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# Acute gentamicin-induced hypercalciuria and hypermagnesiuria in the rat: dose-response relationship and role of renal tubular injury

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**1** Standard renal clearance techniques were used to assess the dose-response relationship between acute gentamicin infusion and the magnitude of hypercalciuria and hypermagnesiuria in the anaesthetized Sprague-Dawley rat. Also investigated were whether these effects occurred independently of renal tubular cell injury.

**2** Acute gentamicin infusion was associated with a significant hypercalciuria and hypermagnesiuria evident within 30 min of drug infusion. The magnitude of these responses was related to the dose of drug infused ($0.14-1.12$ mg kg$^{-1}$ min$^{-1}$). Increased urinary electrolyte losses resulted from a decreased tubular reabsorption of calcium and magnesium.

**3** A rapid dose-related increase in urinary N-acetyl-$\beta$-D-glucosaminidase (NAG) excretion was also observed in response to gentamicin infusion. However, there was no evidence of renal tubular cell injury and no myeloid bodies were observed within the lysosomes of the proximal tubular cells. Gentamicin may thus interfere with the mechanisms for cellular uptake and intracellular processing of NAG causing increased NAG release into the tubular lumen.

**4** The absence of changes in renal cellular morphology indicates that the excessive renal losses of calcium and magnesium were an effect of gentamicin *per se* and not the result of underlying renal tubular injury. The renal effects described in this paper were apparent after administration of relatively low total drug doses, and with plasma concentrations calculated to be within the clinical range. These findings suggest that disturbances of plasma electrolyte homeostasis could occur in the absence of overt renal injury in patients receiving aminoglycoside antibiotics.

**Keywords:** Gentamicin; calcium excretion; magnesium excretion; enzymuria

## Introduction

Aminoglycoside antibiotics are very effective in the treatment of severe gram-negative sepsis. However, their clinical usefulness is tempered by the associated nephrotoxicity which occurs in 10–15% of patients undergoing treatment (Bennett, 1989). The major clinical manifestation of nephrotoxicity is a fall in glomerular filtration rate (GFR) and it is usually this which brings patients to medical attention. The pathogenesis of aminoglycoside-induced nephrotoxicity is well documented (Tulkens, 1989; Kacew, 1990). However, the precise cellular mechanism by which proximal tubular necrosis occurs remains to be elucidated.

A disturbance of electrolyte homeostasis represents a second, potentially serious but less well-documented side-effect of aminoglycoside therapy. A syndrome of hypomagnesaemia, hypocalcaemia and hypokalaemia in patients receiving aminoglycoside therapy has been observed (Kelnar *et al*., 1978; Watson *et al*., 1983; Zaloga *et al*., 1984). A common finding in these studies is that the levels of urinary electrolyte excretion are inappropriately high for their low plasma concentration, indicating a failure of the kidneys to conserve cations adequately.

Clinically it has been very difficult to establish a clear relationship between aminoglycoside treatment and renal electrolyte disturbances because of the concomitant use of other drugs and the presence of underlying disease processes. Animal studies have therefore proved useful. Early animal studies focused on the renal effects of chronic aminoglycoside administration to rats or dogs. Hyperkaliuria (Brinker *et al*., 1981), hypercalciuria and hypermagnesiuria (Chahwala and Harpur, 1983; Harpur *et al*., 1985) have all been seen to accompany long term gentamicin treatment. The major drawback to such studies was that in most cases GFR was depressed and there was histological evidence of proximal tubular necrosis. It was therefore difficult to determine whether the renal functional changes resulted from a direct action of gentamicin or were secondary to the loss of renal tubular integrity. Recent animal studies have overcome this problem by examining renal function during acute aminoglycoside treatment. Foster *et al*. (1992) and Elliott and Patchin (1992) first showed that acute gentamicin infusion into rats would induce a rapid and reversible hypercalciuria. The effect was fully established within one hour and resulted specifically from a reduced tubular reabsorption of calcium. Foster *et al*. (1992) also demonstrated a similar response for magnesium, although this was more transient in nature.

The present study builds on the acute aminoglycoside studies described above. Its aims were two fold. Firstly, to assess whether a dose-response relationship exists between acute gentamicin treatment and the resultant hypercalciuria and/or hypermagnesiuria. Secondly, to investigate whether this effect occurs independently of renal tubular cell injury. The latter objective was investigated directly by histological and ultrastructural evaluation of the kidneys following gentamicin treatment, and indirectly by examination of the urinary excretion of $\gamma$-glutamyl transpeptidase (GGT; EC 2.3.2.2) and N-acetyl-$\beta$-D-glucosaminidase (NAG; EC 3.2.1.30) during gentamicin infusion. These enzymes are located predominantly in the brush border membrane and lysosomes, respectively, and have been selected on the basis that they represent potential cellular targets for gentamicin. Some of the findings have been presented previously in abstract form (Parsons *et al*., 1993; 1994).

