AMINOGLYCOSIDE-INDUCED CHANGES IN THE RENAL HANDLING OF CATIONS IN THE FISCHER 344 RAT

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Disturbances of cation homeostasis, particularly hypomagnesaemia, are a frequent consequence of treatment with aminoglycoside antibiotics. These disturbances are thought to result from renal wasting of cations and administration of gentamicin to rats has been shown to produce hypercalciuria and hypermagnesiuria.

The aims of this study were to attempt to elucidate these responses in anaesthetised rats infused with gentamicin and to use this model to investigate the mechanisms of these effects.

Fischer 344 rats were anaesthetised and surgically prepared for clearance experiments. Infusion of gentamicin in isotonic saline increased urinary output of calcium and magnesium while sodium and potassium output were unaffected. These elevations in calcium and magnesium excretion were explained by reduced tubular reabsorption of these cations. Both the hypercalciuric and hypermagnesiuric responses to gentamicin were extremely rapid and were sustained during drug infusion; when gentamicin infusion ceased both responses were rapidly reversible. Infusion of another aminoglycoside, tobramycin, produced very similar effects to gentamicin. The hypercalciuria and hypermagnesiuria caused by gentamicin infusion were unaffected by parathyroidectomy. The peak increases in calcium and magnesium output brought about by infusion of gentamicin with frusemide were not significantly different to the increases produced by frusemide alone.

The site at which gentamicin interferes with calcium and magnesium reabsorption cannot be firmly deduced from these results. However, the known close association between calcium and sodium reabsorption in the proximal tubule implies that gentamicin is unlikely to change proximal calcium reabsorption without a similar change in proximal sodium reabsorption. The similarity between the hypercalciuric and hypermagnesiuric effects of frusemide alone and the effects of frusemide infused simultaneously with gentamicin suggests that gentamicin may act at the same site as the diuretic, the thick ascending limb of the loop of Henle.

Key words: calcium; magnesium; gentamicin; renal wasting of electrolytes; aminoglycoside nephrotoxicity.

Dedicated, with respect and affection, to my parents.

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ABBREVIATIONS

ADH	Antidiuretic hormone.
C_x	Clearance of x.
Ca	Calcium.
cAMP	Cyclic adenosine-3', 5-monophosphate.
DOCA	Deoxycorticosterone acetate.
E_x	Excretion rate of x.
FE_x	Fractional excretion of x.
GFR	Glomerular filtration rate.
K	Potassium.
Mg	Magnesium.
Na	Sodium.
Na/K-ATPase	Sodium/potasssium adenosine triphosphatase.
P_x	Concentration of x in plasma.
PTH	Parathyroid hormone.
PTX	Parathyroidectomy, parathyroidectomised.
TF_x	Concentration of x in tubular fluid.
TF_x/UF_x	Ratio of concentration of x in tubular fluid to concentration of x in glomerular ultrafiltrate.
TF_x/P_x	Ratio of concentration of x in tubular fluid to concentration of x in plasma.
TPTX	Thyroparathyroidectomy, thyroparathyroidectomised.
U_x	Concentration of x in urine.
UF_x	Concentration of x in glomerular ultrafiltrate.
$\%UF_x$	Concentration of x in glomerular ultrafiltrate expressed as a percentage of the concentration of x in plasma
V	Urine flow rate.

CHAPTER 1: INTRODUCTION

1.1 Aminoglycoside nephrotoxicity

The aminoglycoside antibiotics are widely used in the treatment of serious infections caused by Gram-negative organisms. The usefulness of these agents is limited chiefly by their toxicity to the kidney (Appel and Neu, 1978). The members of this group of drugs are characterised by a chemical structure consisting of one or more amino sugars joined by a glycosidic linkage. They are extremely polar polycations with pKa values of 8.0 or higher and are therefore very water soluble but do not penetrate cell membranes readily.

Aminoglycosides are excreted almost exclusively by the kidney (Schentag and Jusko, 1977). The clearance of gentamicin, the aminoglycoside most commonly used in experimental work and clinical practice, approaches that of inulin (Gyselynck et al, 1971). In clearance experiments in the anaesthetised rat the clearance of gentamicin was 80% of GFR, rising to equal GFR during extracellular volume expansion (Senekjian et al, 1981).

Aminoglycosides are freely filtered at the glomerulus and rapidly taken up by proximal tubular cells (Pastoriza-Munoz et al, 1979; Vandewalle et al, 1981; Senekjian et al, 1981; Just et al, 1977), a process which appears to require energy (Kluwe and Hook, 1978a). Autoradiographic studies suggest that gentamicin is bound to the apical membrane of the proximal tubular cell, taken up by pinocytosis and rapidly transferred to lysosomes (Silverblatt and Kuehn, 1979). This uptake occurs predominantly at the apical, as opposed to the basolateral, membrane (Collier et al, 1979) and the binding sites are apparently composed of anionic phospholipids (Sastrasinh et al, 1982a; Kaloyanides and MacLaughlin, 1983). Binding of aminoglycosides can be inhibited by other

aminoglycosides (Sastrasinh et al, 1982a) and calcium (Humes et al, 1984). The drugs are accumulated in renal proximal tubular cells where they may persist for days (Schentag and Jusko, 1977; Fabre et al, 1976) particularly in the proximal straight tubule. The renal cortical half-life may be 100 hours compared with a serum half-life of 2 hours in humans and 30 minutes in the rat (Luft and Kleit, 1976).

In order to study the functional and morphological changes produced during the development of aminoglycoside nephrotoxicity it has been necessary to develop animal models to eliminate the variables in patients undergoing treatment for various infections. The dose of aminoglycoside required to produce renal failure in dogs or rats is often ten times the therapeutic dose in humans calculated on a body weight basis (Falco et al, 1969; Cronin et al, 1980). However, some strains, such as the Fischer 344 rat, exhibit sensitivity to aminoglycoside nephrotoxicity similar to that of humans (Kosek et al, 1974).

One of the earliest features of experimental aminoglycoside-induced nephrotoxicity is a decrease in urine concentrating capacity (Houghton et al, 1976; Kosek et al, 1974; Cohen et al, 1975; Luft et al, 1975); this appears to mimic the non-oliguric renal failure observed in man (Anderson et al, 1977). Proteinuria (Luft et al, 1975; Soberon et al, 1979) and enzymuria (Wellwood et al, 1976; Luft et al, 1975; Patel et al, 1975; Beck et al, 1977) are also relatively early markers of toxic damage. Other changes include stimulation of organic acid transport (Cohen et al, 1975), glucosuria (Ginsberg et al, 1976) and reduced ammoniagenesis (Kluwe and Hook, 1978b). Depression of GFR is a relatively late manifestation of aminoglycoside nephrotoxicity and appears to be a result of a decrease in the glomerular capillary ultrafiltration coefficient (Baylis et al, 1977).

The histological changes induced by aminoglycosides occur almost

exclusively in the proximal tubule (Houghton et al, 1976; Kosek et al, 1974). The earliest identifiable lesion produced is an increase in the number and size of secondary lysosomes containing myeloid bodies (Kosek et al, 1974). The progression of tubular damage produces decreased height and number of brush border microvillae, mitochondrial swelling and cytoplasmic vacuolisation eventually resulting in total disruption of intracellular organisation and frank cellular necrosis (Houghton et al, 1976).

Factors affecting the severity of the toxic insult produced by aminoglycosides include the drug used (Houghton et al, 1978; Luft et al, 1978a) and the dosage regimen followed (Frame et al, 1977; Bennett et al, 1979). Experimental aminoglycoside nephrotoxicity is also exacerbated by increasing age (Chonko et al, 1979), male gender (Bennett et al, 1982), dietary sodium restriction (Bennett et al, 1976), dietary potassium restriction (Brinker et al, 1981), metabolic acidosis (Elliott et al, 1980; Hsu et al, 1974) and magnesium depletion (Rankin et al, 1984). Conversely, protection from nephrotoxic insult is provided in younger animals and by female gender and sodium, potassium and magnesium repletion. Protection is also afforded by oral calcium loading (Bennett et al, 1982b; Quarum et al, 1984; Humes et al, 1984), parathyroidectomy (Bennett et al, 1985; Elliott et al, 1987) and by streptozotocin-induced diabetes (Texeira et al, 1982).

An interesting feature of the toxic injury produced by aminoglycosides is that, following necrosis and shedding of proximal tubular cells, regeneration of these cells can occur, often coexisting with continuing necrosis (Cuppage et al, 1977; Houghton et al, 1976). There is evidence that these regenerating cells have a raised threshold to injury (Luft et al, 1978b; Gilbert et al, 1979) although the mechanism of this effect is unknown.

The cellular mechanism(s) by which aminoglycosides produce toxic

injury in proximal tubular cells are unknown. The accumulation of these drugs within lysosomes and the increase in their size and number has led to speculation that an effect on these organelles is involved in the mechanism of toxicity. Myeloid bodies are thought to represent an accumulation of undegraded phospholipids (Hruban et al, 1972) which may progress to phospholipidosis since phospholipidosis has been detected concurrently with myeloid body formation in cultured fibroblasts (Aubert-Tulkens et al, 1979) and in the renal cortex (Feldman et al, 1982). Aminoglycoside-induced phospholipidosis may be due to an inhibition of phospholipases (Laurent et al, 1982; Lipsky and Lietman, 1982; Brill and Kaloyanides, 1982). Gentamicin has been reported to have a two-fold effect on lysosomal function (Powell and Reidenberg, 1982) stabilising lysosomal membranes at low concentrations but labilising at higher concentrations, these effects were also produced by the nephrotoxic polyamines, spermine and spermidine. Morin et al (1980) also reported in vivo labilisation of lysosomes following gentamicin administration. These observations have led to the lysosomal dysfunction hypothesis (Kaloyanides and Pastoriza-Munoz, 1980) acording to which interference with normal lysosomal degradation processes may deprive the cell of substrates necessary for the maintenance of cellular integrity. Alternatively, accumulation of aminoglycosides and formation of myeloid bodies may labilise lysosomal membranes leading to the release of potent hydrolases causing cell damage.

Alterations in renal cationic homeostasis have also been reported following aminoglycoside administration. Inhibition of Na/K ATPase has been reported (Williams et al, 1981; Chahwala and Harpur, 1982; Cronin et al, 1982) which resulted in reduced renal cortical content of potassium and magnesium and increased calcium and sodium (Cronin et al, 1982). However, these latter results may simply be a

consequence of cellular overload with predominantly extracellular cations associated with cellular necrosis (Farber, 1981). The significance of effects on renal mitochondria is similarly ambiguous, at low doses reduced mitochondrial uptake of calcium is evident (Sastrasinh et al, 1980b), at higher doses effects on mitochondrial respiration are produced (Weinberg et al, 1980a) and these are apparently related to displacement of magnesium from the inner mitochondrial membrane (Weinberg et al, 1980b). These responses have only been observed in vitro but in vivo stimulation of O_2 consumption has been detected following exposure to gentamicin (Cuppage et al, 1977; Simmons et al, 1980).

1.2 Renal handling of cations

a) Calcium:

Calcium is present in plasma in three fractions: free ionised calcium, calcium complexed to anions and calcium bound to plasma proteins. Only free calcium and complexed calcium can cross the glomerulus and direct measurement of calcium concentrations in the glomerular filtrate in Munich-Wistar rats indicated that approximately 60% of total plasma calcium is filtered (Harris et al, 1974; Lassiter et al, 1963; leGrimellec et al, 1975). This value agrees with data obtained by ultrafiltration of plasma across artificial membranes (leGrimellec et al, 1975; Torribara et al, 1957).

Normally, aproximately 98% of the filtered load of calcium is reabsorbed by the renal tubules, of this about 60% is reabsorbed in the proximal tubule. In this segment the ratio of the calcium concentration in the tubular fluid (TF_{Ca}) to the calcium concentration in plasma ultrafiltrate (UF_{Ca}) remains at a value of approximately 1.1 in rat and dog (Lassiter et al, 1963; Harris et al, 1974; Duarte and Watson, 1967; Morel et al, 1969; Edwards et al, 1971; Buerkert et al, 1972; Agus et al, 1973; deRouffignac et al, 1973) which is consistent with passive reabsorption of calcium through a permeable epithelium. This ratio (TF_{Ca}/UF_{Ca}) remains unchanged during most experimental manoeuvres with some exceptions, during acute calcium loading TF_{Ca}/UF_{Ca} is seen to rise (leGrimellec et al, 1974; Edwards et al, 1974) possibly indicating an increase in the amount of calcium complexed to anions, which is filtered but not reabsorbed. TF_{Ca}/UF_{Ca} falls during osmotic diuresis (Lassiter et al, 1963; Duarte and Watson, 1967) suggesting an active component of proximal calcium reabsorption. Parathyroidectomy has also been suggested to raise proximal TF_{Ca}/UF_{Ca} in the rat (Kuntziger et al, 1974) although this was not confirmed in other studies (Edwards et al, 1972;

Buerkert et al, 1972; Beck and Goldberg, 1973; Agus et al, 1977). As stated above, proximal calcium reabsorption is assumed to be passive and is generally considered to parallel sodium and water reabsorption in this segment (Frick et al, 1965; Brunette and Aras, 1971; Shirley et al, 1976; Lassiter et al, 1963). Microperfusion experiments in the rat have demonstrated a substantial backflux of calcium into the luminal fluid of the proximal tubule indicating high permeability to this solute (Murayama et al, 1972). Evidence for an active component of proximal calcium reabsorption has been provided by stop-flow microperfusion experiments (Ullrich et al, 1976) which showed that this active reabsorptive flux was inhibited by ouabain and low luminal sodium concentration, therefore both passive and active calcium fluxes are apparently sodium-dependent.

Approximately 20-30% of the filtered load of calcium is reabsorbed between the late proximal convoluted tubule and the early distal tubule, this portion of the nephron is composed of the proximal straight tubule, the thin descending and ascending limbs and the thick ascending limb of the loop of Henle.

Experiments in which the papillary tip of the loop of Henle was exposed in the desert rodent, psammomys obesus (deRouffignac et al, 1973) and rat (Jamison et al ,1974) have both shown that, although late proximal TF_{Ca}/UF_{Ca} and TF_{Na}/P_{Na} were approximately equal, TF_{Ca}/UF_{Ca} was significantly lower than TF_{Na}/P_{Na} at the tip of the loop. Since the thin descending limb of the loop is practically impermeable to calcium and has a low permeability to sodium (Rocha et al, 1977) this indicates net addition of sodium in the proximal straight tubule or substantial reabsorption of calcium in this segment. These results may be explained by active calcium transport which has been demonstrated in isolated rabbit proximal straight tubule (Rouse et al, 1980; Almeida et al, 1978) which may represent

component of total calcium reabsorption.

Calcium reabsorption has also been demonstrated in the thick ascending limb of the loop of Henle. This calcium flux was found to be fully explicable by the lumen-positive potential difference generated by active chloride transport in two studies (Bourdeau and Burg, 1979; Shareghi and Stoner, 1978) whereas calcium reabsorption was greater than accounted for by passive forces in other studies (Rocha et al, 1977; Imai, 1978; Suki et al, 1980). This discrepancy is possibly accounted for by marked functional heterogeneity of the cortical and medullary portions of this segment (Suki et al, 1980).

The segments distal to the loop of Henle account for approximately 10% of total calcium reabsorption. The fractions of calcium and sodium reabsorbed up to the early distal tubule are approximately equal (Edwards et al, 1974; Agus et al, 1977); however, dissociation of calcium from sodium reabsorption occurs in the distal nephron (Costanzo and Windhager, 1978; Shareghi and Stoner, 1978). Active calcium reabsorption must occur in the distal convoluted tubule because the transepithelial potential difference is lumen-negative in this segment. The distal tubule is a heterogeneous structure consisting of a 'bright', a 'light' and a 'granular' portion, these portions differ in their response to parathyroid hormone (PTH) and other factors (Morel et al, 1976; Chabardes et al, 1975).

A small but significant proportion of filtered calcium is reabsorbed in the collecting tubule system (Lassiter et al, 1963; leGrimellec et al, 1973; Agus et al, 1977). The collecting duct, like the distal tubule, is a heterogeneous structure composed of a 'granular' and a 'light' portion; active calcium transport has been demonstrated in the 'granular' portion whereas the 'light' portion has a low permeability to calcium (Shareghi and Stoner, 1978).

Those factors known to influence calcium excretion generally exert

their effects in those segments distal to the loop of Henle. While parathyroid hormone is known to enhance overall calcium reabsorption (Kleeman, 1961) it has been shown to increase delivery of calcium to the late proximal tubule (Sutton et al, 1976; Agus et al, 1973). Enhancement of calcium reabsorption by PTH has been reported in the cortical thick ascending limb of the loop of Henle (Suki et al, 1980; Bourdeau and Burg, 1980; Imai, 1981) and in the 'granular' portions of the distal tubule and collecting duct (Shareghi and Stoner, 1978; Imai, 1981) when studied in vitro. This effect may be mediated by cAMP since these sites are reported to possess PTH-sensitive adenylate cyclase activity (Chabardes et al, 1975; Morel et al, 1976; Imai, 1981) and cAMP was shown to mimic the effects of PTH in these segments (Bourdeau and Burg, 1980; Imai, 1981). However, in vivo, the parallel effects of PTH and cAMP have not been consistently found; in the dog (Agus et al, 1973) both PTH and cAMP increased distal delivery of calcium but only PTH reduced fractional excretion of calcium by stimulating calcium reabsorption beyond the distal puncture site. Similarly, cAMP did not reduce the fractional excretion of calcium in parathyroidectomised (PTX) rats (Kuntziger et al, 1974) although cAMP was effective in reducing calcium excretion in the hamster (Burnatowska et al, 1976). There may be important species differences in the response to PTH. Buerkert et al (1972) and Agus et al (1977) demonstrated that TPTX did not affect calcium reabsorption proximal to the distal puncture site but markedly reduced rabsorption beyond this point in the rat. In the TPTX dog, in contrast, Sutton et al (1976) showed an effect of PTH both prior to and, especially, beyond the distal puncture site.

Vitamin D is associated with increased calcium excretion, although this may simply be a response to its primary effect of enhancing intestinal calcium absorption and thereby increasing plasma calcium concentration. However, an increase in calcium excretion without any

change in plasma calcium concentration or GFR has been reported to be produced by vitamin D (Alon et al, 1983). In parathyroid-ectomised (PTX) rats vitamin D has been reported to increase calcium excretion (Rizzoli et al, 1977) and to increase calcium reabsorption (Puschett et al, 1972). Therefore, the direct renal actions of vitamin D are extremely contradictory and investigations of individual nephron segments are required.

Calcitonin, although its extrarenal actions oppose PTH, has similar renal effect in that it has a hypocalciuric action (Sorensen and Hindberg, 1972; Rasmussen et al, 1967). However, in other studies calcium excretion was unchanged (Clark and Kenny, 1969) or increased (Aldred et al, 1970). Microperfusion experiments indicated that the hypocalciuric response was the result of enhanced calcium reabsorption in the loop of Henle (Quamme, 1980a). In isolated perfused preparations calcitonin was found increase calcium reabsorption and stimulate adenylate cyclase in medullary but not cortical portions of the thick ascending limb (Chabardes et al, 1976; Suki and Rouse, 1981).

As stated previously, the renal handling of calcium and sodium are parallel for much of the nephron and have been thought to be interdependent (Walser, 1961). Saline loading produces similar increases in calcium and sodium excretion (Poujeol et al, 1976; Blythe et al, 1968). Dissociation of calcium from sodium clearance was produced by PTH (Agus et al, 1977), mineralocorticoids (Walser, 1971) and chlorothiazide (Costanzo and Windhager, 1978).

Infusion of phosphate reduces calcium excretion (Lau et al, 1982) whereas phosphate depletion has the opposite effect (Goldfarb et al, 1977). The hypercalciuria of phosphate depletion appears to be the result of a proximal and a distal reabsorptive defect (Goldfarb et al, 1977; Quamme et al, 1976). The hypercalciuria can only be partly reversed by administration of PTH (Goldfarb et al, 1977; Quamme et

al, 1976; Coburn and Massry, 1970) suggesting that a mechanism not involving PTH is responsible for at least part of this phenomenon.

Magnesium infusion increases calcium excretion to a greater extent than sodium excretion (Massry et al, 1970) this is partly due to an inhibition of of PTH secretion by hypermagnesaemia (Buckle et al, 1968; Slatopolsky et al, 1976) and partly due to a direct action of magnesium on the medullary thick ascending limb to retard calcium reabsorption (Shareghi et al, 1983).

Alterations in acid/base balance affect calcium excretion. Thus, chronic metabolic acidosis increases calcium clearance relative to sodium (Sutton et al, 1979) whereas alkalosis reverses this effect.

The cellular transport of calcium is an area which is yet to be fully characterised. Briefly, if renal tubular cells are assumed to possess an intracellular calcium concentration of approximately 10^{-7}M, entry of calcium is presumably a passive process since movement into tubular cells wil be down a calcium concentration gradient. However, efflux of calcium at the basolateral membrane must be an active step and two mechanisms have been proposed. A Ca-ATPase has been localised in the basolateral membrane of renal cells (Kinne-Saffran and Kinne, 1974) and a Ca/Na exchange system is thought to exist at the same site (Taylor and Windhager, 1979). This latter system may partially explain the striking similarity of calcium and sodium fluxes in the proximal tubule (Frick et al, 1965) and the apparent dependence of calcium reabsorption on luminal sodium in this segment (Ullrich et al, 1976).

b) Magnesium:

Magnesium, like calcium, is present in three forms in plasma and the ultrafilterable fraction, consisting of free ionised magnesium and magnesium complexed to anions, represents approximately 70-80% of total plasma magnesium as determined by direct sampling from

surface glomeruli (Brunette and Crochet, 1975; leGrimellec et al, 1975).

In contrast to the other major cations luminal magnesium concentration rises with respect to ultrafiltrate concentration along the length of the accessible proximal tubule (Morel et al, 1969; leGrimellec et al, 1973; Quamme et al, 1978; deRouffignac et al, 1973). In the late proximal tubule TF_{Mg}/UF_{Mg} may be as high as 1.5-2.0 indicating that magnesium reabsorption in this segment is not extensive, indeed normally around 20-30% of the filtered load of magnesium is reabsorbed in the proximal tubule. These results suggest low proximal permeability to magnesium and this was confirmed by in vivo microperfusion experiments in the rat (Quamme and Dirks, 1980) and also by the observation that mannitol diuresis does not increase magnesium delivery to the late proximal tubule indicating minimal backflux of magnesium into the lumen (Wong et al, 1979). Magnesium reabsorption follows sodium and water transport but at a lower fractional rate (Poujeol et al, 1976; Wong et al, 1979) and is also directly related to the luminal magnesium concentration (Wong et al, 1983).

Magnesium transport in the proximal straight tubule has been inferred from sampling of tubular fluid at the papillary tip of the loop of Henle in the desert rat, psammomys (deRouffignac et al, 1973; Jamison et al, 1979). These experiments demonstrated that TF_{Mg}/UF_{Mg} was higher at the papillary tip than in the late superficial proximal tubule, presumably due to addition of magnesium to the tubular fluid in the proximal straight tubule or thin descending limb. In contrast, Brunette et al (1974) observed little addition of magnesium to the tubular fluid in normal rats but in magnesium-loaded rats magnesium concentrations which could not be accounted for by water abstraction were found at the bend of the loop (Brunette et al, 1975; Brunette et al, 1978). It was suggested that

magnesium secretion occurred in hypermagnesaemia mediated via the proximal straight tubule or the thin descending limb. However, the concept of magnesium secretion has not been supported by other studies in perfused superficial loops in the rat (Levine et al, 1982; Quamme and Dirks, 1980). Studies in isolated proximal straight tubules of the rabbit have indicated no magnesium flux into magnesium-free perfusate when bath concentrations of magnesium were elevated; furthermore, magnesium reabsorption from magnesium-containing perfusate was less than that of calcium or sodium (Quamme and Smith, 1984). This suggests that the proximal straight tubule has similar properties to the convoluted segment with respect to magnesium transport, although Roy (1985) stated that the straight tubule may be capable of magnesium reabsorption in excess of sodium and water.

Magnesium transport in the thin descending limb of the loop of Henle has not been directly investigated but permeability to magnesium could provide an explanation for the apparent secretion of magnesium into the loop during magnesium loading.

The major site of magnesium reabsorption is the thick ascending limb of the loop of Henle (Morel et al, 1969) 50-60% of filtered magnesium is reabsorbed at this site. The detection of high TF_{Mg}/UF_{Mg} at the papillary tip in the rat during magnesium loading in the rat (Brunette et al, 1975; Brunette et al, 1978) and in psammomys (deRouffignac et al, 1973; Jamison et al, 1979) support this contention since there is very little magnesium remaining in the early distal tubule (Morel et al, 1969; leGrimellec et al, 1973).

In vivo perfusion of the loop of Henle produced reabsorptive rates of magnesium which correlated with luminal magnesium concentration and, in this segment, fractional reabsorption rate was similar to those of calcium and sodium (Quamme and Dirks, 1980). However, increasing magnesium concentration on the basolateral side

of the loop retarded magnesium reabsorption with no effect on sodium chloride transport suggesting that hypermagnesaemia selectively decreases magnesium transport in this segment. This phenomenon led to the apparent maximum for magnesium reabsorption when plasma magnesium was progressively raised in dogs (Massry et al, 1969) magnesium reabsorption was initially stimulated due to the increased filtered load of magnesium then later depressed by the developing hypermagnesaemia. Magnesium transport in this segment is also influenced by sodium chloride flux (Quamme and Dirks, 1983) suggesting that magnesium reabsorption is the result of passive flux driven by the lumen-positive potential difference. This concept has been supported by studies in isolated thick ascending limb of the rabbit (Shareghi and Agus, 1982; Shareghi et al, 1983) and by the observation that inhibition of sodium chloride transport in the loop by frusemide also reduces magnesium reabsorption (Quamme, 1981).

TF_{Mg}/UF_{Mg} increases along the length of the superficial distal tubule and this segment normally reabsorbs only 2-5% of the filtered load of magnesium. Distal magnesium reabsorptive capacity is load-dependent, being increased by high luminal magnesium concentrations in microperfusion experiments (Quamme and Dirks, 1980) and by inhibition of reabsorption in the loop with frusemide (Quamme, 1981). However, distal magnesium reabsorption is normally close to capacity whereas calcium and sodium reabsorption is usually unsaturated.

The collecting ducts apparently play a limited role in magnesium reabsorption (Carney et al, 1980; deRouffiganac et al, 1973). Brunette et al (1978) demonstrated little magnesium transport in exposed colecting ducts in the papilla of the young rat, this was confirmed by Bengele et al (1981) in the rat by microcatheterisation of inner medullary collecting ducts. Shareghi and Agus (1979a) were unable

to detect net magnesium or calcium transport in isolated 'light' portions of rabbit cortical collecting tubule.

PTH is likely to be involved in magnesium homeostasis and PTH administration during magnesium chloride infusion has been shown to decrease the fractional excretion of magnesium in the dog (Massry et al, 1969; Massry and Coburn, 1973). Micropuncture studies have attempted to localise this effect of PTH, in the magnesium-depleted rat (Quamme et al, 1980) and in the golden hamster (Harris et al, 1979) in which the most dramatic responses are produced. In this latter study PTX increased the fractional excretion of magnesium to approximately 20%, administration of PTH promptly reduced this value to normal levels of less than 5%. These effects were mimicked by cAMP and the response was localised prior to the distal tubule, suggesting that the site of action of PTH was in the thick ascending limb, although cAMP was not effective in one study in the rat (Kuntziger et al, 1974). PTH also increases magnesium reabsorption in TPTX rats after reabsorption is initially inhibited by hypermagnesaemia or frusemide (Quamme and Dirks, 1980; Quamme, 1981) suggesting other sites of action for the hormone. The concept of PTH-stimulated magnesium reabsorption in the thick ascending limb has been supported by the detection of PTH-sensitive magnesium transport in isolated sections of rabbit thick ascending limb (Shareghi and Agus, 1979b). PTH secretion may be modulated by plasma magnesium concentration although the parathyroid glands appear to be less sensitive to changes in plasma magnesium than calcium concentration (Harris et al, 1979; Mahafee et al, 1982). Although hypomagnesaemia has been linked to reduced PTH secretion (Rude et al, 1978)) and/or decreased end-organ sensitivity to the hormone (Estep et al, 1969; Rude et al, 1976).

Calcitonin administration to the rat appears to acutely reduce both calcium and magnesium excretion (Poujeol et al, 1980; Sorensen and

Hindberg, 1972) although there appear to be species differences in the magnesium response (Brunette et al, 1979). A calcitonin-sensitive adenylate cyclase has been demonstrated (Chabardes et al, 1976) and it has been suggested that the action of calcitonin is mediated in the thick ascending limb.

Morel et al (1982) have suggested that four peptide hormones, PTH, calcitonin, antidiuretic hormone (ADH), and glucagon may produce similar effects on magnesium reabsorption due to the fact that they act at similar sites and their responses are all mediated by cAMP. For example, ADH has been shown to reduce electrolyte excretion in the TPTX Brattleboro rat (deRouffiganac et al, 1983) and ADH-sensitive adenylate cyclase activity has been detected in the medullary thick ascending limb (Imbert-Teboul et al, 1978). Calcitonin and glucagon also stimulate hormone-sensitive adenylate cyclase in medullary thick ascending limb (Bailly and Amiel, 1982; Bailly et al, 1984; Elalouf et al, 1984a) and PTH is thought to stimulate cAMP production in cortical thick ascending limb (Shareghi and Agus, 1979b). All four hormones may produce the same response, that is a stimulation of cation reabsorption mediated by changes in Na/K/Cl co-transport in the thick ascending limb, affecting sodium, potassium and calcium as well as magnesium in the mouse (Hall and Varney, 1980; Hebert et al, 1981).

A similar situation may pertain in the superficial distal tubule where glucagon, PTH (Bailly et al, 1985), calcitonin (Elalouf et al, 1983) and ADH (Elalouf et al, 1984b) may stimulate generalised electrolyte reabsorption. Hormone-sensitive adenylate cyclase has been localised at this site (Chabardes et al, 1976; Morel et al, 1982). It has been theorised that these hormones acting in concert, rather than the action of a single regulating hormone, are responsible for adjusting renal magnesium homeostasis (Quamme, 1986).

Other factors are thought to influence magnesium homeostasis, such

as vitamin D status (Meintzer and Steenbock, 1955) the adrenocortical steroids (Massry et al, 1967a) growth hormone and the sex hormones, the effects of these are small and are often secondary to other responses (Massry and Coburn, 1973).

Infusion of saline produces increased magnesium excretion (Massry et al, 1967b) this has been shown to be due to reduced proximal magnesium reabsorption (Brunette et al, 1969). The increase in the fractional excretion of magnesium produced by this manoeuvre is approximately double the increase in the fractional excretion of sodium due to the increase in distal delivery of both cations where magnesium reabsorption is overwhelmed but sodium reabsorptive capacity is able to conserve some of the delivered load (Quamme, 1986).

Calcium is known to influence renal magnesium transport, hypercalcaemia inhibits magnesium reabsorption in the dog (Coburn et al, 1970) and the rat (leGrimellec et al, 1974). In vivo microperfusion experiments in the rat (Quamme, 1980b) demonstated that hypercalcaemia inhibited both calcium and magnesium reabsorption without affecting sodium transport in the loop of Henle. Generally, high plasma calcium and magnesium retard magnesium reabsorption more than calcium. These results suggest the existence of a calcium/magnesium interaction at the basolateral membrane of the thick ascending limb by which absorption of either ion is reset by changes in the plasma concentrations of both (Quamme and Dirks, 1980). These observations have been confirmed by studies on isolated rabbit tubules (Shareghi and Agus, 1982).

The cellular aspects of renal magnesium transport remain entirely speculative. Like calcium, magnesium moves down an electrical gradient from lumen to tubular cell, unlike calcium this movement is against a concentration gradient. At the basolateral membrane magnesium must be transported against its electrochemical gradient

and therefore active mechanisms must exist. The existence of a magnesium-activated ATPase has been demonstrated in renal tissue (Proverbio et al, 1975) and an exchange for sodium, similar to the sodium/calcium antiport has been proposed, suggesting similarities between magnesium and calcium at this point. There is apparently no competition between calcium and magnesium at the luminal membrane but the effects of hypercalcaemia and hypermagnesaemia on calcium and magnesium reabsorption suggest an interaction between these cations at the basolateral membrane (Quamme and Dirks, 1980).

c) Sodium:

Sodium is the most important solute in renal homeostatic mechanisms and the processes involved cannot be considered separately from the simultaneous transport of water, chloride, bicarbonate, potassium, glucose, amino acids and many other solutes. However, only those processes necessary for a basic understanding of the renal mechanisms involved in the conservation of urinary sodium are outlined here.

Sodium is freely filtered at the glomerulus since protein binding is negligible. In the early proximal tubule isosmotic reabsorption of water and solutes takes place and the transport of many of these solutes is often linked to that of sodium. Indeed, the net flux of fluid is sodium-dependent since replacement of sodium with lithium or tetramethyl ammonium ions causes a cessation of fluid reabsorption (Giebisch and Windhager, 1973). Proximal reabsorption is thought to be, at least partly, active since sodium transport has been demonstrated even in the presence of the lumen-negative potential difference in the early portion of isolated rabbit proximal tubules (Burg and Orloff, 1970). Sodium transport also occurs in the presence

of osmotic diuretics, such as mannitol, which retard water absorption (Giebisch and Windhager, 1973). Ouabain and removal of luminal potassium reduce sodium reabsorption, both of these manoeuvres reduce the activity of Na/K-ATPase which suggests that this enzyme is responsible for the active component of sodium transport (Chantrelle and Rector, 1980; Schafer et al, 1977). In addition to the sodium-dependence of many solute transport systems sodium reabsorption is dependent upon the movement of other solutes, principally potassium (as noted above) and bicarbonate. Also organic solutes, chiefly glucose, amino acids, lactate and citrate stimulate fluid uptake by a sodium-dependent mechanism (Burg et al, 1976). Indeed, phenylalanine (Foss et al, 1977) and glucose (Beck and Sacktor, 1978) both facilitate sodium reabsorption.

In the later part of the proximal convoluted tubule and the proximal straight tubule the transepithelial potential difference reverses so that the lumen is positive providing a further driving force for sodium transport (Fromter, 1979; Fromter et al, 1973). Approximately 60-70% of filtered sodium is reabsorbed in the proximal segments of the nephron.

The thin limbs of Henle's loop are unique in that they do not show any propensity for active sodium transport when perfused in vitro from either rat, rabbit or hamster (Imai and Kokko, 1974; Kokko, 1970; Imai, 1977). However, there are important differences between the permeabilities of the ascending and descending limbs producing passive sodium fluxes. The most important results gleaned from rat and hamster thin limbs (Imai, 1977) indicated that a) the permeability of the thin descending limb to water was much greater than that of the thin ascending limb, b) the permeability of the thin ascending limb to sodium and urea was greater than that of the descending limb and c) the permeability of the thin ascending limb to sodium was greater than its permeability to urea. During

antidiuresis the papillary environment is hyperosmotic and therefore, as the tubular fluid flows along the thin descending limb, it will also become hyperosmotic due to osmotic movement of water out of the lumen rather than by movement of solutes into the lumen. However, particularly in rodents, some net movement of solute into the tubular fluid does occur in this segment (Jamison et al, 1973; deRouffignac and Morel, 1969). Although the osmolarity of the tubular fluid and the renal interstitium at the papillary tip are similar, the main solute in the tubular fluid is sodium chloride, whereas in the interstitium it is urea. Therefore, as the tubular fluid flows along the thin ascending limb sodium will tend to move out of the tubular lumen by diffusion and urea will tend to diffuse into the lumen, but at a slower rate due to the low permeability of this segment to urea. Thus net movement of solute out of the tubular lumen will occur in this segment, thereby delivering a diluted tubular fluid to the thick ascending limb.

In contrast to the thin portions of the loop, the thick ascending limb is responsible for active uptake of sodium without accompanying water transport (Burg and Green, 1973a; Rocha and Kokko, 1973). There is, apparently, functional heterogeneity between the medullary and cortical portions of this segment (Burg and Green, 1973). At high perfusion rates in isolated rabbit thick ascending limb sodium transport was greater in the medullary than in the cortical portion while at low perfusion rates greater sodium reabsorption occurred in the cortical portion (Burg and Bourdeau, 1978). It has been suggested that the medullary portion is responsible for bulk transport of sodium whereas the cortical portion, which has a greater capacity for transport, creates the concentration gradient for sodium seen at the beginning of the distal tubule. This sodium absorption is thought to be driven by the lumen-positive potential difference in this segment which is, in turn, the result of active chloride transport

(Rocha and Kokko, 1973). Sodium transport has been shown to be passive (Burg and Green, 1973). However, this chloride flux apparently sensitive to ouabain (Rocha and Kokko, 1973; Greger, 1981; Hebert et al, 1981) which is puzzling if chloride flux is the active step and sodium follows passively.

The distal tubules in nondiuretic rats reabsorb some 7% of the filtered load of sodium (Khuri et al, 1975) the reabsorption rate is approximately one third of that in the proximal tubule and decreases towards the end of this segment (Windhager and Costanzo, 1978). The transepithelial potential difference is lumen-negative and therefore sodium transport must be active and is believed to be driven by Na/K-ATPase. The permeability of the distal convoluted tubule to water is very low so that the tubular fluid becomes increasingly dilute as sodium and other solutes are reabsorbed. However, water is reabsorbed from the distal convoluted tubule in the presence of ADH (Woodhall and Tisher, 1973). Sodium reabsorption is also influenced by concentration of urea in the tubular lumen, due to the fact that the distal tubule is impermeable to urea and therefore becomes concentrated in this segment and affects simultaneous sodium and ADH-sensitive water reabsorption (Khuri et al, 1975).

Only some 2% of filtered sodium reaches the collecting system (Ullrich et al, 1963, Windhager and Giebisch, 1961) but important modifications of the tubular fluid occurs in these segments. Rabbit cortical collecting ducts perfused in vitro were found to possess very low permeability to water which was increased by ADH (Grantham and Burg, 1966) and net reabsorption of sodium also occurred (Grantham et al, 1970). In this segment, as in the distal tubule, the transepithelial potential difference is lumen-negative and sodium transport is necessarily active (Grantham et al, 1970; Stoner et al, 1974). Sodium is apparently reabsorbed in exchange for potassium in

the cortical collecting duct and this mechanism is inhibited by amiloride (Stoner et al, 1974) which also reverses the transepithelial polarity. Sodium transport proceeds at a slow rate in this portion and yet large gradients for sodium can be established at low perfusion rates (Grantham et al, 1970) due to the insignificant backflux of sodium into the lumen (Stoner et al, 1974).

In the medullary and papillary collecting duct similar mechanisms with respect to sodium and water reabsorption operate as in the cortical portion (Ullrich, 1960). Only about 3% of filtered sodium is reabsorbed (Diezi et al, 1973) although the luminal concentration of sodium may be substantially decreased, in this segment (Sonnenberg, 1974). Sodium transport gradually decreases to zero in the medullary collecting duct (Stokes et al, 1981) sodium permeability is very low (Stokes, 1982a and b) implying that the very low luminal sodium concentration often achieved proximal to this portion is preserved by this latter property.

Numerous hormones have been shown to influence sodium excretion, most particularly aldosterone which is reported to stimulate sodium reabsorption in the distal convoluted tubule (Hierholzer and Lange, 1974) cortical collecting duct (Schwartz and Burg, 1978) and papillary collecting duct (Ullrich and Pappavassiliou, 1979). The action of aldosterone in sensitive cells apparently involves increased apical permeability to sodium and stimulation of Na/K-ATPase. A direct effect of aldosterone on proximal tubules has not been confirmed (Lynch et al, 1972). However, a direct action in rabbit cortical collecting duct has been confirmed by in vitro perfusion experiments (Schwartz and Burg, 1978; O'Neil and Helman, 1977) in which the artificial mineralocorticoid deoxycorticosterone acetate (DOCA) was shown to increase sodium chloride reabsorption. The actions of aldosterone in other nephron segments have not been so unambiguously confirmed but adrenalectomy increased sodium

concentrations in tubular fluid throughout rat nephron and increased sodium excertion, these effects were reversed by aldosterone (Hierholzer and Lange, 1974).

Renal handling of sodium is also influenced by PTH which reduces proximal reabsorption of sodium in the rat (Bank and Aynedjian, 1976) and dog (Agus et al, 1971) but produces little significant natriuresis. ADH may have a direct action on sodium reabsorption but this is by no means clear, experiments on intact animals generally show an increase in sodium excretion with ADH (Chan, 1971). The existence of a natriuretic hormone has been inferred from the high sodium excretion seen in uraemia (deWardener, 1977; Bricker et al, 1975).

The cellular aspects of renal sodium handling involve a number of interactions with other solutes. The interdependence of sodium and bicarbonate reabsorption is due to the presence of a Na/H antiporter at the luminal membrane, the secreted hydrogen ions react with bicarbonate ions to produce dissolved carbon dioxide which is then absorbed. Na/H exchange has been demonstrated in rat apical membrane vesicles (Murer et al, 1976). Other processes at the luminal membrane include facilitated uptake mechanisms for solutes driven by sodium uptake. As stated previously the driving force for sodium extrusion at the basolateral membrane is the Na/K-ATPase present at this site.

The paracellular transport route is thought to be of major importance in the 'leaky' epithelium of the proximal tubule (Boulpaep, 1972; Diamond and Bossert, 1967).

d) Potassium:

The renal handling of potassium is unusual in that it has been discovered that, even when the rate of excretion is very low, a significant fraction of the potassium appearing in the urine is the

result of secretion from the distal nephron (Berliner et al, 1951; Berliner, 1952). Potassium excretion is remarkably insensitive to variations in filtered load (Davidson et al, 1958) and this was assumed to be due to the secretion of a fixed amount of potassium at a distal site following almost complete reabsorption of filtered potassium in more proximal segments (Berliner, 1961).

Potassium is believed to be freely filtered at the glomerulus, although in one study approximately 10% of serum potassium was found to be bound to plasma proteins (leGrimellec et al, 1975); this result has not been reproduced.

Under normal conditions potassium is reabsorbed in similar proportions to sodium and water in the proximal tubule, thus approximately 60% of filtered potassium is reabsorbed in this segment. Micropuncture studies in the rat (Bloomer et al, 1963; Malnic et al, 1964) and the dog (Beck et al, 1973) have confirmed this assumption. Therefore TF_K/P_K is close to unity along the length of the accessible proximal tubule, although some dissociation from isosmotic sodium and water transport has been noted following acetazolamide administration (Beck et al, 1973) and after unilateral nephrectomy (Diezi et al, 1976).

