Metabolic acidosis does not contribute to chronic renal injury in the rat

D. THROSSELL, J. BROWN, K. P. G. HARRIS and J. WALLS
Department of Nephrology, Leicester General Hospital, Leicester, U.K.

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1. Metabolic acidosis invariably accompanies chronic renal failure, and short periods of metabolic acidosis cause renal growth and proteinuria in normal rats. Rates of ammoniagenesis are increased in chronic renal failure, and it has been suggested that this contributes to disease progression. This study assessed (i) whether prolonged acidosis causes chronic renal injury in the normal kidney and (ii) whether abrogation of acidosis slows disease progression in the remnant kidney.

2. Metabolic acidosis was induced in normal rats by dietary hydrochloric acid. Urinary excretion of total protein, lysozyme and albumin increased, peaking at week 8 but returning to baseline by week 14. At killing after 14 weeks, kidney weights, glomerular filtration rates and serum creatinine were the same in both groups, but kidney/body weight and kidney/heart weight ratios were greater in the acidotic group. All kidneys were normal by light microscopy.

3. Rats subjected to five-sixths nephrectomy were given sufficient dietary bicarbonate to abolish uraemic acidosis, and their outcome was compared with that of non-alkalinized remnants (controls). Proteinuria, glomerular filtration rates, blood pressure, histological injury and time to the development of terminal uraemia were no better in bicarbonate-supplemented animals than in controls.

4. These data demonstrate that metabolic acidosis neither causes nor exacerbates chronic renal injury. We conclude that the treatment of uraemic acidosis is unlikely to influence disease progression in patients with chronic renal failure.

INTRODUCTION

Metabolic acidosis (MA) is an invariable feature of chronic renal disease, and results from inadequate ammonium excretion and decreased tubular bicarbonate reabsorption [1]. It contributes to the uraemic syndrome by exacerbating renal bone disease [2] and promoting skeletal muscle catabolism [3], and evidence from animal studies suggests that it may also promote the progression of chronic renal injury by altering rates of ammoniagenesis. Although total urinary ammonia excretion is reduced in both human and experimental renal disease, ammonium production per residual nephron increases [4]. Nath et al. [5], using the remnant model of chronic renal failure in the rat, demonstrated that proteinuria and tubulointerstitial injury could be mitigated by dietary supplementation with sufficient sodium bicarbonate to reduce renal ammoniagenesis. They suggested that cortical ammonia resulting from up-regulation of ammoniagenesis activates the alternative complement pathway, causing tubulointerstitial inflammation and thereby compounding renal injury.

In addition to a possible role in the progression of established renal disease, MA causes abnormal renal growth and proteinuria in animals with normal renal function. Renal hypertrophy and hyperplasia were first described in acidotic rats by Lotspeich [6], and in vitro studies have since demonstrated hypertrophy in proximal tubular cells exposed to ammonia [7] or low pH [8]. Renal growth has also been identified in several animal models of chronic renal disease (reviewed in [9]), raising the possibility that it is a prerequisite for the development of renal injury. Early reports of a correlation between urinary hydrogen ion content and protein excretion in human subjects [10] have been confirmed in rats, which develop proteinuria when made acidotic for short periods by dietary ammonium chloride (NH₄Cl) [11]. It was originally proposed that this proteinuria resulted from increased protein filtration at the glomerulus, but recent data from this laboratory have shown that short-term acidosis causes a significant increase in urinary lysozyme and N-acetylglucosaminidase, while excretion of immunoglobulin G remains relatively unchanged [12]. This profile suggests tubular, rather than glomerular, injury and is consistent with immunohistochemical studies of kidneys from rats given dietary hydrochloric acid supplements for 14 days. Sections stained with antibody to Tamm-Horsfall protein (THP) show less prominent staining of tubular cells than controls, but also contain...
numerous THP-positive tubular casts [12]. Such changes also occur in biopsies from patients with renal impairment, when they develop before significant light microscopic abnormalities appear, and have therefore been proposed as an index of early tubular damage and renal injury [13]. The demonstration of similar immunohistochemical changes in both renal impairment and acidosis lends further weight to the hypothesis that MA contributes to renal injury.