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## Methods

### Animals

Male Sprague Dawley rats (200–360 g) were purchased from Charles River Laboratories (Kent, U.K.), maintained at 21–23°C and exposed to a normal 12 h light cycle (lights on at 08 h 00 min). Animals had free access to food (CRM Labsure; Dorset, U.K.) and water before the experimental procedures.

### Surgical preparation

Rats were anaesthetized with an i.p. injection of sodium-5-ethyl-5(1-methyl propyl)-2-thiobarbiturate (Inactin; Byk-Gulden, Konstanz, Germany) at a dose of 110 mg $kg^{-1}$ and placed on a thermostatically-controlled heated operating table. Body temperature was maintained at $37.0 \pm 1.0$°C. Polyethylene catheters were placed in the jugular vein (for infusion), carotid artery (for blood pressure monitoring and collection of blood samples) and bladder (for collecting urine). A tracheostomy ensured a clear airway.

### Experimental protocol

Animals received a priming dose of 0.3 ml 0.9% (w/v) NaCl containing 6 $\mu$Ci[$^3$H]-inulin followed by a continuous infusion at 200 $\mu$l $min^{-1}$ of 0.9% NaCl containing 0.5 $\mu$Ci $ml^{-1}$ [$^3$H]-inulin. After 2 h the rate was reduced to 100 $\mu$l $min^{-1}$ and remained thus for 5 h. The [$^3$H]-inulin content of the lower infusion rate was 1.0 $\mu$Ci $ml^{-1}$. All animals received 0.9% NaCl for the first 4 h. The first 3 h of infusion served for equilibration and the following hour as a control period. During the final 3 h (experimental period) the infusate was either kept to 0.9% NaCl (control animals, $n=8$) or changed to one containing gentamicin base (Cidomycin; Roussel Laboratories Ltd., Uxbridge, U.K.) in 0.9% saline. Gentamicin was infused at a rate of 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$ ($n=8$ rats in each group). These doses provided a total drug delivery over the experimental period of 25, 50, 100 and 200 mg $kg^{-1}$, respectively. During control and experimental periods 30 min urine samples were collected into pre-weighed plastic containers and urine volume was assessed gravimetrically. Blood samples (150 $\mu$l) were collected hourly from the carotid artery into heparin-treated microhaematocrit tubes. These were centrifuged and the plasma immediately separated for determination of [$^3$H]-inulin activity. At the end of the experiment a terminal blood sample was collected from the carotid artery, centrifuged and the plasma used for determination of cation concentration and osmolality. An ultrafiltrate of terminal plasma was prepared by use of an Amicon Centrifree Micropartition System (Amicon Inc., Beverley, M.A., U.S.A.).

### Urinary enzyme excretion

In some animals urinary excretions of GGT and NAG were assessed throughout control and experimental periods. Enzyme activities were initially determined only for animals infused with the highest dose of gentamicin (for GGT $n=4$ and NAG $n=3$). Further experimental groups were then studied ($n=3$). Aliquots of urine (0.5 ml) were passed through separation columns containing Sephadex G-25 medium (Pharmacia NAP-5 Columns; Pharmacia LKB, Sweden) to remove salts and inhibitory substances. The urine was then eluted from the columns with 1.0 ml of 0.9% NaCl and stored at 4°C before analysis which was always performed within 48 h of urine collection.

### Light and electron microscopy

At the end of the experiment the kidneys from a number of animals were fixed by *in situ* perfusion. The abdominal aorta was tied off at a point above the renal arteries and a catheter attached to a 19 gauge needle inserted into the aorta at a point below the kidneys. This catheter was attached to a 50 ml syringe containing a solution of sterile 0.9% NaCl with 25 iu $ml^{-1}$ ammonium heparin (Sigma Chemical Company, Dorset, U.K.) and 0.4 mg $ml^{-1}$ lignocaine hydrochloride (Astra Pharmaceuticals, Langley, U.K.). The kidneys were perfused with this solution at 8.5 ml $min^{-1}$ to clear them of blood. The vena cava was severed to allow drainage of perfusate from the kidneys. The kidneys were then perfused at the same rate with 30 ml of Karnovsky's fixative. This comprised a solution of 0.1 M Sorenson's phosphate buffer (0.1 M sodium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate; pH 7.4) containing 2% paraformaldehyde and 2.5% glutaraldehyde (Sigma Chemical Company, Dorset, U.K.). The left kidney was removed and sectioned. For this, a 2 mm mid-coronal slice was taken and tissue samples (1 $mm^3$) from the outer and mid-cortex placed in Karnovsky's fixative for 30 min. Samples were then washed in phosphate buffer (pH 7.4), post-fixed for one hour in 1% osmium tetroxide (Johnson Matthey Materials, Herts, U.K.) in phosphate buffer (pH 7.4), rapidly dehydrated in graded ethanol solutions and embedded in Araldite CY 212 resin (Agar Scientific Ltd., Essex, U.K.).