The concentration of potassium in the tubular fluid increases markedly between the end of the accessible tubule and the papillary tip of the loop of Henle in rat and psammomys (Battilana et al, 1978; deRouffignac and Morel, 1969; Jamison et al, 1976). This increase is due to potassium secretion into the tubular lumen and is accentuated by potassium loading (Battilana et al, 1978) and reduced by potassium restriction (Dobyan et al, 1979). The source of the secreted potassium is not known precisely but it has been suggested that the medullary collecting duct reabsorbs potassium which is recycled into the descending limbs of adjacent nephrons (deRouffignac and Morel, 1969).

Only 5-15% of filtered potassium reaches the early distal tubule (Malnic et al, 1964; Malnic et al, 1966) and this figure varies very little with potassium repletion or deprivation. This low distal potassium delivery indicates substantial reabsorption in the ascending limb, probably the thick ascending limb since frusemide and other loop diuretics increase the delivery of potassium to the early distal tubule (Burg et al, 1973; Burg and Green, 1973b).

Micropuncture studies of rat nephron have indicated that the late accessible distal convoluted tubule is capable of potassium secretion during dietary potassium supplementation (Malnic et al, 1964). The potassium concentration in late distal tubular fluid can rise to twenty times the simultaneous plasma concentration. In contrast to the proximal tubule, where TF_K/P_K is unaffected by potassium balance, the potasium in the late distal tubule varies drastically depending on whether conservation or secretion of potassium is necessary (Malnic et al, 1964;).

There is functional heterogeneity in the collecting duct system with respect to potassium transport, potassium secretion continues in the cortical collecting duct followed by net reabsorption in the medullary region (Reineck et al, 1975; Reineck et al, 1978). Isolated rabbit cortical collecting tubules have been shown to secrete potassium against a steep concentration gradient (Grantham et al, 1970) this process was inhibited by amiloride (Stoner et al, 1974) and was stimulated in tubules isolated from DOCA-treated rabbits (O'Neil and Helman, 1977). The medullary collecting tubule was shown to reabsorb potassium in microcatheterisation experiments (Sonnenberg, 1974). Only during maximal kaliuresis (Hierholzer, 1961) or administration of diuretics (Sonnenberg, 1978) was potassium secretion observed in this segment. Potassium reabsorption in medullary collecting tubules is dependent on tubular fluid flow rate, reabsorption demonstrated in hydropenic rats

disappeared when the animals were volume-expanded with saline (Reineck et al, 1975).

Most factors affecting potassium excretion do so by modifying the secretory processes in late distal tubules and cortical collecting tubule. Aldosterone increases potassium and decreases sodium excretion (Barger et al, 1958) and the effect on potassium is dependent on an adequate luminal sodium concentration (Relman and Schwartz, 1952; Howell and Davis, 1954). The site of action of aldosterone is apparently in the distal regions of the nephron since it is in distal puncture sites that potassium concentration is seen to decrease in adrenalectomised rats (Cortney, 1969; Hierholzer et al, 1965) and where potassium secretory capacity is restored following aldosterone administration. Mineralocorticoids increase the potassium secretory capacity of isolated rabbit cortical collecting tubules (Schwartz and Burg, 1978). The cellular mechanism of action involves both changes in the transepithelial potential difference (which favours potassium secretion) and the potassium permeability of sensitive segments; both properties are reduced by adrenalectomy and increased by aldosterone (Wiederholt et al, 1972; Wiederholt et al, 1974).

It is clear that potassium excretion is dependent on an adequate supply of sodium and this dependence is most marked in the cortical collecting tubule. This was elegantly demonstrated in stop-flow experiments by Vander (1961). Fluid obtained from the region of the cortical collecting tubule possessed a high potassium concentration due to secretion in this segment and increasing contact time with the tubular epithelium produced an increasing peak in potassium concentration from this site. However, when contact time exceeded 2.5 minutes this peak decreased and was eventually replaced by a trough, this was due to a gradual reduction in the luminal sodium concentration with contact time consequent upon sodium

reabsorption at this site. The sodium-dependent potassium secretion therefore decreased with luminal sodium concentration and the peak of potassium concentration disappeared. This effect of sodium supply on potassium secretion may be due to either the decrease in the transepithelial voltage caused by sodium depletion or a reduction in the activity of the proposed Na/K exchange system in the luminal membrane (Grantham et al, 1970). It should be pointed out that the ratio of sodium reabsorbed to potassium secreted in this segment varies, therefore sodium availability is not the limiting factor for potassium secretion.

Distal potassium handling is also influenced by acid-base balance (Malnic et al, 1971) some diuretics (Duarte et al, 1971) and the adaptive changes following loss of renal mass (Diezi et al, 1976).

Movement of potassium across the tubular epithelium varies greatly from a reabsorptive flux in the proximal tubule and thick ascending limb to a secretory flux in the late distal tubule and collecting system.

In the proximal tubule the TF_K/P_K of one or slightly less (Malnic et al, 1964) indicates an active component of potassium reabsorption, it is unlikely that the marginally lumen-positive potential difference is responsible for this movement. Furthermore, acetazolamide which abolishes this potential difference (Boulpaep, 1976) does not reduce proximal potassium reabsorption (Beck et al, 1973). However, reabsorptive movement of potassium by a transcellular route would necessitate active steps at both the luminal and basolateral membranes (Edelman et al, 1979; Fujimoto et al, 1977). There is therefore a large body of opinion in favour of the concept that substantial potassium fluxes occur via a paracellular route in the proximal tubule (Whittembury and Rawlins, 1971; Boulpaep, 1976). Potassium reabsorption is presumably entrained in sodium and water flux and this contention is supported by the observation that

inhibition of bulk proximal fluid reabsorption by extracellular volume expansion or osmotic diuresis also inhibits potassium reabsorption (Malnic et al, 1964).

In the late distal and cortical collecting tubule, where net secretion of potassium takes place, passive forces favour this movement, both the lumen-negative voltage and the concentration gradient for potassium. As discussed earlier the transepithelial potential difference is important in the mechanism of potassium secretion and the action of aldosterone since changes imposed on distal potential difference also affect potassium secretion, luminal potassium concentration was shown to increase as the luminal potential was made more negative (Garcia-Filho et al, 1980).

In the late distal tubule potassium secretion does not exceed what would be predicted by passive movement to achieve electrochemical equilibrium. In contrast, in the cortical collecting tubule active secretion occurs under certain conditions (Grantham et al, 1970). This potassium flux is electrogenic and is therefore capable of reducing or even reversing the luminal potential (Stoner et al, 1974). The active mechanism responsible for this phenomenon is probably located in the luminal membrane.

1.3 Effects of aminoglycoside antibiotics on the renal handling of cations

The earliest report of aminoglycoside-induced disturbances of electrolyte homeostasis (Holmes et al, 1970) concerned the development of severe hypokalaemic, hypomagnesaemic alkalosis in patients undergoing treatment for pulmonary tuberculosis. The only common features to all four patients were the administration of ethambutol and gentamicin. Plasma renin and aldosterone levels were raised in all patients and the electrolyte imbalance was thought to be due to secondary hyperaldosteronism, although no obvious stimulus for raised aldosterone secretion was evident (only one patient was hyponatraemic).

Further clinical studies revealed a fairly consistent syndrome characterised by hypomagnesaemia, hypokalaemia and hypocalcaemia, often associated with inappropriately high excretion rates of these cations and inappropriately low levels of circulating PTH following the administration of large doses of aminoglycosides (Bar et al, 1975; Keating et al, 1977; Bamford and Jones, 1978; Kelnar et al, 1978; Patel and Savage, 1979; Freedman et al, 1982; Goodhart and Handelsman, 1985; Wilkinson et al, 1986). In contrast to the findings of Holmes et al (1970) circulating renin and aldosterone, when measured, were found to be normal (Bar et al, 1975; Kelnar et al, 1978) except in one study where plasma renin was found to be raised (Patel and Savage, 1979).

In other studies isolated changes in cation homeostasis have been reported, such as the transient hypokalaemia reported following combined gentamicin and cephalothin therapy (Hansen and Kaaber, 1977). Streptomycin was reported to have a hypocalcaemic effect in malignant hypercalcaemia (Roediger et al, 1975) Aminoglycoside-induced hypocalcaemia was detected in five patients (Sethi et al,

1975) in whom circulating PTH was low and calcitonin levels were normal, a direct toxic effect of aminoglycosides on the parathyroid glands was hypothesised by the authors. Plasma calcitonin was measured in one other study and was found to be within normal limits (Keating et al, 1977).

The mechanisms operating in these instances are believed to result from a primary renal wasting of magnesium brought about by aminoglycosides, this results in hypomagnesaemia since the kidney is almost wholly responsible for magnesium homeostasis. Hypomagnesaemia may result in reduced PTH secretion (Rude et al, 1978) and in impaired end organ sensitivity to the hormone (Estep et al, 1969) thereby precipitating hypocalcaemia. Hypokalaemia resulting from renal wasting of potassium may occur in hypomagnesaemia (Shils, 1969) also a syndrome asssociated with renal loss of magnesium and potassium has been described (Gitelman et al, 1966) suggesting an association between the renal handling of these cations.

The aetiology of these effects is complicated by the variety of disease states in which aminoglycosides have been implicated as a cause of electrolyte disturbances. Cystic fibrosis may be associated with hypomagnesaemia (Orenstein and Orenstein, 1983) and appears to exacerbate the hypomagnesaemic effect of aminoglycosides (Green et al, 1985). Leukaemia may also predispose to hypomagnesaemia (Keating et al, 1977; Freedman et al, 1982; Davey et al, 1985) due to a preponderance of factors likely to precipitate electrolyte wasting (Jaffe et al, 1972). In recent reports (Zaloga et al, 1984; Davey et al, 1985) asymptomatic hypomagnesaemia was reported to be a relatively common consequence of aminoglycoside treatment.

Studies relating to the effects of aminoglycosides on electrolyte handling in experimental animals have been infrequent and have yielded inconclusive results. The most common association revealed

from such experiments was the appearance of hyperkaliuria following aminoglycosides, in the rat (Luft et al, 1978a; Chiu et al, 1977; Smith et al, 1981; Pastoriza et al, 1983) the dog (Brinker et al, 1981, Cronin et al, 1980) and in the sheep (Bennett et al, 1983). In most of these studies high doses of aminoglycosides administered over long periods were used, resulting in the appearance of overt nephrotoxicity which would be assumed to disrupt renal mechanisms to such an extent that reduced reabsorption of most solutes would occur. However, aminoglycosides were administered acutely in the studies of Pastoriza et al (1983) and Bennett et al (1983) and no depression of GFR was produced in the experiments of Smith et al (1981). Interestingly, in a third study in which gentamicin was infused into dogs (Chiu et al, 1976) both GFR and the fractional excretion of potassium were unaffected.

Sodium excretion was also found to be altered in some of these studies, although, the results were variable; sodium excretion was demonstrated to increase in rats (Luft et al, 1978a; Smith et al, 1981; Pastoriza et al, 1983) but was decreased in sheep (Bennett et al, 1983) and unaffected in dogs (Chiu et al, 1976). The mechanisms of these effects are unknown, as noted above the co-existence of nephrotoxic changes may non-selectively reduce cation reabsorption, as is seen with low molecular weight proteins (Davey et al, 1984). Smith et al (1981) demonstrated that the increase in sodium excretion produced by gentamicin was prevented by adrenalectomy whereas the increase in potassium excretion was not, suggesting a dependence of the natriuretic effect on aldosterone secretion and also indicating that this effect was not simply the result of a severe toxic insult but rather a specific effect on renal cation handling. Mitchell et al (1977) perfused isolated rat kidneys with gentamicin and detected no change in sodium reabsorption but a significant increase in the fractional excretion of potassium which was inhibited

by amiloride, suggesting a selective effect on the potassium secretory process of the distal nephron.

Effects of aminoglycosides on divalent cations were only occasionally demonstrated in early studies. Galante (1970) produced hypocalcaemia with streptomycin and other aminoglycosides in rats which was apparently due to stimulation of of calcitonin release, the response did not involve renal mechanisms since nephrectomy did not prevent the drop in plasma calcium concentration produced by streptomycin. Crawford and Teske (1978) administered neomycin to dogs and stimulated calcium excretion, although they did not report whether this was accompanied by nephrotoxic effects. Increased magnesium excretion was demonstrated in sheep during gentamicin infusion (Bennett et al, 1983) although this result was gleaned from only two animals. More recently a sensitive and specific effect of gentamicin on the excretion of divalent cations, particularly calcium, has been detected in rats. Administration of 40 mg/kg/day gentamicin for seven days produced a gradual rise in calcium excretion accompanied by an inconsistent effect on magnesium and no significant effect on sodium or potassium excretion (Chahwala and Harpur, 1983). Despite the fact that these effects were accompanied by extensive necrotic damage the specificity of the response is noteworthy. Harpur et al (1985a) reported an increase in 24-hour calcium excretion after a single dose of 10 mg/kg gentamicin without accompanying changes in urinary markers of nephrotoxicity. Changes in magnesium excretion were transient; sodium and potassium were not studied. Toxic damage was unlikely to be extensive in this study and therefore this response is a specific and sensitive effect of gentamicin. In a further study (Harpur et al, 1985b) increases in both calcium and magnesium excretion were seen during treatment with 20 mg/kg/day gentamicin and these responses persisted for several days after the last drug injection after other urinary markers

had returned to basal levels. Cronin and Newman (1985) also demonstrated a rise in calcium excretion after 60 mg/kg/day gentamicin given to rats for 8 days, this was accompanied by a decrease in potassium excretion a slight fall in sodium excretion and, curiously, a rise in the plasma calcium concentration. Similar effects were produced in two other studies (Bennett et al, 1985; Elliott et al, 1987); in the first of these sodium excretion was unaffected whereas in the second sodium excretion declined simultaneously with the increase in calcium excretion.

The consequences of these changes in cation excretion on whole body homeostasis are uncertain. Due to the supplementation of most animal diets with electrolytes the development of measurable deficiencies in any of the major electrolytes in animals is unlikely, even following extensive renal wasting of these elements. Hypokalaemia was demonstrated in dogs receiving 30 mg/kg/day gentamicin or netilmicin in association with renal wasting of potassium; hypocalcaemia was also demonstrated in the gentamicin group but significant nephrotoxicity was also evident in these animals (Cronin et al, 1980). Finton et al (1983) reported a significant drop in plasma magnesium concentration in baboons given gentamicin (5 mg/kg); these animals were excreting inappropriately high levels of magnesium but there was no evidence of gross nephrotoxic changes in these animals. It was possible to demonstrate hypocalcaemia in rats as a consequence of renal wasting of calcium during gentamicin administration in one study (Elliott et al, 1987), due to the provision of a calcium-restricted diet.

The effect of aminoglycoside-induced changes in electrolyte homeostasis on renal physiology and their possible relationship to nephrotoxicity have been difficult to define. Gentamicin administration led to a decline in the renal cortical content of potassium in rats (Weinberg et al, 1983) and dogs (Cronin et al,

1982) but in the latter study toxicity only occurred in potassium-depleted dogs despite the fact that the renal concentration of potassium was decreased in potassium-depleted and potassium-supplemented animals. Decreased magnesium content was also a feature of the early stages of aminoglycoside nephrotoxicity in these studies. The importance of potassium and magnesium loss from renal tissue in the development of toxicity is highlighted by the exacerbation of toxicity following potassium (Cronin et al, 1982; Brinker et al, 1981) or magnesium depletion (Rankin et al, 1984).

Calcium supplementation ameliorates gentamicin nephrotoxicity (Bennett et al, 1982; Quarum et al, 1984; Humes et al, 1984) although the mechanism by which calcium supplementation reduces gentamicin toxicity is not precisely known. No measurable increase in plasma calcium concentration accompanies this manoeuvre which essentially refutes the theory that an increase in the filtered load of calcium is responsible for the protection by reducing renal gentamicin uptake into renal cells (Humes et al, 1984). Furthermore, McCarron et al (1984) showed no protection from nephrotoxicity in spontaneously hypertensive rats which were also hypercalciuric. The possibility that calcium repletion afforded protection from toxicity by suppression of PTH has been explored. PTH stimulates turnover of renal anionic phospholipids (Farese etal, 1980; Bidot-Lopez et al, 1981) which are thought to provide the initial binding site for aminoglycosides (Sastrasinh et al, 1982a); therefore PTH suppression could reduce aminoglycoside binding and uptake. However, calcium supplementation does not necessarily reduce the renal accumulation of gentamicin (Humes et al, 1984) although a delay in gentamicin accumulation was found in other studies (Bennett et al, 1982; Quarum et al, 1984). Parthyroidectomy (PTX) was found to reduce gentamicin nephrotoxicity in rats when compared with PTH-stimulated controls (Bennett et al, 1985; Elliott et al, 1987);

sensitivity to the toxic insult was restored by PTH administration (Bennett et al, 1985). Therefore, PTH levels appear to alter sensitivity to gentamicin nephrotoxicity and calcium repletion almost certainly protects from nephrotoxicity by suppression of PTH. The exact mechanism by which PTH alters the injury threshold is difficult to interpret. Protection from gentamicin nephrotoxicity by parathyroidectomy was accompanied by lower renal gentamicin concentrations in one PTX study (Elliott et al, 1987) but not in the other (Bennett et al, 1985). Furthermore, PTH increased renal accumulation of gentamicin in rats but this effect was not due to an increase in the number of binding sites at the cell membrane (Holohan et al, 1987), therefore the cellular site of action of PTH, in its effect on gentamicin toxicity, cannot be determined from these results.

In summary, aminoglycosides produce a complex disturbance of cation homeostasis in humans, probably resulting from renal wasting of magnesium. Animal studies indicate a sensitive effect on the renal handling of calcium, at least in the rat, and probably effects on magnesium and potassium, in addition. These effects may be related to toxicity since depletion of potassium and magnesium and supplementation with calcium influence the severity of the toxic insult. The mechanisms of these effects remain to be fully elucidated.

1.5 Aims and objectives

The primary objective of this study was to reproduce the early, sensitive hypercalciuric response to gentamicin administration seen in conscious Fischer 344 rats (Harpur et al, 1985a) in a different model. This model involved standard clearance techniques applied to an anaesthetised Fischer rat undergoing saline diuresis. The renal handling of the four major cations could then be studied during infusion of saline alone and during infusion of saline containing gentamicin. The mechanisms of any changes in renal cation handling could be studied, for example, any change in the renal clearance of a solute without a simultaneous change in GFR would indicate that the response was due to alterations in whole kidney handling of that solute. Also, any change in the fractional excretion of a solute would indicate that this change was due to alterations in the tubular handling of that solute, rather than any change in the filtered load. A second objective was to attempt to define the physiological mechanisms responsible for any changes in cation handling produced by gentamicin, particularly with regard to the role of parathyroid hormone in the hypercalciuric response to gentamicin (Bennett et al, 1985; Elliott et al, 1987).

A further, broader objective was to attempt to relate the changes in cation handling seen in the Fischer rat to the hypomagnesaemic hypocalcaemia and hypokalaemia seen in man following aminoglycoside therapy (Zaloga et al, 1984) which is thought to be the result of renal wasting of these electrolytes. Whereas the best-defined response to gentamicin in the rat is the appearance of hypercalciuria (Harpur et al, 1985a) disturbances of monovalent cation homeostasis have frequently been reported in man (Holmes et al, 1970; Keating et al, 1977; Hansen and Kaaber, 1977) following aminoglycosides. Changes in sodium and potassium excretion rate

were not seen in the Wistar rat following gentamicin administration (Chahwala and Harpur, 1983). Therefore, it was deemed necessary to catalogue the effect of infused gentamicin on all four major cations.

The elevation in calcium excretion rate reported by Harpur et al (1985a) appeared to be related to the dose of drug received, rats receiving 30 mg/kg/day excreted more calcium than those receiving 10 mg/kg/day. Therefore, a study to further elucidate the possibility that the degree of gentamicin-induced hypercalciuria was related to the amount of drug delivered was also included.

Changes in cation excretion rate have also been reported following neomycin (Crawford and Teske, 1978) and netilmicin (Pastoriza et al, 1983) in animal models, thus to determine the effects of other aminoglycosides on the renal handling of cations was a further aim.

CHAPTER 2: MATERIALS

2.1 Chemicals:

Heparin (sodium salt, activity: 147U/mg), Lanthanum chloride, tobramycin (base, potency: 945μg/mg), bovine parathyroid hormone (1-34 fragment) and methyl hydroxybenzoate obtained from Sigma Ltd., Poole, Dorset, U.K.

Sodium chloride, potassium chloride, Optiphase MP scintillation fluid and sodium metabisulphite obtained from F.S.A. Laboratory Supplies, Loughbourough, U.K.

Calcium nitrate and magnesium nitrate atomic absorption standards and E.D.T.A. obtained from B.D.H. Ltd., Poole, Dorset, U.K.

Propyl hydroxybenzoate obtained from Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.

^{3}H-Inulin (specific activity: 1.0-5.0 Ci/mmol) obtained from Amersham International, Amersham, Bucks., U.K.

Inactin (5-ethyl-5[1'-methyl-1-propyl]-2-thiobarbiturate powder) obtained from Byk Gulden, Konstanz, F.R.G.

Gentamicin sulphate (Cidomycin injectable, 2ml vials each containing 80mg gentamicin base) obtained from Roussel Ltd., Wembley, U.K.

Frusemide (Lasix, 2ml ampoules each containing 20mg frusemide) obtained from Hoechst Ltd., Hounslow, Middlesex, U.K.

2.2 Apparatus:

Polyethylene tubing (P50, P90 and P200) obtained from Portex Ltd., Hythe, Kent, U.K.

Small operating table and universally variable infusion pump obtained from Harvard Apparatus Ltd., Edenbridge, Kent, U.K.

Pressure transducer obtained from Bell and Howell Ltd., Basingstoke, U.K.

Chart recorder (Devices M-2) obtained from Ormed Engineering Ltd., Welwyn Garden City, Herts., U.K.

Cautery apparatus, used for all operations other than selective parathyroidectomy obtained from Downs Surgical, Mitchum, Surrey, U.K.

Cautery apparatus used to perform selective parathyroidectomy (Elektrotom 60) obtained from Gebruder Martin, Tuttlingen, F.R.G.

Microhaematocrit tubes and centrifuge obtained from Hawksley and Sons Ltd., Lancing, Sussex, U.K.

Positive displacement syringe obtained from Kloehn Co. Inc., Whittier, California, U.S.A.

Sterile universal containers obtained from Sterilin Ltd., Hounslow, Middlesex, U.K.

Atomic absorption spectrophotometers (Perkin-Elmer 560 and 373) obtained from Perkin-Elmer Corp., Norwalk, Connecticut, U.S.A.

Scintillation counter (2000CA liquid scintillation counter) obtained from Packard Instruments Ltd., Reading, Berks., U.K.

Ultrafiltration cones (Centrifree) obtained from Amicon Corp., Danvers, Massachusetts, U.S.A.

Fixed rotor refrigerated centrifuge (Beckman J2-21) obtained from Beckman Ltd., Glenrothes, Fife, U.K.

Calibrated capillary tubes (Microcaps) obtained from Drummond Scientific, Broomall, Pennsylvania, U.S.A.

Air displacement pipettes obtained from Gilson Medical Electronics, Villiers-le-Bel, France.

2.3 Experimental animals:

Fischer 344 rats obtained from Harlan Olac Ltd., Bicester, Oxon., U.K.

or Charles River U.K. Ltd., Margate, Kent, U.K.

2.4 Animal diet:

Pilsbury's rat and mouse breeding diet obtained from Heygate and Sons Ltd., Northampton, U.K.

Stated ionic content: Calcium - 1.72%
Magnesium - 0.28%
Sodium - 0.28%
Potassium - 0.56%
Phosphate - 1.09%

CHAPTER 3: METHODS

3.1 Housing:

Male Fischer 344 rats, body weight 190-310g, were used in all experiments. Rats were housed in cages containing never more than 6 individuals and were permitted unlimited access to tap water and food. Temperature was maintained at 20-25°C and a normal light cycle (12 hours light, 08.00-20.00h) was used. Rats were acclimatised to this environment for at least 7 days before clearance experiments were performed.

3.2 Surgery:

a) Rats were anaesthetised with an i.p. injection of Inactin freshly dissolved in 0.9% (w/v) NaCl at a dose of 120 mg/kg body weight. A thermostatically-controlled, heated operating table was used to maintain body temperature; monitored by a rectal thermometer; at 37.0 $\pm$ 0.5°C.

b) An incision was made in the neck and the trachea exposed by blunt surgery. A tracheostomy was performed by tying a short length (approx. 8-10mm) of slightly tapering polyethylene tubing (P200) into an aperture made in the trachea.

c) The left jugular vein was cannulated with polyethylene tubing (P50). This cannula was connected to a 50ml plastic syringe containing 0.9% (w/v) NaCl, mounted on a continuously variable infusion pump. Infusion was initiated immediately after tying the cannula into the vein.

d) The right carotid artery was cannulated with P50 polyethylene tubing connected to a pressure transducer filled with 0.9% (w/v) NaCl containing 10U/ml heparin. A chart recorder was used to record arterial blood pressure. Rats which had an initial systolic blood pressure below 100 mmHg were not used. The arterial cannula was in two sections connected by a hollow stainless steel tube (modified hypodermic needle) allowing the two sections to be disconnected to obtain arterial blood samples.

The pressure transducer was calibrated by linking the pressure sensitive unit to a sphygnomanometer by which pressure was raised to 100mmHg, the pen deflection produced was then marked on the chart recorder.

e) An incision was made in the abdomen and the abdominal muscle and peritoneum were pierced by electrocautery. A slightly flared polyethylene catheter (P90) was inserted into a cauterised aperture in the urinary bladder and tied in place. The flared opening of the catheter situated within the bladder prevented it from being dislodged by respiratory movements. Urine was collected continuously in plastic vials.

3.3 Experimental protocol:

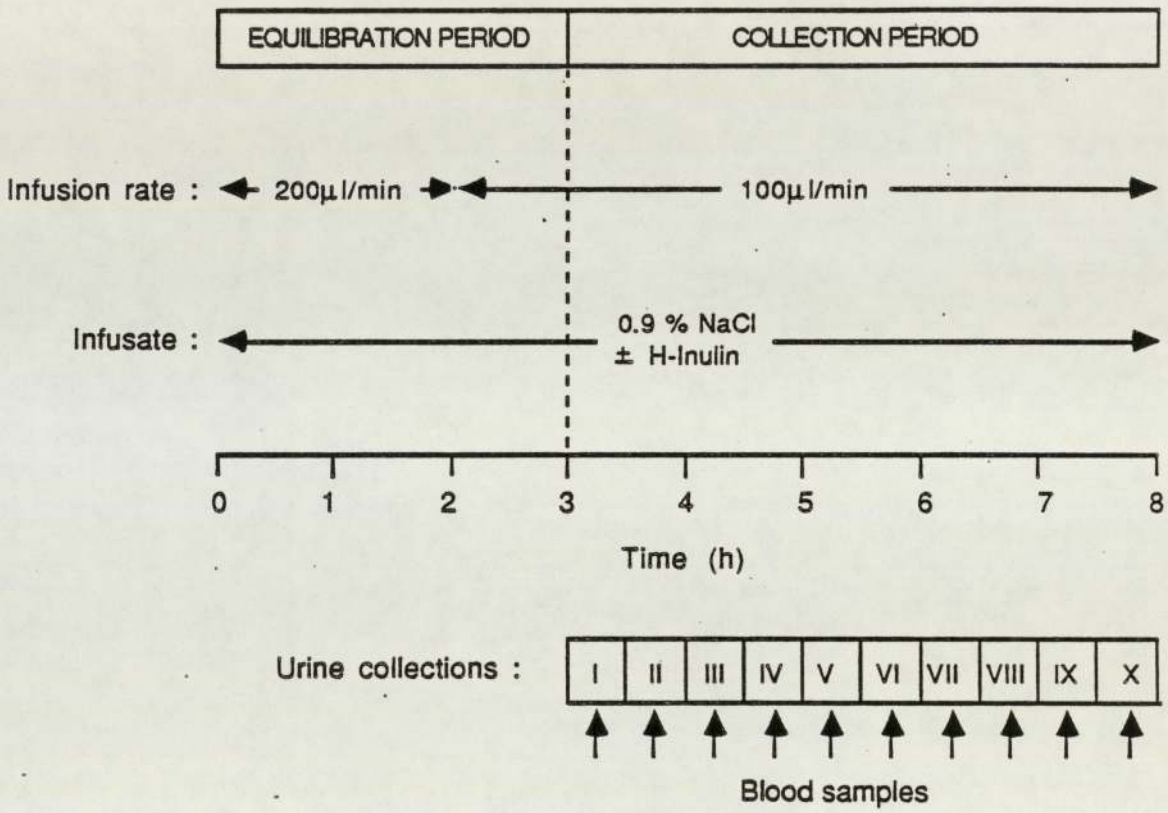
a) In all experiments the first three hours of infusion constituted an equilibration period during which 0.9% (w/v) NaCl (usually containing 1μCi/ml ^{3}H-Inulin) was infused at a rate of 200μl/min for the first two hours and 100μl/min for the third hour. This procedure initiated a diuresis which stabilised to produce a urine flow rate approximately equal to the infusion rate during the ensuing five hour collection period (Figure 1). The total volume of urine recovered in eight hours was equal to 86.4 $\pm$ 3.7% of the total volume of

infusate (n=9).

b) The subsequent five hours of infusion constituted the collection period. The infusion rate was 100µl/min throughout, except in those experiments in which frusemide was infused (Figures 99 and 105). In these experiments the infusion rate was adjusted to compensate for the considerable urinary losses sustained during infusion of frusemide (see Section 4.8).

c) In all experiments ten, consecutive 30 minute urine samples were collected into weighed plastic vials during the collection period. The vials were re-weighed after each collection and urine volume estimated by weight (1ml of urine was assumed to weigh 1g). At the mid-point of each urine collection an arterial blood sample was withdrawn from the carotid. The arterial cannula was clamped near to the needle coupling and the two sections of cannula were disconnected at this point. The clamp was released and the heparinised solution in the arterial cannula was drawn off into a 1ml syringe. Arterial blood was then allowed to run into two heparinised microhaematocrit tubes each with a capacity of 75µl. The cannula was clamped and re-connected and the clamp released to allow recording of arterial blood pressure to continue. The microhaematocrit tubes were sealed with plasticene® and centrifuged in a microhaematocrit centrifuge for 3 minutes. The tubes were broken above the buffy coat layer and the sections containing packed red blood cells were discarded. Plasma was removed in two aliquots from the remaining halves of the tubes containing plasma: 10µl of plasma was removed into a third capillary tube for determination of ^{3}H-Inulin activity. A total of 40µl was drawn from the remaining plasma into a 50µl positive displacement syringe for determination of cation concentrations. Identical volumes of urine were pipetted

Figure 1. General experimental protocol used in clearance experiments.



from each urine sample for the same determinations.

d) At the end of each experiment the animal was killed by intravenous administration of a lethal dose of Inactin or by air embolism.

3.4 Analytical determinations:

a) Calcium:

Urine and plasma were diluted with 0.1% (w/v) lanthanum chloride acidified with 5mM HCl. Plasma and urine were diluted by adding 40µl of either to 4ml of diluent giving a dilution of 1:101.

Total calcium (Ca) concentration was then measured by atomic absorption spectrophotometry in an air:acetylene flame. The wavelength for Ca determinations was 422.7nm, the sensitivity of the determination was 0.08µg/ml and the relationship between Ca concentration and absorbance was linear up to a Ca concentration of 5µg/ml. Distilled water was used to provide a zero Ca reading and a one point calibration of 5µg/ml was used. The standard used to give this calibration was made up from a combined Ca/Mg stock solution containing $Ca(NO_3)_2$ and $Mg(NO_3)_2$ atomic absorption standards (1mg/ml and 10mg/ml respectively) diluted in distilled water or lanthanum chloride. All atomic absorption standards were freshly made up in bottles which were washed, rinsed in distilled water and thoroughly dried before use.

b) Magnesium:

Magnesium (Mg) was measured in the same diluted samples used for Ca measurements. Total Mg was also measured by atomic absorption spectrophotometry in an air:acetylene flame. The wavelength for Mg determinations was 285.5nm, the sensitivity of

the determination was 0.007µg/ml and absorbance was linear with respect to concentration up to a Mg concentration of 0.5µg/ml. Distilled water was used to provide a zero Mg reading and a one point calibration of 0.5µg/ml Mg was used. The standard used to provide this calibration was made up from the same combined Ca/Mg stock solution used to calibrate Ca determination.

c) Sodium:

Urine and plasma were diluted with distilled water, a final dilution of approximately 1:5000 was necessary for both samples which was achieved as follows: 40µl of either urine or plasma were added to 4ml of distilled water giving a 1:101 dilution. This was further diluted by adding 100µl of this 1:101 dilution to 4.9ml of distilled water to give a final dilution of 1:5050. Sodium (Na) concentration was measured by atomic absorption spectrophotometry in an air: acetylene flame. The wavelength for Na determinations was 589.0nm, the sensitivity of the determination was 0.015 µg/ml and the relationship between absorbance and Na concentration was linear up to 1µg/ml. Distilled water was used to provide a zero Na reading and a one point calibration of 1µg/ml Na was used. The standard used to provide this calibration was a combined Na/K standard made up from a stock solution containing 100µg/ml of both NaCl and KCl dissolved in distilled water.

d) Potassium:

Plasma and urine were diluted with distilled water, a final dilution of approximately 1:100 was necessary for plasma samples and 1:1000 for urine. The initial 1:101 dilutions used for diluting samples for Na determinations were also used to provide dilutions for potassium (K) determinations. The 1:101 plasma dilutions were used directly and 0.5ml of the the urine dilutions were added to 4.5ml of

distilled water to give a final dilution of 1:1010. K was also measured by atomic absorption spectrophotometry in an air:acetylene flame. The wavelength for K determination was 766.5nm, the sensitivity of the determination was 0.04μg/ml and the relationship between absorbance and concentration was linear up to a K concentration of 2μg/ml. Distilled water was used to provide a zero K reading and a one point calibration of 2μg/ml K was used. The standard used to provide this calibration was made up from the same combined Na/K stock solution used to calibrate Na determination.

e) Phosphate:

Phosphate (PO_4) was determined at the Dept. of Physiological Sciences, University of Manchester by an automated method on a Technicon Auto Analyser II system. Urine was dialysed against 0.72M H_2SO_4 (aq). The dialysed urine was then mixed with an acidic ammonium molybdate solution forming phosphomolybdic acid, which was immediately reduced by stannous chloride hydrazine. The absorbance of the analytic stream was measured at 660nm in a 15mm flow cell.

f) ^{3}H-Inulin:

10μl of urine or plasma were added to 5ml or 2ml of Optiphase MP scintillation fluid in either glass scintillation vials or plastic vial inserts. ^{3}H activity was measured on a liquid scintillation analyzer.

g) Concentration of ultrafilterable calcium and magnesium in plasma:

The ultrafilterable fraction of Ca and Mg in plasma (UF_{Ca}, UF_{Mg}) was measured in terminal blood samples obtained by cardiac puncture, or by draining arterial blood from the carotid cannula immediately before killing the animal. Blood was collected into lithium heparin coated tubes to inhibit coagulation. The plasma was separated by

centrifugation and approximately 1ml of plasma was pipetted into each of two ultrafiltration cones. These were then centrifuged at 2500r.p.m. in a fixed rotor centrifuge at 4°C.

40µl of the plasma ultrafiltrate produced by this process was diluted as appropriate for determination of Ca and Mg concentrations. The Ca and Mg concentrations in plasma ultrafiltrate were determined as described above.

In all calculations of Ca and Mg clearance and fractional excretion the concentration of ultrafilterable Ca or Mg in plasma was used where an expression for the concentration of solute in plasma was required.

3.5 Definition and calculation of terms used to study renal function:

a) Excretion rate:

The excretion rate of a solute is the quantity of that solute excreted in urine in unit time.

For any solute the following equation applies:

$$\text{Quantity} = \text{Concentration} \times \text{Volume}$$

Therefore, in the case of a solute excreted in urine:

$$\text{Quantity excreted per unit time} = \text{Concentration in urine} \times \text{Volume of urine per unit time}$$

Therefore, for a solute, x, the following equation applies:

$$Q_x = U_x \cdot V$$

Where: Q_x = Quantity of x excreted in unit time.

U_x = Concentration of x in urine.

V = Volume of urine produced in unit time.

Q_x equals the excretion rate of x, denoted by E_x.

Commonly:

$$E_x \text{ (mg/min)} = U_x \text{ (mg/ml)} \cdot V \text{ (ml/min)}$$

b) Clearance:

The clearance of a solute is the volume of plasma completely cleared of that solute in unit time.

In early studies of renal physiology it was found that altering the concentration in plasma of an excreted solute, such as urea, produced proportionate changes in its rate of excretion. From this it was concluded that the solute contained in a fixed volume of plasma was being completely 'cleared' in unit time, this quantity of solute subsequently appearing in urine. This volume, or 'clearance', was assumed to be the volume of glomerular filtrate formed in unit time. However, it was quickly discovered that the clearances of different solutes in any individual could vary by orders of magnitude. Obviously, with certain important exceptions, the clearance of a solute does not represent the rate of production of glomerular filtrate.

Clearly, the opening definition of clearance does not relate to a real volume of plasma, rather it is a virtual volume. However, the clearance does equal the volume of plasma containing the amount of solute excreted in unit time and, as such, is a useful physiological parameter.

Since the quantity of solute excreted in unit time equals the quantity contained in a volume of plasma equal to that solute's clearance, the following can be derived:

As above:

Quantity excreted per unit time = Concentration in urine x Volume of urine per unit time

A similar equation can be constructed with regard to clearance:

Quantity contained in volume of plasma equal to clearance = Concentration in plasma x Clearance

For a solute, x:

$$Q_x = U_x \cdot V$$

Also:

$$Q_x = P_x \cdot C_x$$

Where: P_x = Concentration of x in plasma.

C_x = Clearance of x.

Therefore:

$$U_x \cdot V = P_x \cdot C_x$$

This can be rearranged to give an equation for clearance:

$$C_x = \frac{U_x \cdot V}{P_x}$$

If V is expressed as a volume per unit time, C_x must also be a volume per unit time, commonly:

$$C_x \text{ (ml/min)} = \frac{U_x \text{ (mg/ml)} \,.\, V \text{ (ml/min)}}{P_x \text{ (mg/ml)}}$$

N.B. In all calculations of calcium and magnesium clearances the concentrations of ultrafilterable calcium and magnesium in plasma were used to provide values for the terms P_{Ca} and P_{Mg} respectively.

c) Glomerular filtration rate:

Glomerular filtration rate (GFR) is the volume of glomerular filtrate formed in unit time, and is a measurement of the bulk flow of solvent across the glomerular filter.

If a freely filtered solute is neither reabsorbed nor secreted along the nephron, the quantity excreted in urine in unit time must equal the quantity filtered at the glomerulus in unit time.

The best example of such a solute is the polysaccharide, inulin.

Therefore:

Rate of excretion of inulin = Rate of filtration of inulin

Substituting symbols:

$$\text{Rate of excretion of inulin} = U_{In} \,.\, V$$
$$\text{Rate of filtration of inulin} = P_{In} \,.\, GFR$$

Where: U_{In} = Concentration of inulin in urine.

P_{In} = Concentration of inulin in plasma.

V = Volume of urine produced in unit time.

GFR = Glomerular filtration rate.

Therefore:

$$U_{In} . V = P_{In} . GFR$$

This can be rearranged to give an equation for GFR :

$$GFR = \frac{U_{In} . V}{P_{In}}$$

d) Fractional excretion:

Fractional excretion (FE) is the the rate of excretion of a solute expressed as a fraction of its rate of filtration at the glomerulus.

The rate of filtration of any freely filtered solute can be determined if a simultaneous measurement of GFR is made:

Rate of filtration = Concentration in plasma x GFR

Therefore:

$$\text{Rate of filtration of x} = P_x . GFR$$

This parameter is known as the filtered load.

As above:

$$\text{Rate of excretion of x } (E_x) = U_x . V$$

Therefore:

$$\text{Fractional excretion of x } (FE_x) = \frac{U_x . V}{P_x . GFR}$$

If a solute undergoes net reabsorption in its passage through the kidney, its rate of excretion will be less than its rate of filtration and its FE will therefore be less than one. If it undergoes net secretion, its rate of excretion will exceed its rate of filtration and its FE will be greater than one.

FE can be expressed as a fraction or, more commonly, as a percentage.

The expression for FE can be further simplified.

Since:

$$\frac{U_x . V}{P_x} = C_x$$

Then:

$$FE_x = \frac{C_x}{GFR}$$

3.6 Statistical analysis:

All statistical comparisons were carried out on the BBC microcomputer utilising software written by Dr. K. Wilson (Dept. of Pharmaceutical Sciences, University of Aston). Student's 't' test for small groups (unpaired data) was used in all comparisons with the exceptions noted below. Statistical comparisons on each parameter were carried out on mean data from coincident collection periods drawn from different groups of experimental animals.

Student's 't' test for paired data was used to compare temporal changes within groups of experimental animals, using the data illustrated in Figures 32, 56, 71 and 98. The data are shown as the

mean percentage increase in excretion rate or clearance for each group. Each pair of data points was drawn from one experimental animal, for example the clearance of magnesium during collection IV and the magnesium clearance during collection V in the same animal would be a representative data pair used in such a comparison. However, the data in these comparisons were expressed as percentage increases, therefore the data pair actually consisted of a zero baseline value (collection IV) and the percentage increase above this value in the same animal at a later collection (V or VI).

CHAPTER 4: RESULTS

4.1 The effect of infusion of saline and 'Cidomycin' excipients on the renal handling of cations

a) Introduction:

The standard clearance methods employed in this study required the infusion of 60ml of 0.9% (w/v) sodium chloride (saline). This is necessary to promote a high urine flow rate, which will provide adequate sample volumes for the various analytical procedures to be carried out. Therefore experiments to study the changes with time during saline infusion alone were necessary.

At intervals during the period of study represented in this work a total of 11 rats were infused with saline to assess the stability with time of the parameters measured. This total included seven rats in which calcium and magnesium handling was studied, which was divided into four rats used in clearance experiments near the beginning (control group A), and three rats used towards the end (control group B) of the period of study. ^{3}H-Inulin was included in the infusate delivered to group B but not group A. Comparing the effects of saline infusion on the renal handling of calcium and magnesium between groups A and B of rats would indicate whether there were any changes in these effects during the intervening period.

'Cidomycin', the gentamicin preparation used in sections, 4.2, 4.3, 4.4, 4.6, 4.7 and, 4.8 contained the following excipients: Sodium metabisulphite, methyl hydroxybenzoate, propyl hydroxybenzoate, and, EDTA. To determine whether these contributed to the response to 'Cidomycin' infusion three rats were infused with excipients alone (control group C). Renal handling of calcium and magnesium was studied in this group and the results from these rats were compared

with results from group A. ^{3}H-Inulin was included in the infusate delivered to this group.