Although previous studies suggested that acidosis may be implicated in renal injury, their conclusions were based on short experimental protocols. Studies of acidosis in normal kidneys considered periods of only 14 days, thereby failing to establish whether the hypertrophy and proteinuria seen after short-term acidosis are forerunners of more significant structural and functional abnormalities when acidosis is prolonged. Similarly, in their study of alkalized remnants, Nath et al. analysed proteinuria and histological injury only 4-6 weeks after nephrectomy. Several groups have reported that the renal function of remnant rats steadily improves from the time of surgery until around the eighth post-operative week [14], a phenomenon attributed to the combined effects of recovery from peripерoperative acute renal failure and the development of compensatory hypertrophy in residual renal tissue. The period of bicarbonate supplementation previously studied therefore corresponds to this recovery phase, rather than to a stable period of progressive renal injury. Since patients with chronic renal failure are exposed to MA for months or years, any study assessing the contribution of acidosis to progression must continue for a prolonged period. The present study therefore aimed to evaluate further the role of acidosis in promoting chronic renal injury by assessing the effects of prolonged acidosis (14 weeks) on renal structure and function in the normal rat, and the effects of prolonged alkalinization (from the time of surgery to the development of terminal uraemia) on proteinuria, renal excretory function, histological injury, collagen deposition and survival in rats subjected to five-sixths nephrectomy.

MATERIALS AND METHODS

The study comprised two experimental protocols as described below.

Protocol I: acidosis in normal rats

Fifty-three female Wistar rats (initial weight approximately 200 g), housed in individual cages with constant temperature and humidity and a 12-h light/dark cycle, were fed a powdered diet (ICN no. 960259) containing 20% casein (w/w), offered as a paste mixed with water (1:1, w/w) and methylcellulose (2 g/100 g). After a 2-week induction period, 32 animals had the standard paste replaced by feed reconstituted with 0.5 mol/l hydrochloric acid instead of water, while 21 pair-fed controls continued on the standard diet without acid supplementation. All animals had free access to tap water. Food consumption was recorded daily, body weights were measured weekly and at 2-weekly intervals animals were placed in metabolic cages for collection of 24-h urine samples.

After 14 weeks, glomerular filtration rate (GFR) was measured using the method of Nankivel et al. [15]. An intraperitoneal injection of 20 MBq of technetium-99m-labelled diethylenetriaminepentaacetic acid (DTPA) in 0.5 ml of normal saline was administered at time 0, and samples of heparinized blood were withdrawn from a tail vein under light ether anaesthesia at 45 and 90 min. Tc activity of 50-μl aliquots of plasma was then counted using a Cobra autogamma 5002 counter (Canberra Packard, Pangbourne, U.K.), and GFR was calculated using the slope–intercept method. Animals were then weighed and killed by exsanguination under anaesthesia (2.7 ml/kg Hypnorm/Hypnovel/water, 1:1:2 by vol., intraperitoneally). Aortic blood was collected in a heparinized, air-free syringe for blood gas determination, and serum was analysed for urea and creatinine. Kidneys were removed, weighed and bisected coronally. One half of each kidney was fixed in formal saline and the other half in Carnoy’s solution. Sections of formalin-fixed tissue were stained with haematoxylin and eosin and examined by light microscopy. Kidneys fixed in Carnoy’s solution were used to quantify infiltrating macrophages as follows. Sections of 3 μm thickness were incubated with ED1 mouse monoclonal antibodies (Serotec, Oxford, U.K.), then biotinylated rabbit anti-mouse immunoglobulin G (Zymed, San Francisco, CA, U.S.A.). Demonstration was with streptavidin–peroxidase followed by aminoethyl carbazole and hydrogen peroxide. For each section, the number of ED1-positive nuclei falling within five randomly selected high-power fields was recorded.