For light microscopy, semi-thin (1 $\mu$m) sections were cut by use of a Leica ultramicrotome and stained with basic fuchsin and methylene blue (Sigma Chemical Company, Dorset, U.K.). For electron microscopy ultra-thin (60–90 nm) sections were cut and stained with saturated alcoholic uranyl acetate and Reynold's lead citrate (Agar Scientific Ltd., Essex, U.K.).

### Analytical techniques

Urine, terminal plasma and plasma ultrafiltrates were analysed for calcium and magnesium by atomic absorption spectrophotometry (Perkin Elmer 3100; Norwalk, C.T., U.S.A.). Sodium and potassium concentrations were determined by flame emission spectrophotometry (Corning 480; Corning Medical and Scientific Ltd., Essex, U.K.). Plasma osmolality was determined by freezing point depression (Roebling Camlab; Cambridge, U.K.). Urinary enzyme activities were determined colorimetrically by use of commercially available kits (Boehringer-Mannheim, Sussex, U.K.) and a centrifugal analyser (Cobas Fara 2; Roche Diagnostics, Herts, U.K.). The substrates for the GGT and NAG assays were $\gamma$-glutamylcarboxy-4-nitroanilide and 3,3′ dichlorophenol sulphophthalein-N-acetyl-$\beta$-D-glucosaminidine, respectively. [$^3$H]-inulin in urine and small plasma samples was assessed by liquid scintillation counting by use of Optiphase MP (FSA Laboratory Supplies, Leicester, U.K.) as scintillant. Appropriate corrections were made for quenching by means of the transformed spectral index of the external standard (tSIE) as the quench indicating parameter.

### Calculations and statistics

All data are presented as means ± s.e.mean. Glomerular filtration rate (GFR) was calculated as the clearance of inulin $C$in = Uin.$V$/Pin and fractional solute excretion as Ux.Pin/Uin.Px where U and P are the urinary and plasma concentrations of inulin (in) or solute (x), respectively, and $V$ is urine flow rate. The recovery of urine (%) was calculated as urine flow rate/infusion rate × 100. Urinary enzyme activity was calculated as (UENZ.$V$)/(BW/100), where UENZ = measured enzyme activity in urine eluates and BW = body weight.

All statistical analyses were performed by use of the Statistical Package for Social Sciences (SPSS). For urine and blood pressure data a multivariate analysis of variance (MANOVA) for repeated measures was used for statistical comparison between the groups over control and experimental periods. Significant differences between control and gentamicin infused groups during the experimental period were further analysed by a planned contrast MANOVA. Terminal plasma

and body weight data were analysed by one way analysis of variance (ANOVA) with Scheffe's *post hoc* test.

## Results

### *General data*

Mean body weights were comparable between the five groups of rats at the start of the experiment. Values were (g): 259±11, 251±9, 269±13, 286±8 and 272±18 for controls and rats infused with increasing drug doses, respectively. Mean arterial blood pressure was stable throughout the experimental protocol and was not significantly affected by any dose of gentamicin infusion (Table 1). Gentamicin infusion also had no significant effect on urine flow rate. Flow rates were comparable between the five groups during both control and experimental periods. Data for the final clearance period are shown in Table 1. In terms of the volume of saline infused the mean recovery of urine from all experiments during the control and experimental periods was 104±5.6% ($n=40$). In control animals GFR was stable throughout the experimental protocol (Figure 1). Moreover, there were no significant differences in GFR between any of the groups during the control period. However, during the experimental period GFR was significantly ($P<0.05$) depressed by approximately 10–20% in animals infused with gentamicin at the highest dose. At lower doses no significant changes were apparent.

### *Urinary calcium and magnesium excretion*

Figure 2 presents data for urinary calcium excretion. There were no significant differences in the fractional excretion of calcium between any of the groups during the control period. However, in all gentamicin infused groups there was a significant increase in fractional calcium excretion during the experimental period when compared to the control group. This hypercalciuric response was evident even after 30 min of infusion with the lowest dose of gentamicin. The hypercalciuria was fully established within 60 min of gentamicin infusion and, with the exception of the highest gentamicin dose, remained stable thereafter. It can be seen from Figure 2 that the magnitude of hypercalciuria was clearly related to the dose of gentamicin infused. Data for fractional magnesium excretion are presented in Figure 3. The response for magnesium was less pronounced and more variable than that for calcium. There