The four remaining rats were those in which sodium and potassium handling was studied (control group D), ^{3}H-Inulin was included in the infusate delivered to this group.

b) Methods:

When saline alone was infused (groups A, B and D) the protocol outlined in Figure 2 was followed. For the purposes of this chapter the equilibration period is generally excluded from protocol diagrams and only the five hour collection period is shown. When 'Cidomycin' excipients were infused (group C) the procedure illustrated in (Figure 3) was followed. A solution identical to the aqueous vehicle for gentamicin in 'Cidomycin' was prepared. Infusion of this solution in saline resulted in the following infusion rates of each excipient:

Sodium metabisulphite - 0.045 mg/kg/min
Methyl hydroxybenzoate - 0.025 mg/kg/min
Propyl hydroxybenzoate - 0.003 mg/kg/min
EDTA - 0.001 mg/kg/min

An infusion of 'Cidomycin' in saline which would produce identical infusion rates of excipients would produce an infusion rate of gentamicin of 0.56 mg/kg/min.

c) Results:

In control groups A and B mean urine flow rate (Figure 4), GFR (Figure 12, group B only) and the mean plasma calcium and magnesium concentrations (Figures 5a and 5b) were essentially stable for the duration of the collection period.

In contrast, a gradual fall in calciuria was seen during much of the collection period in both groups. The mean excretion rate (Figure 6a) and clearance (Figure 7a) of calcium fell during collections I-VI or I-

VII reaching a nadir of approximately 50% of initial values before stabilising for the remainder of the collection period. In group B, in which GFR was measured, these changes could be explained by a parallel decrease in the mean fractional excretion of calcium (Figure 13a), indicating increased tubular reabsorption of this cation.

A similar decrease in magnesiuria also occurred during saline infusion; mean excretion rate (Figure 6b) and clearance (Figure 7b) reaching approximately 70% of initial values at the end of collection VI. A slight rise in magnesiuria was seen in the final hour of the collection period. These changes could also be explained by decreased mean fractional excretion of magnesium in group B rats (Figure 13b), indicating increased magnesium reabsorption.

In control group D mean GFR (Figure 14b) and the mean plasma sodium and potassium concentrations (Figures 15a and 15b) were essentially stable throughout the collection period. A small drop in mean urine flow rate (Figure 14a) was seen during collections VIII-X, final values approximating 80% of initial values, however considering that no such change was seen in groups A and B this is unlikely to be a significant finding.

In this group mean sodium excretion rate (Figure 16a) and clearance (Figure 17a) were stable throughout the collection period.

Mean excretion rate (Figure 16b) and clearance (Figure 17b) of potassium were stable during collections I-IV then fell steadily for the remainder of the collection period to approximately 60-65% of initial values. The mean fractional excretion of potassium (Figure 18b) changed in parallel with excretion rate and clearance indicating that increased net tubular potassium reabsorption was responsible for these findings.

Mean fractional excretion of sodium (Figure 18a) was stable throughout the collection period.

In control group C mean urine flow rate (Figure 8), GFR (Figure 12)

and the mean plasma calcium and magnesium concentrations (Figures 9a and 9b) were essentially stable for the full duration of the collection period. In group C a decline in calciuria, similar to that observed in saline infusion, was produced. Mean calcium excretion rate (Figure 10a) and clearance (Figure 11a) fell to approximately 50% of peak values. These changes could be explained by a parallel decrease in the fractional excretion of calcium (Figure 13a).

Magnesiuria followed a similar pattern to calciuria. The mean excretion rate (Figure 10b) and clearance (Figure 11b) of magnesium dropped to approximately 65% of peak values. These changes could be explained by a parallel decrease in the mean fractional excretion of magnesium (Figure 13b).

d) Discussion:

In all four control groups the following general features were evident:

Mean urine flow rate, GFR (when measured) and the mean plasma calcium, magnesium, sodium and potassium concentrations were relatively stable during the collection period. The lack of change in GFR and urine flow rate indicates that no gross disturbances of renal function were produced by the experimental procedures used. The stability of plasma cation concentrations indicates that there was no significant volume expansion of experimental animals, which would have led to a generalised decrease in the concentrations of solutes in plasma including cations.

In contrast, the mean excretion rates, clearances and fractional excretions (when measured) of calcium, magnesium and potassium all decreased during the collection period. Mean excretion rate, clearance and fractional excretion of sodium were essentially stable over the same period. The renal handling of sodium and calcium have been reported to be very closely associated in the rat and dog

during saline loading (Walser, 1961; Massry and Kleeman, 1972; Agus et al, 1977) although dissociation of calcium from sodium clearance can be produced by acute thyroparathyroidectomy (Agus et al, 1977). In the present study values of calcium and sodium clearance were similar during collections I-III (in separate experiments), calcium clearance then declined reaching values which were approximately half of the corresponding sodium clearance. However, saline infusion in the cited studies was of a shorter duration than in the present study, therefore the long-term responses to saline infusion may differ from the short-term responses. Although the site and mechanism of this dissociation is unknown it must occur in the distal tubule since no dissociation of calcium and sodium reabsorption has been reported in the proximal tubule. Such dissociation generally involves the action of PTH, as noted above, but since no significant hypocalcaemia was produced in these experiments stimulation of PTH secretion cannot be inferred.

The renal handling of magnesium is less closely associated with sodium than the renal handling of calcium, for example, the superficial proximal tubule is less permeable to magnesium than it is to either sodium or calcium (Quamme and Dirks, 1980). Nevertheless, magnesium reabsorption does follow sodium reabsorption in the proximal tubule, although at a lower fractional rate (Massry et al, 1967b; Brunette et al, 1969) and in the loop of Henle (Massry and Coburn, 1973; Quamme and Dirks, 1983) at a higher fractional rate. There is also a marked interaction between magnesium and calcium reabsorption, particularly in the ascending limb of the loop of Henle (Coburn et al, 1970; Massry and Coburn, 1973; Walser, 1973). Magnesium, as well as calcium, reabsorption is dissociated from sodium in the distal tubule. Therefore the arguments pertaining to the dissociation of the renal handling of calcium from sodium also apply to magnesium.

The renal handling of potassium is also closely linked to sodium, unlike calcium and magnesium this linkage also extends to the distal convoluted tubule where potassium secretion is dependent, in part, on luminal sodium concentration (Good and Wright, 1979). Therefore, it is difficult to suggest a mechanism by which the renal handling of potassium could escape from sodium.

A reduction in calcium, magnesium and potassium outputs concurrent with increasing sodium output has been reported in experiments of similar design to those presently under discussion (Shafik, 1984; Blacklock et al, 1985), although these depressions were mild in comparison with those seen in the present results no attempt was made to explain this anomaly.

There were no consistent differences in mean urine flow rate, GFR or the excretion rates, clearances or plasma concentrations of calcium and magnesium between groups A and B. This would suggest that the effects of saline infusion on these parameters were unchanged during the interval between these sets of experiments.

There were no consistent differences in these parameters between groups A and C. Infusion of excipients produced no changes in the renal handling of calcium or magnesium, urine flow rate or GFR which were not produced by saline alone. Thus, any effects produced by infusion of 'Cidomycin' must result entirely from the presence of gentamicin.

All three groups of rats were used to provide control data in statistical comparisons with the experimental data yielded in the following studies.

Figure 2. Protocol used in those experiments in which saline alone was infused (control groups A, B and D).

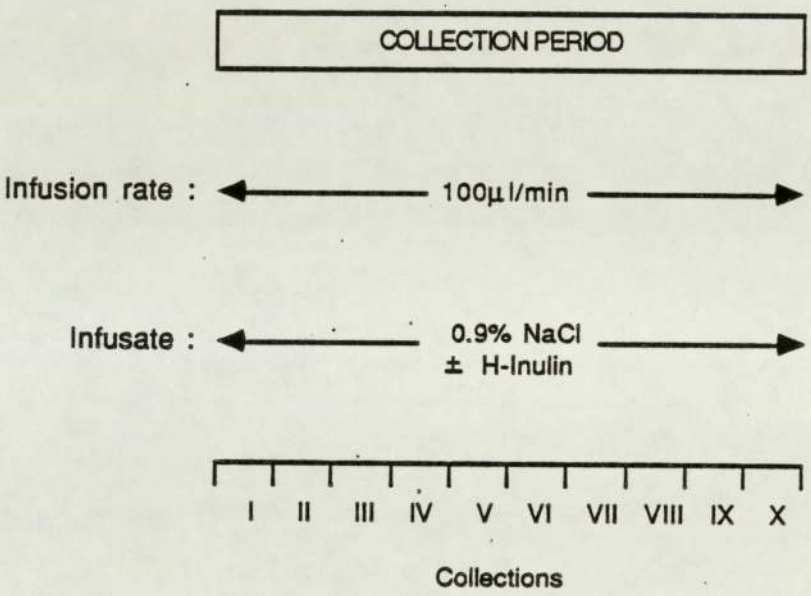
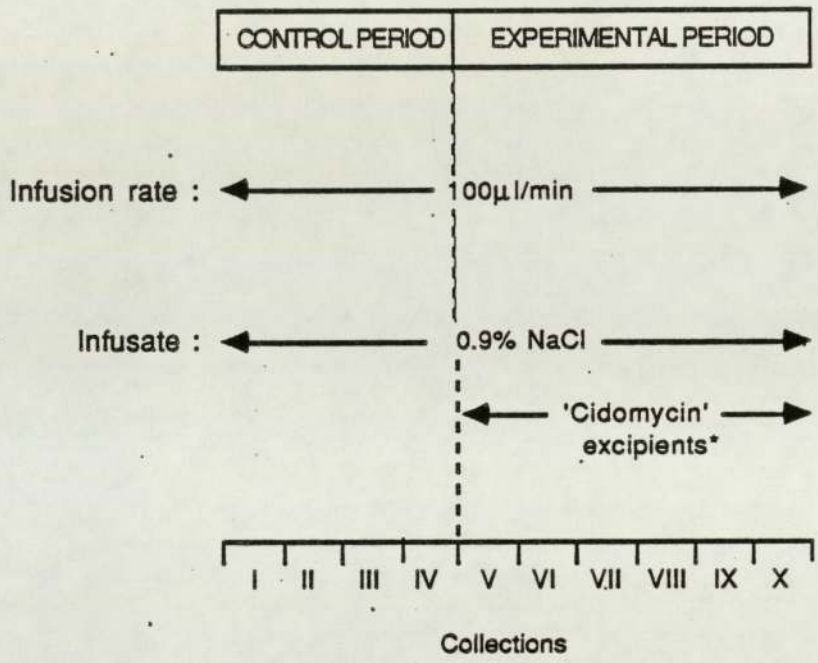


Figure 3. Protocol used in those experiments in which 'Cidomycin' excipients were infused (control group C).



* Added to infusate according to protocol.

Figure 4. Urine flow rate in control groups A and B expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

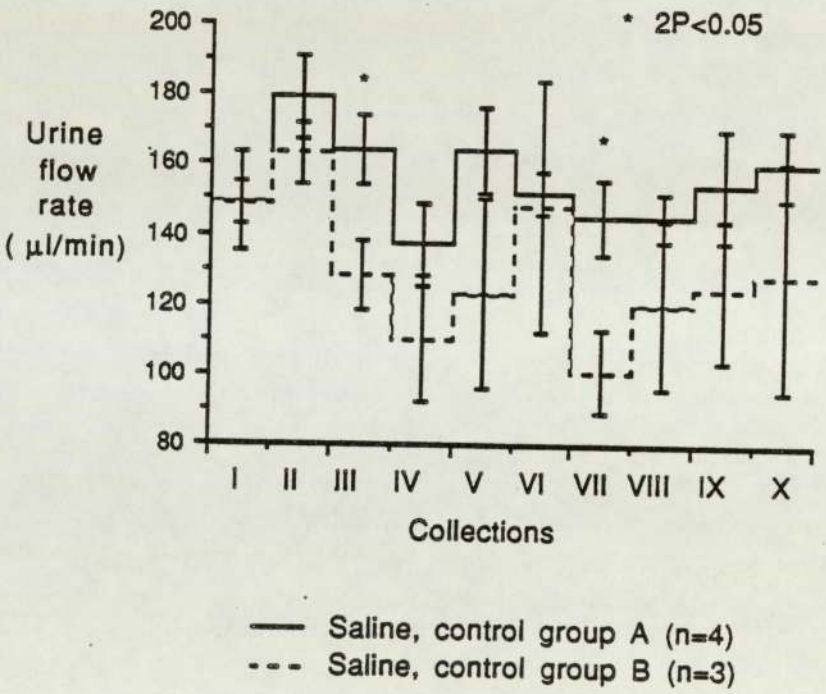


Figure 5. Concentrations of cations in plasma in control groups A and B expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

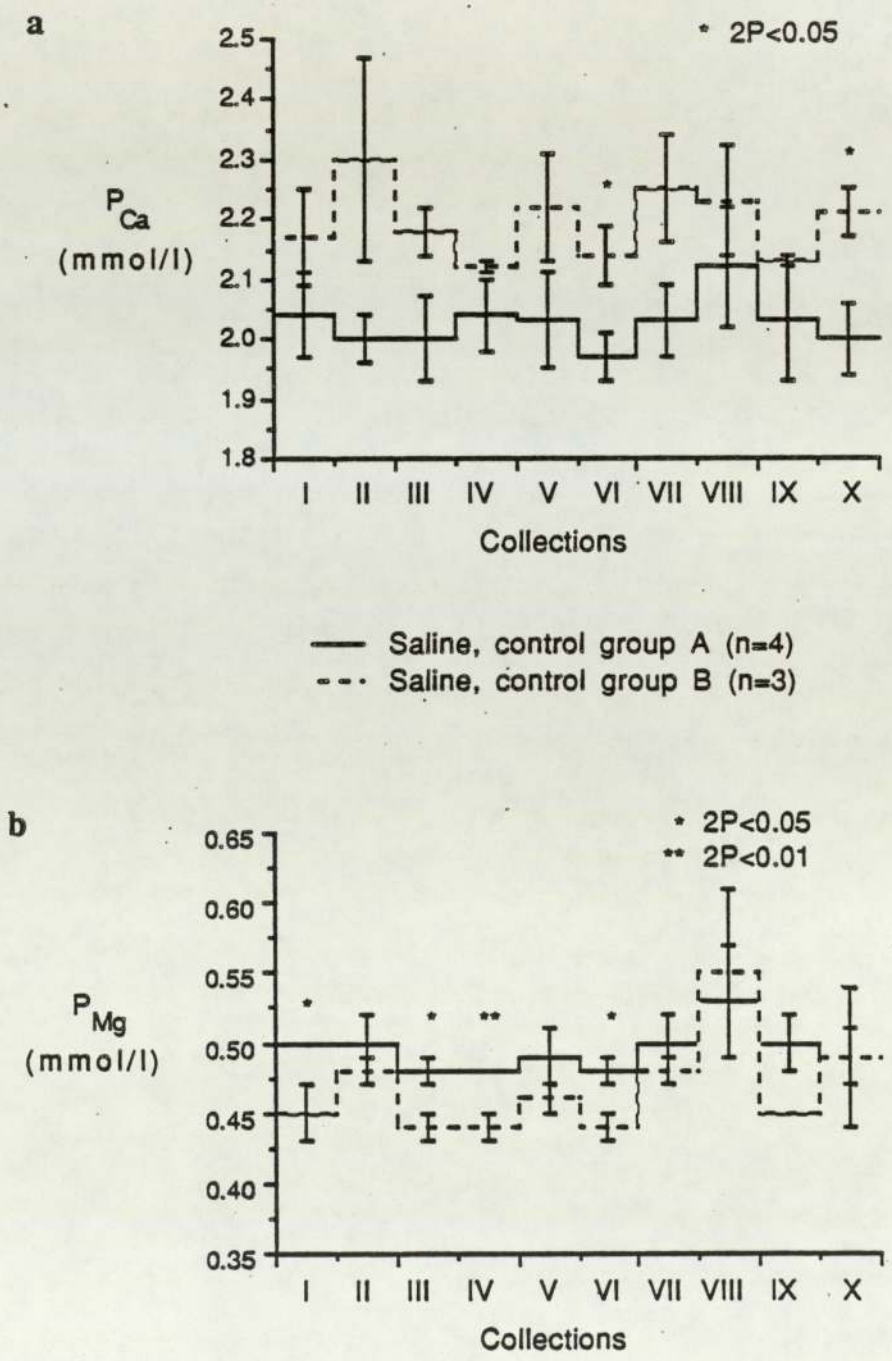


Figure 6. Excretion rates of cations in control groups A and B expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

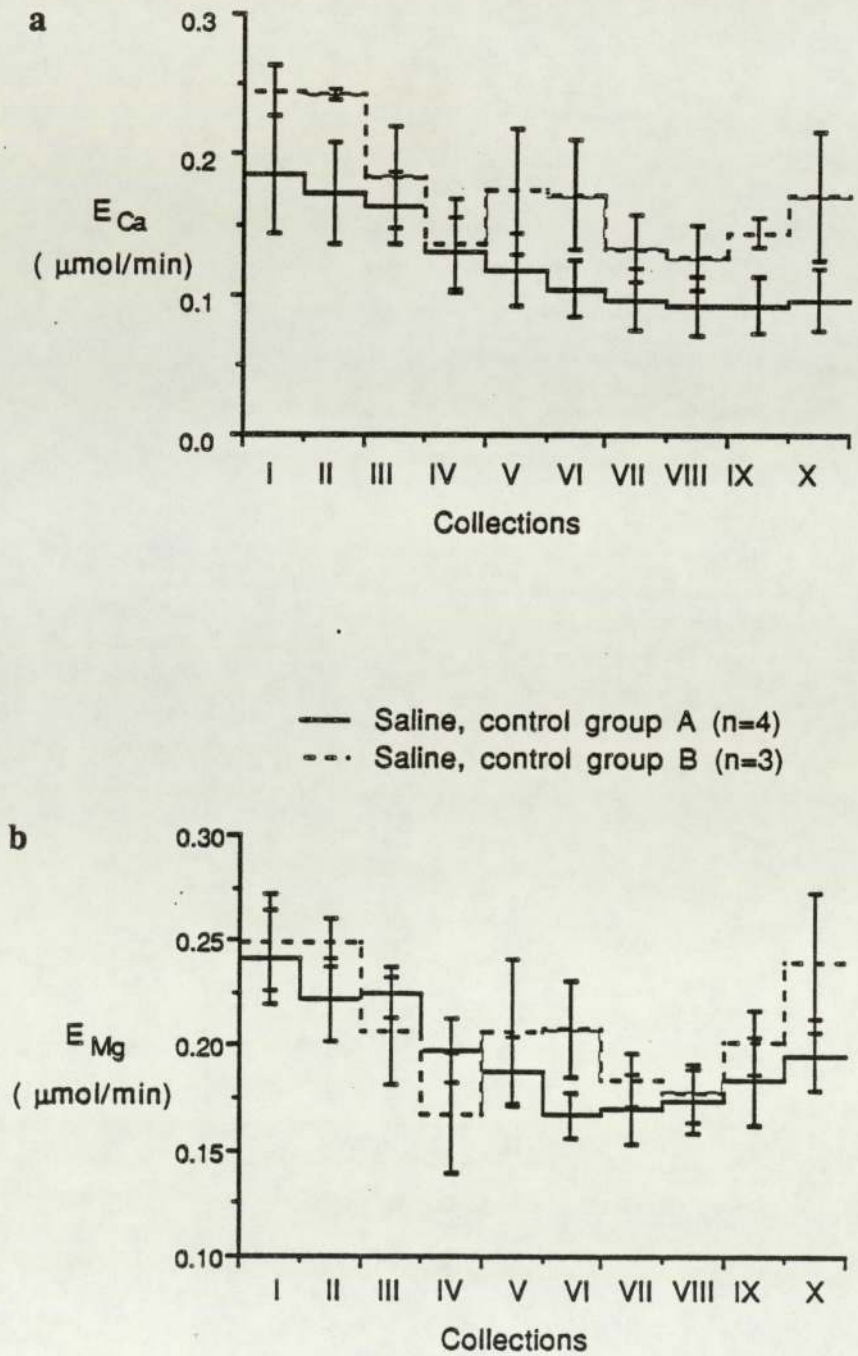


Figure 7. Clearances of cations in control groups A and B expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

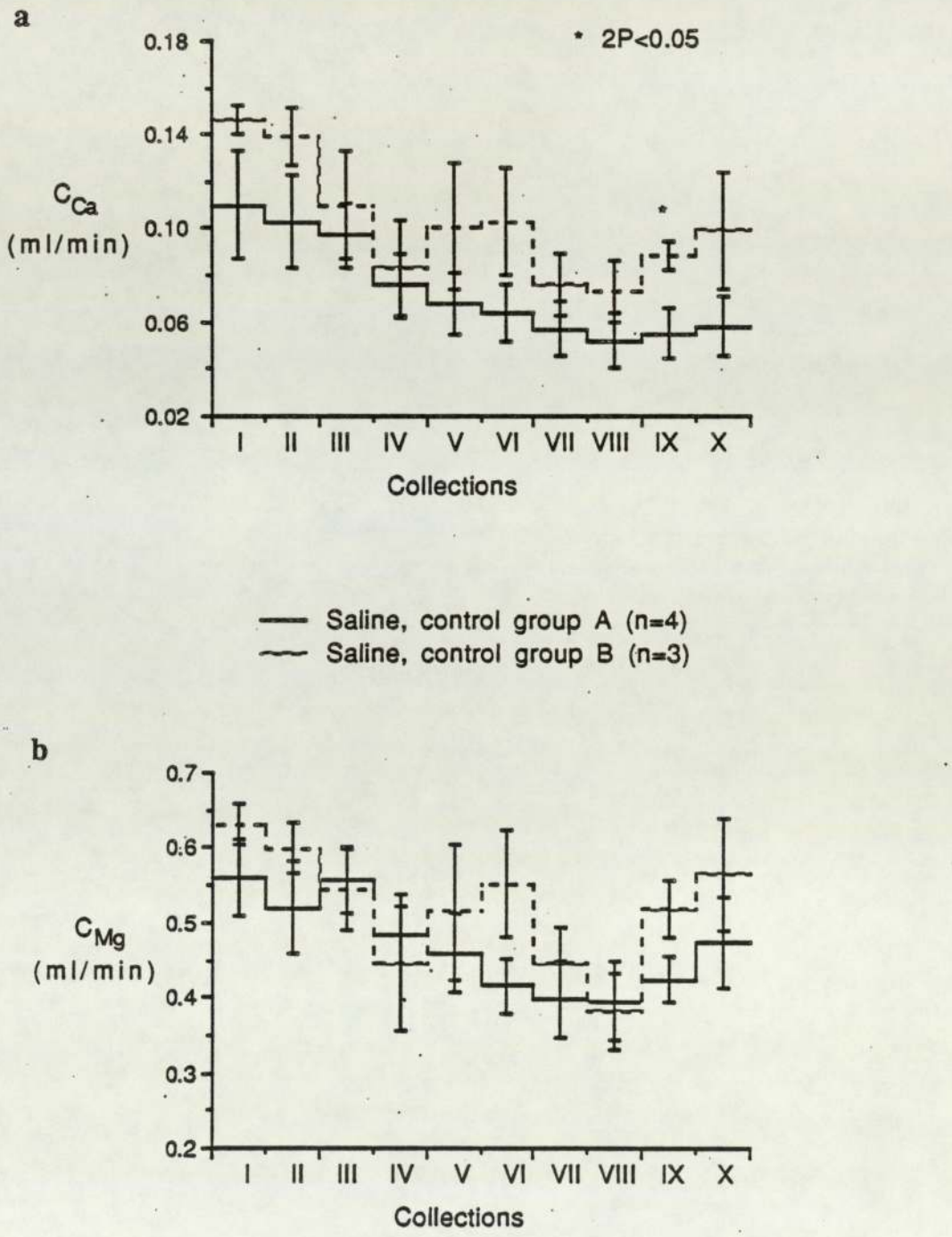


Figure 8. Urine flow rate in control groups A and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

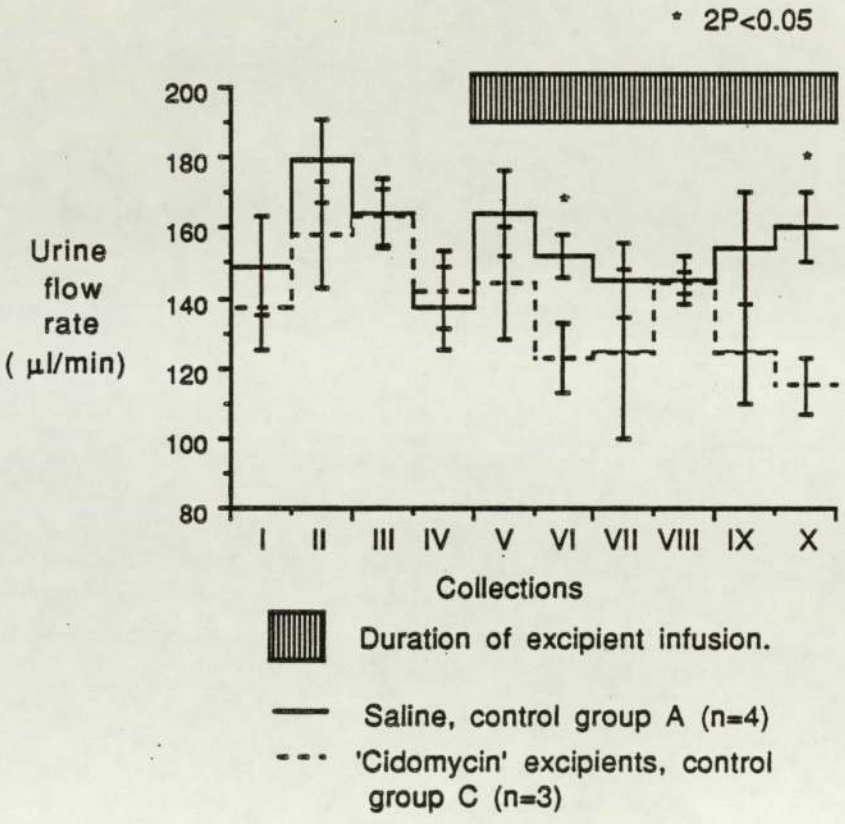


Figure 9. Concentrations of cations in plasma in control groups A and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium..

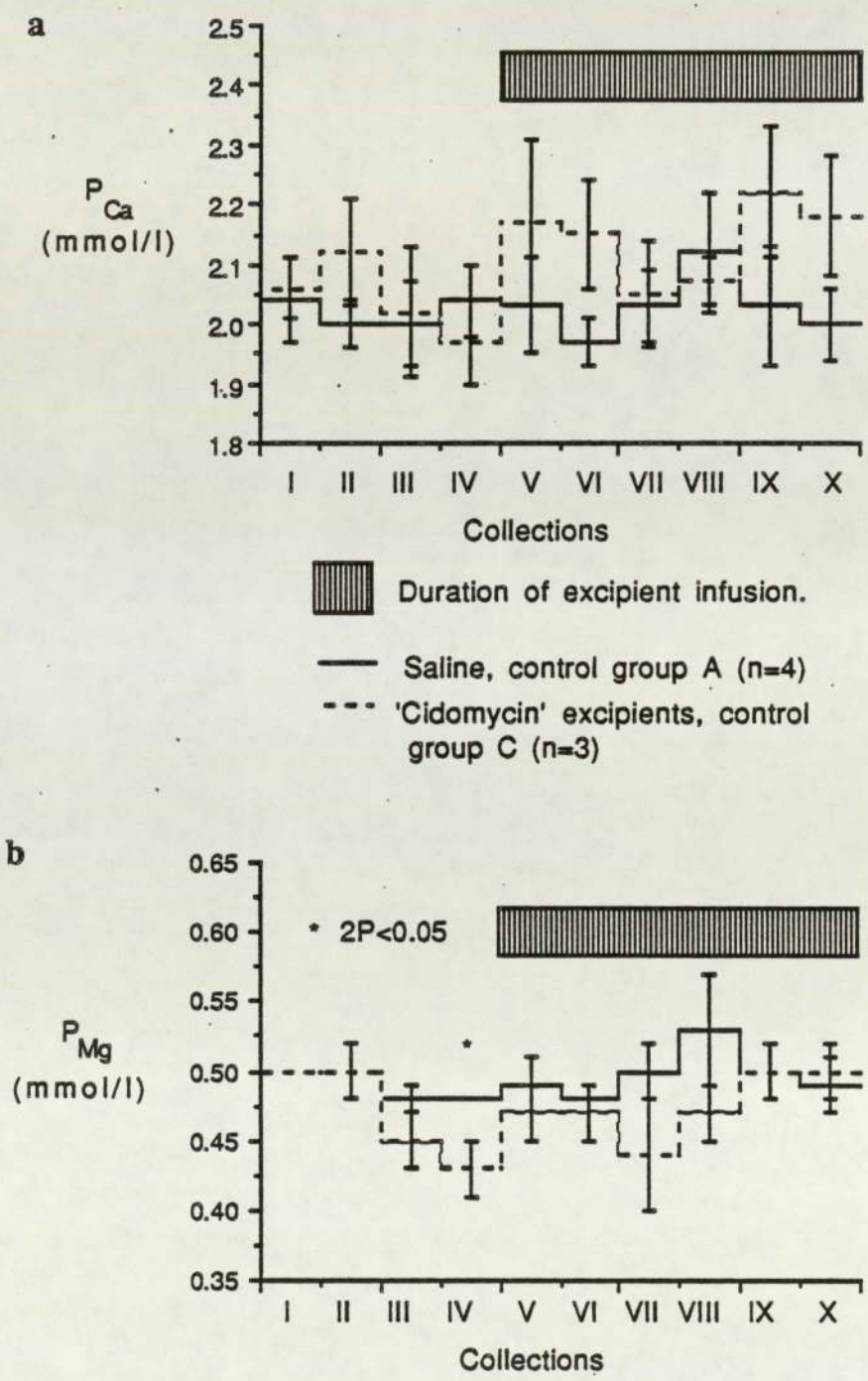


Figure 10. Excretion rates of cations in control groups A and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

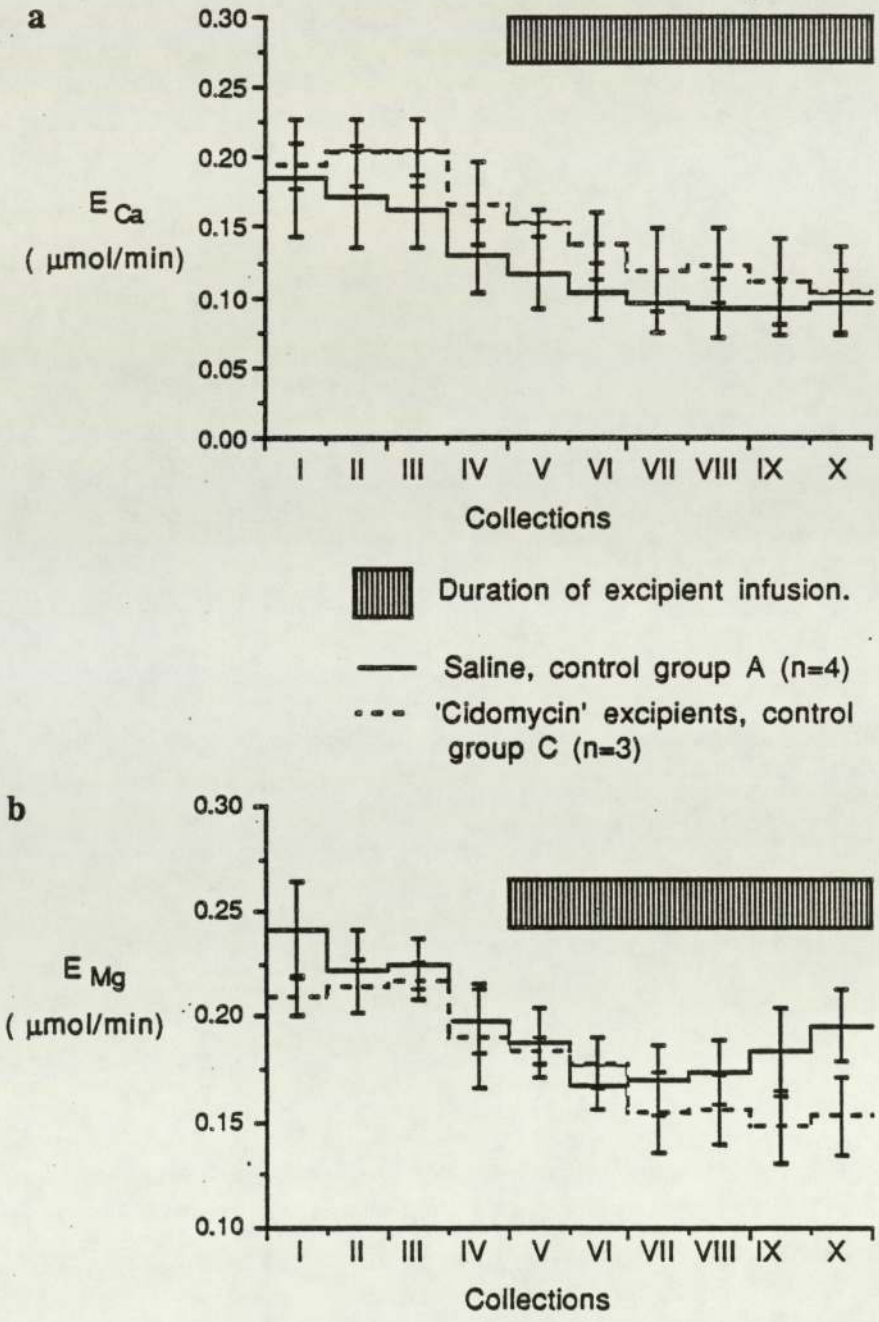


Figure 11. Clearances of cations in control groups A and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

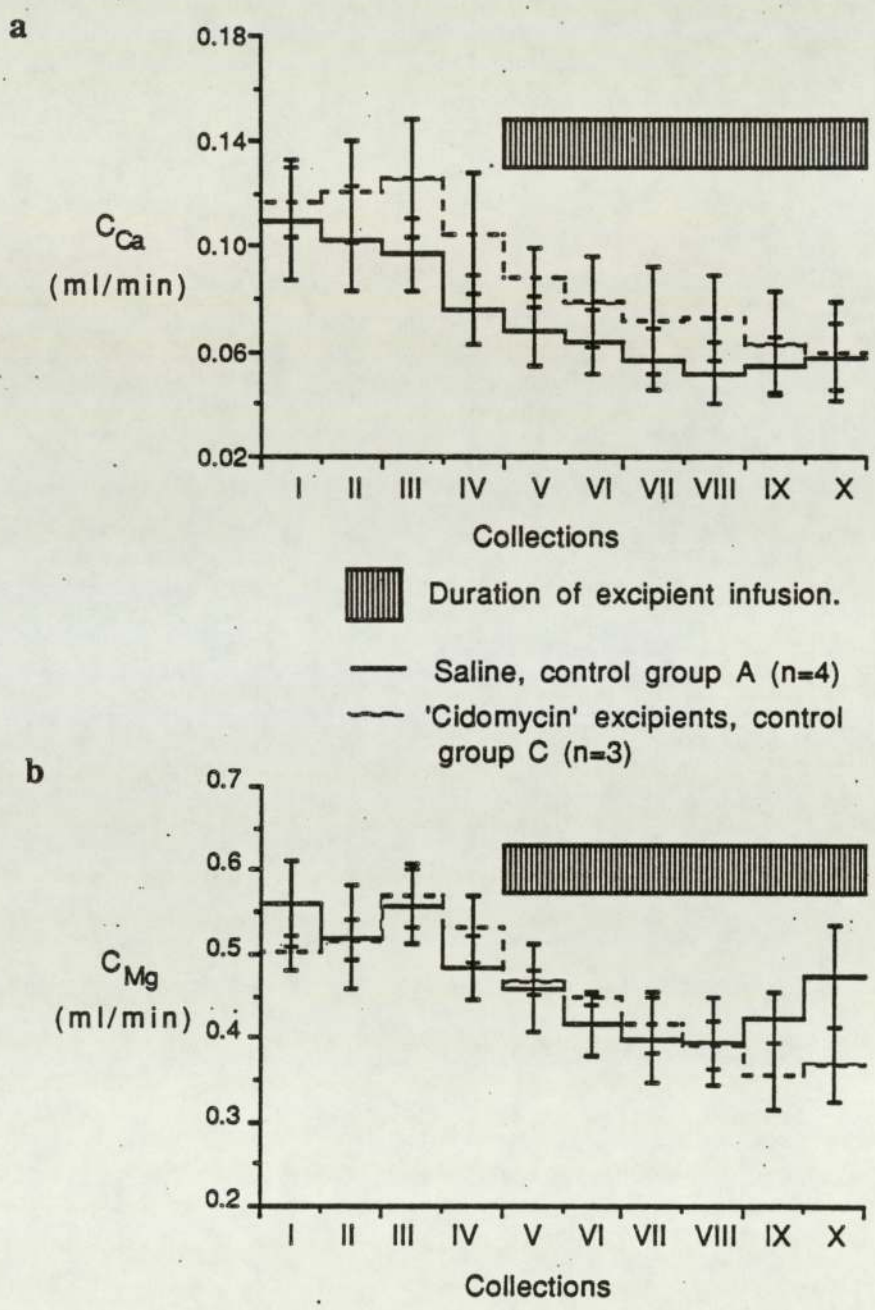


Figure 12. GFR in control groups B and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

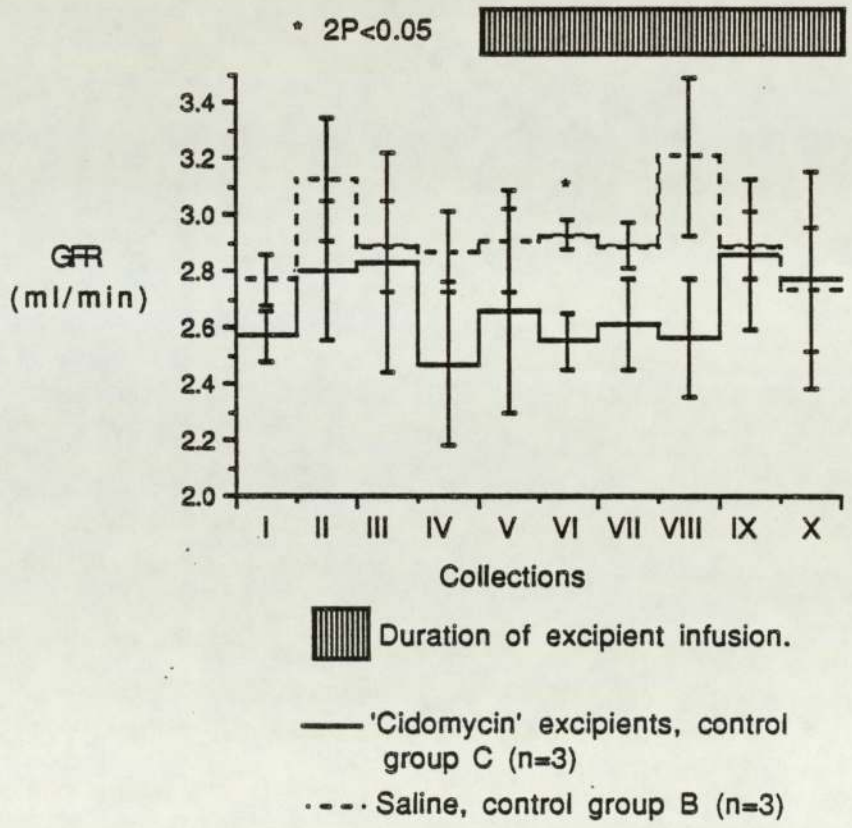


Figure 13. Fractional excretions of cations in control groups B and C expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

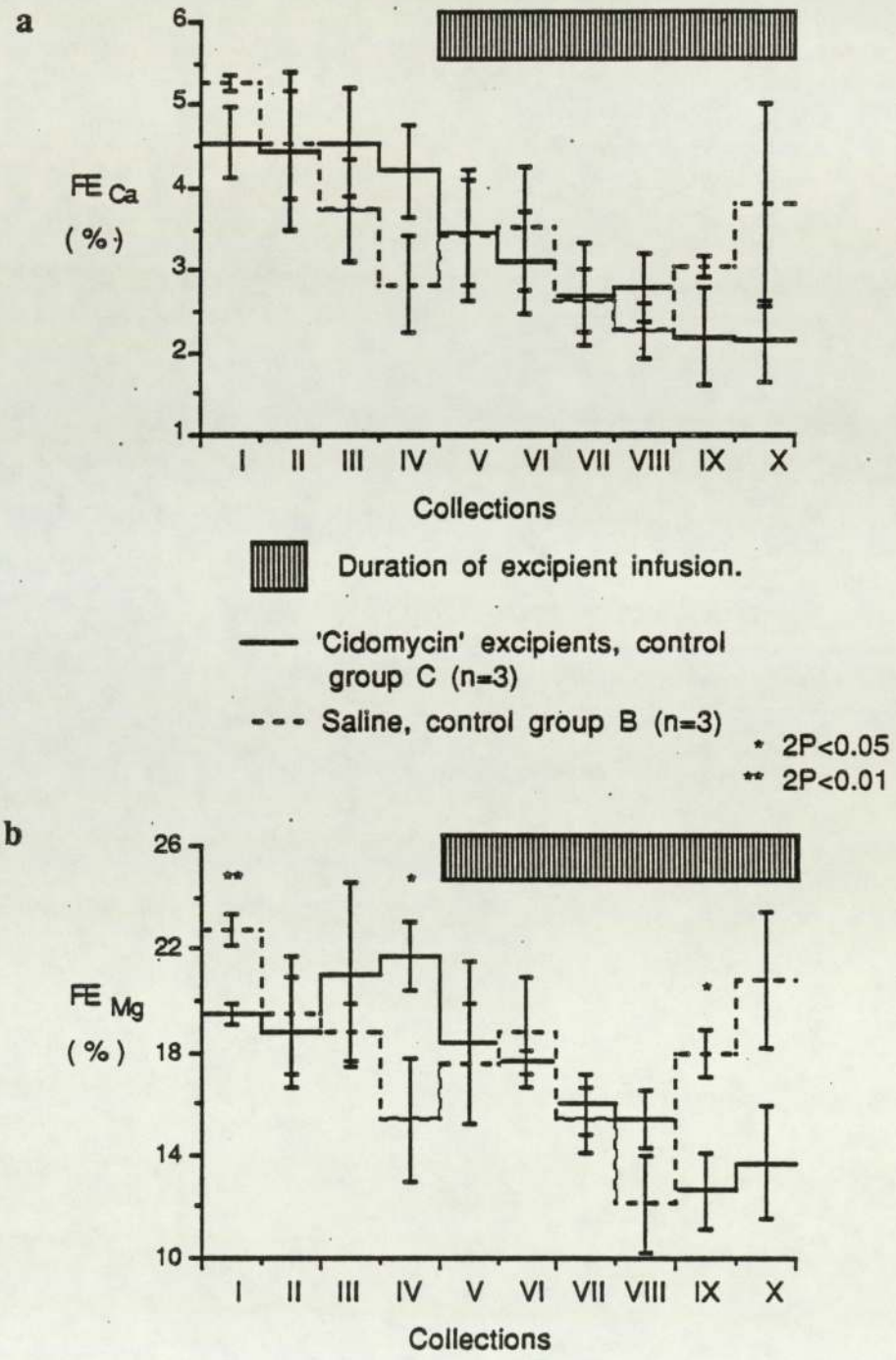


Figure 14. a. Urine flow rate in control group D. **b.** GFR in control group D. Both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

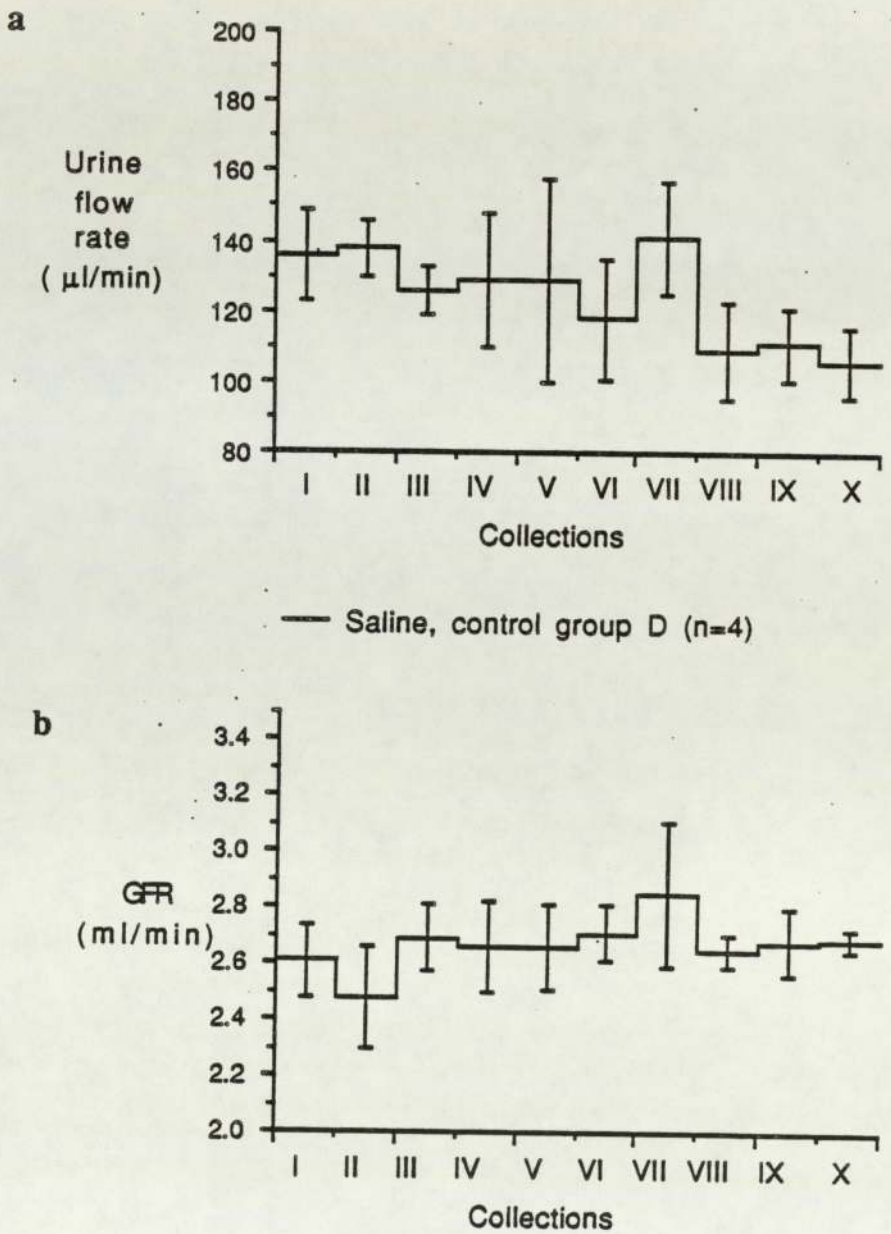
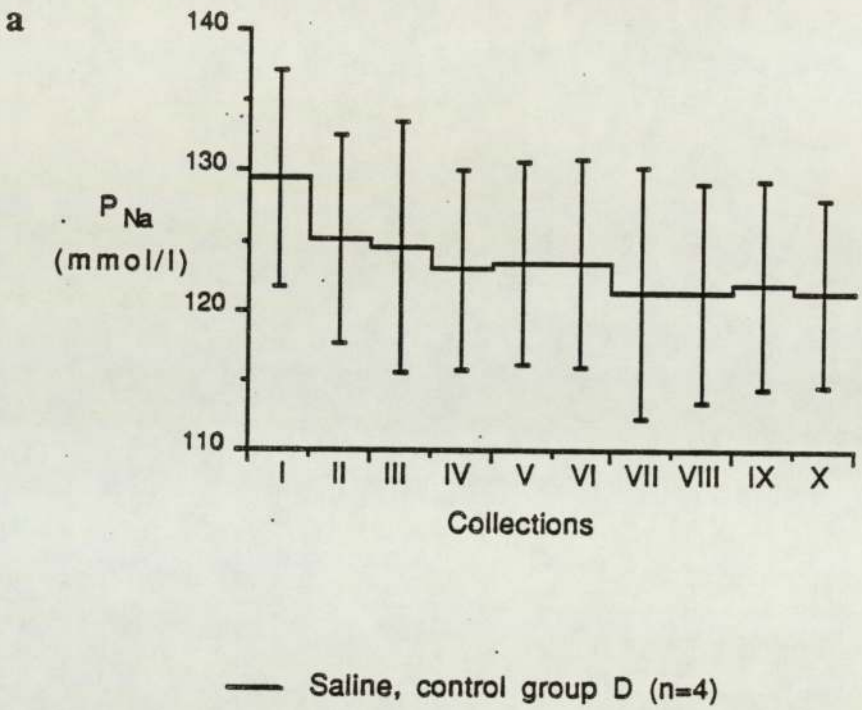


Figure 15. Concentrations of cations in plasma in control group D expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.