Urinary lysozyme was measured by a turbidimetric assay [16] adapted for automated analysis using a one-point standard method on an ŠPS Vitatron system. Urine was incubated with a suspension of 60 mg% Micrococcus lysodeikticus (Sigma, Poole, U.K.) and the reduction in turbidity resulting from subsequent cell lysis was quantified spectrophotometrically at 540 nm. Crystalline hen egg white lysozyme (Sigma) was used as standard. Urinary albumin and immunoglobulin G (IgG) concentration were determined by a solid-phase ELISA developed in our laboratory. Disposable polystyrene microtitre plates (Nunc Immunoplate, Denmark) were coated with 100 μl of either rabbit anti-rat albumin (10 mg/ml, Sigma) or rabbit anti-rat IgG (5 μg/ml, Cappel, Turnhout, Belgium), dissolved in coating buffer (9:1 mixture of molar sodium bicarbonate and sodium carbonate pH 9.6). After washing, plates were blocked with BSA as an irrelevant protein for 1 h and washed again. The standards (rat
albumin and rat IgG, Sigma) and samples were then added in duplicate after appropriate dilution and the plate was incubated overnight at 4°C. After further washing, horseradish peroxidase-labelled rabbit anti-rat albumin or IgG (1:2000 and 1:3000 respectively) was added and the plate incubated for 1 h at room temperature. The colour was developed using 50 µl of a 1,2-phenylenediamine detection system {0.03 mol/1 citric acid, 0.07 mol/l disodium hydrogen orthophosphate at pH 5.0, 2-µg 1,2-phenylenediamine hydrochloride (OPD) tablet (Dakopatts, Denmark) and 1.25 µl of 30% hydrogen peroxide per 3 ml of solution}. The reaction was stopped after 5 min by adding 75 µl of 1 mol/l sulphuric acid to each well and the plate was then read on a Titertek Multiscan photometer (Lab-systems, Finland) at 492 nm.

Protocol 2: bicarbonate supplementation in remnant rats

Twenty-four female Wistar rats (initial weight approximately 160 g) underwent right nephrectomy and ligation of two left renal artery branches as a single procedure under general anaesthesia (2.7 ml/kg Hypnorm/Hypnovel/water, 1:1:2 by vol., intraperitoneally). Post-operatively, they were fed 20% casein feed (ICN 960259), offered as a paste mixed with water (1:1, w/w) and methylcellulose (2 g/100 g). After 2 weeks, all animals were placed in metabolic cages for collection of 24-h urine specimens, and blood samples were subsequently withdrawn from a tail vein under light ether anaesthesia. Animals were ranked for severity of injury based on proteinuria and serum urea and creatinine, and were then allocated alternately to one of two groups. Group 1 rats (alkalinized remnants) were fed a sodium-deficient diet (ICN 960231) offered as a paste mixed with water (1:1, w/w) and methylcellulose (2 g/100 g) and supplemented with 1.9 g NaHCO₃ per 100 g. Group 2 rats (control remnants) were pair fed the same diet, which, to ensure equal sodium intake, was supplemented with 1.3 g NaCl per 100 g. Food consumption was recorded daily, body weights were measured weekly and at 2-weekly intervals animals were placed in metabolic cages to quantify 24-h urinary protein excretion. After 1, 2 and 3 months, systolic blood pressure was recorded using a Harvard tail cuff sphygmomanometer. At 6 and 12 weeks, GFR was measured using the same technique as in protocol 1.

Animals were individually examined on a daily basis, and were killed by exsanguination under anaesthesia (2.7 ml/kg Hypnorm/Hypnovel/water, 1:1:2 by vol., intraperitoneally) when they developed terminal uraemia, characterized by lethargy, anorexia and failure to gain weight. Arterial blood was collected in a heparinized, air-free syringe for blood gas determination, and serum was analysed for urea and creatinine. Whole-body and kidney weights were recorded, and kidneys were then sectioned coronally. The first half of each kidney was fixed in formal saline for histological examination, and the second half was assayed for collagen as described below.

Histological assessment was carried out on 3-µm kidney sections stained with haematoxylin and eosin. Each was graded 0–5 for severity of injury based on a blinded assessment of glomerulosclerosis, tubular dilatation, cast formation and interstitial fibrosis. In addition, cast formation was quantified by recording the number of casts contained within 500 tubules, starting counting in the outer cortex of the mid-pole and moving in a corticomedullary direction.