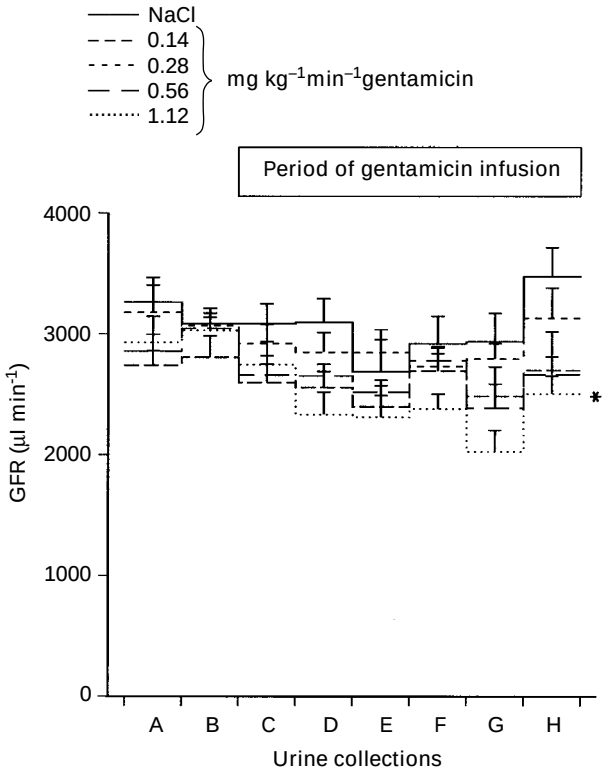


**Figure 1** Glomerular filtration rate (GFR) during control (A and B) and experimental (C–H) clearances in animals infused with 0.9% NaCl or gentamicin at 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$. Drug-infused groups received gentamicin during clearances C–H (shown by bar on figure). *$P<0.05$ (MANOVA) for gentamicin vs NaCl rats during drug infusion.

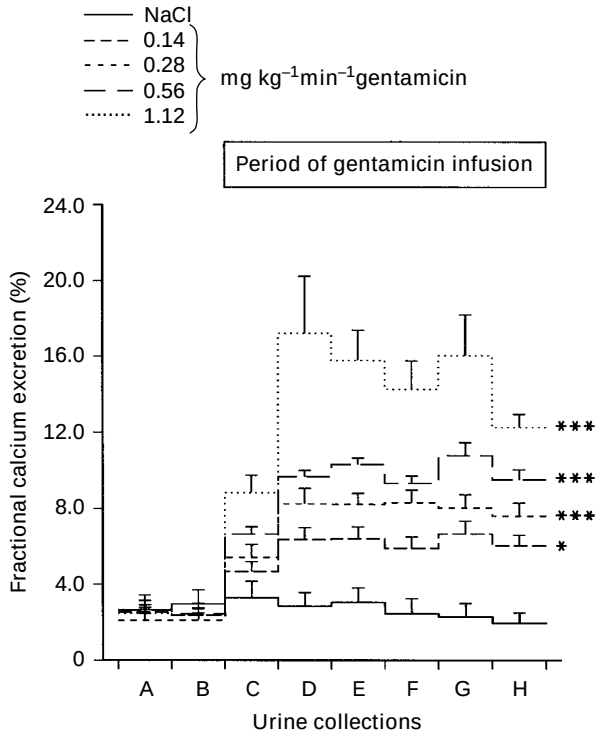


**Figure 2** Fractional calcium excretion during control (A and B) and experimental (C–H) clearances in animals infused with 0.9% NaCl or gentamicin at 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$. Drug-infused groups received gentamicin during clearances C–H (shown by bar on figure). ***$P<0.001$; *$P<0.05$ (MANOVA) for gentamicin vs NaCl rats during drug infusion.

**Table 1** Mean arterial blood pressure, urine flow rate and fractional sodium and potassium excretion for rats infused with saline or gentamicin (Gen) at 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$

| | *Saline* | *Gen 0.14* | *Gen 0.28* | *Gen 0.56* | *Gen 1.12* |
|---|---|---|---|---|---|
| MABP (mmHg) | 120±8 | 109±5 | 123±5 | 117±4 | 123±7 |
| Urine flow rate (µl $min^{-1}$) | 88.3±8.2 | 84.1±7.9 | 96.5±4.5 | 94.6±9.7 | 89.3±7.2 |
| Fractional sodium excretion (%) | 2.9±0.1 | 3.1±0.2 | 3.3±0.3 | 3.5±0.3 | 3.4±0.2 |
| Fractional potassium excretion (%) | 20.4±1.1 | 23.2±2.8 | 23.4±2.3 | 26.5±1.2 | 24.9±2.6 |

Data are presented as the mean of 3 time points during the experimental period (for blood pressure) or for the last clearance period of the experimental phase (H) for urine values. Data are means±s.e.mean. MABP: mean arterial blood pressure.

was some evidence for a dose-response relationship, although significant differences between gentamicin and control groups were seen only at higher drug doses.

### *Urinary sodium and potassium excretion and terminal plasma data*

Fractional sodium and potassium excretion were not significantly affected by gentamicin infusion. Data for the final clearance period are shown in Table 1. Terminal plasma and ultrafiltrable cation concentrations were also comparable between the five groups as was plasma osmolality (Table 2).