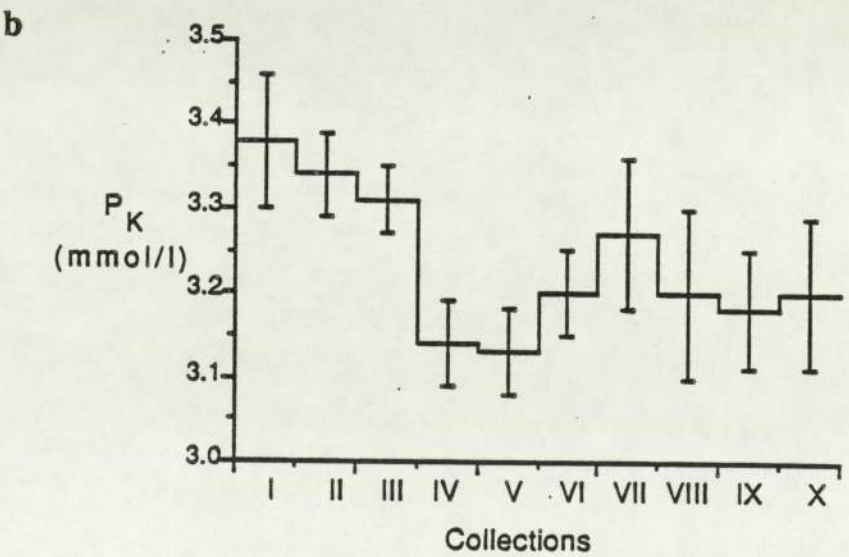
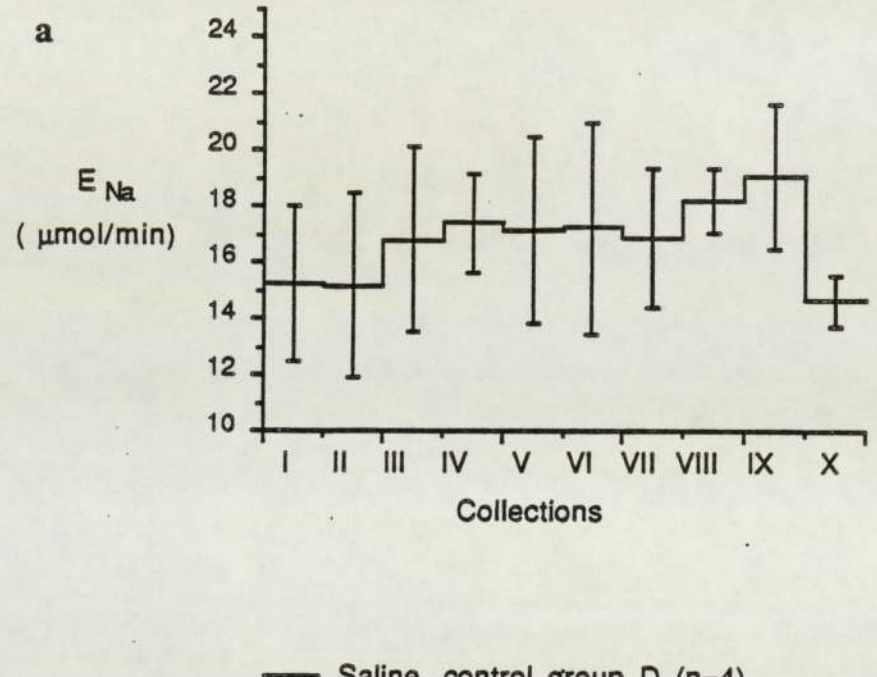


Figure 16. Excretion rates of cations in control group D expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.



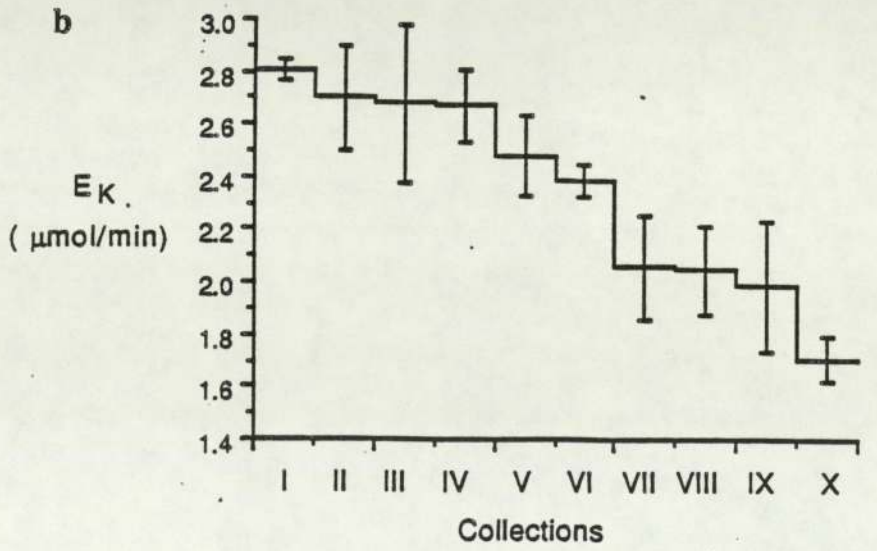


Figure 17. Clearances of cations in control group D expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.

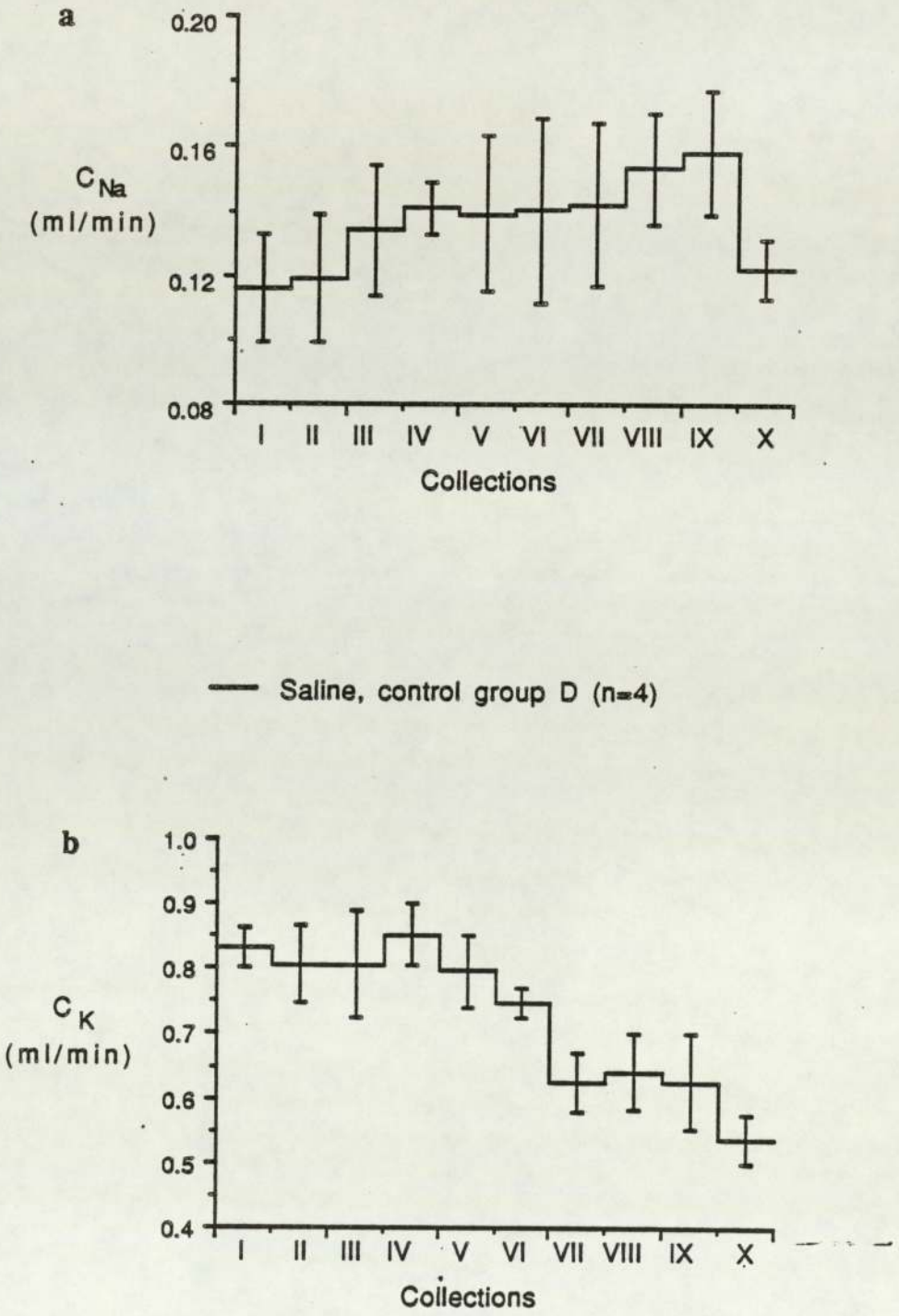
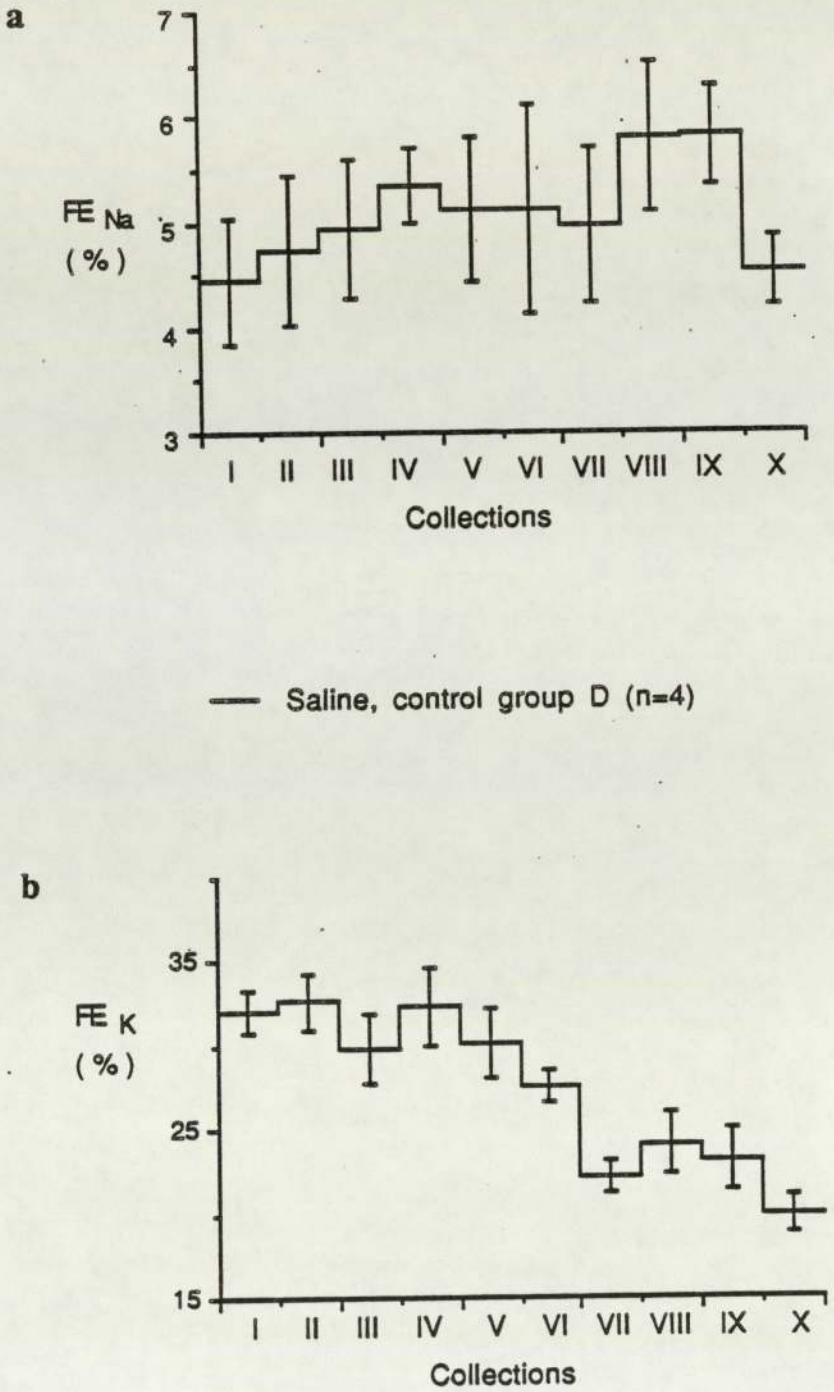


Figure 18. Fractional excretions of cations in control group D expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.



4.2 The effect of infusion of gentamicin on the renal handling of calcium and magnesium

a) Introduction:

Administration of gentamicin to conscious Wistar (Chawhala and Harpur, 1983) and Fischer 344 (Harpur et al, 1985a) rats is reported to result in a dose-related increase in calcium excretion rate. Less well-defined changes in magnesium excretion rate were also produced whereas sodium and potassium excretion rates were largely unaffected.

The present study was designed to define the origin of these effects. An increase in calcium output could be brought about by effects of gentamicin not involving the kidney. For example, an increase in the concentration of ultrafilterable calcium or an increase in glomerular filtration rate would elevate filtered load, and therefore excretion rate, without any effect on the renal mechanisms responsible for reabsorbing calcium. Alternatively, if no change in filtered load was produced an increase in excretion rate would indicate some interference with renal calcium reabsorptive mechanisms.

Therefore, if gentamicin infusion produced an elevation of calcium excretion rate without a simultaneous elevation in filtered load, this would indicate impaired renal reabsorption of calcium.

b) Methods:

The experimental procedure followed in these experiments is outlined in Figure 19. Gentamicin (as 'Cidomycin') was diluted in saline and administered by infusion during the experimental period as indicated. The rate of infusion of gentamicin base was 0.28 mg/kg/min or 0.56 mg/kg/min. The renal handling of calcium and magnesium handling was studied in six rats which received 0.28 mg/kg/min gentamicin and a second group of six rats which received

0.56 mg/kg/min gentamicin. Statistical comparisons were made with control group A, as described in the previous section.

c) Results:

i) 0.28 mg/kg/min gentamicin:

In rats infused with 0.28 mg/kg/min gentamicin mean urine flow rate did not differ significantly from the mean flow rate in saline-infused control rats at any time during the control or experimental periods (Figure 20). Mean plasma calcium and magnesium concentrations in these rats did not differ consistently from control rats (Figures 21a and 21b).

During the control period neither the mean excretion rate (Figure 22a) nor clearance (Figure 23a) of calcium in rats infused with 0.28 mg/kg/min gentamicin differed significantly from saline-infused control animals. Introduction of infused gentamicin at the beginning of the experimental period produced an immediate rise in both parameters to approximately 200% of initial values. This increase was highly significant ($2P<0.001$) during collections VI-IX and both mean excretion rate and mean clearance of calcium were sustained at stable, significantly raised levels for the full duration of this period.

Mean magnesium excretion rate (Figure 22b) and clearance (Figure 23b) were not different from saline-infused controls during the control period. Introduction of gentamicin to the infusate produced less marked increases in these parameters than in calcium excretion rate or clearance, both magnesium excretion rate and clearance rising to only 110-115% of initial values, these changes were significant only during collections V-IX and the significance level never exceeded $2P<0.01$. Both mean excretion rate and mean clearance of magnesium declined to values which were not significantly different from controls in the final hour of the experimental period.

ii) 0.56 mg/kg/min gentamicin:

Mean urine flow rate was consistently significantly lower in rats infused with 0.56 mg/kg/min gentamicin ($2P<0.05$ for much of the collection period) than in saline-infused controls (Figure 24). However, this was true during both the control and experimental periods and therefore cannot be considered a response to gentamicin infusion. Also, no significant depression of urine flow rate was seen in other experiments in which an identical experimental protocol was used (see Figure 28) therefore this is unlikely to be a significant finding.

The mean plasma calcium concentration (Figure 25a) was not consistently significantly different from saline-infused controls whereas the mean plasma magnesium concentration (Figure 25b) was significantly lower than controls during most of the experimental period at a significance level of not less than $2P<0.01$ (see Discussion).

Mean calcium excretion rate (Figure 26a) and clearance (Figure 27a) in those rats infused with 0.56 mg/kg/min gentamicin did not differ significantly from saline-infused controls during the control period. Introduction of infused gentamicin caused a rise in both parameters which was less immediate ($2P<0.05$ during collection V) but more marked than that produced by 0.28 mg/kg/min. Both mean excretion rate and clearance of calcium reached a peak of over 300% of initial values after one hour of gentamicin infusion. This response was sustained at a significantly raised ($2P<0.001$), stable level for the remainder of the experimental period.

Neither mean magnesium excretion rate (Figure 26b) nor clearance (Figure 27b) were significantly different from saline-infused controls during the control period. Introduction of gentamicin to the infusate caused a larger increase in mean magnesium clearance than that

produced by 0.28 mg/kg/min ($2P<0.001$ during collections VI-IX) but a similar increase in mean excretion rate. Mean magnesium clearance reached a peak of approximately 125% of initial values after one hour of gentamicin infusion, mean excretion rate reached a peak of approximately 110% of initial values after the same interval.

d) Discussion:

Infusion of gentamicin in saline at 0.28 and 0.56 mg/kg/min produced significant hypercalciuria and hypermagnesiuria There were no concomitant increases in plasma calcium and magnesium concentrations and, although GFR was not measured in these experiments, it seems unlikely that increases in the filtered loads of calcium or magnesium were responsible for these changes. Furthermore, in later experiments in which 0.56 mg/kg/min gentamicin was infused, GFR was found to be stable, as were the concentrations of calcium and magnesium in plasma (see Section 4.4). Also, similar elevations of calcium and magnesium output in these experiments were reflected by elevations in the fractional excretion of these cations indicating depression of tubular reabsorption of calcium and magnesium by gentamicin.

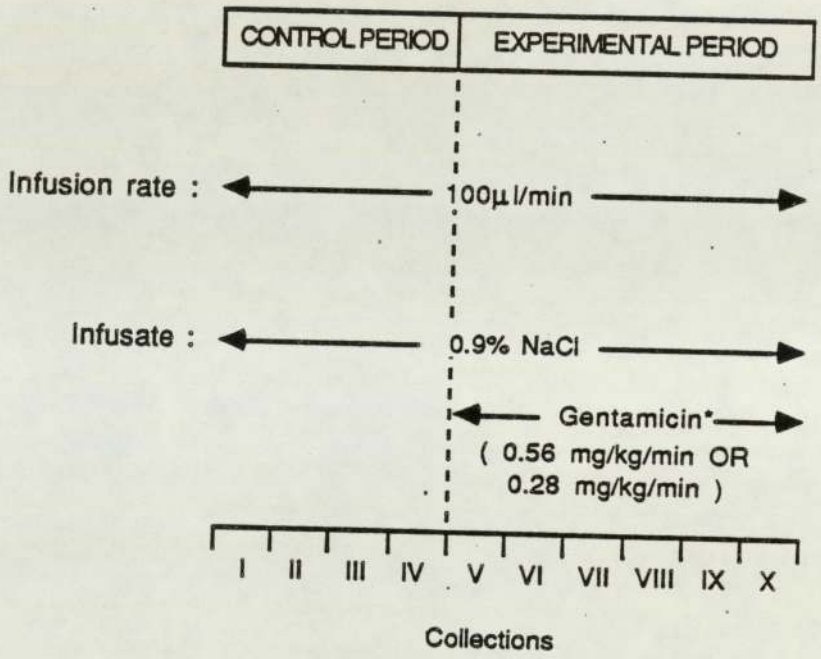
The hypercalciuric and hypermagnesiuric responses to gentamicin infusion were extremely rapid; occurring within 30 minutes of introduction of the drug to the infusate. However, the effect on calcium was consistently larger and more stable than the effect on magnesium (see Figure 32).

These effects were apparently related to the infusion rate of gentamicin, the hypercalciuric response, in particular, was greater at the higher infusion rate.

A notable finding was a significant reduction in mean plasma magnesium concentration in rats infused with 0.56 mg/kg/min gentamicin which occurred simultaneously with the elevations in

calciuria and magnesiuria. This did not occur in those rats infused with 0.28 mg/kg/min gentamicin, despite similar increases in magnesium output in the two groups, suggesting that renal wasting of magnesium was not responsible for the appearance of hypomagnesaemia in only one of these groups. Possibly the significant difference in mean urine flow rate between rats in control group A and rats infused with 0.56 mg/kg/min gentamicin led to a significant volume expansion of the gentamicin-infused group relative to the controls. This would in turn lead to a relative dilution of plasma solutes in the gentamicin-infused rats including plasma calcium and magnesium. Plasma calcium concentration is extremely closely regulated and therefore homeostatic mechanisms would be called into play to reverse any hypocalcaemia whereas the plasma concentration of magnesium would be permitted to fall.

Figure 19. Protocol used in clearance experiments in which gentamicin ('Cidomycin') was infused for three hours following a two hour saline infusion.



* Added to infusate according to protocol.

Figure 20. Urine flow rate in rats infused with 0.28 mg/kg/min gentamicin over a period of three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

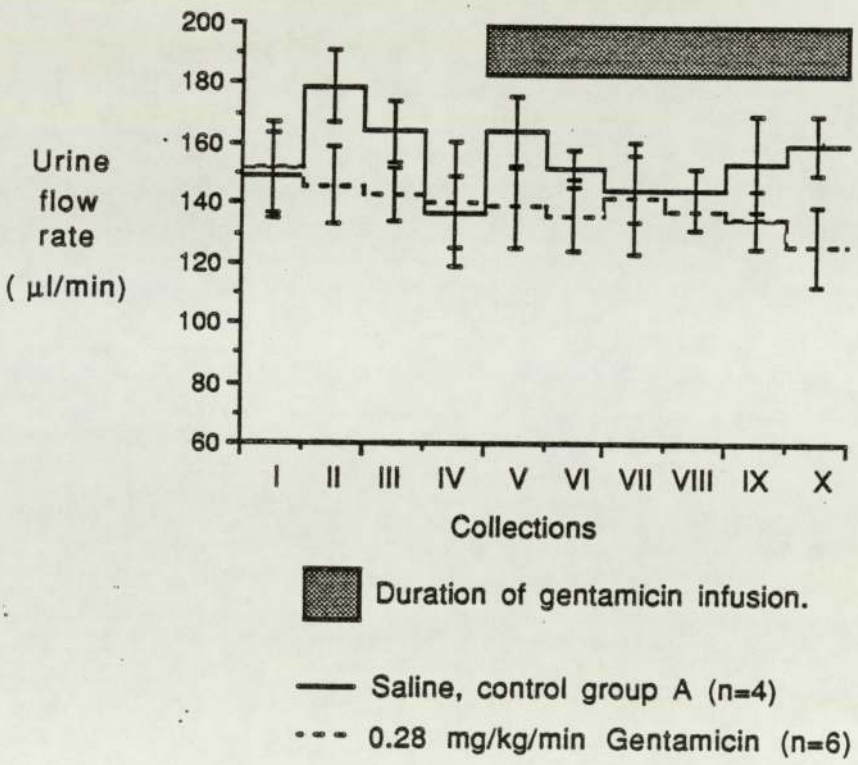


Figure 21. Concentrations of cations in plasma in rats infused with 0.28 mg/kg/min gentamicin over three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

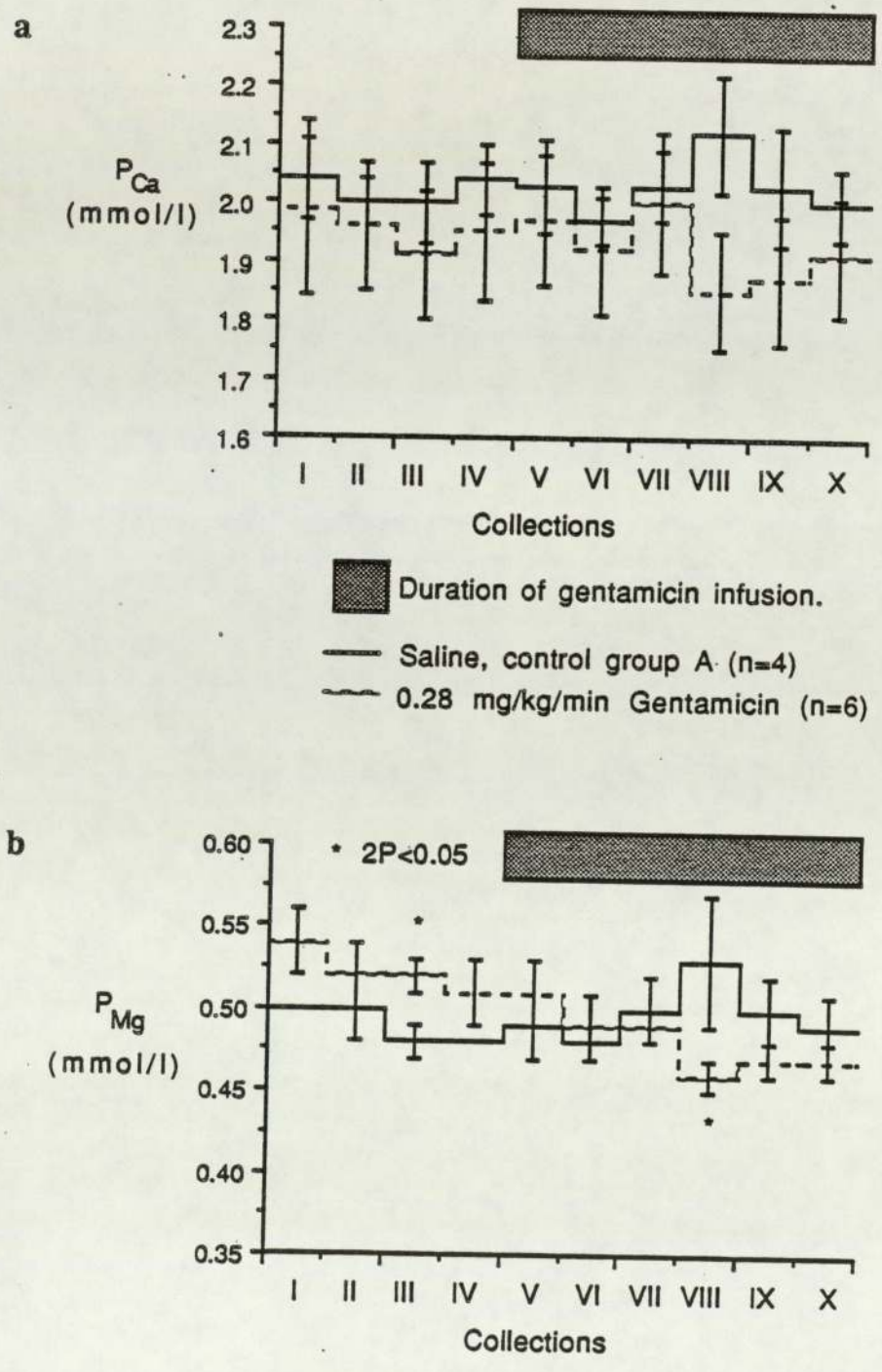


Figure 22. Excretion rates of cations in rats infused with 0.28 mg/kg/min gentamicin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

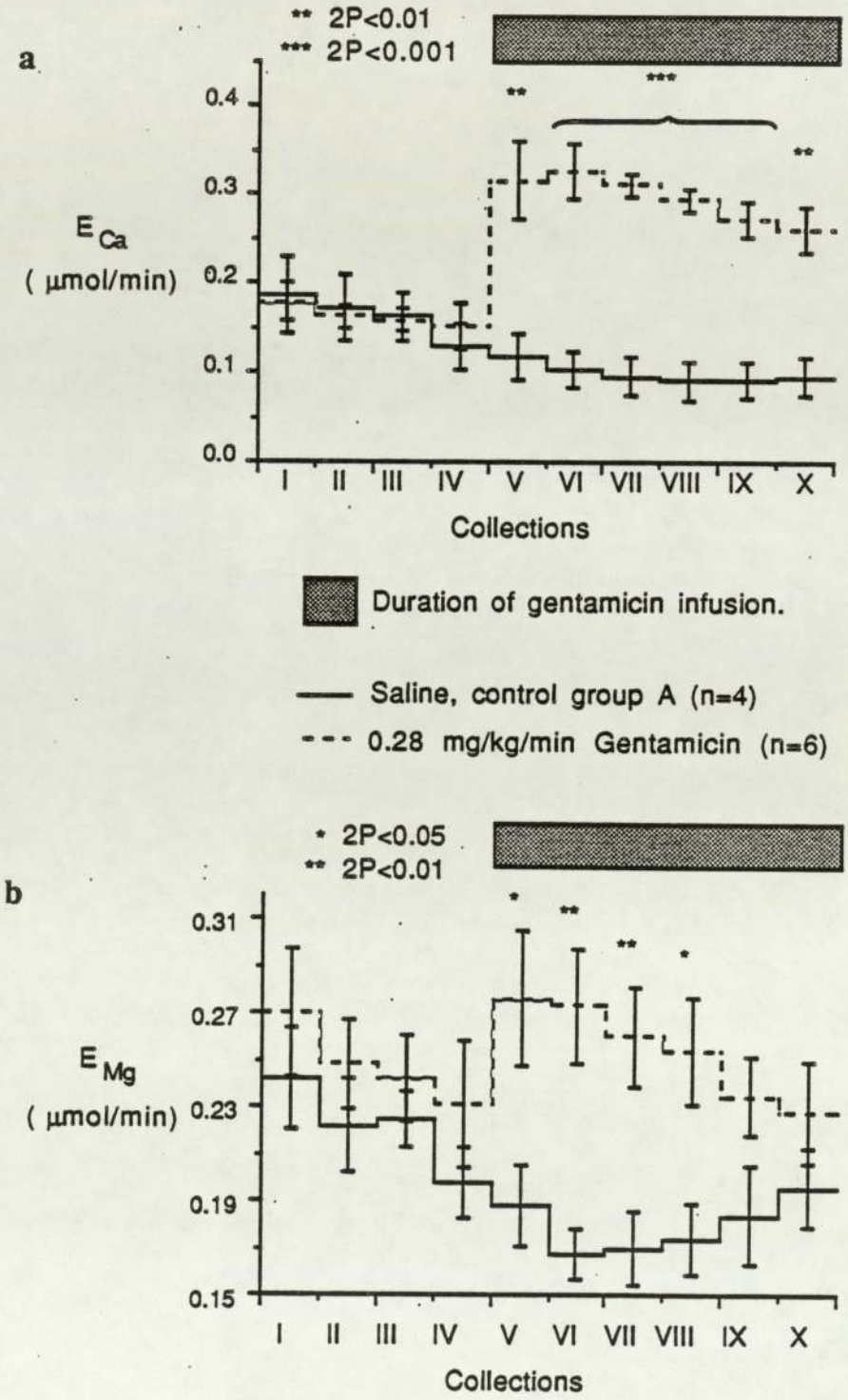


Figure 23. Clearances of cations in rats infused with 0.28 mg/kg/min gentamicin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

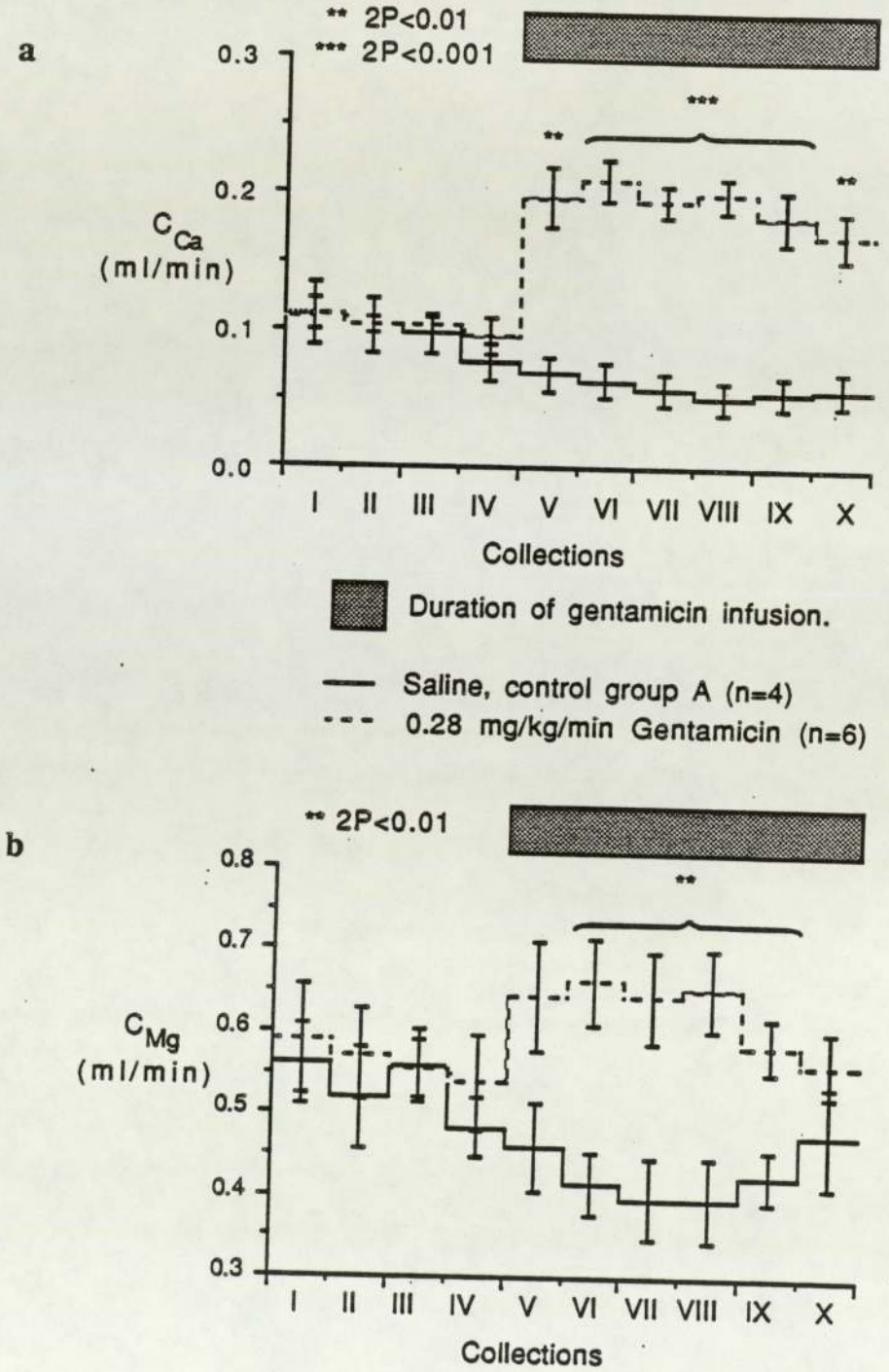


Figure 24. Urine flow rate in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

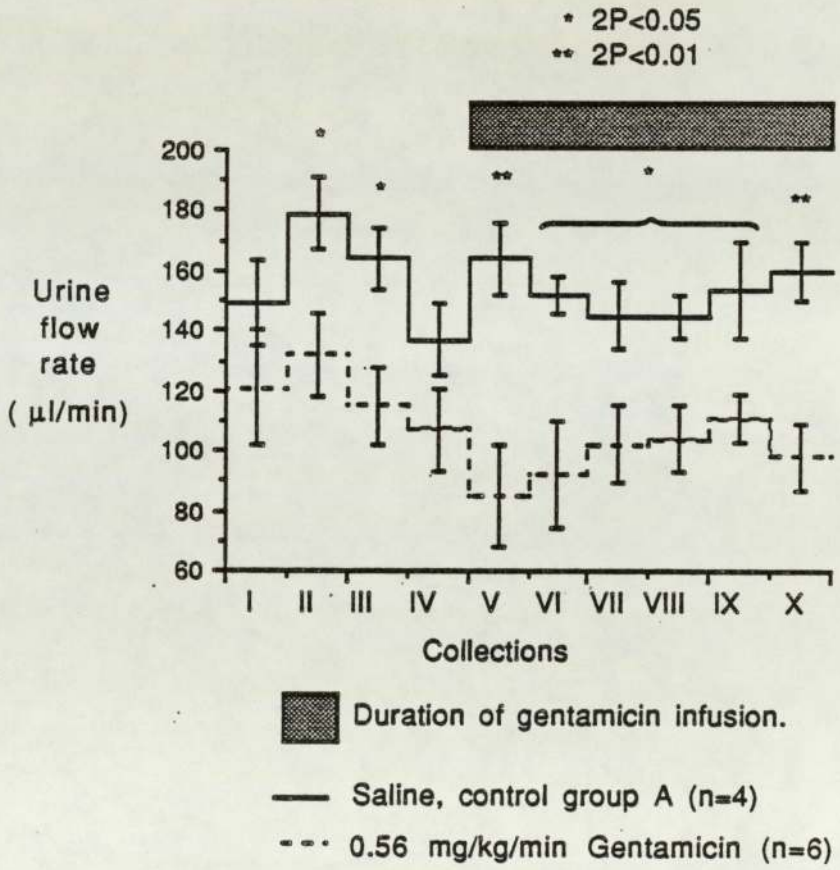


Figure 25. Concentrations of cations in plasma in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