Total kidney collagen content was determined using a hydroxyproline assay as follows. The portion of each kidney retained for collagen analysis was weighed and cut into small (approximately 1 mm³) pieces before being reduced to a pulp in a ground-glass organ grinder. The resulting tissue was hydrolysed in 6 mol/l HCl at 110°C for 16 h, evaporated to dryness, suspended in deionized water, lyophilized and then resuspended in 2 ml of buffer. The hydroxyproline concentration of this solution was determined by the method of Stegemann & Stalder [17] as follows. One millilitre of the sample was mixed with 0.5 ml of a 0.05 mol/l solution of chloramine T, then left at room temperature for 20 min to oxidize. After oxidation, 1 ml of a 1.2 mol/l solution of Ehrlichs aldehyde reagent (dimethylaminobenzaldehyde) in propan-1-01 (70%) and perchloric acid (30%) was added, and the resulting solution was incubated at 60°C for 15 min. After cooling, absorbance of the solution was determined at 550 nm using a Cecil CE 2040 spectrophotometer, and hydroxyproline concentration was then calculated by reference to a standard curve. The collagen content of the hydrolysate was estimated by multiplying its hydroxyproline content by 7.42 [18], and total kidney collagen was then derived in each case from the tissue collagen concentration and original kidney weight.

Biochemical analyses

Arterial blood gases were measured using a Corning 238 blood gas analyser. Urea was measured by urease reagent and creatinine by the Jaffe reaction using a Vitatron SPS analyser. Urine total protein was measured by a dye-binding assay using pyrogallol red (Randox Laboratories, U.K.).

Statistics

Values are expressed as means±SEM. In protocol 1, serial urine chemistries within each group were compared by two-way analysis of variance (ANOVA). In both protocols, urinary indices at each time point and data at killing were compared by unpaired t-test. Histological scores were analysed by Wilcoxon rank sum test, and in protocol 2 numbers of casts per 500 tubules were compared by unpaired t-test. P-values of less than 0.05 were considered significant.
RESULTS

Protocol I

Somatic growth was significantly impaired by dietary acid, and at the end of the study the mean weight of the experimental group was 251 ± 4 g compared with 313 ± 10 g in controls (P < 0.001). Overall daily food consumption was 41 ± 0.4 g in acidotic animals and 38 ± 0.5 g in controls; acidotic animals therefore received a mean daily acid load of 10.3 mmol. Urine volume after 14 weeks was 30 ± 2 ml/24 h in acidotic animals compared with 19 ± 2 in controls (P < 0.001), and urine pH was at least 0.5 lower in the acidotic group at each time point (Fig. 1), progressively falling from 5.67 ± 0.04 at week 2 to 5.47 ± 0.03 at week 14 (P < 0.001). There was no significant change in the pH of control urine. In the acidotic group, urinary protein excretion rose significantly, reaching a maximum of 10.0 ± 0.9 mg/day at week 8 before returning to levels no greater than controls by week 14 (Fig. 2). Excretion of lysozyme and albumin followed a similar pattern. Urinary lysozyme increased from undetectable levels at baseline to a peak of 176 ± 36 µg/day at week 8 (P < 0.001) before again becoming undetectable at week 12. Urinary albumin also peaked at week 8, when its excretion was 4.5 times greater than in controls (P < 0.001), but by week 12 excretion had returned to baseline. Acidosis caused only a slight increase in the excretion of IgG, such that at 8 weeks excretion in acidotic animals exceeded that in controls by a factor of 1.7 (P < 0.05).

At killing, mean arterial pH was lower in the acid-supplemented group (7.35 ± 0.02 versus 7.41 ± 0.01; P < 0.05). There was no significant difference in serum creatinine concentration between acidotic and control rats (58 ± 4 versus 60 ± 4 µmol/l), and GFR, measured in 10 animals from each group, was 2.2 ± 0.2 ml/min in acidotic versus 2.4 ± 0.1 ml/min in controls (not significant). Kidney weights were very similar in both groups, but kidney/body weight ratios were greater in acidotic animals (Table 1). In contrast, heart weights, recorded as an index of growth of organs other than the kidney, were lower in acidotic animals, such that kidney/heart weight ratios were higher in the acidotic group (Table 1).