### *Urinary enzyme excretion*

Figure 4 presents data for urinary GGT excretion. During the experimental period the excretion of GGT was significantly ($P<0.01$) elevated in animals infused with the highest dose of gentamicin. However, a similar difference between control and experimental animals was also apparent during the control period. Excretion rates of GGT were not investigated at lower gentamicin infusion rates. Figure 5 presents data for the urinary excretion rate of NAG. During the control period NAG excretion rate was comparable between saline and gentamicin infused animals. Infusion of gentamicin was associated with a dose-dependent increase in NAG excretion which was significantly different from the control group in all except the lowest drug group.

### *Light and electron microscopy*

Figure 6 presents photomicrographs of representative semithin and ultra-thin sections taken from the mid-cortex of animals infused with the highest dose (1.12 mg $kg^{-1}$ $min^{-1}$) of gentamicin. No abnormalities in renal tubular cell morphology were observed by light or electron microscopy and morphology was comparable to that of control animals. There was no evidence for any of the morphological changes normally associated with chronic gentamicin administration (eg: proximal tubular necrosis) and no cytosegrosomes or myeloid bodies were observed ultrastructurally within proximal tubular cells. As no morphological changes were observed at the highest gentamicin infusion rate, tissues from animals infused at lower drug concentrations were not examined.

**Table 2** Terminal plasma data for rats infused with saline or gentamicin (Gen) at 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$

| | *Saline* | *Gen 0.14* | *Gen 0.28* | *Gen 0.56* | *Gen 1.12* |
|---|---|---|---|---|---|
| Sodium (mmol $l^{-1}$) | 144±1 | 145±1 | 145±1 | 146±1 | 144±1 |
| Potassium (mmol $l^{-1}$) | 3.70±0.09 | 4.08±0.11 | 3.42±0.08 | 3.72±0.05 | 3.65±0.11 |
| Calcium (mmol $l^{-1}$) | 2.35±0.10 | 2.29±0.08 | 2.18±0.07 | 2.25±0.09 | 2.15±0.07 |
| Ultrafilterable Calcium (mmol $l^{-1}$) | 1.52±0.16 | 1.53±0.06 | 1.38±0.05 | 1.54±0.06 | 1.39±0.11 |
| Magnesium (mmol $l^{-1}$) | 0.59±0.03 | 0.57±0.02 | 0.54±0.04 | 0.57±0.04 | 0.50±0.04 |
| Ultrafilterable magnesium (mmol $l^{-1}$) | 0.47±0.03 | 0.44±0.03 | 0.43±0.05 | 0.42±0.03 | 0.38±0.04 |
| Osmolality (mosmol $kg^{-1}$ $H_2O$) | 291±1 | 293±1 | 293±3 | 293±2 | 294±2 |

Data are presented as means±s.e.mean.

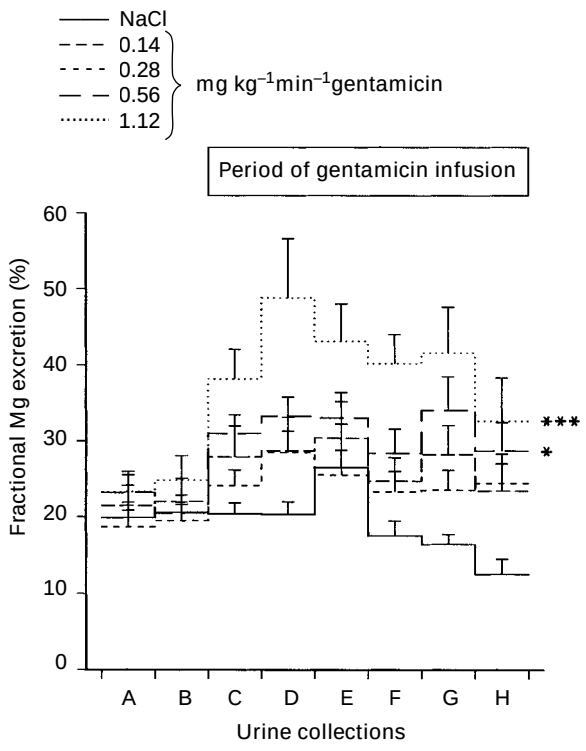


**Figure 3** Fractional magnesium excretion during control (A and B) and experimental (C–H) clearances in animals infused with 0.9% NaCl or gentamicin at 0.14, 0.28, 0.56 or 1.12 mg $kg^{-1}$ $min^{-1}$. Drug-infused groups received gentamicin during clearances C–H (shown by bar on figure). ***$P<0.001$; *$P<0.05$ (MANOVA) for gentamicin vs NaCl rats during drug infusion.