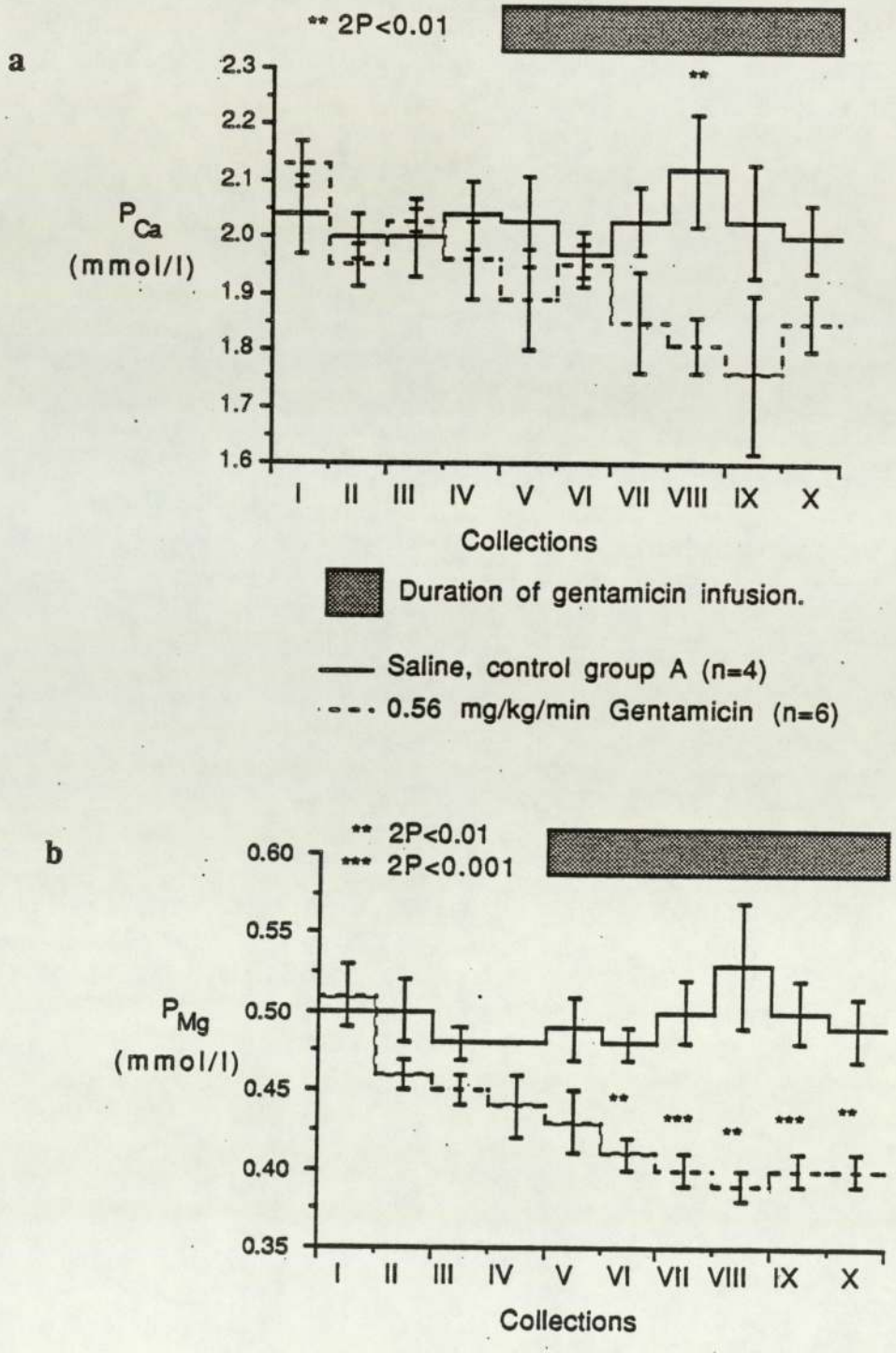


Figure 26. Excretion rates in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

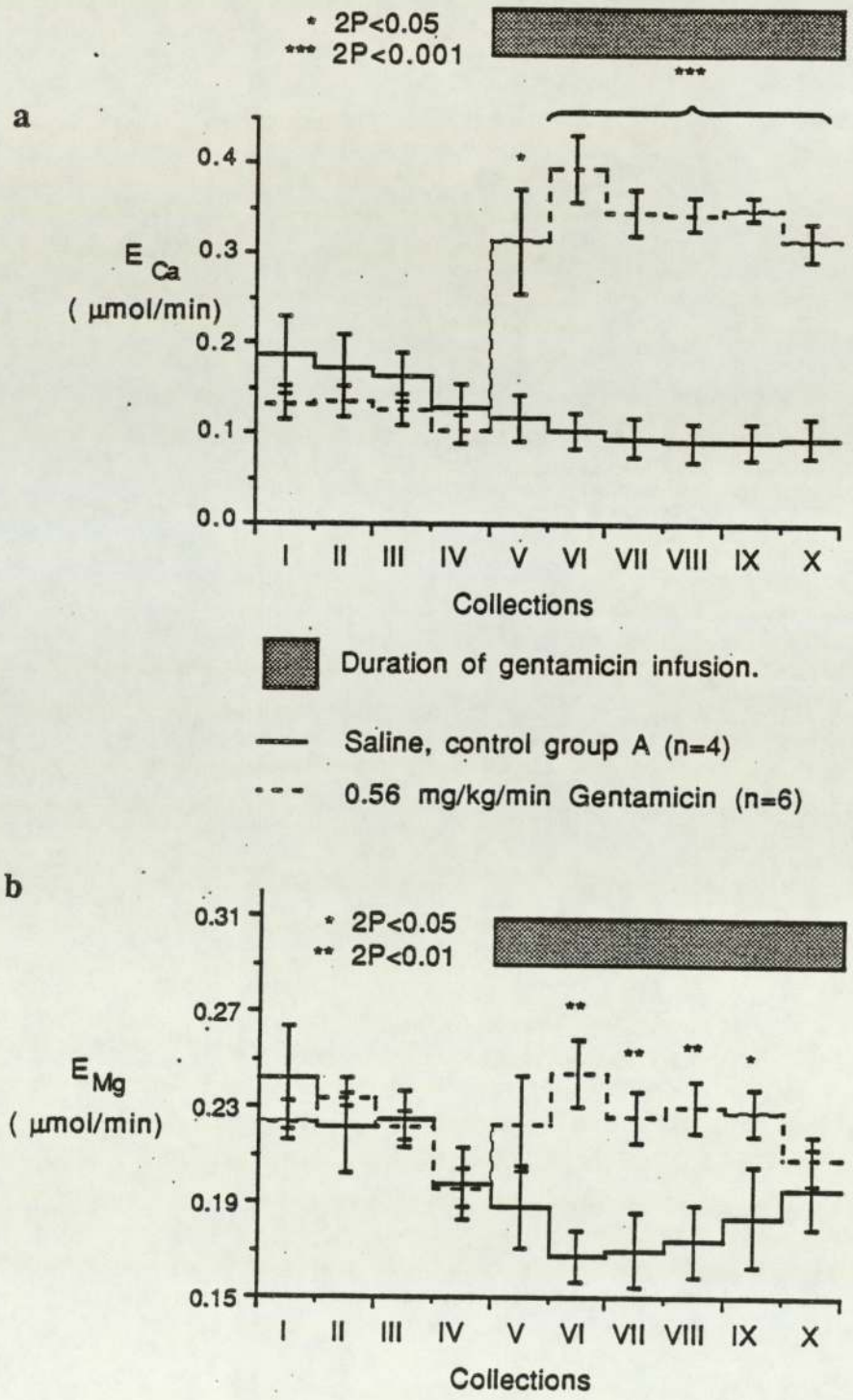
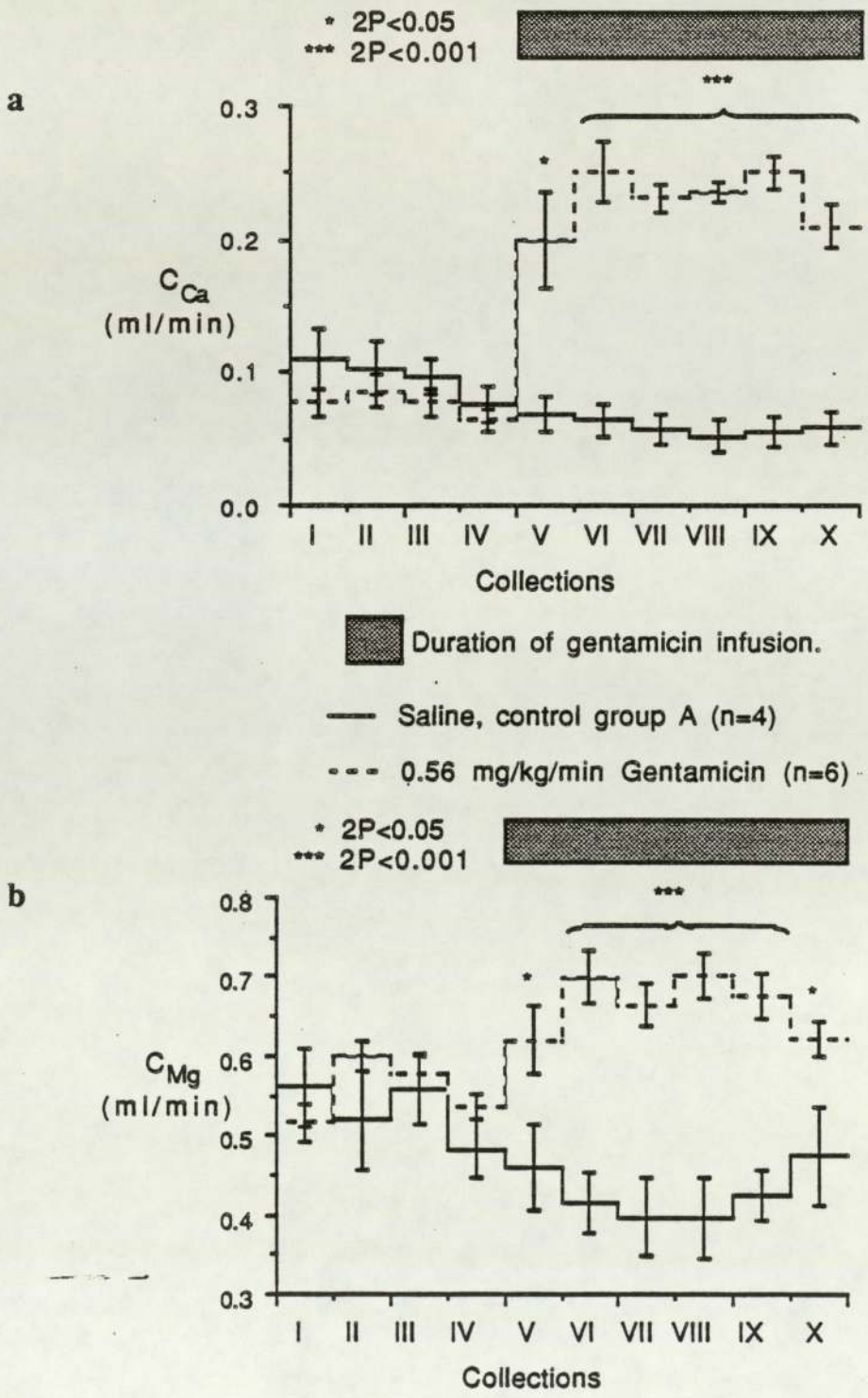


Figure 27. Clearances of cations in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



4.3 The effect of infusion of gentamicin on the renal handling of sodium and potassium

a) Introduction:

Changes in the renal handling of potassium have been reported as a response to the administration of aminoglycosides in various animal models. In most cases an increase in potassium output was the result (Mitchell et al, 1977; Luft et al, 1978a; Smith et al, 1981; Brinker et al, 1981; Cronin et al, 1980; Bennett et al, 1983; Pastoriza et al, 1983) although in a small number of studies no change in potassium output was seen (Chiu et al, 1976; Chahwala and Harpur, 1983; Cronin and Newman, 1985). Reports of changes in sodium output have been less consistent in their findings, increases (Luft et al, 1978a; Smith et al, 1981; Pastoriza et al, 1983) decreases (Bennett et al, 1983; Elliott et al, 1987) and no change (Chiu et al, 1976; Mitchell et al, 1977; Chahwala and Harpur, 1983; Cronin and Newman, 1985; Bennett et al, 1985) have all been demonstrated.

Therefore a study to detect any changes in the renal handling of sodium and potassium in the present model was imperative.

b) Methods:

The experimental protocol followed in these experiments is outlined in Figure 19. Cidomycin was diluted in saline and infused during the experimental period as shown. The renal handling of sodium and potassium was studied in five rats which received 0.56 mg/kg/min gentamicin base, statistical comparisons were made with control group D.

c) Results:

Neither mean urine flow rate (Figure 28) nor mean plasma sodium concentration (Figure 29a) in gentamicin-infused rats were

consistently different from saline-infused controls during the control or experimental periods.

Mean plasma potassium concentration was significantly higher in rats infused with gentamicin between collections IV and VII (Figure 29b). However, mean excretion rate (Figure 30b) and clearance (Figure 31b) of potassium in gentamicin-infused rats did not differ from controls except for a transient fall in excretion rate ($2P<0.05$) and clearance ($2P<0.01$) during collection IV. Thus an increase in plasma concentration, and presumably filtered load, of potassium did not result in increased urinary output of this cation, indicating that the renal handling of potassium was probably unimpaired. The elevation in mean plasma potassium concentration first occurred during collection IV, 30 minutes before infusion of gentamicin was initiated, therefore this result was probably not a response to the aminoglycoside.

Mean excretion rate (Figure 30a) and clearance (Figure 31a) of sodium did not differ significantly from saline-infused controls during the control or experimental periods except for a small, temporary rise in sodium clearance ($2P<0.05$) during collection V. GFR was not measured in the gentamicin-infused rats and so whether this change was the result of increased filtered load or increased fractional excretion of sodium cannot be discerned. As stated above, no significant change in plasma sodium concentration occurred in gentamicin-infused rats, therefore elevated filtered load of sodium could only be responsible for the increase in sodium clearance by means of an increase in GFR.

d) Discussion:

Infusion of 0.56 mg/kg/min gentamicin produces no apparent change in the renal handling of potassium in this model and is unlikely to contribute to an elevation in mean plasma potassium

concentration seen in this experiment.

Gentamicin infusion may produce a small, transient rise in sodium clearance immediately after introduction of infused gentamicin, the mechanism of this occurrence is not clear since no coincident changes in sodium excretion rate or plasma concentration were seen. This isolated and minimal event is probably of no consequence and, indeed, may not be a response to the infusion of gentamicin.

Therefore, effects on the renal handling of cations produced by gentamicin infusion are restricted to the divalent cations, particularly calcium (Figure 32).

Figure 28. Urine flow rate in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

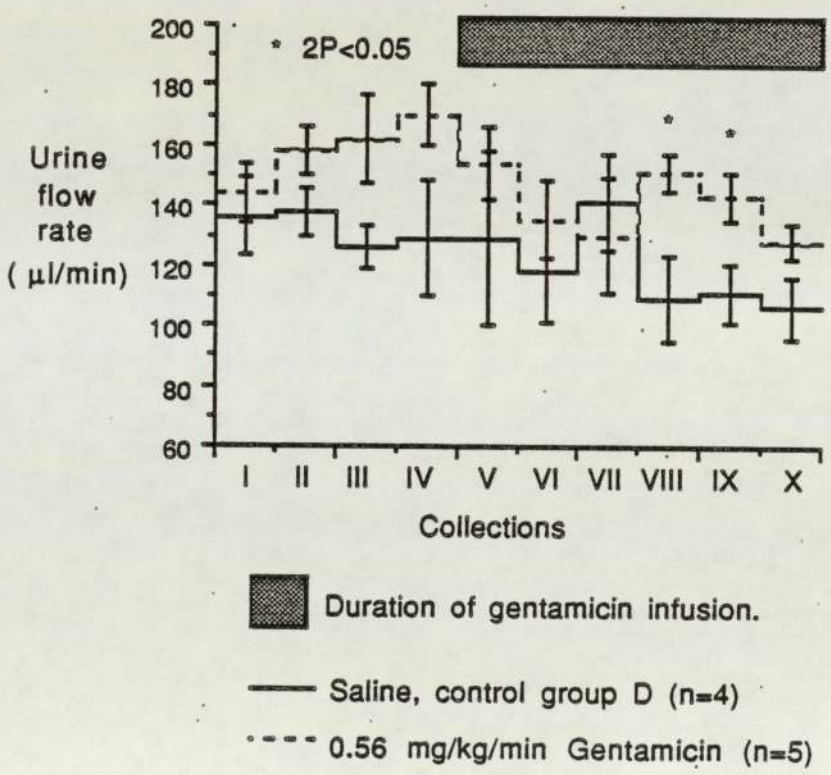


Figure 29. Concentrations of cations in plasma in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.

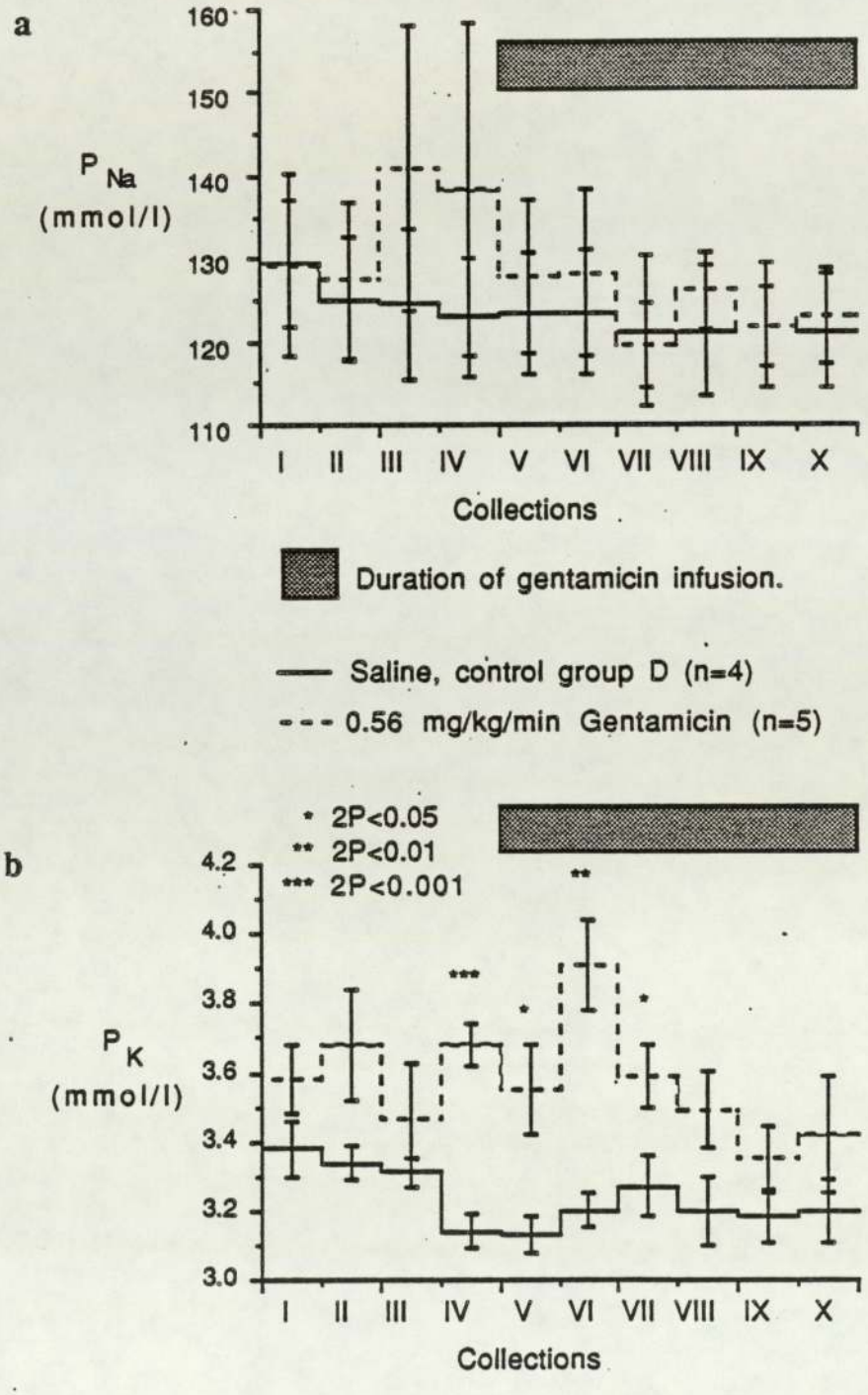


Figure 30. Excretion rates of cations in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.

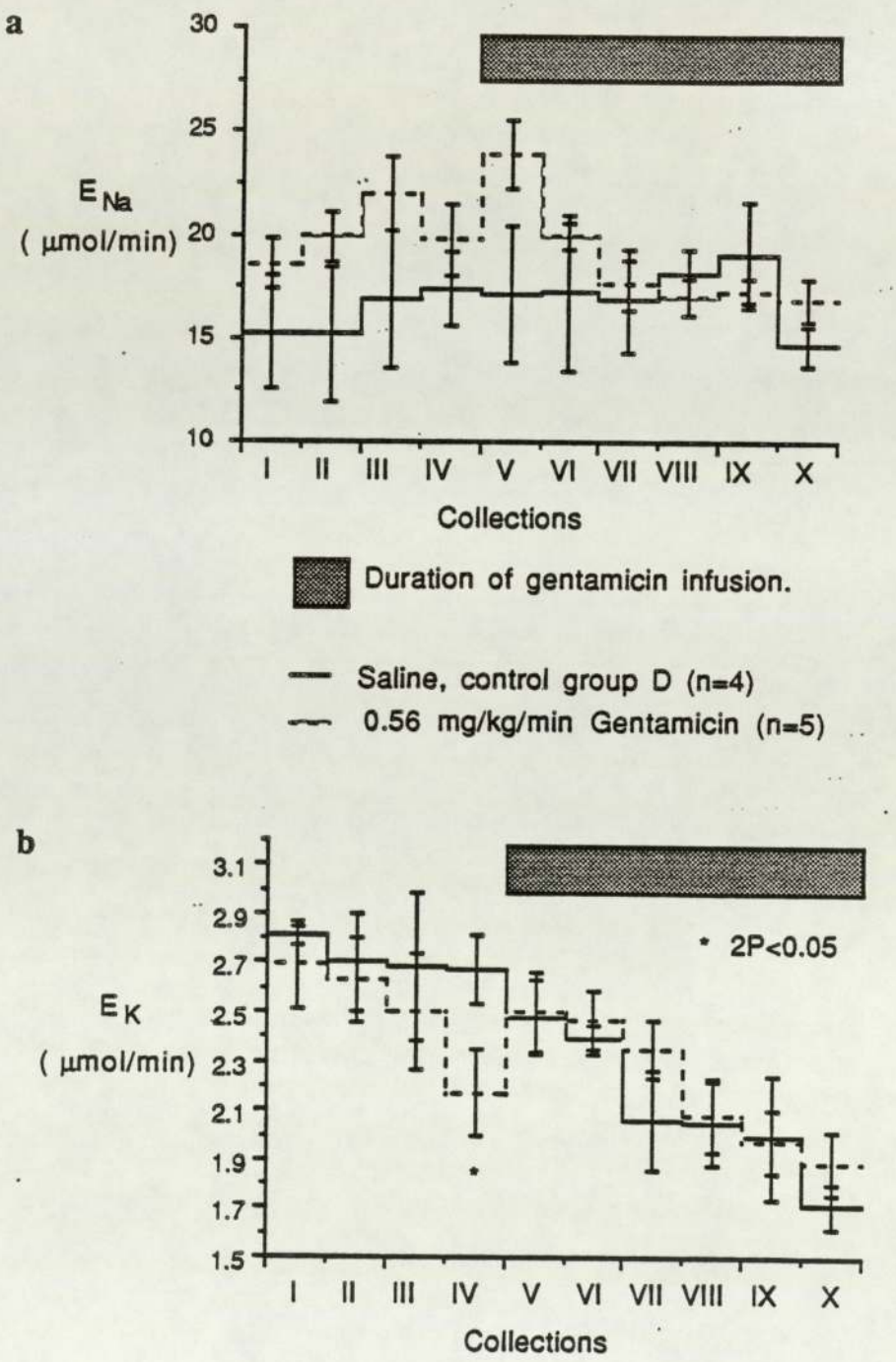


Figure 31. Clearances of cations in rats infused with 0.56 mg/kg/min gentamicin for three hours expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Sodium. **b.** Potassium.

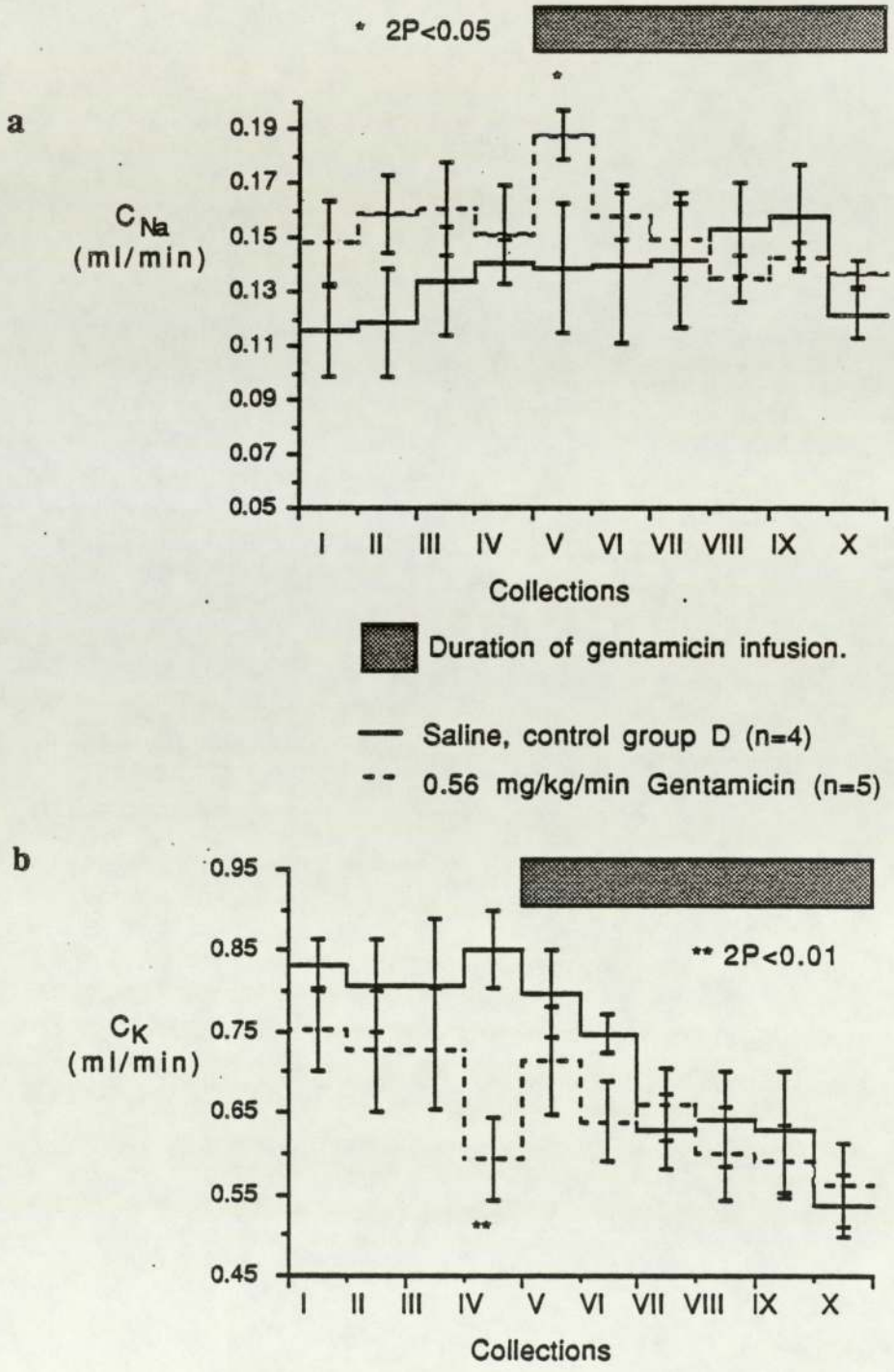
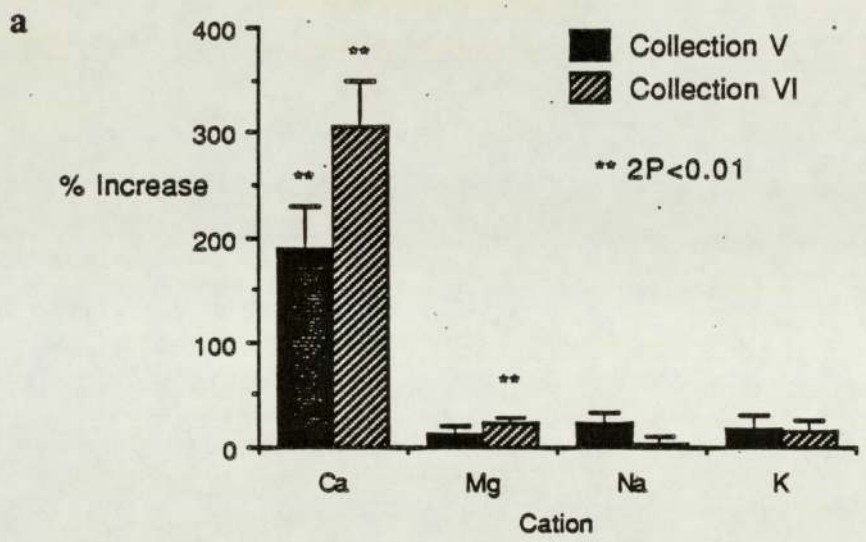
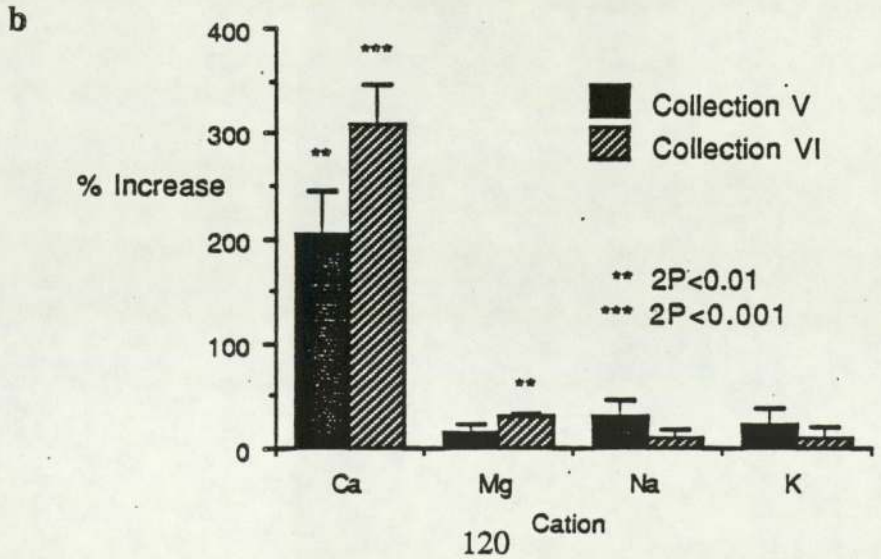


Figure 32. Histograms illustrating the effect of 0.56 mg/kg/min gentamicin on the excretion rates and clearances of the four major cations. Each column represents the mean of the individual percentage increases in excretion rate or clearance at collection V or VI over values at collection IV from the same individuals ± s.e.m. Statistical comparisons were made using Student's 't' test for paired data. **a.** Excretion rates. **b.** Clearances.





4.4 Reversibility of gentamicin-induced alterations in the renal handling of calcium and magnesium

a) Introduction:

In the study of Chahwala and Harpur (1983) the elevation in calcium excretion rate prompted by seven days of gentamicin administration (40 mg/kg/day) in rats was still evident six days after the last drug injection. However, in the study of Harpur et al (1985a) the elevation of calcium excretion rate produced by eight days of gentamicin (10-30 mg/kg/day) returned to control levels within seven days of the last injection. A further investigation (Harpur et al, 1985b) in which a moderate dose (20 mg/kg/day) of gentamicin was used produced contrasting results. Following eighty one days of gentamicin injection calcium excretion rate, which was consistently raised during this period, persisted at elevated levels for a further twenty days. In this study magnesium excretion rate was also raised in drug-treated rats, a response which had not been found consistently in earlier experiments. Magnesium, unlike calcium, excretion rate returned to control levels within twenty days of the last injection. This body of work prompted an investigation into the reversibility of the acute hypercalciuria and hypermagnesiuria produced by gentamicin infusion in anaesthetised rats.

b) Methods:

The experimental procedures followed in this investigation are outlined in Figures 33 and 39. three rats were infused with 0.56 mg/kg/min gentamicin for two hours followed by three hours of saline infusion. A further three rats were infused with 0.56 mg/kg/min gentamicin for one hour followed by four hours of saline infusion.

Since previous experiments failed to show any effect of gentamicin on the renal handling of sodium or potassium, only the renal handling of calcium and magnesium were studied in these and future experiments. In statistical tests both groups were compared with control group C.

c) Results:

i) 2 hour gentamicin infusion:

Mean urine flow rate (Figure 34a), GFR (Figure 34b) and the mean plasma calcium and magnesium concentrations (Figures 35a and 35b) in these rats were not significantly different from controls at any time during the experimental and recovery periods.

The mean excretion rate (Figure 36a) and clearance (Figure 37a) of calcium were both significantly (generally $2P<0.01$) elevated with respect to controls during the experimental period. Both parameters were stable during this period but fell throughout the recovery period. These changes could be explained by parallel alterations in the mean fractional excretion of calcium (Figure 38a), indicating that the elevation of calcium output and clearance promoted by gentamicin infusion were brought about by a reduction in the tubular reabsorption of calcium. Mean calcium excretion rate was still significantly ($2P<0.05$) higher than controls at the end of the recovery period. Only mean calcium clearance fell to a value which was not significantly different from controls in the final 30 minutes of the recovery period. Fractional excretion of calcium was still significantly raised ($2P<0.05$) at the end of the recovery period indicating that the impairment of calcium reabsorption caused by gentamicin had not fully recovered three hours after gentamicin infusion had ceased.

Mean magnesium clearance (Figure 37b) was significantly higher (generally $2P<0.05$) than controls for the full duration of the

experimental period, in contrast, the mean excretion rate (Figure 36b) of magnesium was only significantly ($2P<0.05$) higher than controls during the first 30 minutes of gentamicin infusion but remained above control values for the remainder of the experimental period. Mean fractional excretion of magnesium (Figure 38b) was not significantly elevated during the experimental period except during the first 30 minutes ($2P<0.05$) but was raised with respect to controls for the remainder of the experimental period. This suggests that reduced tubular magnesium reabsorption was probably responsible for the elevation of magnesium clearance. Termination of drug infusion resulted in a more rapid decline in magnesuria than calciuria. Mean magnesium clearance was indistinguishable from control values after only 60 minutes of recovery, mean excretion rate also declined at a similar rate. Mean fractional excretion of magnesium decreased as rapidly as excretion rate indicating that the interference with magnesium reabsorption caused by gentamicin was fully reversed within the recovery period. Unlike the gentamicin-induced impairment of calcium conservation which persisted for the full duration of the recovery period.

ii) 1 hour gentamicin infusion:

Mean urine flow rate (Figure 40a), GFR (Figure 40b) and the mean plasma magnesium concentration (Figure 41b) were not consistently significantly different from control values. The mean concentration of calcium in plasma (Figure 41a) was significantly (generally $2P<0.05$) higher than controls in 50% of arterial blood samples, however, the points at which elevated plasma calcium concentration appeared were not coincident with hypercalciuria, therefore this result probably does not represent a response to gentamicin.

Mean calcium excretion rate (Figure 42a) and clearance (Figure 43a) were both significantly raised ($2P<0.01$) with respect to controls

during the experimental period. Both parameters fell during the recovery period, reaching values which were not significantly different from controls 2-3 hours after the end of gentamicin infusion. These responses could be explained by parallel alterations in the fractional excretion of calcium (Figure 44a) which was significantly ($2P<0.01$) elevated during the experimental period indicating an impairment of calcium reabsorption in the presence of gentamicin. Mean fractional excretion of calcium then fell, reaching values indistinguishable from controls 2.5 hours after gentamicin infusion had ceased, indicating recovery of tubular calcium reabsorptive capacity to control levels in this interval.

Mean magnesium excretion rate (Figure 42b) and clearance (Figure 43b) were both significantly higher ($2P<0.05$ or $2P<0.01$) than controls during the experimental period. This response could be explained by a significant ($2P<0.05$) elevation of mean fractional excretion of magnesium (Figure 44b) indicating reduction in the tubular reabsorption of this cation caused by gentamicin. Again, termination of drug infusion resulted in a more rapid decrease in magnesuria than in calciuria, mean magnesium excretion rate and clearance were indistinguishable from control values within the first hour of recovery. Mean fractional excretion of magnesium returned to control values within the 30 minutes of cessation of drug infusion indicating again that the impairment of magnesium reabsorption produced by gentamicin was very rapidly reversible.

d) Discussion:

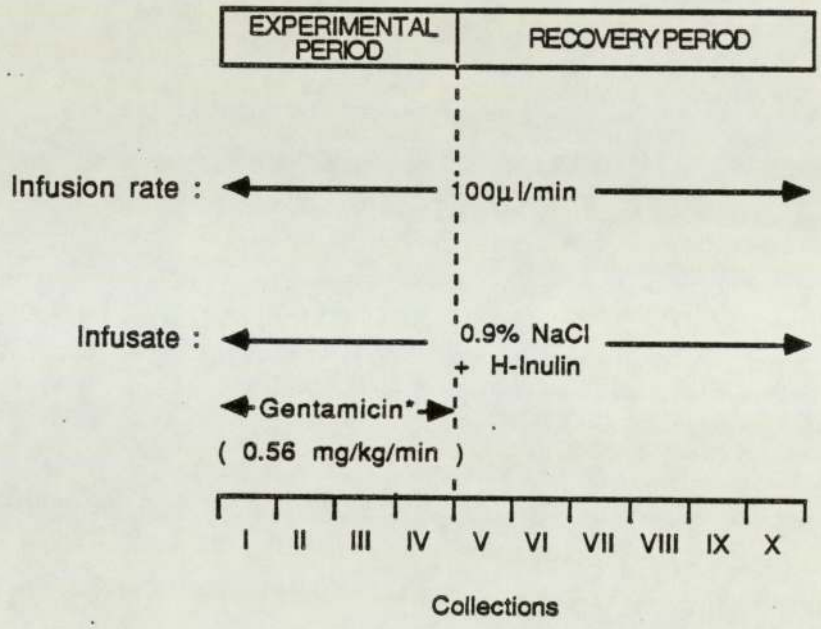
It is clear from this study that the increases in calcium and magnesium output stimulated by gentamicin infusion were produced by a decrease in renal tubular reabsorption since the fractional excretions of both cations were also elevated during drug infusion.

The hypercalciuric and hypermagnesiuric responses to gentamicin

infusion are apparently rapidly reversible when gentamicin infusion ceases. Calciuria returned to control levels approximately three hours after the end of gentamicin infusion. In contrast, magnesiuria recovered to control values within an hour of recovery.

Possibly, the acute impairment of calcium and magnesium reabsorption demonstrated in the anaesthetised model develops into a chronic renal 'leak' of divalent cations with prolonged exposure to gentamicin. Evidence in support of this theory is provided by the reversibility of hypercalciuria after only eight days of gentamicin injection (Harpur et al, 1985a) in contrast with the lack of reversibility after eighty days of the drug (Harpur et al, 1985b). It is also noteworthy that in this latter study hypermagnesiuria was apparently more rapidly reversible than hypercalciuria which is a conclusion also drawn from the present investigation, albeit in a different model.

Figure 33. Protocol used in clearance experiments in which the reversibility of the changes produced by gentamicin was studied, gentamicin was infused for two hours followed by a three hour saline infusion.



* Added to infusate according to protocol.

