubscript{3} the reduced response of the osteocytes to PTH is further decreased by the inhibition of carbonic anhydrase. Administration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} may diminish the acidifying effect of acetazolamide by improving the PTH-mediated bone buffering. In fact, 1,25-(OH)\textsubscript{2}D\textsubscript{3}, in the presence of PTH, is able to increase the affinity of the transport systems for hydrogen ions subsequent to a reactivation of the osteocyte metabolism [24]. However, it is even possible that the effect of 1,25-(OH)\textsubscript{2}D\textsubscript{3} in diminishing acetazolamide-induced acidosis may be due in part to an increased intestinal absorption of phosphate and an increased buffering capacity.

Effect of acetazolamide on serum concentration of potassium

The serum potassium concentration underwent opposite changes after acetazolamide treatment in healthy subjects and haemodialysis patients. In healthy subjects the reduced serum potassium level was accompanied by an excessive urinary loss, thus confirming previous studies [1, 2]. Conversely, the haemodialysis patients showed a significant rise that may be ascribed to the shift of potassium from the intracellular to the extracellular fluid secondary to the worsening of metabolic acidosis. Uraemic patients given acetazolamide alone, who had a more severe metabolic acidosis, showed a greater rise in serum potassium concentration when compared with uraemic patients pretreated with 1,25-(OH)\textsubscript{2}D\textsubscript{3}. It is possible that the elevated extracellular hydrogen ion concentration produced by acetazolamide may favour the movement of hydrogen ions into cells in exchange for potassium.

Effect of acetazolamide on calcium metabolism

In uraemic patients on maintenance haemodialysis acetazolamide induced some disturbances in calcium metabolism and bone turnover reflected by significant increases in the serum concentration of inorganic phosphorus, PTH and bone isoenzyme of alkaline phosphatase, and by distinct changes in serum total and ionized calcium. The elevation in inorganic phosphorus concentration was not accounted for by extracellular volume contraction since neither packed cell volume nor body weight changed during acetazolamide treatment. These findings suggest that the acetazolamide-induced hyperphosphataemia may be attributed to a change in calcium metabolism. However, the greater rise in serum inorganic phosphorus in the patients treated with 1,25-(OH)\textsubscript{2}D\textsubscript{3} may be due in part to the combined actions of PTH and 1,25-(OH)\textsubscript{2}D\textsubscript{3} on bone resorption. It is well established that PTH increases the dissolution of hydroxyapatite which releases calcium, phosphorus and bicarbonate into the extracellular fluid, and such an action of PTH requires the presence of 1,25-(OH)\textsubscript{2}D\textsubscript{3} [27, 28]. The opposite changes in serum calcium concentration induced by acetazolamide in the two treated groups of haemodialysis patients seem to support this hypothesis. Data from uraemic patients given acetazolamide without 1,25-(OH)\textsubscript{2}D\textsubscript{3} demonstrate that the administration of the carbonic anhydrase inhibitor, in spite of the rise in serum PTH, results in a fall in both serum total and ionized calcium. It is therefore conceivable that in these patients the hypercalcaemic response to PTH is blocked by acetazolamide, as previously demonstrated by Waite et al. [29] in parathyroidectomized-nephrectomized rats. Our findings indicate that the administration of acetazolamide in renal failure leads to a dissociation of the effects of PTH, with stimulation of bone turnover by the increase in serum PTH, but a failure of the serum calcium to respond to the higher PTH levels. This may be due to separate actions of PTH on bone turnover (resorption/formation) and on the release of calcium from a labile bone pool, and might suggest that one action is more dependent on carbonic anhydrase than the other. Data from uraemic patients given 1,25-(OH)\textsubscript{2}D\textsubscript{3} demonstrate that the administration of acetazolamide results in a rise in serum levels of PTH and bone isoenzyme of alkaline phosphatase. However, whereas acetazolamide alone results in a fall in serum calcium, the combined treatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3} induces a rise in serum calcium, sometimes to hypercalcaemic levels. It is possible that such a rise may be due in part to the combined effect of PTH and 1,25-(OH)\textsubscript{2}D\textsubscript{3} on intestinal absorption of calcium. It is well established that the effect of PTH on intestinal calcium transport is only indirect and is due to the renal PTH activation of 1x-hydroxylase and consequently of 1,25-(OH)\textsubscript{2}D\textsubscript{3} synthesis [30]. However, in patients treated with 1,25-(OH)\textsubscript{2}D\textsubscript{3} we did not observe any significant increase in serum 1,25-(OH)\textsubscript{2}D\textsubscript{3} during the treatment with acetazol-
aminoxylic acid, despite the rise in serum calcium concentration. Therefore, we may hypothesize that in these patients the increase in serum calcium induced by acetazolamide administration may be due to the combined effect of PTH and \( 1,25-(OH)_2D_3 \) on bone resorption and calcium release. In patients receiving \( 1,25-(OH)_2D_3 \) adequate amounts of \( 1,25-(OH)_2D_3 \) might be available at bone level. As \( 1,25-(OH)_2D_3 \) must be present for PTH to cause calcium release from bone [27, 28], it is conceivable that such an action of \( 1,25-(OH)_2D_3 \) may overwhelm the hypocalcaemic effect of acetazolamide. Thus, in these patients the exacerbation of secondary hyperparathyroidism induced by the administration of the carbonic anhydrase inhibitor should lead to the increase in bone turnover and calcium release. This hypothesis is consistent with the concept that hormonally induced bone resorption and calcium release are mediated, at least in part, by the action of carbonic anhydrase, as previously reported by Hall & Kenny [31, 32], who demonstrated that carbonic anhydrase plays a role in the action of both PTH and \( 1,25-(OH)_2D_3 \) on bone resorption.

CONCLUDING REMARKS

This study indicates that the administration of acetazolamide in renal failure leads to a severe metabolic acidosis as well as the exacerbation of secondary hyperparathyroidism. How the inhibition of carbonic anhydrase may increase serum PTH is unclear. However, a reasonable conclusion is that acidosis may be a factor in the hypersecretion of PTH independent of changes in extracellular calcium, as suggested by the increase in both serum calcium and PTH in haemodialysis patients given \( 1,25-(OH)_2D_3 \) and acetazolamide.

REFERENCES