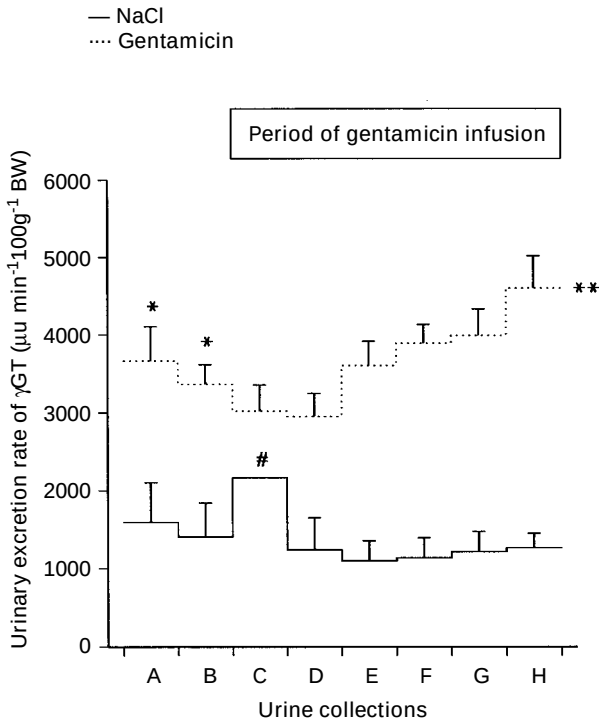


**Figure 4** Urinary excretion rate of γ-glutamyl transpeptidase during control (A and B) and experimental (C–H) clearances in animals infused with 0.9% NaCl or gentamicin at 1.12 mg $kg^{-1}$ $min^{-1}$. The latter group received gentamicin during clearances C–H (shown by bar on figure). **$P<0.01$ for gentamicin vs NaCl rats during drug infusion; *$P<0.05$ for similar comparison during control period. #This result was derived from only one observation.

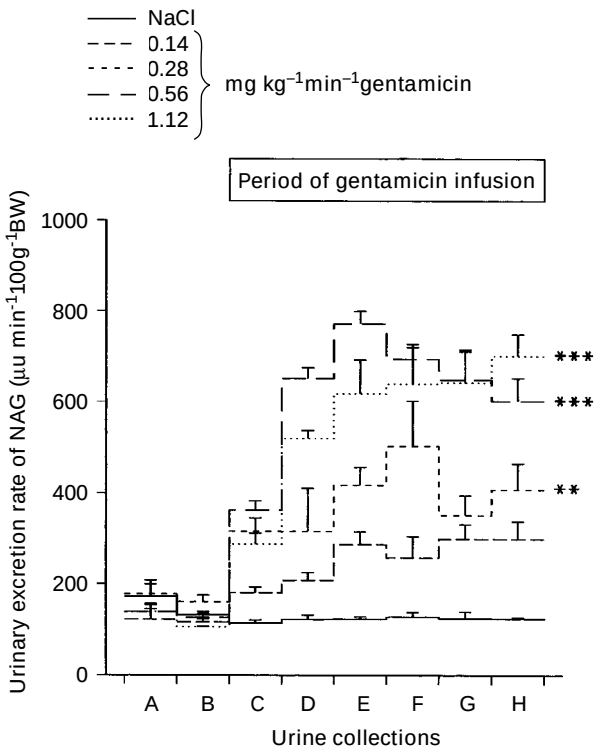


**Figure 5** Urinary excretion rate of N-acetyl-$\beta$-D-glucosaminidase (NAG) during the control (A and B) and experimental (C–H) clearances in animals infused with 0.9% NaCl or gentamicin at 0.14, 0.28, 0.56 or 1.12 mg kg$^{-1}$ min$^{-1}$. Drug-infused groups received gentamicin during clearances C–H (shown by bar on figure). ***$P<0.001$; **$P<0.01$ (MANOVA) for gentamicin vs NaCl rats during drug infusion.