Figure 34. a. Urine flow rate in reversal experiments. **b.** GFR in reversal experiments. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

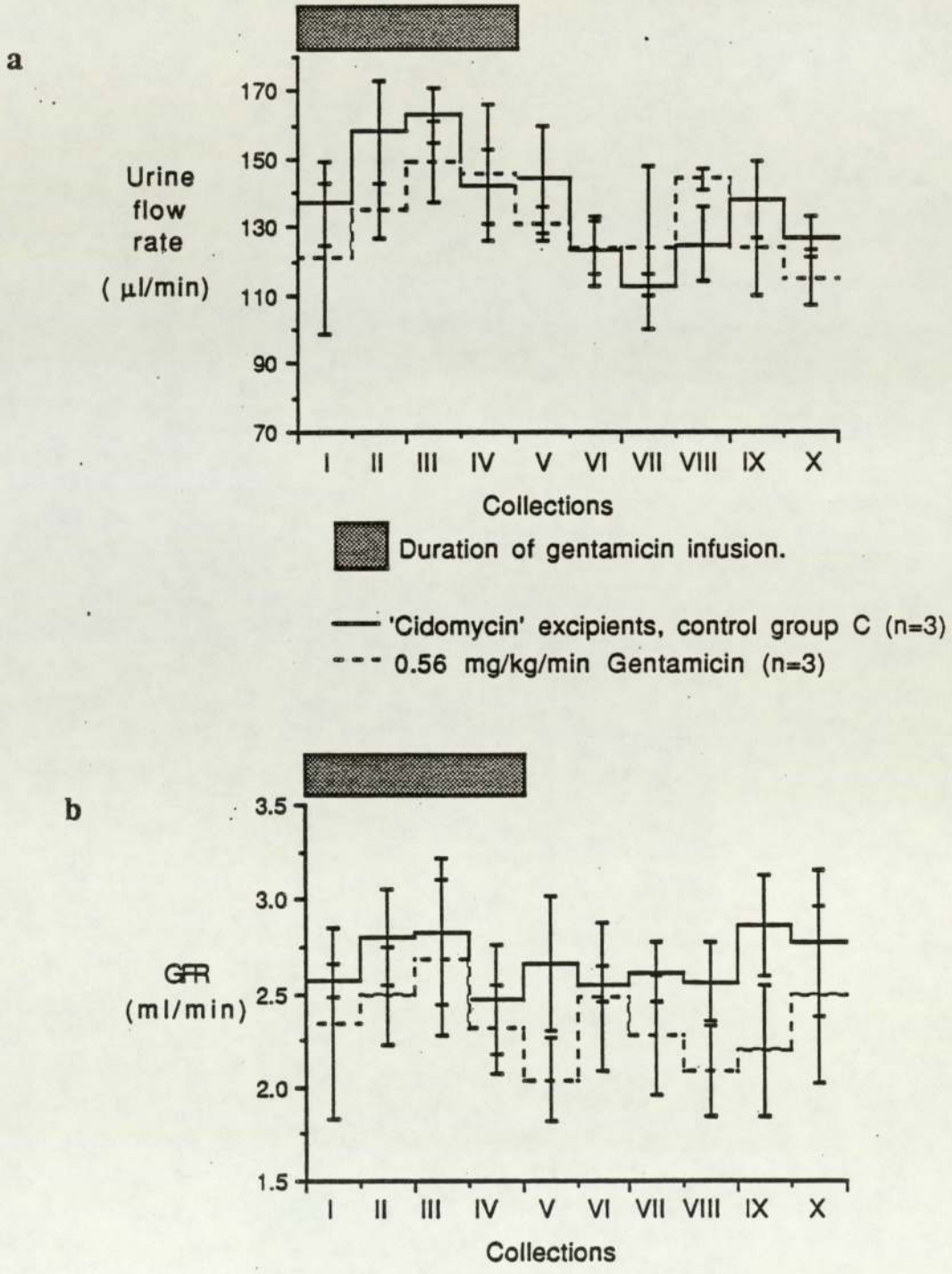


Figure 35. Concentrations of cations in plasma in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

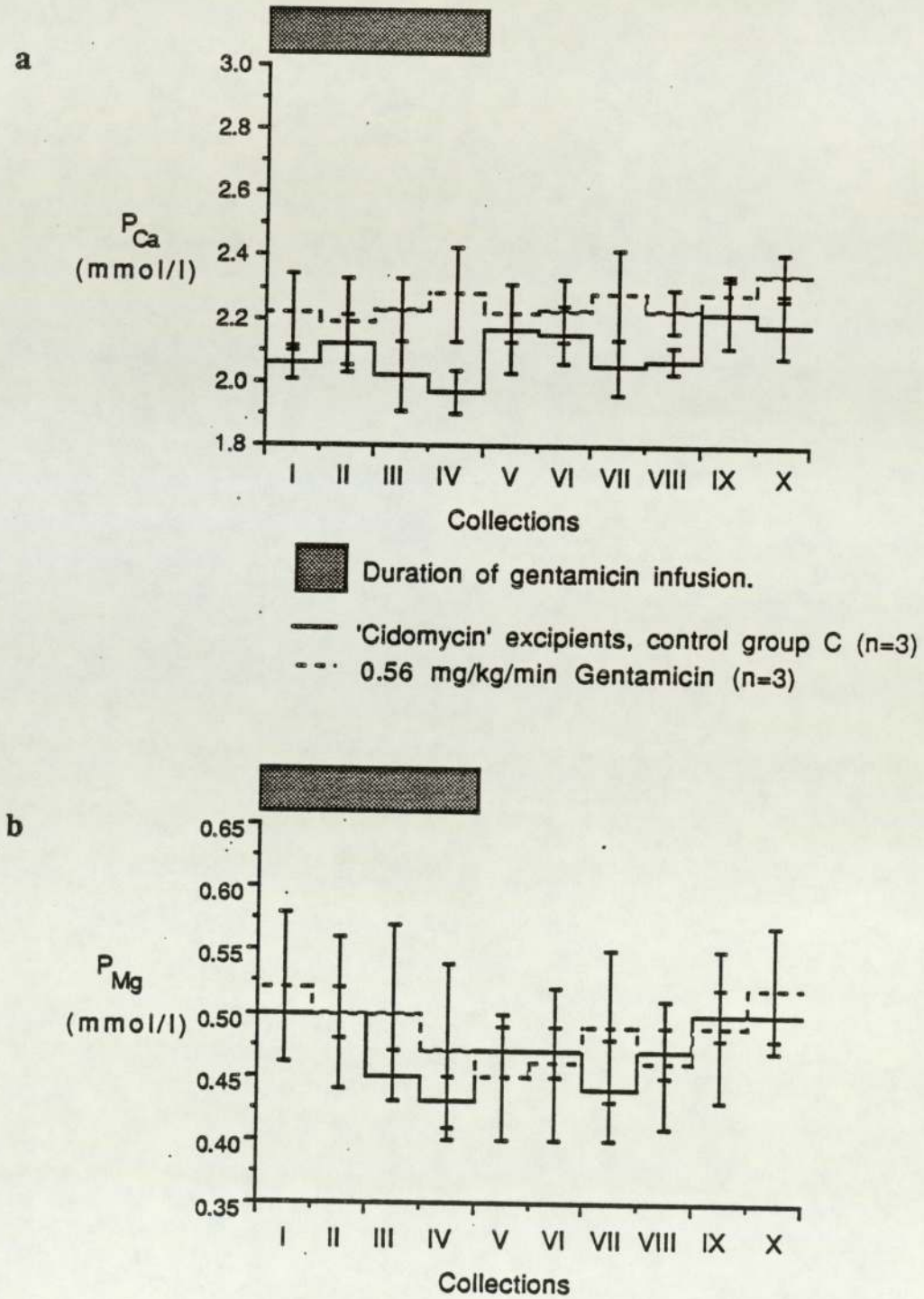
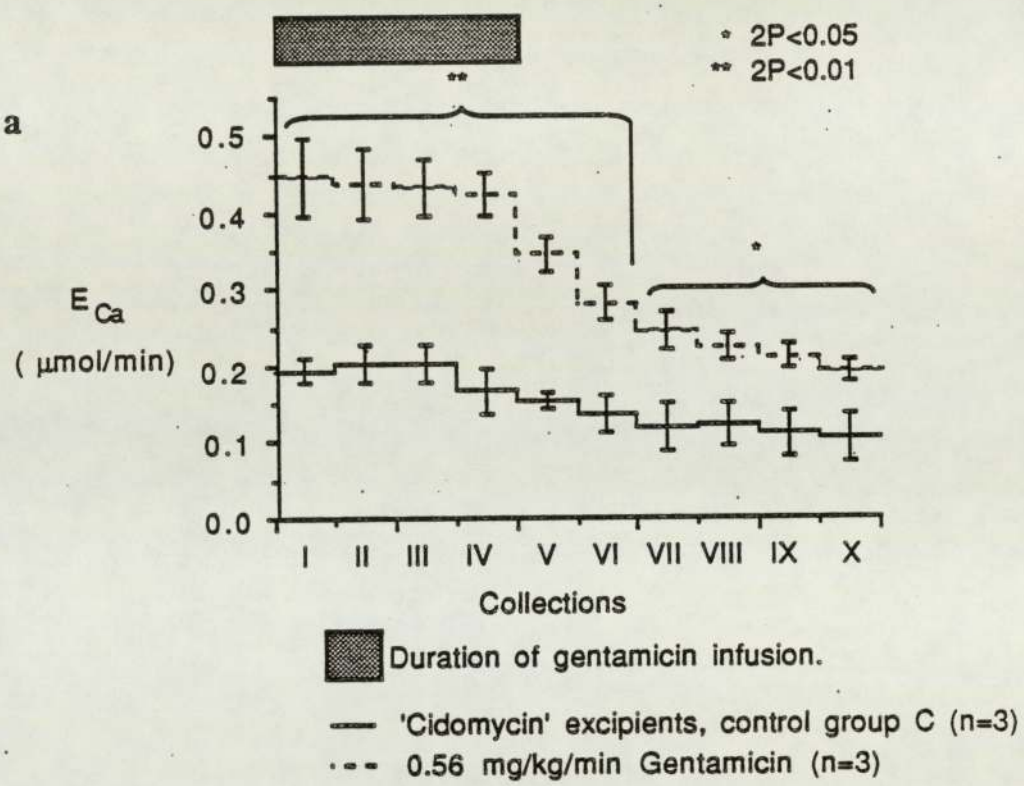


Figure 36. Excretion rates of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



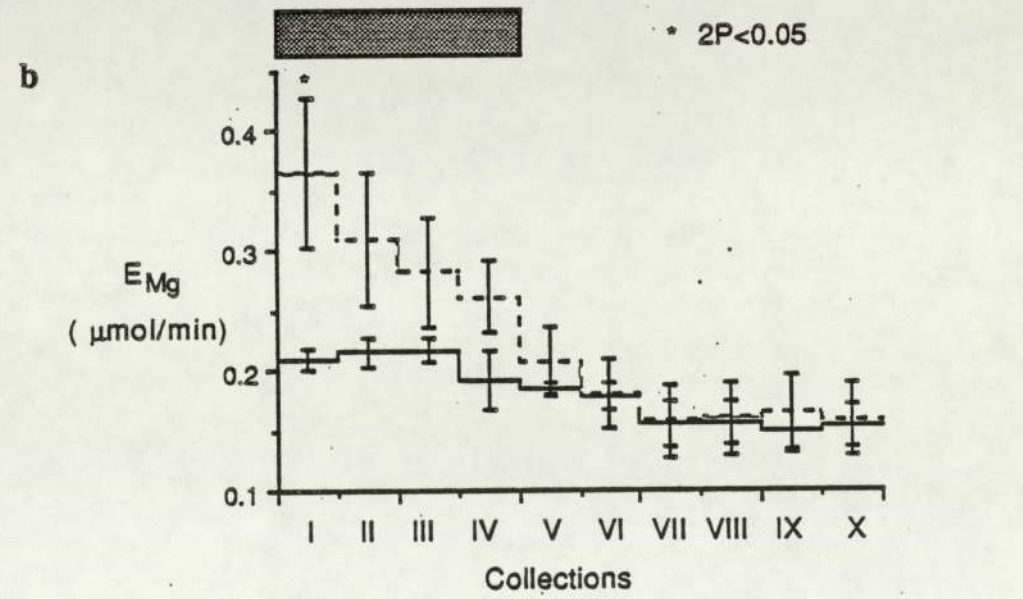


Figure 37. Clearances of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

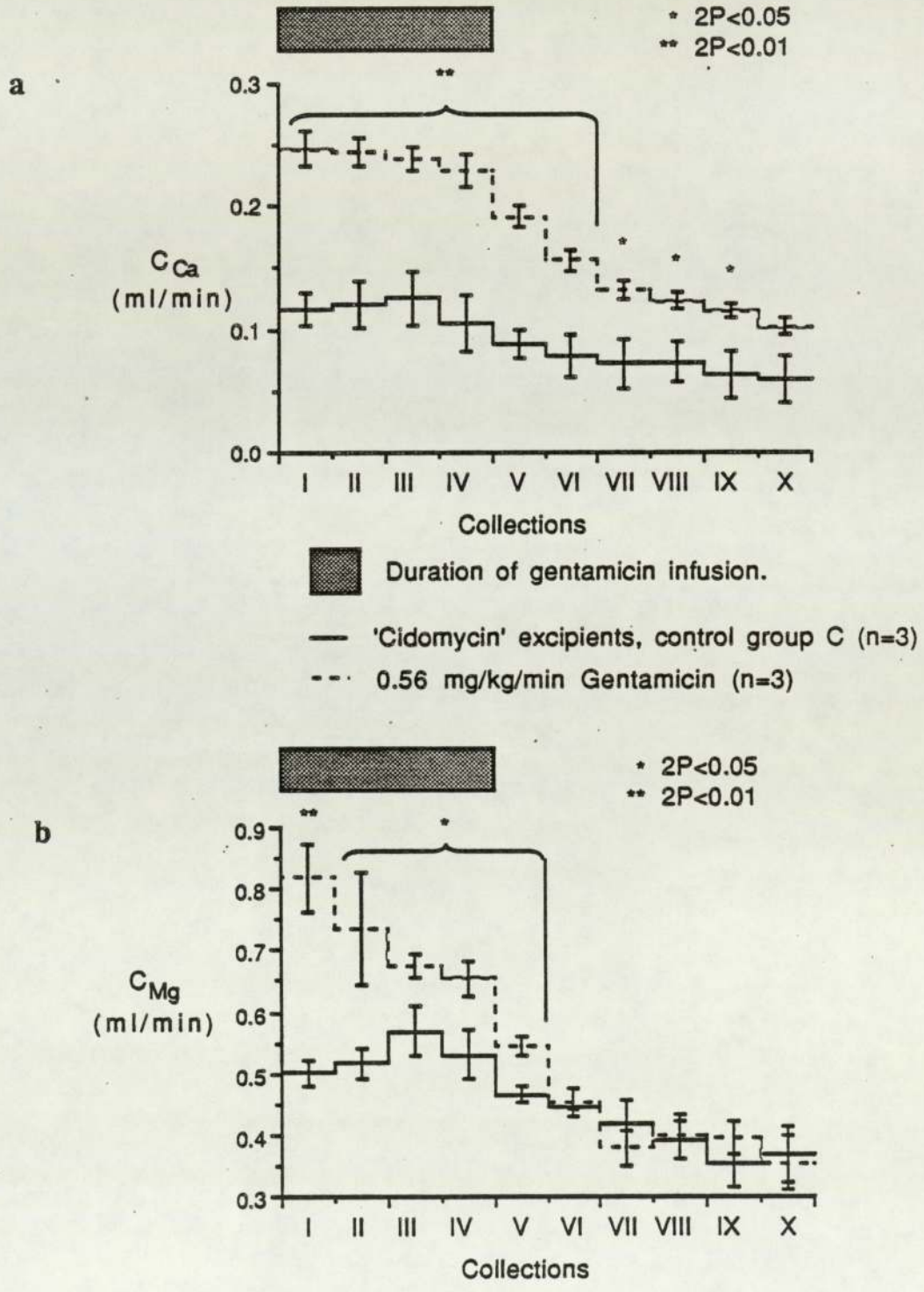


Figure 38. Fractional excretions of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

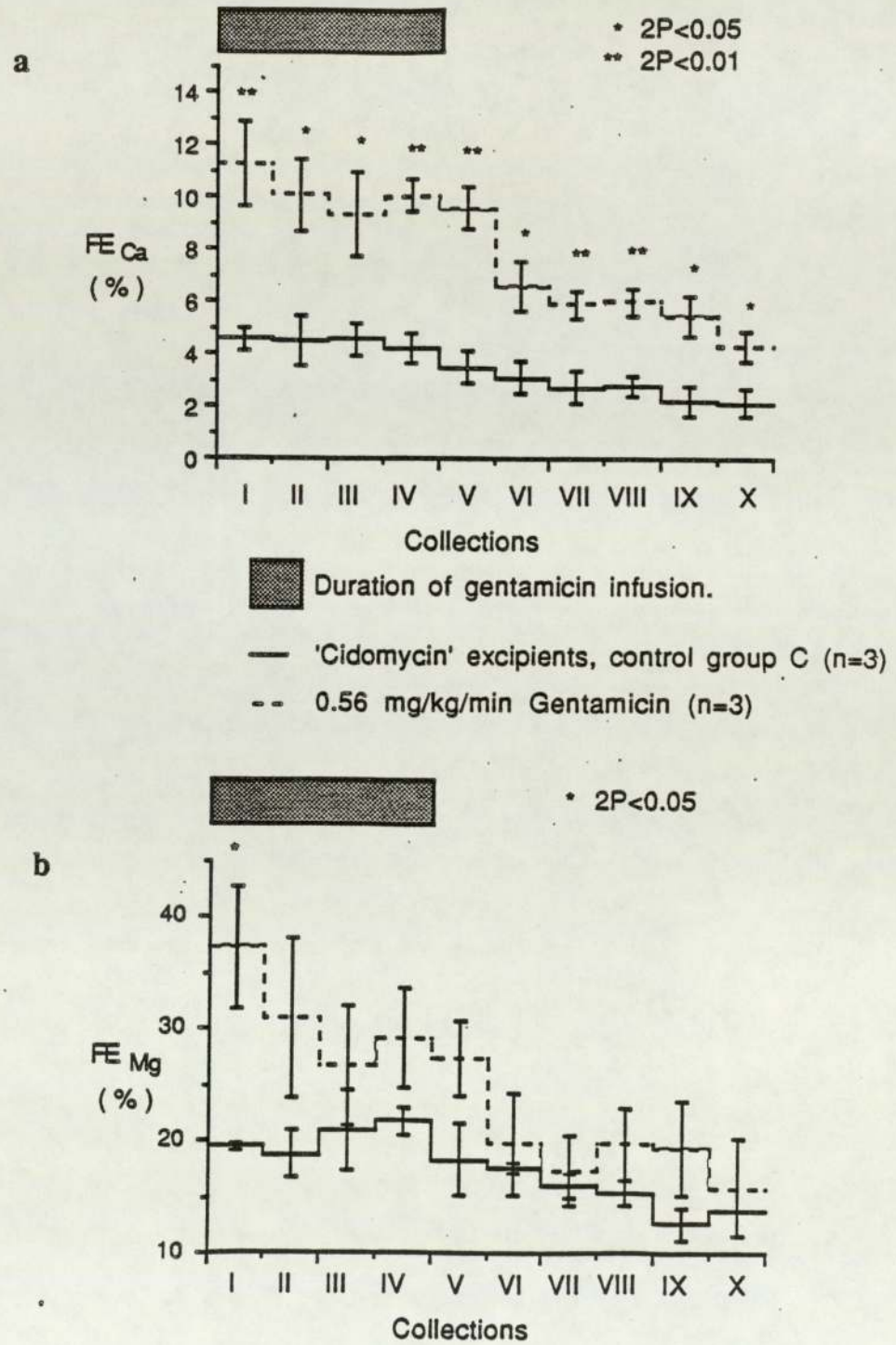


Figure 39. Protocol used in clearance experiments in which the reversibility of the changes produced by gentamicin was studied, gentamicin was infused for one hour followed by a four hour saline infusion.

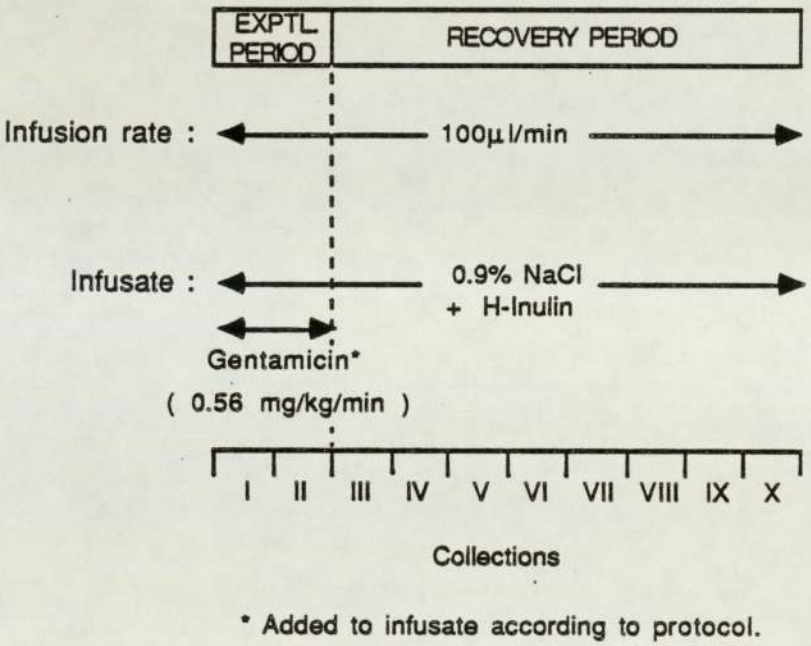


Figure 40. a. Urine flow rate in reversal experiments. **b.** GFR in reversal experiments. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

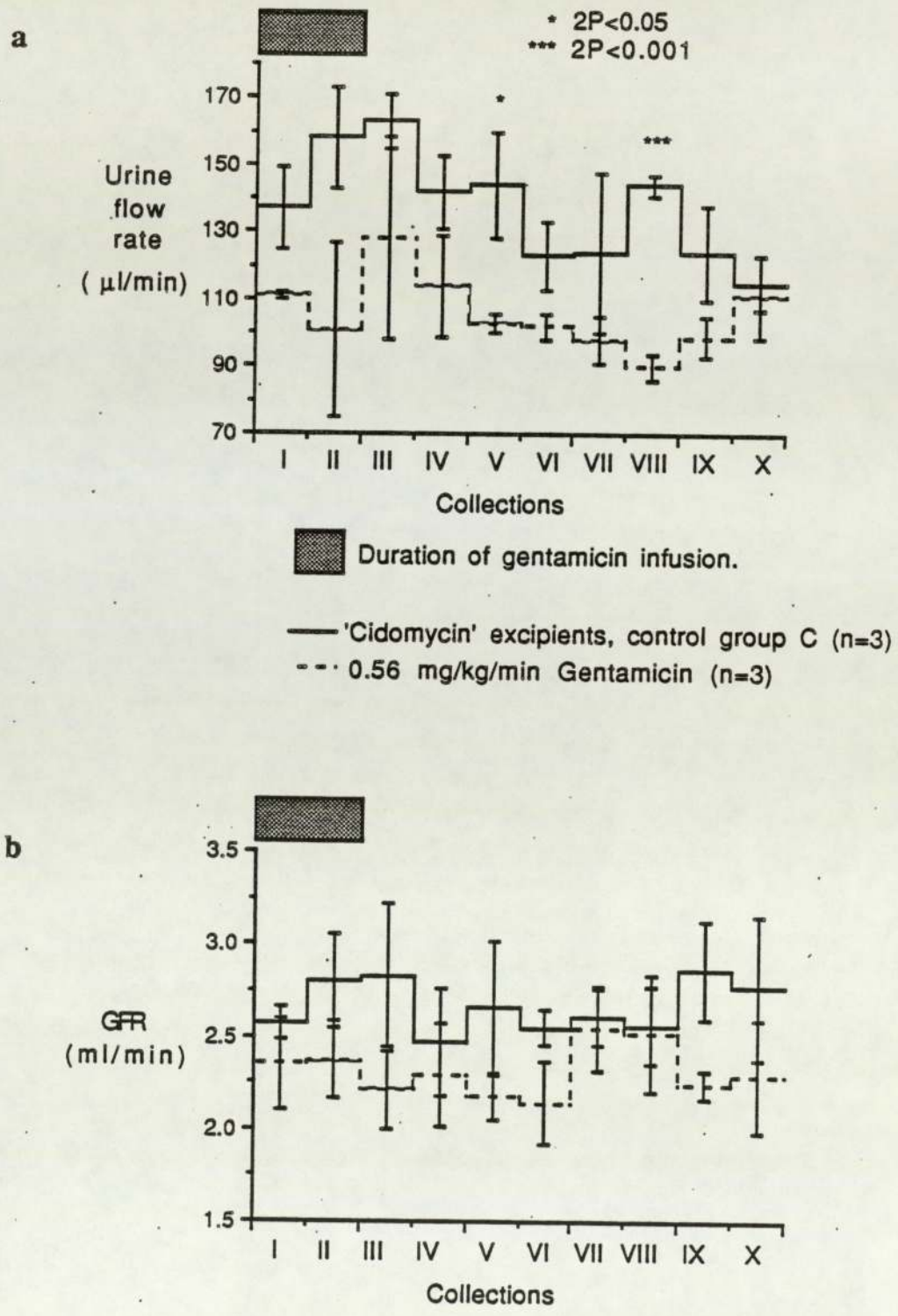


Figure 41. Concentrations of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

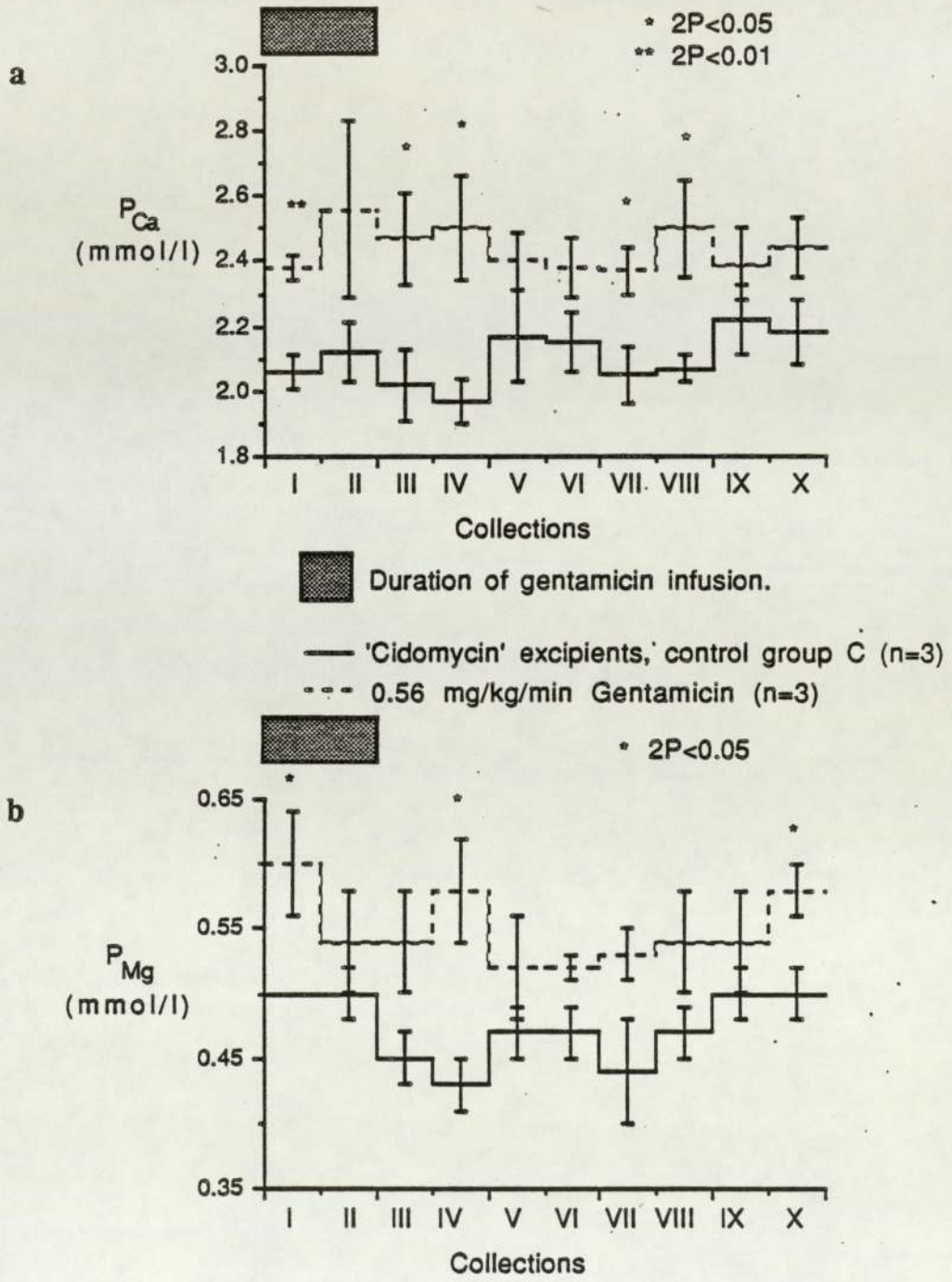


Figure 42. Excretion rates of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

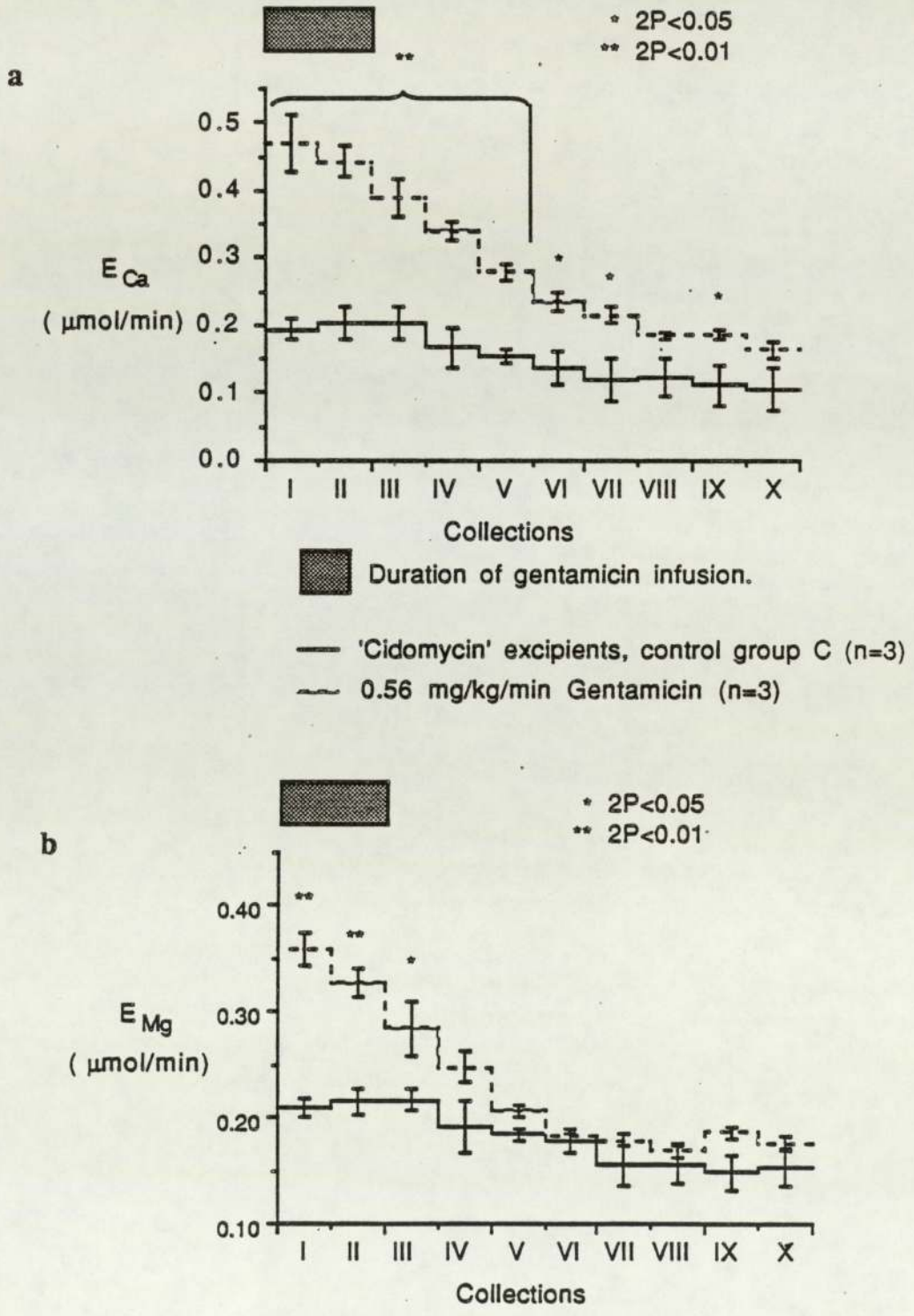


Figure 43. Clearances of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

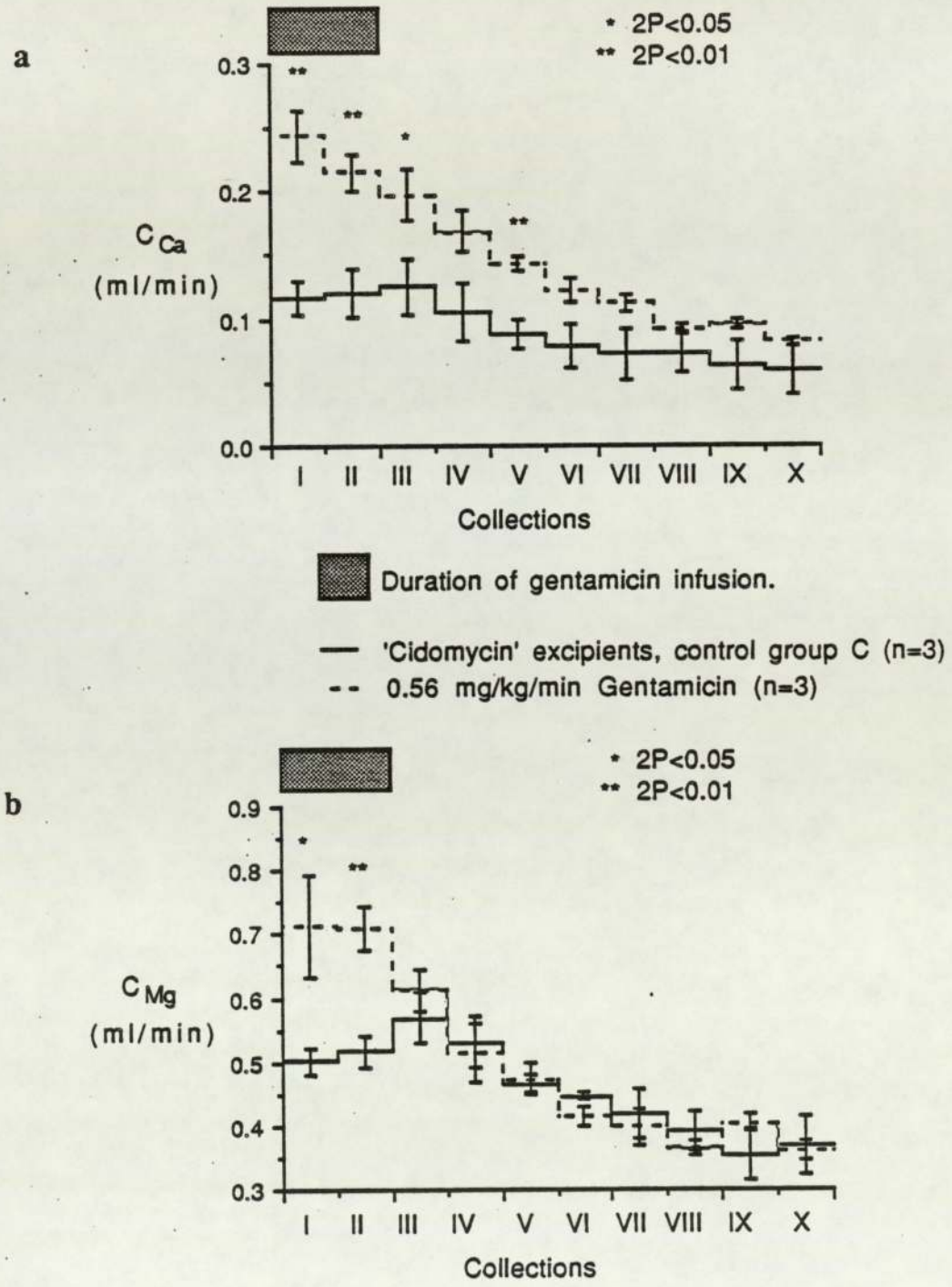
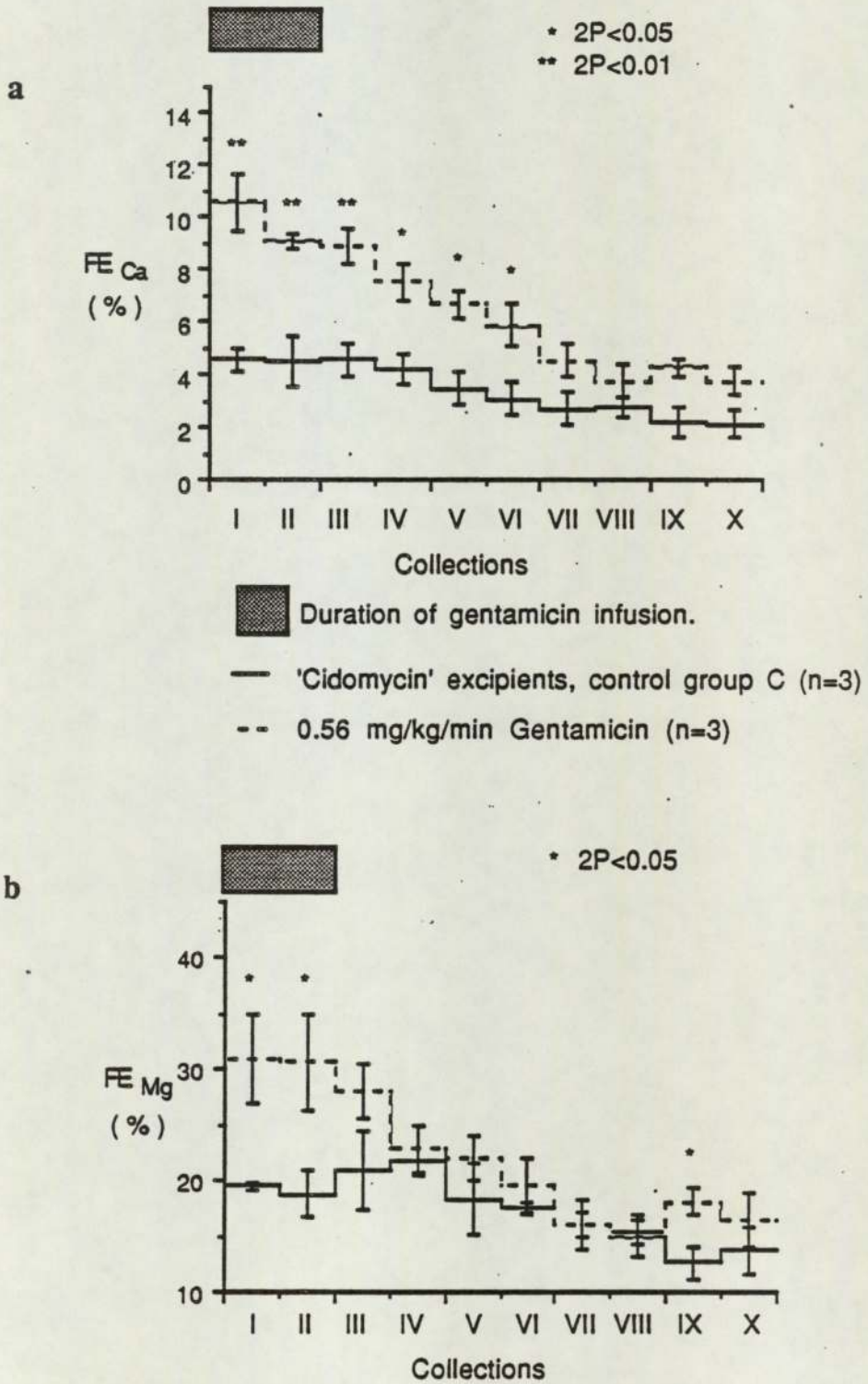


Figure 44. Fractional excretions of cations in reversal experiments expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



4.5 Effect of tobramycin infusion on the renal handling of calcium and magnesium

a) Introduction:

Whether the effect of gentamicin on the renal handling of divalent cations is related to its nephrotoxic potential is presently unknown. However, other aminoglycoside antibiotics have been reported to cause electrolyte disturbances in humans (Zaloga et al, 1984) and infusion of netilmicin into rats has been reported to increase fractional excretion of all four major cations (Pastoriza et al, 1983). Such actions, therefore, seem to be shared with other aminoglycosides.

Other nephrotoxic agents, such as cisplatin, are also reported to produce hypomagnesaemia and renal wasting of divalent cations (Schilsky et al, 1980; Mavichak et al, 1985).

An investigation in which another aminoglycoside, tobramycin, was infused into anaesthetised rats was therefore carried out, to confirm that the acute responses to gentamicin infusion were not unique to that agent.

Also a secondary objective could be partially addressed because tobramycin has consistently been reported to have a lower nephrotoxic potential than gentamicin (Houghton et al, 1978; Luft et al, 1978a, Soberon et al, 1979). Therefore, if a correlation between nephrotoxic potential and disturbance of renal calcium and magnesium conservation exists, tobramycin might be expected to produce a smaller effect on calciuria and magnesiuria than gentamicin.

Thus, the reasons for investigating the acute effects of tobramycin infusion were two-fold:

To determine if acute hypercalciuria and hypermagnesiuria are responses unique to gentamicin or if they are shared by another

aminoglycoside. Secondly, to investigate whether renal wasting of calcium and magnesium are related to the nephrotoxic potential of the causative agent.

b) Methods:

The experimental procedure used in this investigation is outlined in Figure 45. four rats were infused with 0.56 mg/kg/min tobramycin (base) as indicated. Statistical comparisons were made with control group A and with rats infused with 0.56 mg/kg/min gentamicin (as described in section 4.2).

c) Results:

Mean urine flow rate (Figure 46) and mean plasma calcium and magnesium concentrations (Figures 47a and 47b) in tobramycin-infused rats did not differ substantially from saline-infused controls. GFR was stable in tobramycin-infused rats (Figure 50).

Mean calcium excretion rate (Figure 48a) and clearance (Figure 49a) were not significantly different from saline-infused controls during the control period. Introduction of infused tobramycin caused an immediate increase in both parameters to approximately 200% of initial values ($2P<0.001$). Mean excretion rate and clearance were essentially stable at these elevated values but declined slightly during the final collection of the experimental period. These changes could be explained by a parallel increase in mean fractional excretion of calcium (Figure 51a), indicating that this response to tobramycin was due to reduced tubular calcium reabsorption.

Mean excretion rate (Figure 48b) and clearance (Figure 49b) of magnesium were not significantly different from controls during the control period. Introduction of infused tobramycin produced an immediate increase in both parameters to approximately 110-125% of initial values ($2P<0.01$). Magnesiuria was rather unstable during

both the control and experimental periods but there did not appear to be a decline in either excretion rate or clearance in the final minutes of the experimental period. These increases were reflected by a similar rise in mean fractional excretion of magnesium (Figure 51b) indicating that the hypermagnesiuria prompted by tobramycin was the result of reduced reabsorption of this cation.

Rats infused with 0.56 mg/kg/min tobramycin when compared with rats infused with 0.56 mg/kg/min gentamicin over the same period showed the following features:

Mean urine flow rate (Figure 52) and the mean plasma calcium and magnesium concentrations (Figures 53a and 53b) did not differ substantially between gentamicin and tobramycin-infused rats.

Mean calcium excretion rate (Figure 54a) was not significantly different between gentamicin and tobramycin-infused rats during the control period, but mean calcium clearance (Figure 55a) was significantly ($2P<0.05$) higher in tobramycin-infused rats at two points. During collections V-VII neither calcium excretion rate nor clearance differed significantly between the two groups. However, during collections VIII-X both parameters were higher in gentamicin-infused than tobramycin-infused rats ($2P<0.05$ generally).

Mean magnesium excretion rate (Figure 54b) was not substantially different between gentamicin and tobramycin-infused rats during the control period, although mean magnesium clearance (Figure 55b) was significantly ($2P<0.01$) lower in tobramycin-infused rats at two points. Neither parameter differed substantially between the two groups during the experimental period.