Histological examination of sections stained with haematoxylin and eosin showed no light microscopic abnormalities in kidneys from acidotic or control groups. ED1 staining of Carnoy’s-fixed sections demonstrated no significant difference in macrophage density between acidotic and control groups (median 5 versus 8 positive cells per five high-power fields).
Acidosis does not promote renal injury

Protocol 2

The division of the rats into two comparable groups on the basis of post—operative ranking for serum and urine biochemistry ensured similar initial values of serum creatinine (94.4 ± 6.3 versus 93.7 ± 6.8 mmol/l, not significant), urea (21.6 ± 2.1 versus 22.2 ± 2.4 mmol/l, not significant) and 24-h urinary protein excretion (26.5 ± 11.3 versus 31.7 ± 8.3 mg/24 h, not significant) in control and bicarbonate-supplemented animals. Two animals died within 2 weeks of surgery and were therefore excluded from the analysis. Food intake was similar in both groups (overall means 33.4 ± 0.7 versus 32.7 ± 0.5 g/day, not significant), and mean bicarbonate intake in the alkali-supplemented group was 3.7 mmol/day. In every 24-h urine collection from the fourth week onwards, urine pH was significantly higher in the bicarbonate-supplemented group than in controls (Fig. 3). All animals developed proteinuria, which progressively increased from the time of surgery until a preterminal decline approximately 2 weeks before death. Because of this pattern of proteinuria, the peak and overall mean protein excretion was recorded for each animal in addition to calculations of group means from each 2-weekly 24-h urine collection. There was no significant difference in 24-h protein excretion between the two groups at any time (Fig. 4), and peak proteinuria (222 ± 23 versus 199 ± 16 mg/24 h, not significant) and overall mean proteinuria (93 ± 8 versus 100 ± 8 mg/24 h, not significant) were also similar. Systolic blood pressure rose to a similar extent in both groups (Fig. 5), and glomerular filtration rates were 0.77 ± 0.10 versus 0.64 ± 0.10 ml/min at 1 month, and 0.62 ± 0.12 versus 0.66 ± 0.10 at 2 months (both not significant).

There was no statistically significant increase in survival in the bicarbonate-treated group, and the difference in average time from surgery to death between the two groups was less than 1 week (Table 2). Because of variable sensitivity to general anaesthesia, which resulted in a range of values for PCO₂ in arterial blood at the time of sacrifice, terminal arterial pH is expressed both as ‘raw pH’, and as a ‘corrected pH’, adjusted using the Henderson—Hasselbalch equation to represent the pH value at a standard PCO₂ of 5.6. Using both these indices, pH was markedly higher in bicarbonate-supplemented animals than in controls (Table 2). Alkalized
animals had a negligible base deficit, demonstrating that bicarbonate supplementation had adequately abolished uraemic acidosis. Serum urea and creatinine were both lower in controls than in treated animals, and for urea this difference achieved statistical significance (Table 2). Final body weights were the same in both groups, but kidney weights of control and bicarbonate-supplemented kidneys were higher in controls than in the bicarbonate-supplemented group ($P = 0.053$ and $P < 0.05$ respectively). Despite this difference in kidney weights, total kidney collagen content was the same in both groups (Table 2). All kidney sections showed advanced chronic injury, characterized by tubular dilation, cast deposition, glomerulosclerosis and interstitial fibrosis. The severity of injury was the same in both groups, and with the single exception of one control kidney, which scored grade 3, all sections scored grade 5 on the 0–5 histological injury scale. There was no statistical difference in the number of casts per 500 tubules in control and bicarbonate-supplemented kidneys (Table 2).