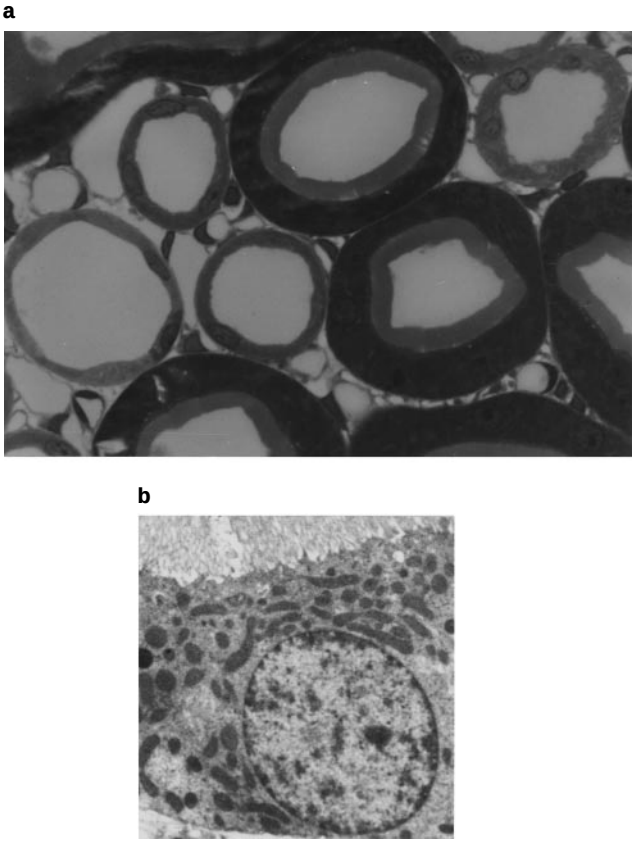


**Figure 6** (a) Basic fuchsin/methylene blue stained semi-thin section from mid-cortex of animal infused with gentamicin at 1.12 mg kg$^{-1}$ min$^{-1}$ (× 450 magnification). Proximal and distal tubular cell morphology is comparable to saline-infused controls. (b) Electron micrograph of proximal tubular cell from animal infused with gentamicin at 1.12 mg kg$^{-1}$ min$^{-1}$ (×11,500). Cellular morphology appears normal and no myeloid bodies are evident.

## Discussion

The rapid increase in urinary calcium and magnesium excretion seen here within 30 min of the onset of gentamicin infusion confirms the observations of Foster *et al.* (1992) and Elliott and Patchin (1992). However, our study is the first to demonstrate a dose-response relationship between the magnitude of divalent cation excretion and dose of gentamicin infused. The dose-response curve for calcium was more pronounced than that for magnesium. Indeed, for the latter ion, significant differences between gentamicin and control groups were seen only with higher doses of the drug. Moreover, the magnesiuric response to gentamicin was less pronounced, demonstrated a higher threshold and was not as sustained as that for calcium. The lack of effect of any dose of gentamicin on renal sodium and potassium handling supports earlier suggestions (Foster *et al.*, 1992; Elliott and Patchin, 1992) that the acute effects of gentamicin on renal electrolyte handling may be specific for the divalent cations.

The acute urinary calcium and magnesium losses observed in the present study may have implications for the decreased plasma levels of these ions observed in some patients receiving aminoglycoside therapy. Although no decrease in plasma electrolyte levels was recorded in the present study, previous acute studies in our laboratory (Foster *et al.*, 1992; Garland *et al.*, 1994) have demonstrated hypomagnesaemia after three hours of gentamicin infusion. Unpublished data from our laboratory have found steady state serum gentamicin concentrations of 32.6 $\mu$g ml$^{-1}$ in animals infused with gentamicin at 0.28 mg kg$^{-1}$ min$^{-1}$. Assuming linear pharmacokinetics, steady state serum values should be approximately half this value with a 0.14 mg kg$^{-1}$ min$^{-1}$ drug infusion. Given a half-life of 30 min for gentamicin (Garland *et al.*, 1994), after a one hour infusion at this lower dose, serum gentamicin levels should be 75% of steady state values, i.e. 12.2 $\mu$g ml$^{-1}$. The present study demonstrated a two fold increase in fractional calcium excretion by this time (Figure 2 urine collection D). In patients receiving gentamicin therapy, a peak serum gentamicin concentration of 10 $\mu$g ml$^{-1}$ can readily be attained. This value therefore approaches that which produced a two fold increase in fractional calcium excretion in our rat model.

The present study is the first to demonstrate unequivocally that gentamicin-induced changes in renal electrolyte handling can occur independently of and before the development of proximal cell injury. Using both light and electron microscopic evaluation, we have observed no change in renal tubular cell morphology after infusing 200 mg kg$^{-1}$ gentamicin over three hours, despite a significant hypercalciuria evident within the first 30 min. The histological findings of the present study are in agreement with those of Williams *et al.* (1981) who, using light microscopy, observed no change in proximal tubular cell morphology of rats 1.5 h after a single i.p. injection of 50–350 mg kg$^{-1}$ gentamicin. However, these authors did show an increased number of cytosegrosomes within proximal tubular cells examined by electron microscopy. Such structures were not observed in the present study in animals infused with gentamicin at a rate of 1.12 mg kg$^{-1}$ min$^{-1}$. Differences between the two studies may result from different experimental protocols. Thus, in the present study, anaesthesia and/or the high rate of saline infusion may have afforded protection against cytosegrosome formation. The histological and ultrastructural findings in the present study nevertheless support the use of this experimental model to distinguish between the primary pharmacological actions of gentamicin on the kidney and those occurring secondary to renal tubular injury. Since an

acute renal response to gentamicin (particularly for calcium) can be detected before the development of nephrotoxicity, it is possible that an early disruption of renal electrolyte handling may contribute to subsequent renal cell injury. Such a hypothesis was first proposed by Harpur *et al*. (1985) in the absence of any histological evidence.