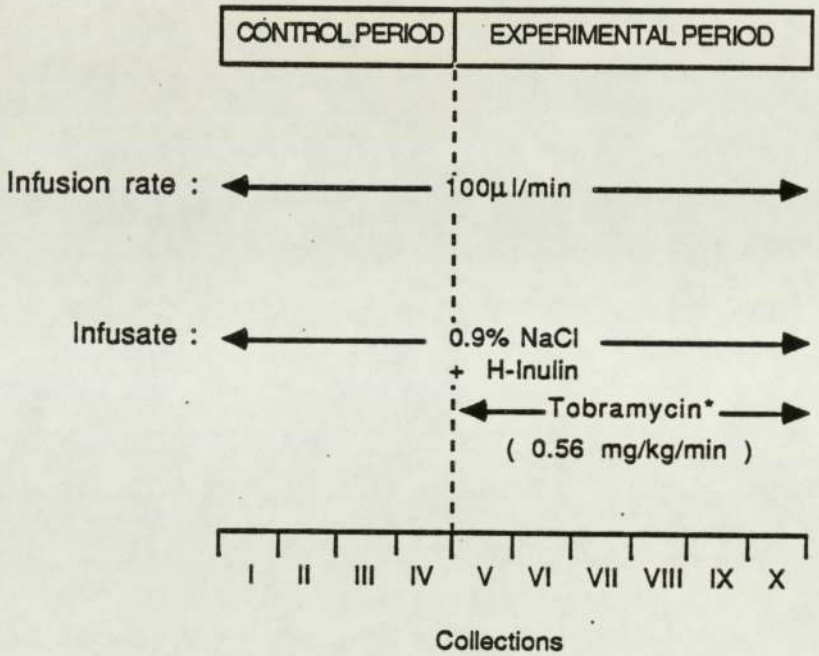
d) Discussion:

Infusion of tobramycin produced significant hypercalciuria and hypermagnesiuria, therefore such responses are not unique to

gentamicin. These reponses were reflected by increases in the fractional excretions of these cations indicating that decreased tubular reabsorption was responsible for the increased output of calcium and magnesium. Comparison between the results from tobramycin-infused rats and rats infused with gentamicin at the same rate for the same interval revealed similar responses to the two drugs (Figure 56).

This would appear to refute any clear relationship between nephrotoxic potential and disturbance of divalent cation conservation in this model, in the specific case of gentamicin versus tobramycin.

Figure 45. Protocol used in clearance experiments in which tobramycin was infused for three hours following a two hour saline infusion.



* Added to infusate according to protocol.

Figure 46. Urine flow rate in rats infused with tobramycin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

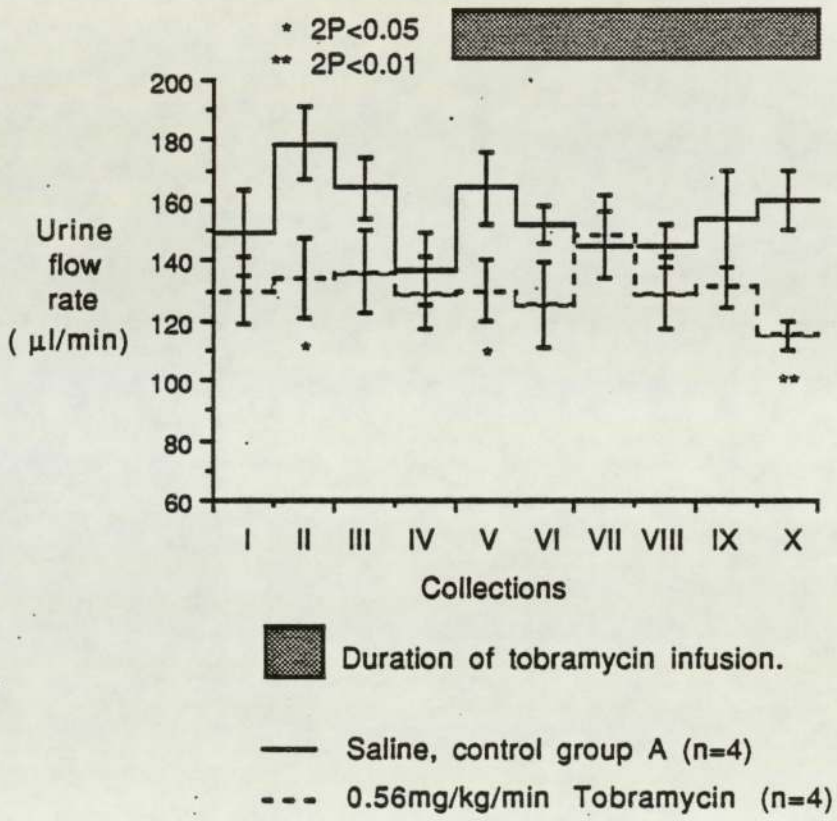


Figure 47. Concentrations of cations in plasma in rats infused with tobramycin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

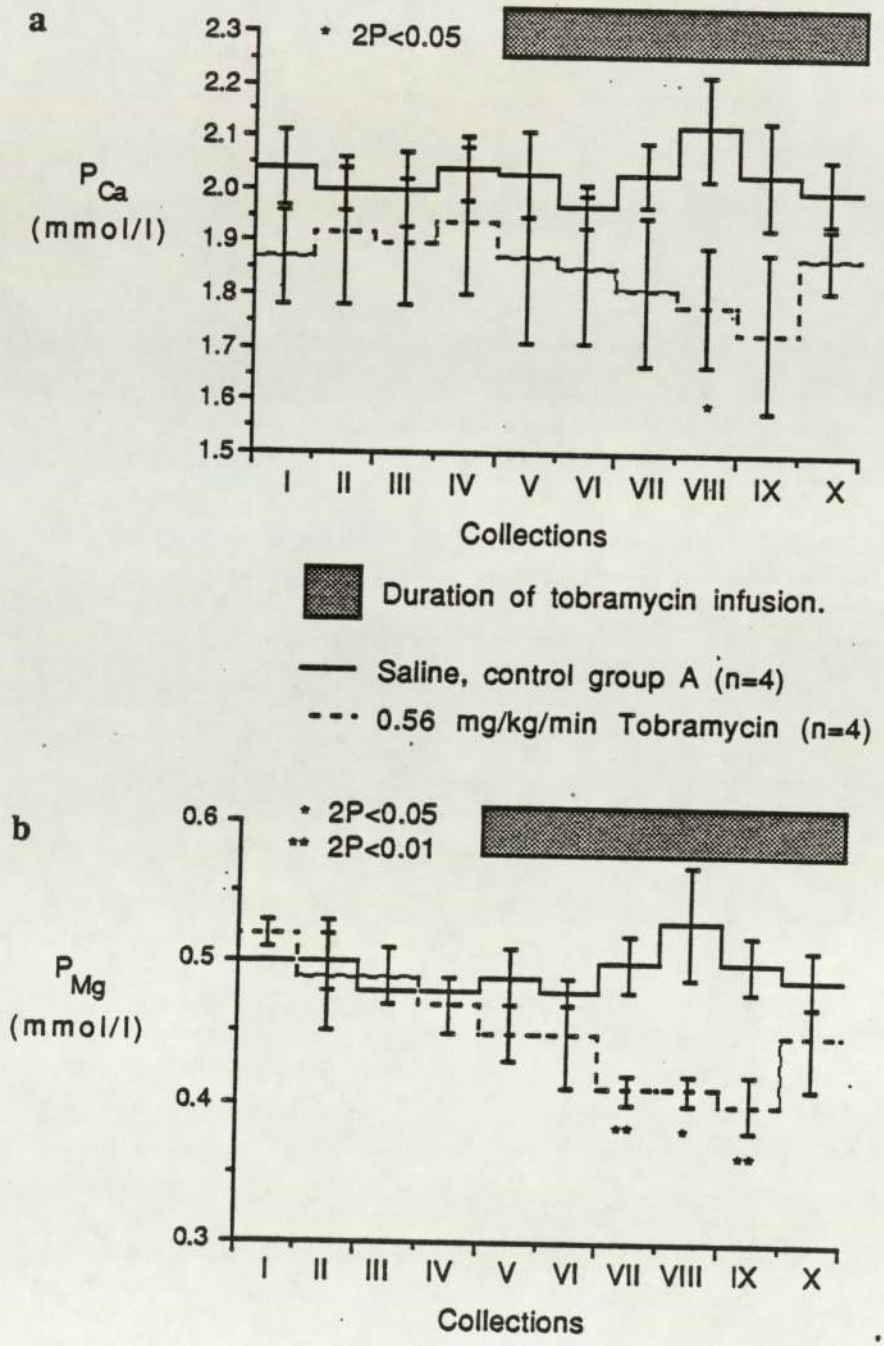


Figure 48. Excretion rates in rats infused with tobramycin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

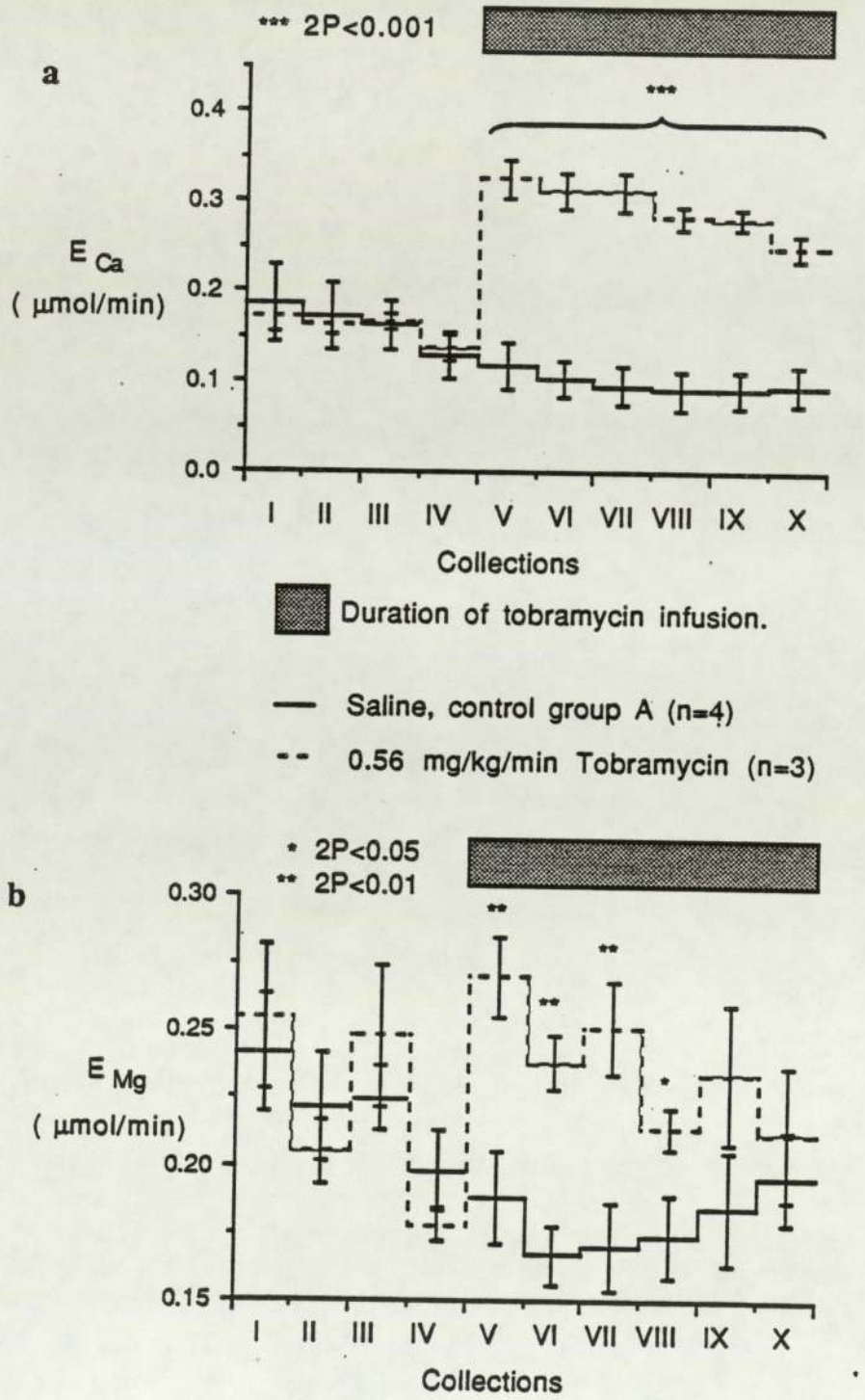


Figure 49. Clearances of cations in rats infused with tobramycin expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

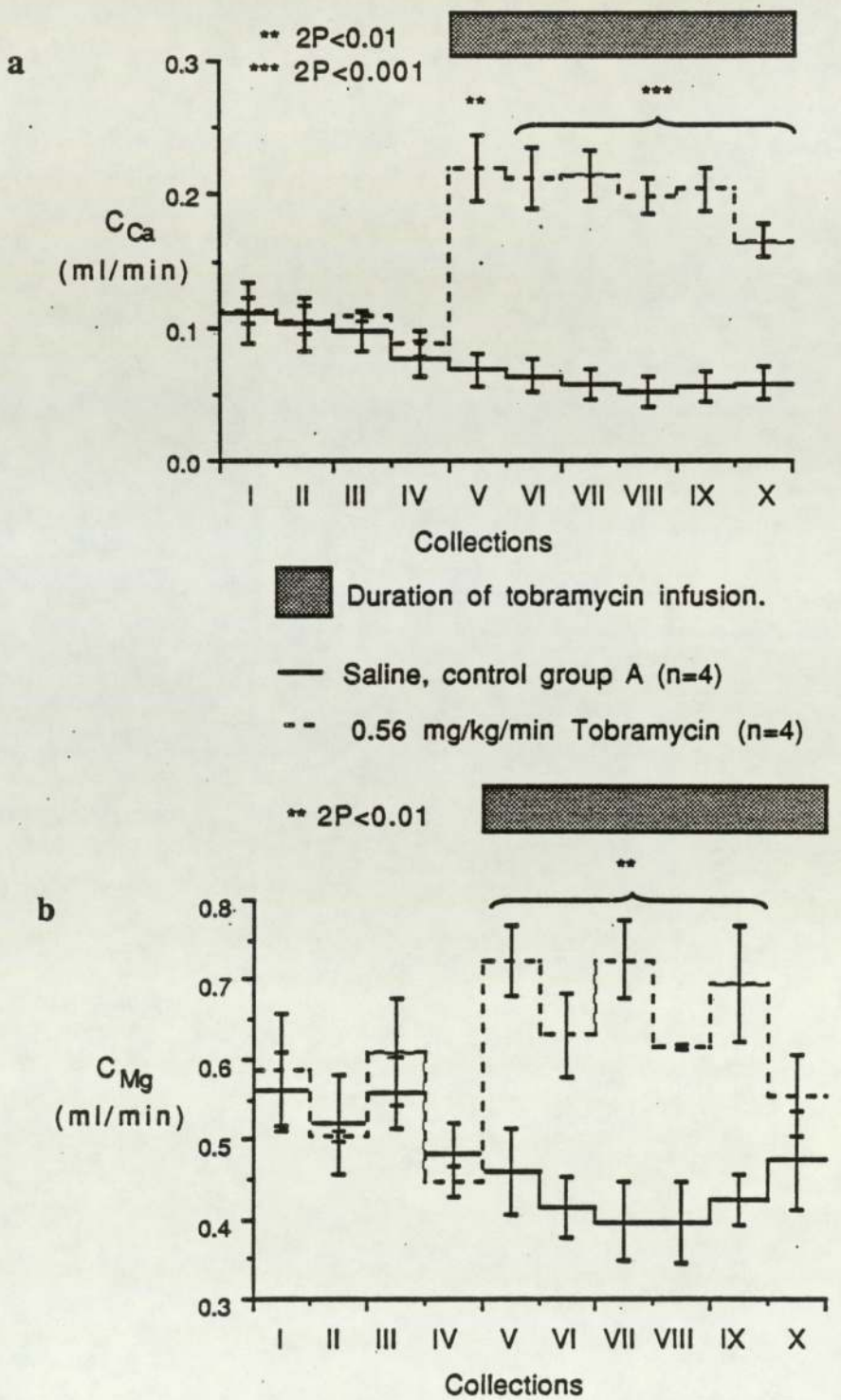


Figure 50. GFR in rats infused with tobramycin expressed as mean ± s.e.m.

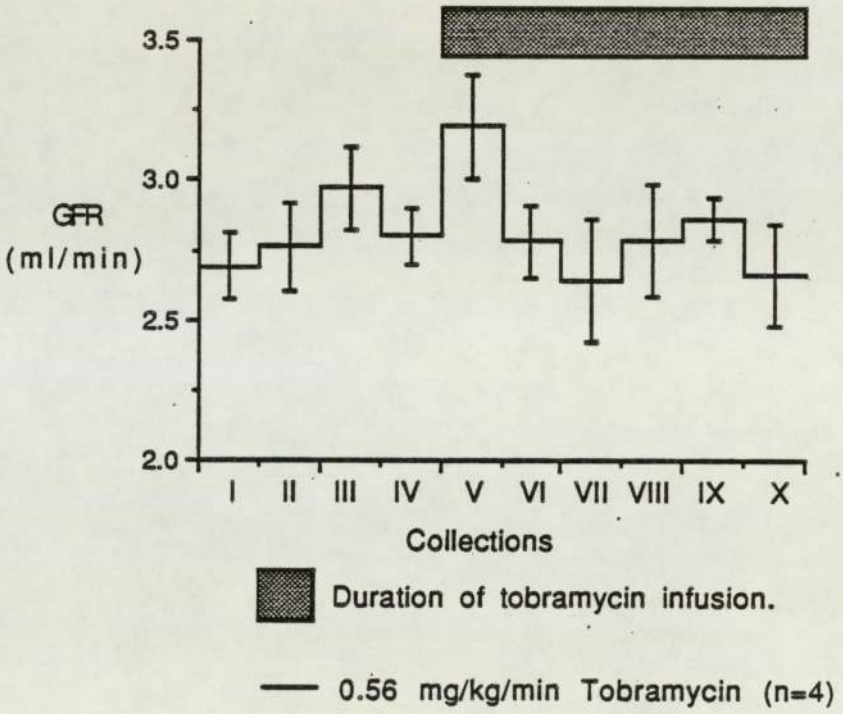


Figure 51. Fractional excretions of cations in rats infused with tobramycin expressed as mean ± s.e.m. **a.** Calcium. **b.** Magnesium.

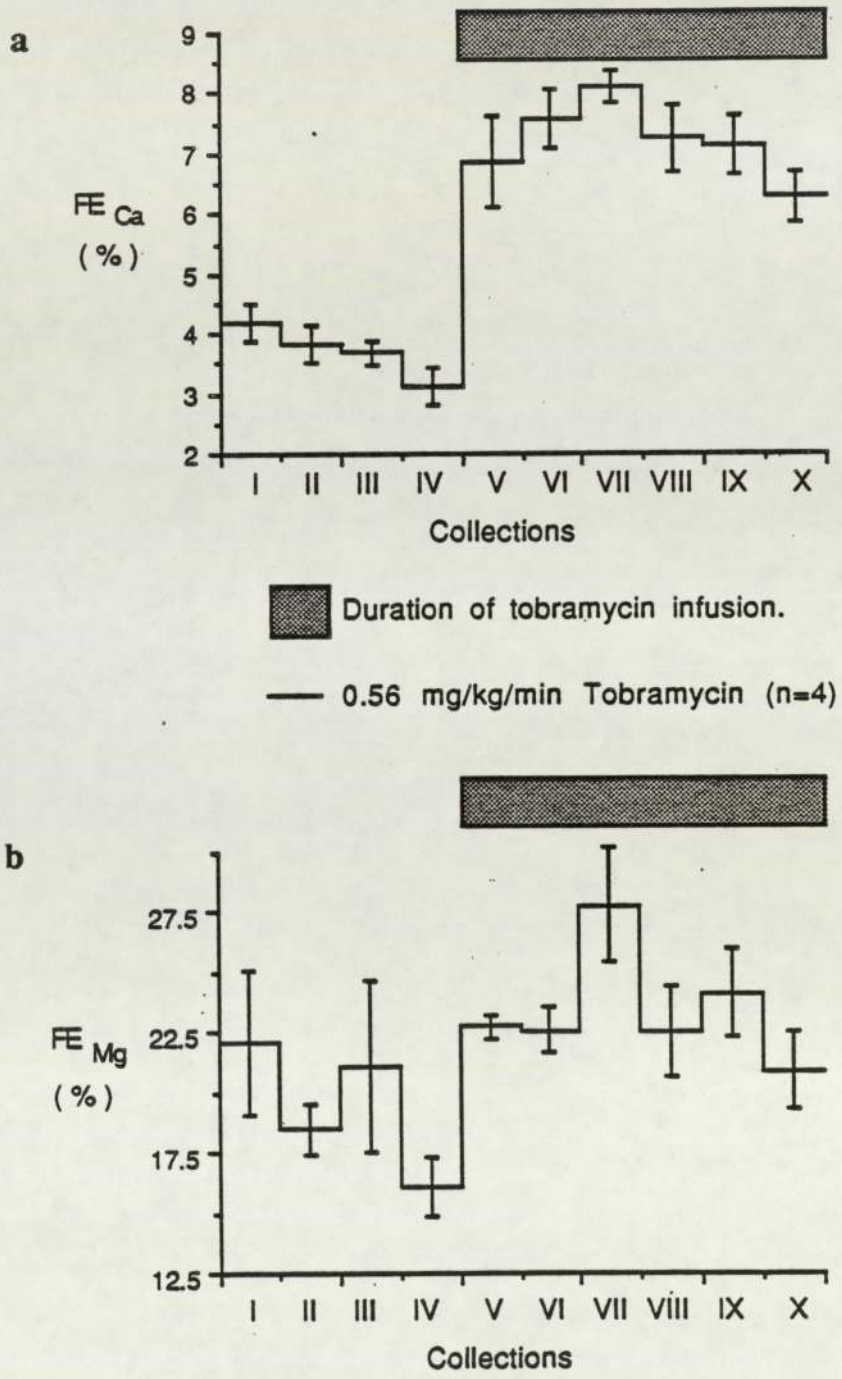


Figure 52. Comparison of urine flow rate between rats infused with gentamicin and rats infused with tobramycin at an equal rate, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

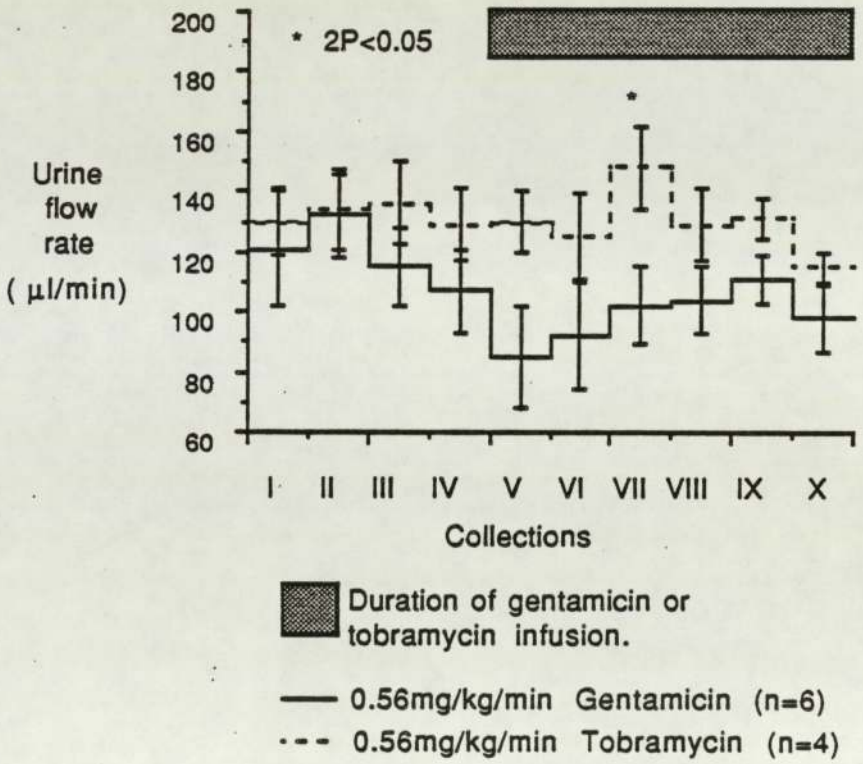


Figure 53. Comparison of concentrations of cations in plasma between rats infused with gentamicin and rats infused with tobramycin at an equal rate, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

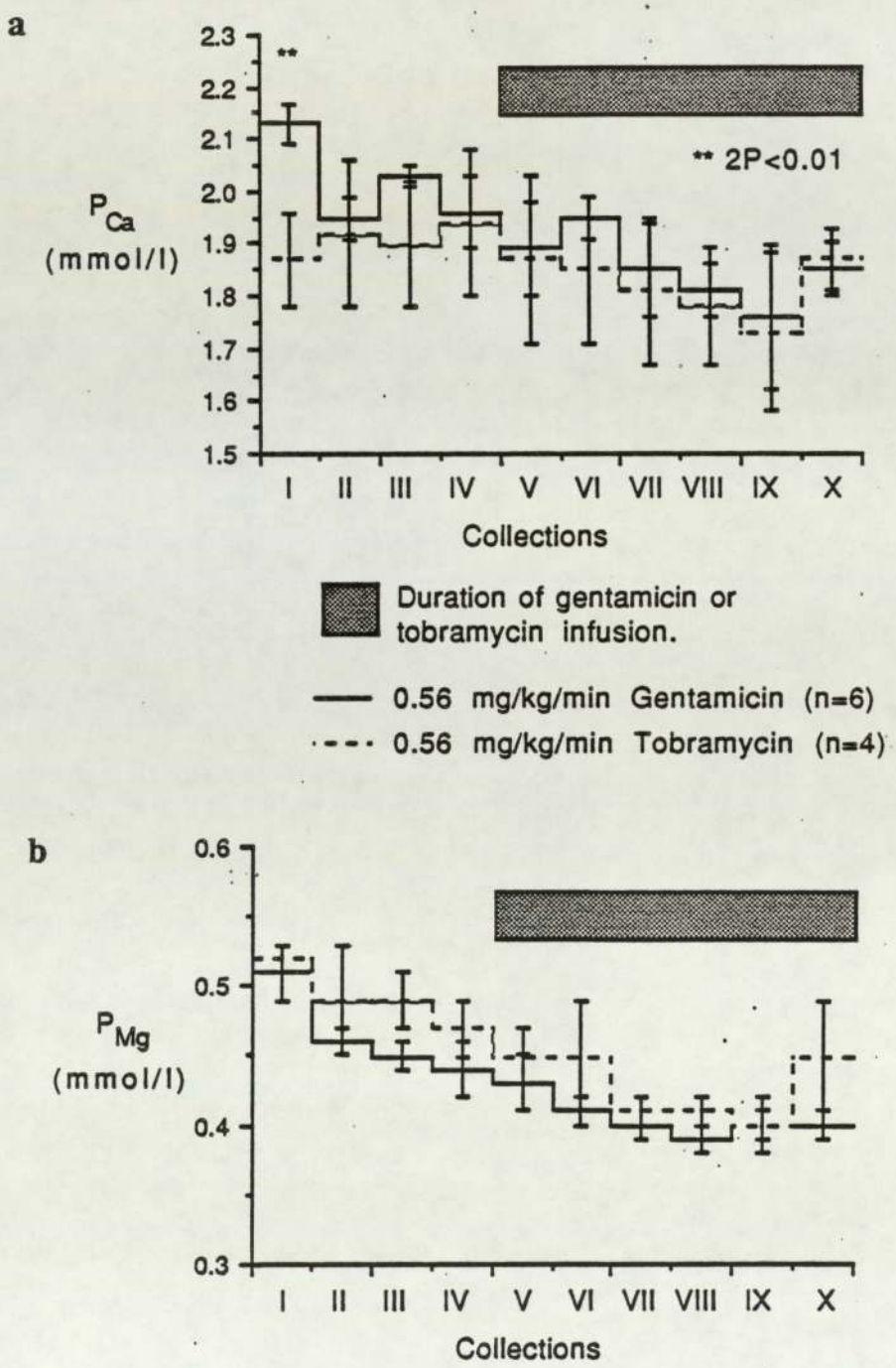


Figure 54. Comparison of excretion rates of cations between rats infused with gentamicin and rats infused with tobramycin at an equal rate, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

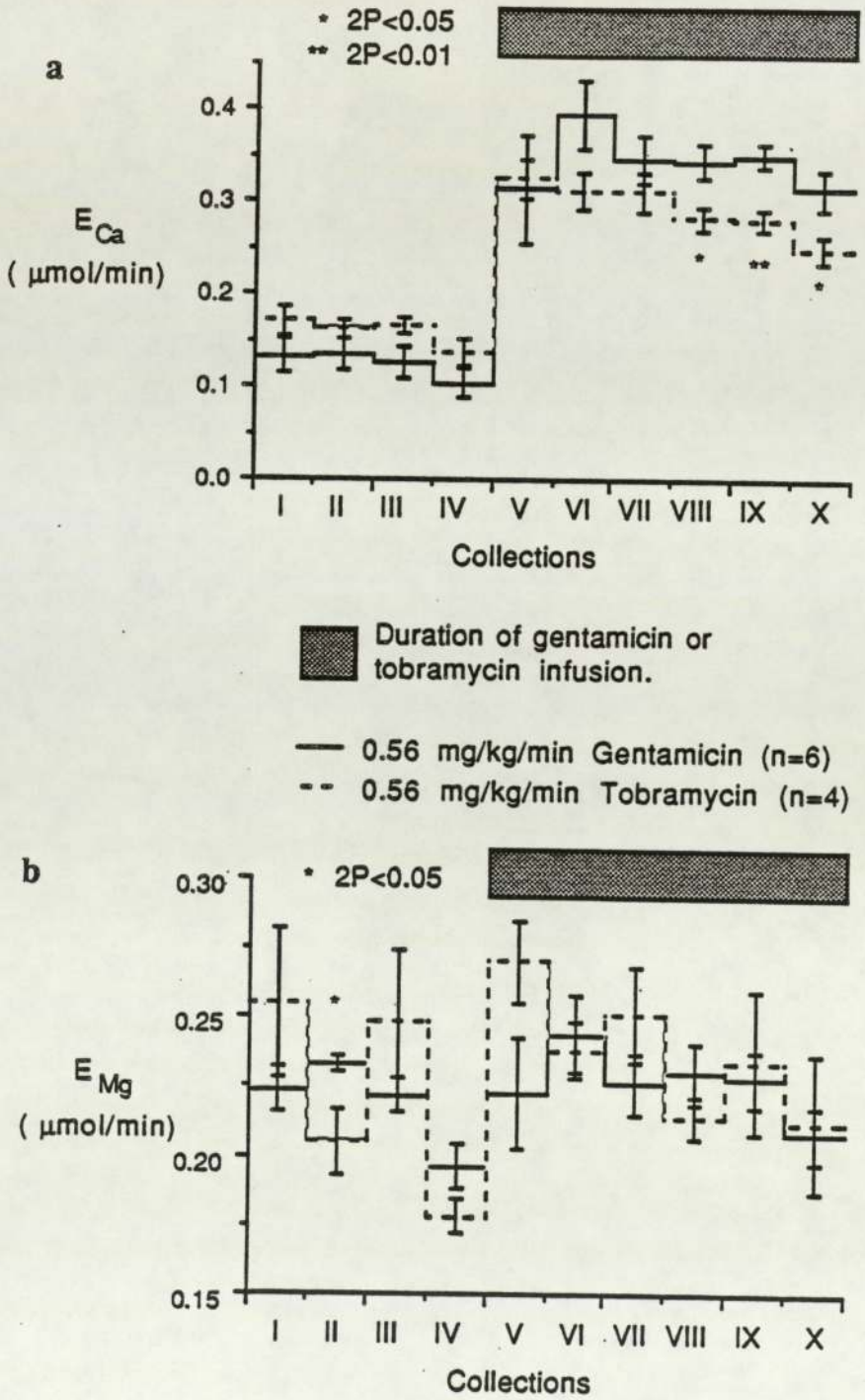


Figure 55. Comparison of clearances of cations between rats infused with gentamicin and rats infused with tobramycin at an equal rate, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

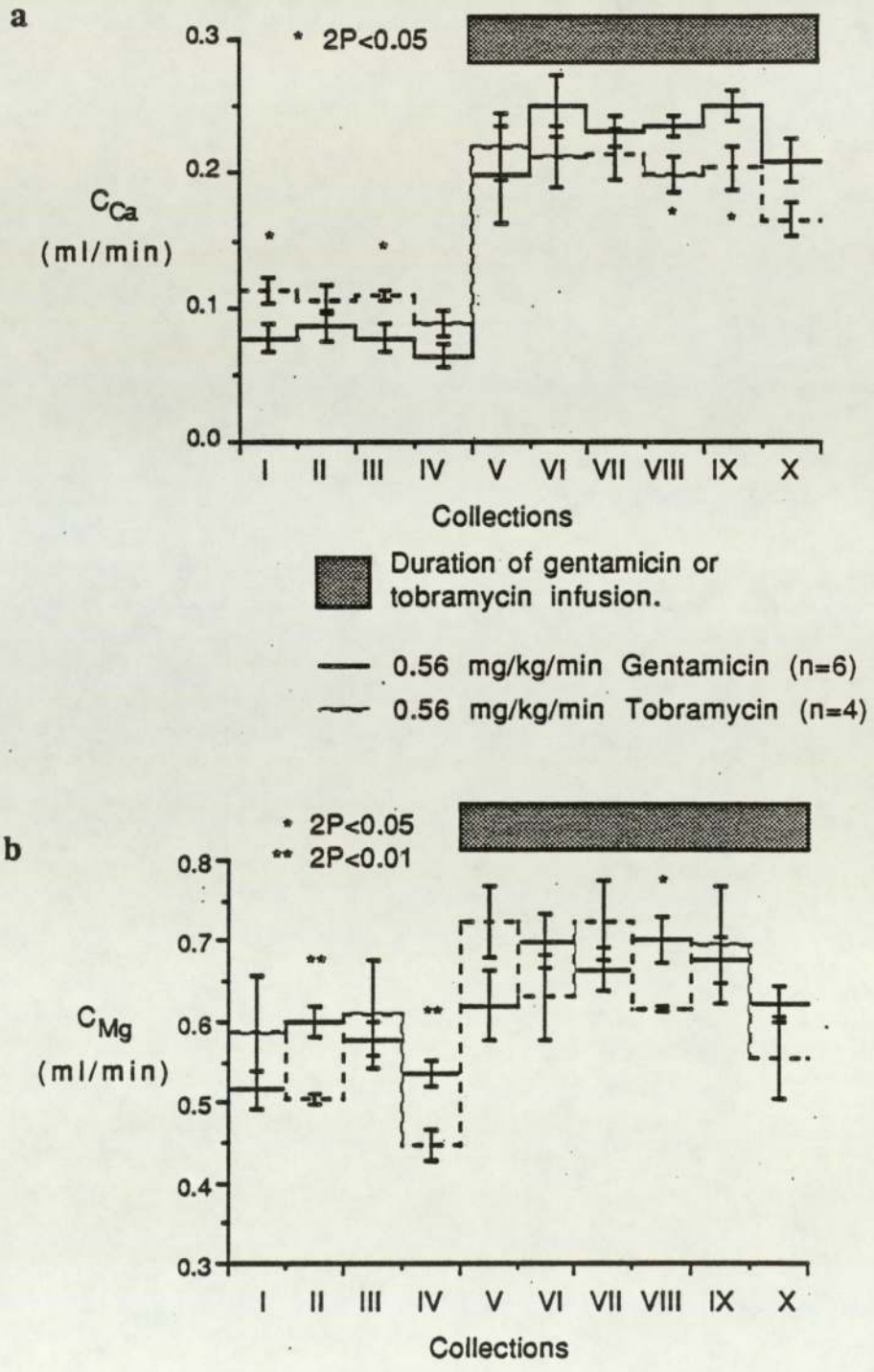
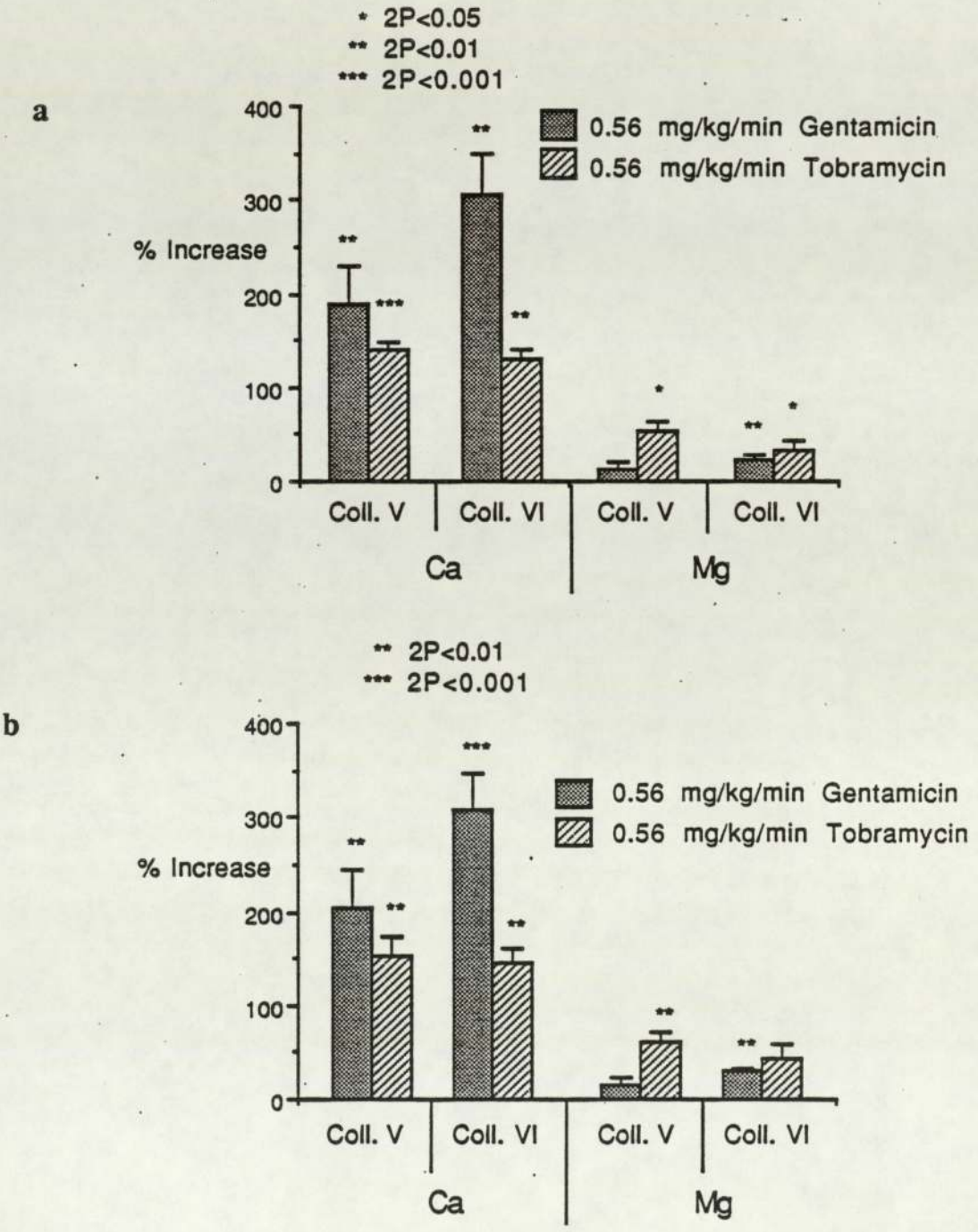


Figure 56. Histograms illustrating the effects of gentamicin and tobramycin infusion on the excretion rates and clearances of calcium and magnesium. Each column represents the mean of the individual percentage increases in excretion rate or clearance at collection V or VI over values at collection IV from the same individuals ± s.e.m. Statistical comparisons were made using Student's 't' test for paired data. **a.** Excretion rates. **b.** Clearances.



4.6 The effect of acute thyroparathyroidectomy (TPTX) on the renal handling of calcium and magnesium in the presence and absence of infused gentamicin

a) Introduction:

The precise mechanism by which gentamicin and tobramycin infusion produce acute hypercalciuria and hypermagnesiuria and in which segment of the nephron reabsorption of these cations is impaired is unknown.

Suppression of parathyroid hormone (PTH) activity is known to promote hypercalciuria, therefore acute impairment of PTH activity by gentamicin could conceivably produce the observed responses.

Therefore, prior depletion of PTH by removal of the parathyroid glands, if such a mechanism is correct, would obliterate these responses to gentamicin. Thus the effect of acute thyroparathyroidectomy on the hypercalciuric and hypermagnesiuric responses to gentamicin infusion was determined.

b) Methods:

The experimental procedure outlined in Figure 57 was followed in these experiments. Acute thyroparathyroidectomy (TPTX), involving removal of the entire thyroid axis by blunt surgery and electrocautery, was carried out immediately after the venous cannulation as described in 3.2 c). The half-life of PTH in the TPTX rat is approximately 24 minutes (Melick et al, 1965) and therefore circulating PTH levels should be minimal in these rats by the end of the 3 hour equilibration period.

Five TPTX rats received an infusion of saline throughout, a further Five received an infusion of 0.56 mg/kg/min gentamicin during the experimental period as indicated in Figure 57. TPTX rats infused with saline alone were compared with control group A in statistical tests,

TPTX rats infused with 0.56 mg/kg/min gentamicin were compared with intact rats infused with 0.56 mg/kg/min gentamicin (as described in section 4.2).

c) Results:

i) Saline infusion:

Mean plasma magnesium concentration in TPTX rats was not consistently different from intact controls (Figure 59b). Mean plasma calcium concentration (Figure 59a) fell significantly ($2P<0.05$, 0.01 or 0.001) in TPTX rats between collections IV and X, presumably indicating a hypocalcaemic effect of TPTX. Mean urine flow rate (Figure 58) was significantly lower in TPTX rats during much of the collection period, however, gentamicin-infused TPTX rats had comparable urine flow rates with intact gentamicin-infused rats (Figure 62) therefore this result is probably of little importance. GFR was essentially stable throughout the collection period (Figure 66b).

Mean calcium excretion rate (Figure 60a) and clearance (Figure 61a) were not significantly different between TPTX and intact rats infused with saline at any point. Mean fractional excretion of calcium in TPTX rats explained the changes in excretion rate and clearance (Figure 70a).

Mean magnesium excretion rate (Figure 60b) and clearance (Figure 61b) were significantly ($2P<0.05$ or 0.01) lower in TPTX rats for the majority of the collection period, between collections IV or V and X. Mean fractional excretion of magnesium in TPTX rats explained these changes (Figure 70b).

ii) Gentamicin infusion:

Mean urine flow rate (Figure 62) and the mean plasma calcium concentration (Figure 63a) were not consistently different between intact and TPTX rats receiving gentamicin at the same infusion rate.

The concentration of magnesium in plasma (Figure 63b) was significantly ($2P<0.05$ or 0.01) higher in 60% of samples from TPTX rats. GFR was essentially stable in gentamicin-infused TPTX rats (Figure 66b). Further, there were no consistent differences in mean urine flow rate (Figure 66a), GFR (Figure 66b) or the concentrations of calcium or magnesium in plasma (Figures 67a and 67b) between TPTX rats infused with gentamicin and TPTX rats infused with saline.