### DISCUSSION

This study demonstrates that metabolic acidosis neither causes nor exacerbates chronic renal injury. Evidence for this conclusion comes from both experimental protocols. In protocol 1, renal histology and GFR remained normal despite 14 weeks of acid supplementation, and the proteinuria previously reported in short-term acidosis resolved spontaneously by the end of the experiment. If, as previously proposed, ammonia causes renal injury by activating the alternative complement pathway [5, 19], it would be expected that kidneys in these chronically acidic animals, in which ammoniagenesis is increased at least threefold [20], would develop progressive tubulointerstitial disease. Since histological sections from acidic kidneys showed neither macrophage infiltration (a consistent feature of glomerular and tubulointerstitial inflammation in several models of experimental renal disease [21]) nor structural abnormalities, it can be concluded that high cortical ammonia concentrations do not directly cause injury in normal renal tissue. In protocol 2, abolition of uraemic acidosis by oral bicarbonate produced no improvement in GFR, serum creatinine, proteinuria, blood pressure, histological injury or fibrosis, and the time from surgery to development of terminal uraemia was the same in bicarbonate-supplemented animals as in non-alkalinized controls. If ammonia contributed significantly to the pathogenesis of tubulointerstitial injury in the remnant, then it would be predicted that alkalinization, which reduces ammoniagenesis, would slow disease progression in this model. Since structural and functional parameters were the same in alkalinized and non-alkalinized remnants, it follows that neither acidosis nor ammoniagenesis promotes the progression of established renal disease.

Urinary protein profiles in protocol 1 are consistent with previous data from this laboratory, and suggest a tubular origin for the proteinuria of acidosis. Large increases were seen in excretion of the freely filtered but normally completely reabsorbed low molecular weight protein lysozyme, with smaller increases in albumin excretion, but only a minor rise in excretion of immunoglobulin G. A novel finding was that total protein and lysozyme excretion progressively increased during the first 8 weeks of acidosis, but subsequently declined, returning to control levels by week 14. The mechanism of acidosis-induced proteinuria remains open to speculation. Since tubular cells were histologically normal at the time of sacrifice, the development of tubular proteinuria could be explained either by transient tubular damage that resolved before sacrifice or by a short-lived reduction in endocytosis or intralysosomal degradation of tubular proteins. Studies examining the effect of pH on endocytosis by proximal tubular cells have yielded conflicting results. Using a microperfusion technique in rat proximal tubules, Christensen & Bjerke [22] demonstrated that reducing the pH of perfusion fluid from 7.4 to 6.0 or 4.5 resulted in a 15% increase in albumin reabsorption. In contrast, Schwegler et al. [23] reported that cultured opossum kidney (OK) cells responded to increased or decreased pH by reducing absorption of fluorescein-labelled albumin [23]. Although their specific findings disagreed, these studies established the principle that protein reabsorption by tubular cells can be influenced by alterations in pH. With respect to intralysosomal protein degradation, Golchini et al. [7] reported that NH$_4$Cl given to cultured proximal tubular cells caused swelling of lysosomes and a reduction in activity of lysosomal proteases. It has been suggested that ammonia, acting as a weak base, raises intralysosomal pH and prevents targeting of mannose 6-phosphate-coupled enzymes to lysosomes, thereby reducing intralysosomal protein degradation [24]. Since acidosis is accompanied by large increases in cortical ammonium production, the resulting high local concentration of ammonium ions might influence degradation of tubular proteins in a similar manner.
Although mechanisms such as these might increase protein excretion in acidosis, they do not account for the spontaneous resolution of proteinuria seen in this study. If renal ammonia were responsible for proteinuria, protein excretion should have increased during continued acidosis, since urine pH gradually fell (Fig. 1), and therefore by implication cortical ammonia concentration rose, as the study proceeded. An association between protein excretion and systemic acidosis, rather than intrarenal ammonia, would be more consistent with the data. The progressive fall in urine pH in the face of constant intake of dietary protein and acid suggests increasing efficiency of urinary acid disposal, and arterial pH must consequently have risen as the study progressed. One explanation of the data would be that proteinuria occurs below a threshold arterial pH, resolving when this is exceeded after upregulation of mechanisms compensating for acidosis.

Proximal tubular bicarbonate reabsorption is regulated by systemic pH [25], and protein reabsorption may be regulated in a similar manner. Exactly how systemic acidosis might influence intracellular proteolysis, however, is not clear, since proximal tubular intracellular pH is resistant to external pH changes [26], and the luminal aspects of tubular cells continue to be bathed in acid urine after compensatory mechanisms have increased blood pH towards normal.