In the present study there were no differences in terminal plasma cation levels between control and gentamicin infused animals and, with the exception of the highest gentamicin dose, there were no differences in GFR between the two groups. Thus, the filtered loads of calcium and magnesium were not altered by gentamicin infusion, confirming previous studies (Foster *et al*., 1992; Elliott and Patchin, 1992) that the hypercalciuria and hypermagnesiuria result from a decreased tubular reabsorption of these ions. The precise nephron site where gentamicin acts to reduce renal tubular calcium transport has recently been located at the level of the early distal tubule (Parsons *et al*., 1995). Comparable information is not yet available for magnesium. Also unknown is the cellular mechanism whereby gentamicin inhibits the reabsorption of the two ions. Perhaps the simplest mechanistic explanation for gentamicin-induced hypercalciuria would be chelation of calcium by gentamicin within the tubular lumen thereby preventing luminal uptake. However, gentamicin has been shown not to chelate calcium in phosphate buffered saline or human serum (Kohlepp *et al*., 1982), suggesting that this may not occur *in vivo*. Aminoglycosides have been shown to inhibit calcium channels in other tissues (Adams and Durrett, 1978; Mack *et al*., 1992). Luminal calcium channels have been shown to mediate calcium uptake by distal tubular epithelial cells (Bacskai and Friedman 1990). It is possible, therefore, that gentamicin may induce hypercalciuria by inhibiting calcium channels demonstrated to be present within the luminal membrane of the distal tubule (Friedman and Gesek, 1994). The mechanisms for basolateral calcium extrusion from distal cells include calcium ATPase and sodium-calcium exchange. There is no evidence for a direct effect of gentamicin upon either transport system. However, the activity of the sodium-calcium exchanger is dependent upon the gradient for sodium maintained by the activity of the sodium/potassium ATPase pump. Gentamicin can inhibit sodium/potassium ATPase activity in renal tissue (Cronin *et al*., 1982; Williams *et al*., 1984) and sodium-dependent calcium reabsorption is decreased under such conditions (Ullrich *et al*., 1976). It is possible, therefore, that gentamicin may inhibit sodium/potassium ATPase and thus reduce the gradient for sodium driving basolateral sodium-calcium exchange. Given the putative location of the sodium-calcium exchanger within the nephron (Ramachandran & Brunette, 1989), such a mechanism may occur distally.

Our study is the first to measure urinary enzyme excretion during acute gentamicin infusion. There is no apparent explanation for the significant difference in urinary excretion rate of GGT between saline and gentamicin infused animals during the control period, when both groups received identical saline infusions. However, this does preclude attributing the significant effect observed during the experimental period to an action of gentamicin *per se*. The present study has demonstrated for the first time that acute gentamicin infusion induces a rapid dose-related increase in urinary NAG excretion. Given that NAG is not filtered at the glomerulus and that gentamicin did not alter glomerular morphology or, with the exception of the highest drug dose, GFR, then the increased NAG excretion must be of tubular origin. Although NAG is located predominantly within the proximal tubule (Mellman *et al*., 1986), no conclusions can be drawn from the present study regarding the precise tubular origin of the enzyme. An increased urinary NAG excretion is normally interpreted as indirect evidence of tubular injury and has been shown to occur in patients within 2–3 days of the onset of aminoglycoside therapy (Gibey *et al*., 1981). In the present study, an increased urinary NAG excretion occurred within hours, rather than days, of the onset of drug treatment. When an increased urinary NAG excretion is seen before the development of histologically detectable tubular injury it is usually interpreted as the biochemical harbinger of morphological injury. However, when it occurs in the absence of ultrastructural changes, a possible explanation may be that gentamicin is simply interfering with the cellular cycling of the enzyme. For example, it has been demonstrated in human cultured fibroblasts that weak bases interfere with the acid pH-dependent dissociation of lysosomal enzyme-receptor complexes (Gonzalez-Noriega *et al*., 1980) and are, therefore, unable to process endogenous, or internalize exogenous, lysosomal enzymes. These changes are associated with an increased release of NAG into the tubular medium which is analogous to an increased release of NAG into the tubular lumen *in vivo*. The increase in urinary calcium output in the present study appeared to parallel that of NAG excretion, suggesting a mechanistic link between the two processes. However, since the site of decreased calcium reabsorption appears to be in the early distal tubule (Parsons *et al*., 1995) and NAG is located predominantly proximally (Mellman *et al*., 1986), it is possible that the effects on calcium and NAG, although concomitant, may occur at independent sites within the nephron.

In conclusion, therefore, this study has demonstrated that the magnitude of hypercalciuria and hypermagnesiuria following acute gentamicin infusion is related to the dose of drug infused. Such responses are independent of renal tubular cell injury and represent an effect of gentamicin *per se* on renal calcium and magnesium handling. A rapid increase in urinary NAG excretion also occurs in response to gentamicin infusion and, in the absence of any changes in lysosomal morphology, may indicate an effect of gentamicin on the cellular uptake or intracellular transport of NAG. These observations indicate the potential for rapid biochemical responses to gentamicin administration and may have implications for patients receiving this class of antibiotic.

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