Body and kidney weight data from both protocols confirm previous reports that acidosis causes renal growth. In protocol 1, acidic animals had higher kidney/body weight and kidney/heart weight ratios than controls, and in protocol 2 animals with uncorrected uraemic acidosis had higher absolute kidney weights and kidney/body weight ratios than those given bicarbonate supplements. It has previously been suggested that renal growth is a prerequisite for the development of progressive renal injury [19]. Although renal hypertrophy has been reported after only 2 weeks of acid supplementation [6], kidneys from acidic animals in protocol 1 showed no evidence of structural or functional abnormality after being exposed to the hypertrophic influence of acidosis for over 3 months. This suggests that, while renal growth may be a prerequisite for injury in the kidney, progressive tubulointerstitial damage is not an inevitable consequence of renal growth. Similarly, although kidney weights were greater in non-alkalinized remnants than in those given bicarbonate supplements, the severity of renal injury was identical in the two groups, demonstrating that manoeuvres that reduce renal hypertrophy do not necessarily mitigate the associated renal damage.

An important aim of this study was to establish whether the early benefits of bicarbonate supplementation reported in remnant rats by Nath et al. [5] would continue into the later phase of progressive renal impairment, thereby slowing the rate of fall of GFR and delaying the onset of end-stage renal failure. The present study not only demonstrated no long-term benefit from alkalinization, as discussed above, but also failed to reproduce the early effects of alkalinization reported in the previous study. In their original experiments, Nath et al. administered a similar dose of bicarbonate to that used here (2.5 mequiv./100 g feed compared with 2.3 mequiv./100 g in this study) and reported that protein excretion at 4–6 weeks was almost halved by alkalinization, and that tubular casts, interstitial infiltration and tubular dilatation were less pronounced in the bicarbonate-supplemented group. Although historical data relating to this period are not available from our study, histological appearances at the time of death were similar in both groups, and protein excretion at both 4 and 6 weeks was identical in bicarbonate-supplemented animals and controls (Fig. 4). It is noteworthy that in the earlier study, GFR at 4–6 weeks was lower (overall mean 0.16 ml min⁻¹ 100 g⁻¹ body weight) than was seen here (overall mean 0.35 ml min⁻¹ 100 g⁻¹ body weight at 6 weeks), consistent with the more extensive renal lesion (seven-eighths versus five-sixths nephrectomy respectively). In the remnant rat, uraemic acidosis is most profound during the first postoperative month, but then improves until a second decline in the preterminal period [27]. The 4–6 week GFR reported by Nath et al., which is approximately 16% of normal, would be sufficient to induce uraemic acidosis, and, although arterial pH and base excess were not measured in their study, plasma bicarbonate was low-normal (20 mequiv./l) in the non-alkalinized group, suggesting acidosis. Since we have shown that acidosis induces transient tubular proteinuria (from protocol 1), it is possible that uraemic acidosis contributed to the early proteinuria in Nath et al.’s non-alkalinized remnants by a similar mechanism. This would be consistent with the significant proportion (approximately 40%) of total urinary protein that was of low molecular weight, and with the impairment of tubular transport function reported in this group. In contrast, the 6-week GFR in the present study (35% of normal) would be insufficient to induce acidosis, thus explaining the similarity in protein excretion between the bicarbonate-supplemented remnants and controls. The subsequent decline in renal function occurred at varying rates in different animals (reflected in the range of survival times), and any proteinuria caused by preterminal uraemic acidosis would consequently have been insufficient to influence significantly the mean protein excretion at any single time point.

The use in protocol 2 of an end point based partly on a subjective diagnosis of terminal uraemia is open to criticism. The alternative approach, however, of continuing the experiments until uraemic death, does not allow the collection of blood samples or the fixation of renal tissue for histological examination. Although objective measurements including weight loss and decreased food
intake were taken into account, the lower serum urea and creatinine in non-alkalinized remnants at killing suggest that these animals were culled slightly earlier in the course of their chronic renal disease than the bicarbonate-supplemented group, presumably because their clinical appearance was made worse by acidosis. Despite this effect, however, the survival of alkalized animals still did not significantly exceed that of controls, consistent with the conclusion that bicarbonate supplementation is of no benefit in the remnant rat.

In conclusion, this study demonstrates that chronic metabolic acidosis does not cause chronic renal injury, or promote the progression of established renal disease, in the rat. The treatment of uraemic acidosis, while it may confer symptomatic relief and improve renal bone disease, is therefore unlikely to influence rates of disease progression in patients with chronic renal failure.

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