Mean calcium excretion rate (Figure 64a) and clearance (Figure 65a) were significantly ($2P<0.01$) higher in TPTX rats than intact rats during the control period. This hypercalciuria may indicate reduced calcium reabsorption in response to TPTX. Both calcium excretion rate and clearance were elevated by introduction of infused gentamicin to approximately 140-160% of initial values in TPTX rats. Although both parameters dropped gradually during the remainder of the experimental period there were no significant differences between TPTX and intact rats in either mean excretion rate or clearance during gentamicin infusion. Mean fractional excretion of calcium in TPTX rats (Figure 70a) was elevated during infusion of gentamicin indicating that the hypercalciuria produced was the result of reduced tubular reabsorption of calcium. Mean calcium excretion rate and clearance were significantly ($2P<0.05$ or 0.01) higher in TPTX rats receiving gentamicin than in saline-infused TPTX rats during the control period (Figures 68a and 69a). Both parameters rose to highly significantly ($2P<0.001$) elevated levels for the full duration of the gentamicin infusion in TPTX rats infused with the drug in comparison with TPTX controls. These observations were explained by parallel changes in the mean fractional excretion of calcium (Figure 70a).

Mean magnesium excretion rate (Figure 64b) was not significantly different between TPTX and intact rats throughout the control period. Introduction of infused gentamicin increased mean

magnesium excretion rate to a peak of approximately 115% of initial values in TPTX rats, excretion rate did not differ significantly between TPTX and intact rats during the experimental period. Mean magnesium clearance (Figure 65b), in contrast, was consistently lower in TPTX rats during the control period. Mean magnesium clearance rose in response to gentamicin infusion in TPTX rats but remained significantly lower ($2P<0.01$ or $2P<0.05$) than intact gentamicin-infused rats throughout the experimental period. Mean fractional excretion of magnesium in TPTX rats (Figure 70b) rose during gentamicin infusion indicating that the increases in excretion rate and clearance were the result of decreased reabsorption of magnesium. Mean magnesium excretion rate and clearance in TPTX rats infused with gentamicin were significantly higher ($2P<0.05$ or 0.01) than saline-infused TPTX rats at some points during the control period (Figures 68b and 69b). However, the fractional excretion of magnesium was not significantly higher in TPTX rats (Figure 70b) indicating that there was no difference in the tubular handling of magnesium between the two TPTX groups during this period. Infusion of gentamicin produced elevation of both excretion rate and clearance which was highly significant ($2P<0.001$) in comparison with TPTX controls. This magnesuria was explicable by a similar rise in the fractional excretion of magnesium (Figure 70b).

d) Discussion:

TPTX in saline-infused rats reduced mean plasma calcium concentration with respect to intact saline-infused rats. Mean plasma magnesium, in contrast, remained similar to intact controls. GFR was stable and, although mean urine flow rate was lower than in intact saline-infused rats, no such difference was seen between intact and TPTX gentamicin-infused rats suggesting that this is not a consistent effect of this manoeuvre. The renal handling of calcium in TPTX rats

infused with saline did not differ from intact saline-infused rats, whereas magnesium excretion rate and clearance were significantly depressed in TPTX rats.

Paradoxically, TPTX in gentamicin-infused rats did not significantly reduce mean plasma calcium concentration with respect to intact gentamicin-infused controls. However mean plasma calcium was slightly lower in these intact gentamicin-infused animals than in other intact rats, therefore any significant differences with slightly hypocalcaemic PTX rats might be obscured. Mean plasma magnesium concentration was slightly elevated in the TPTX group. GFR was stable and urine flow rate was not consistently different from intact gentamicin-infused rats, as stated above. Both calcium excretion rate and clearance in TPTX rats were elevated during the initial saline infusion when compared with intact rats, but both parameters were similar between TPTX and intact rats during the subsequent gentamicin infusion. Magnesiuria was depressed in TPTX rats during both saline and gentamicin infusion in comparison with intact gentamicin-infused rats.

The effects on plasma cation concentrations following TPTX are contradictory, plasma calcium concentration was depressed in saline-infused rats but unaffected in gentamicin-infused rats, plasma magnesium was elevated in gentamicin-infused rats but not in saline-infused rats. The effect on plasma magnesium was small and may be discounted but the effect on plasma calcium was more pronounced and apparently progressive. Possibly the removal of parathyroid tissue was not complete in some of the gentamicin-infused animals thereby pre-empting the hypocalcaemic response seen in saline-infused rats.

That TPTX per se did not appear to promote hypercalciuria (except perhaps during the control period in gentamicin-infused rats) is surprising given the marked increase in calcium clearance reported

following this manoeuvre in experiments of similar design (Agus et al, 1977). The hypercalciuric effect of PTH depletion may simply be masked by the high calcium output produced by saline infusion in this model.

Magnesiuria was apparently depressed in both TPTX groups. The mechanism of this effect of TPTX on the renal handling of magnesium is unknown. However, plasma calcium concentration is known to influence the renal handling of magnesium (Coburn et al, 1970; leGrimellec et al, 1974) which has led to the suggestion that hypocalcaemia may enhance magnesium reabsorption in the loop of Henle (Quamme and Dirks, 1980). Therefore, the reduction in magnesiuria in TPTX saline-infused rats may be the result of the hypocalcaemia in these animals

Above all, it is clear that the hypercalciuric and hypermagnesiuric responses to gentamicin infusion were retained following acute thyroparathyroidectomy (Figure 71). Although there were differences in these responses between intact and TPTX rats, this would seem to refute PTH suppression as the mechanism responsible.

Figure 57. Protocol used in clearance experiments in which acute thyroparathyroidectomy (TPTX) was carried out.

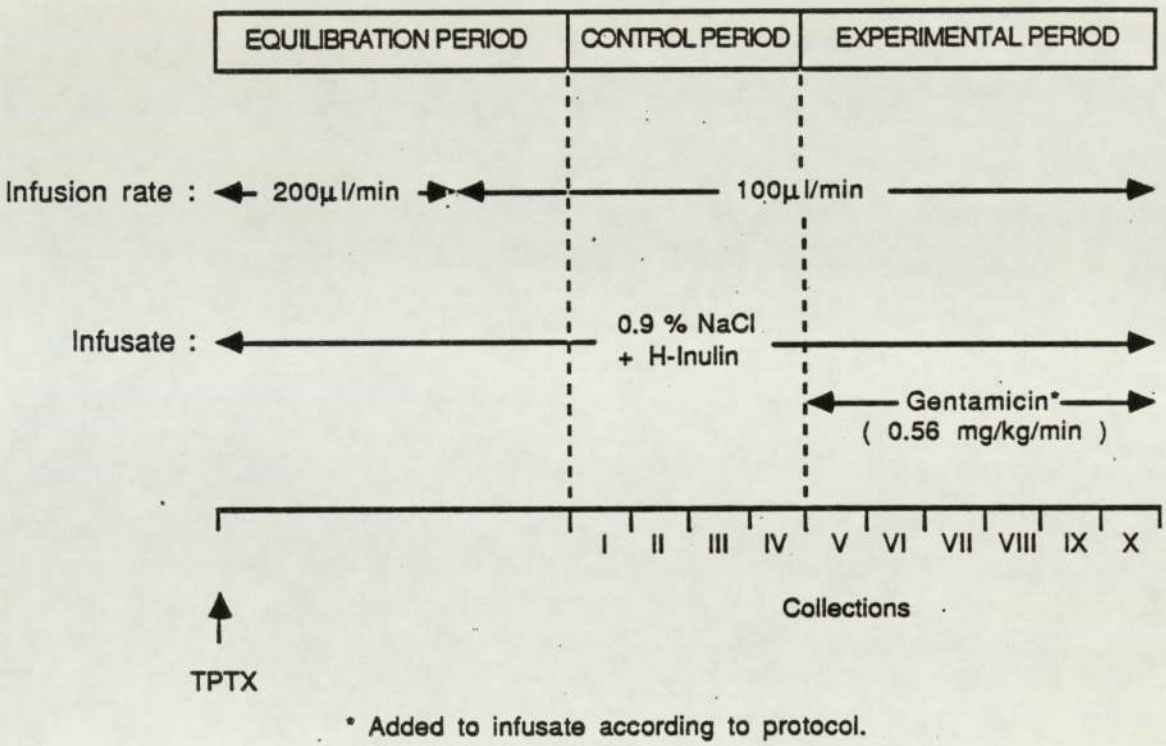


Figure 58. Comparison of urine flow rate between TPTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

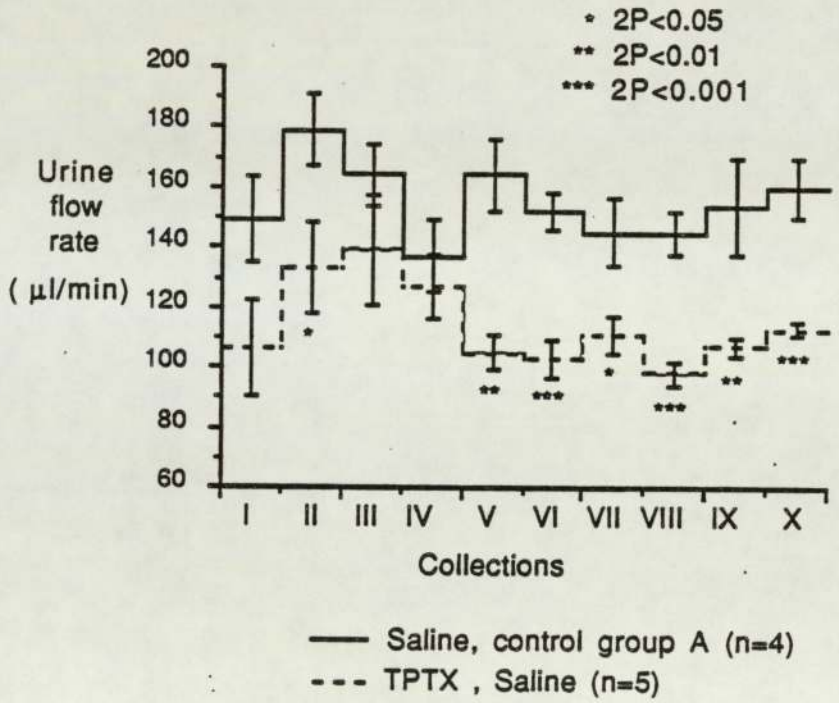


Figure 59. Comparison of concentrations of cations in plasma between TPTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

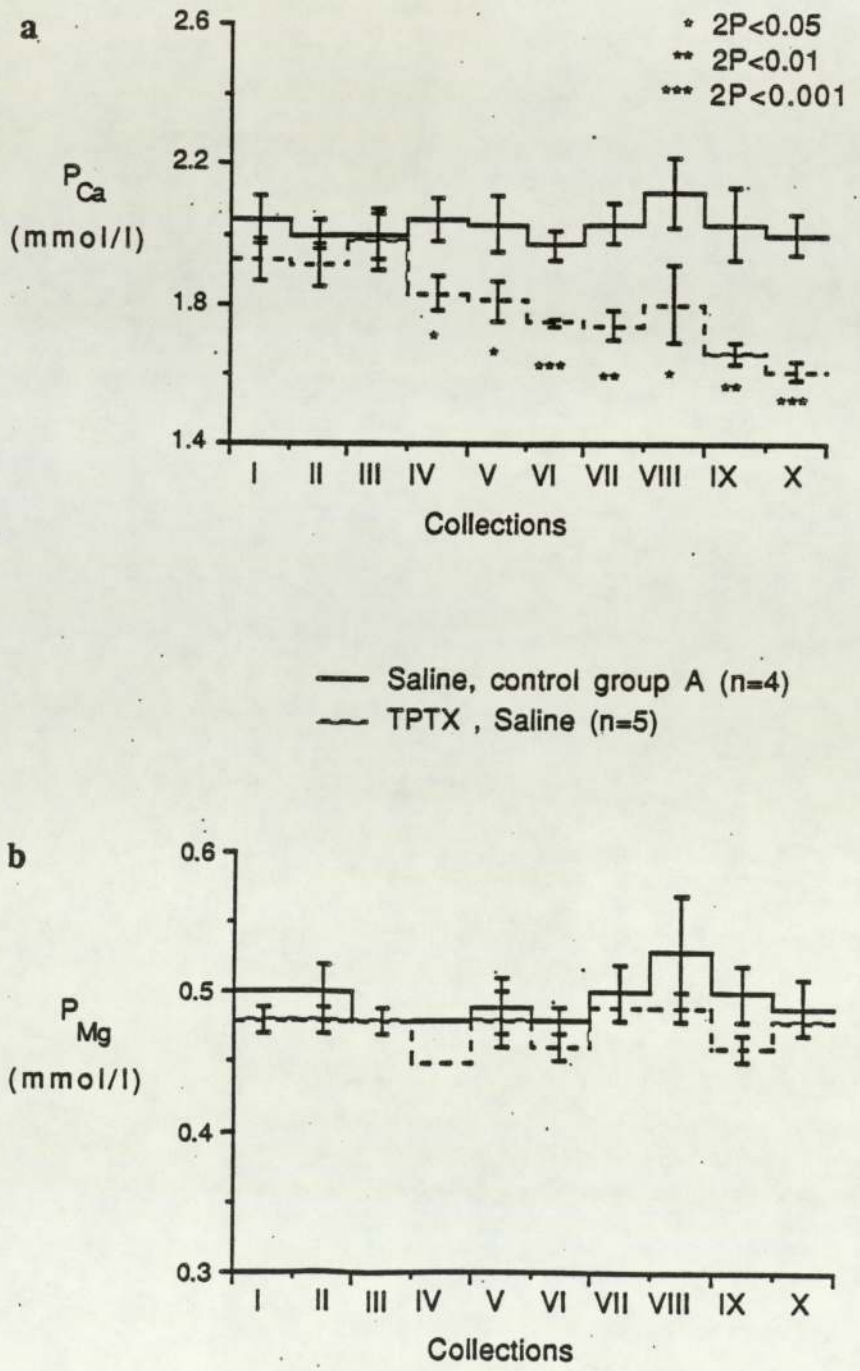
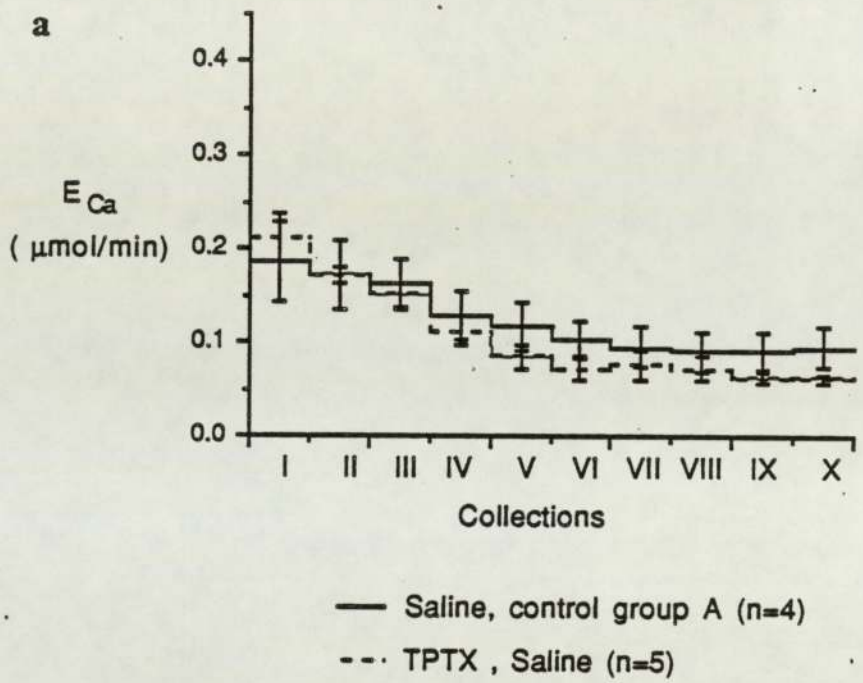


Figure 60. Comparison of excretion rates of cations between TPTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



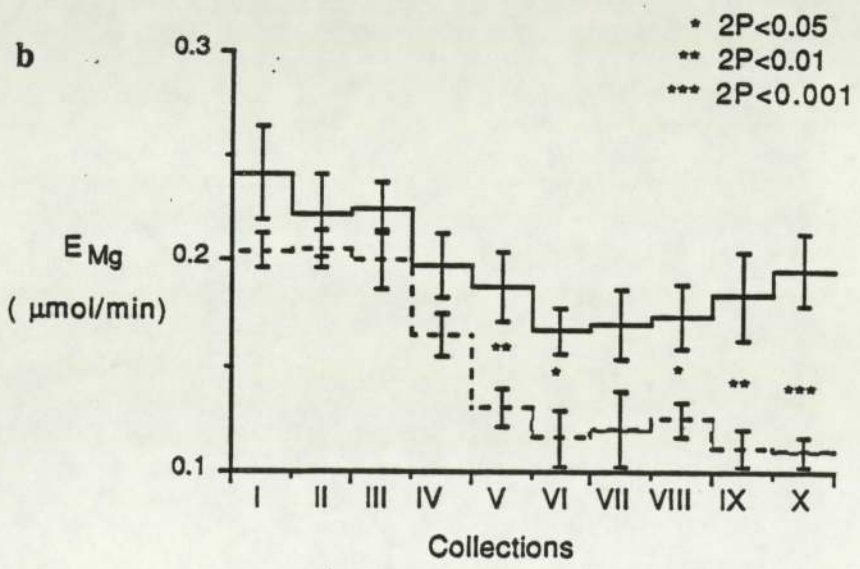
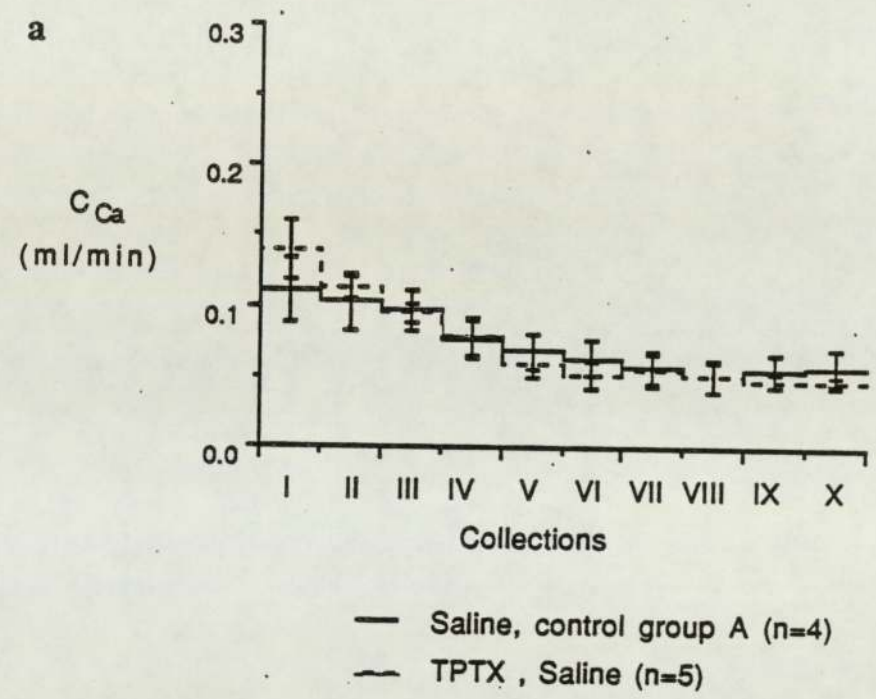


Figure 61. Comparison of clearances of cations between TPTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



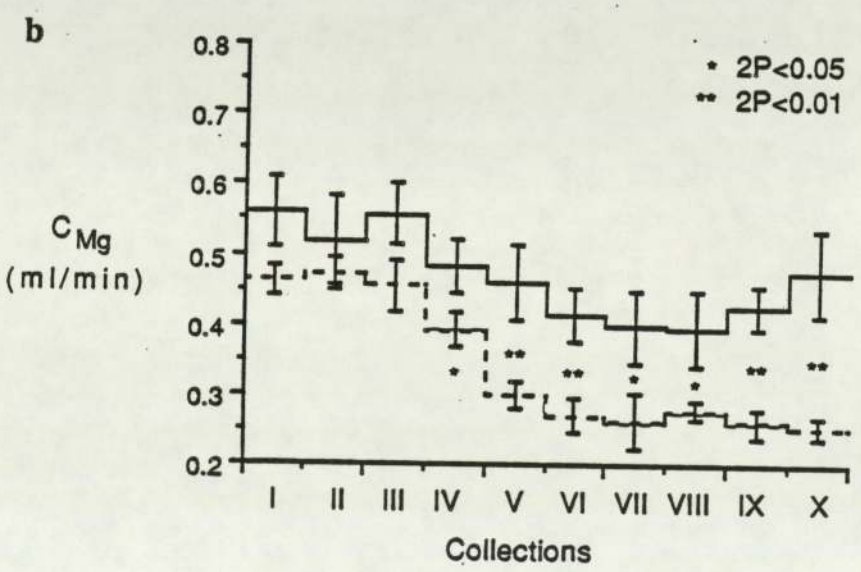


Figure 62. Comparison of urine flow rate between TPTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

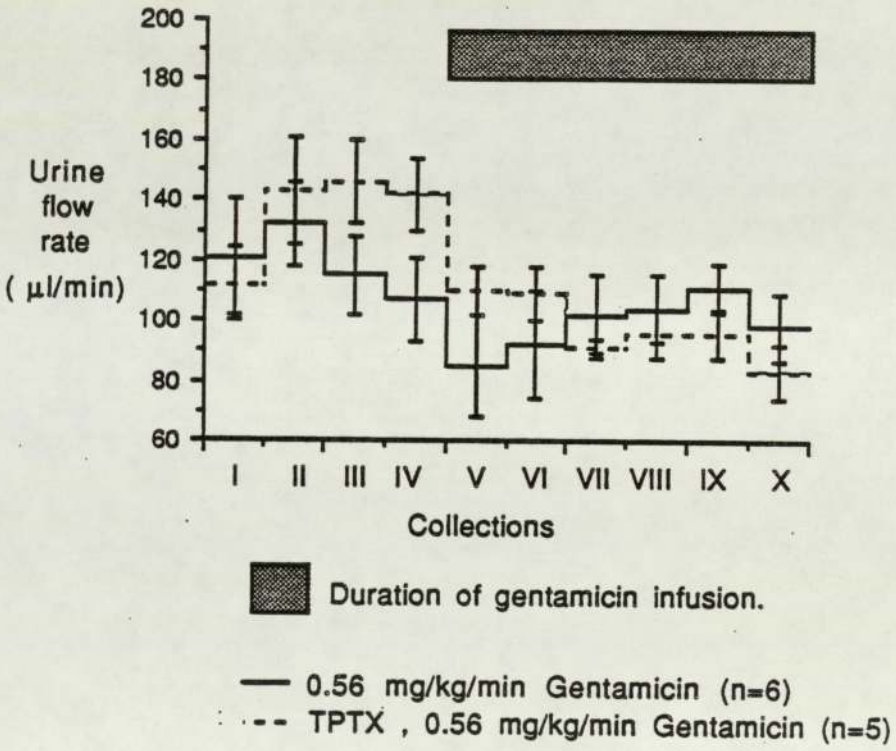
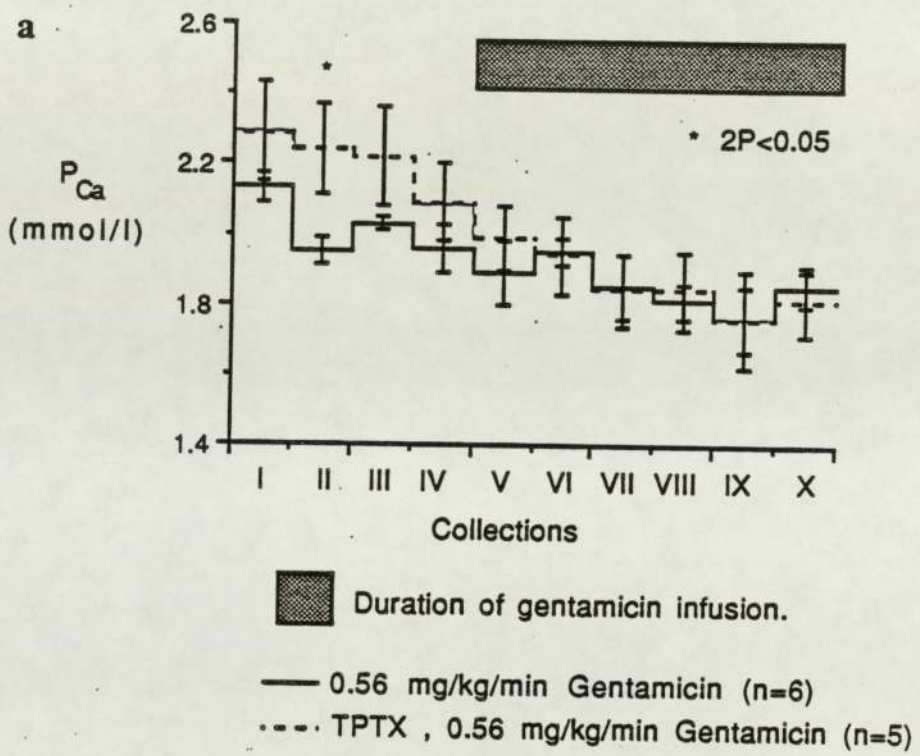


Figure 63. Comparison of concentrations of cations in plasma between TPTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



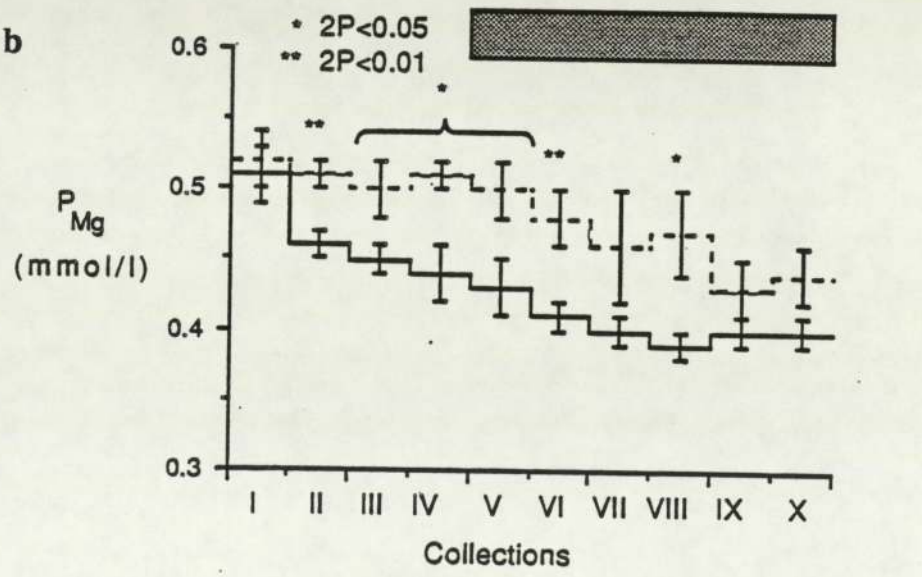


Figure 64. Comparison of excretion rates of cations between TPTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

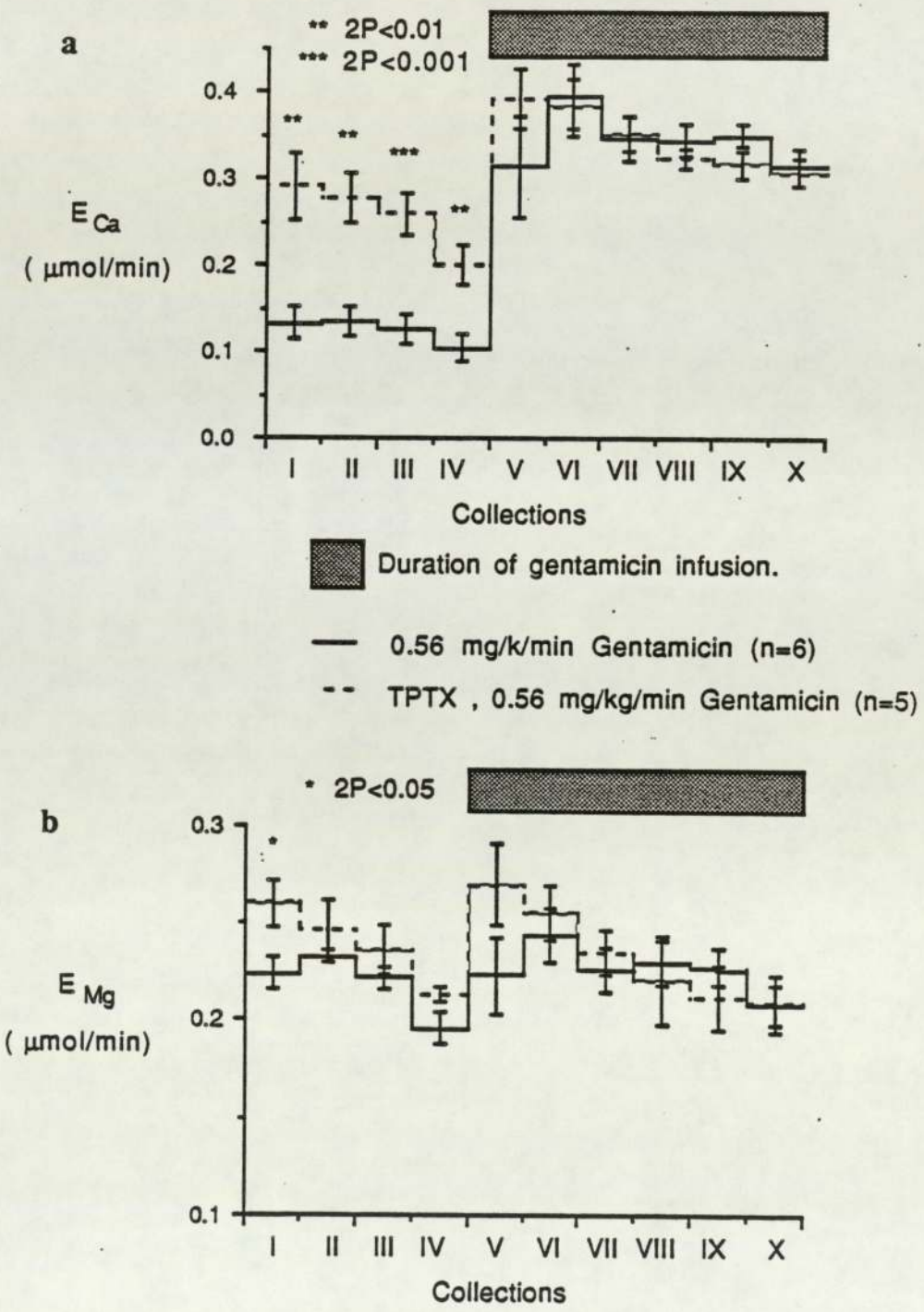
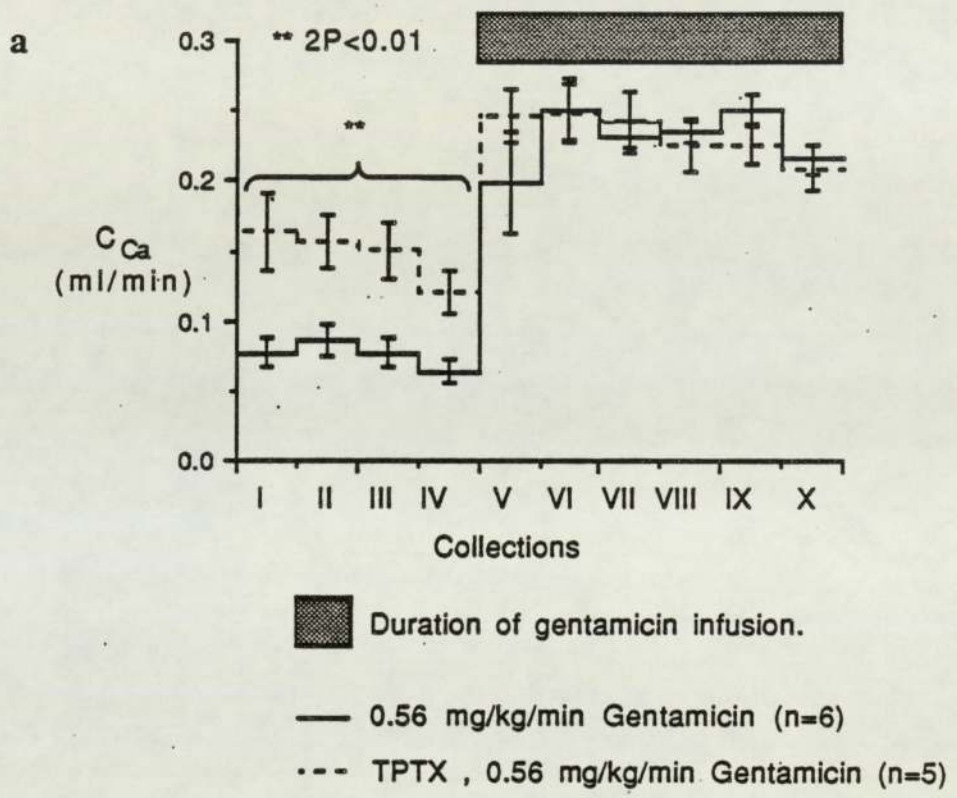


Figure 65. Comparison of clearances of cations between TPTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



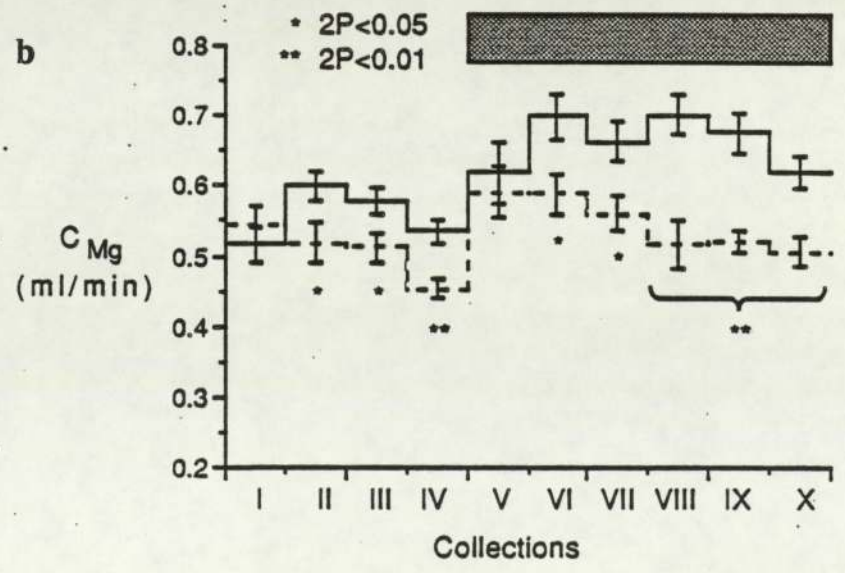


Figure 66. Comparison of **a.** urine flow rate and **b.** GFR between TPTX rats infused with saline and TPTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

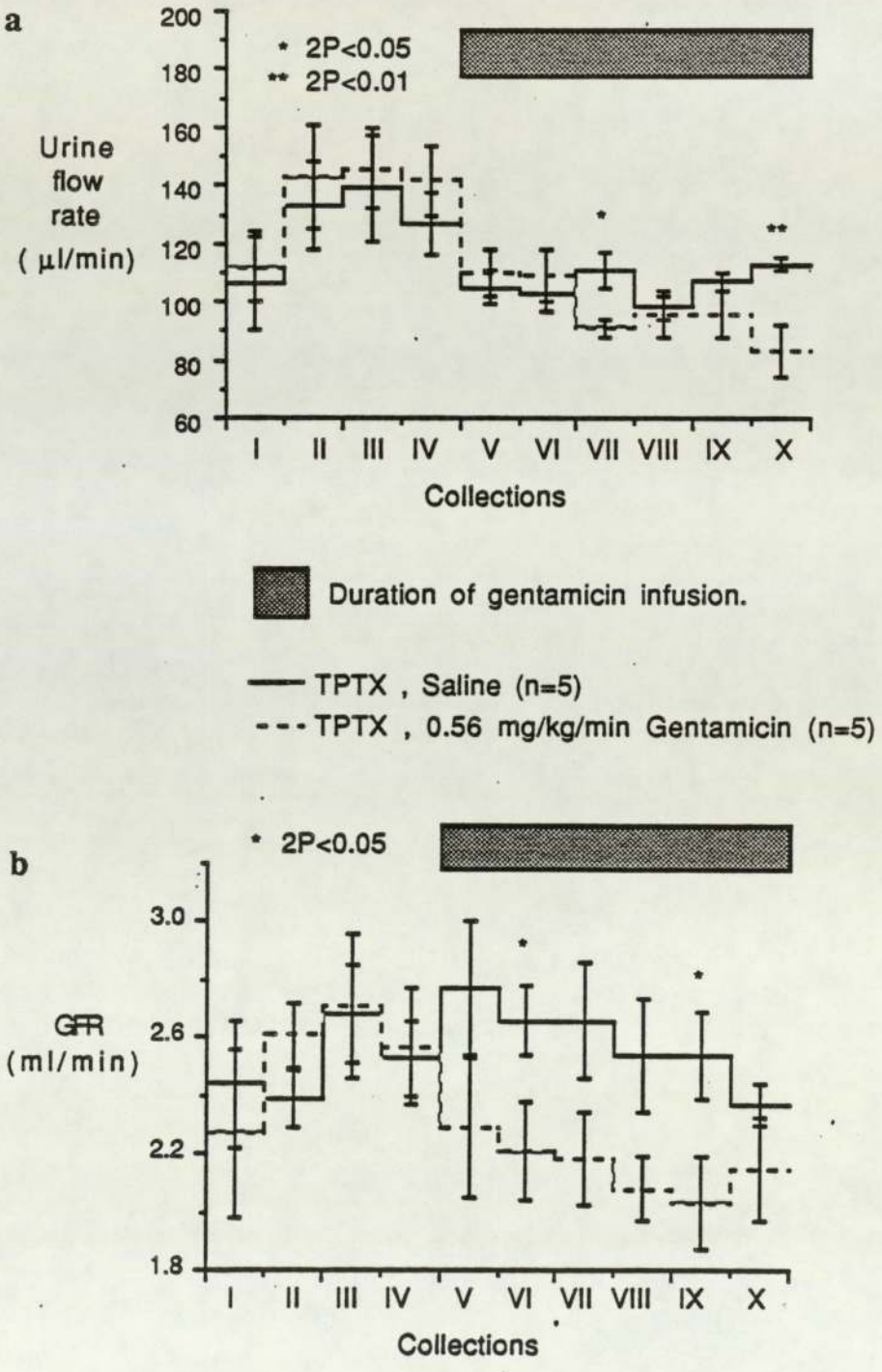


Figure 67. Comparison of concentrations of cations in plasma between TPTX rats infused with saline and TPTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

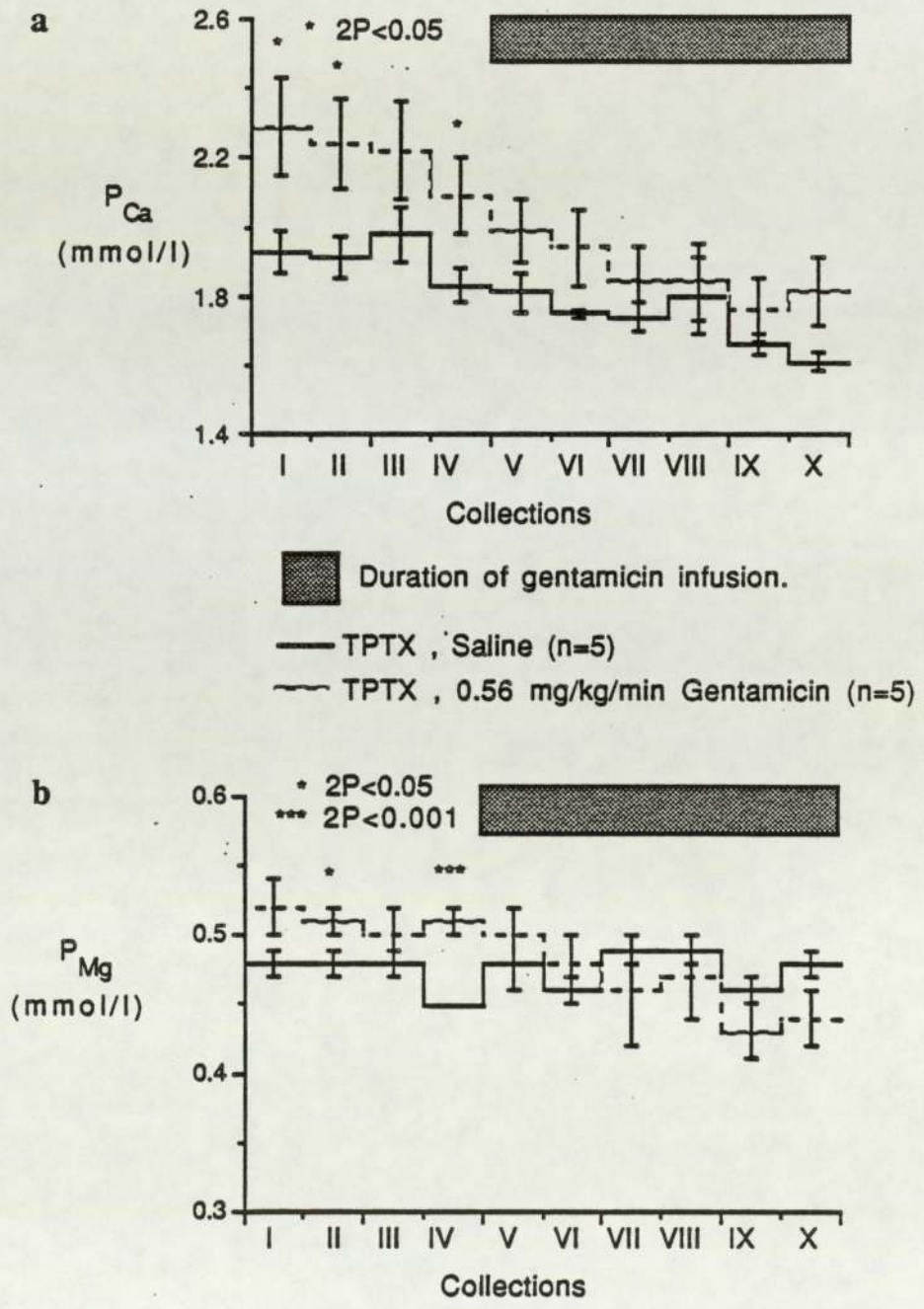


Figure 68. Comparison of excretion rates of cations between TPTX rats infused with saline and TPTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

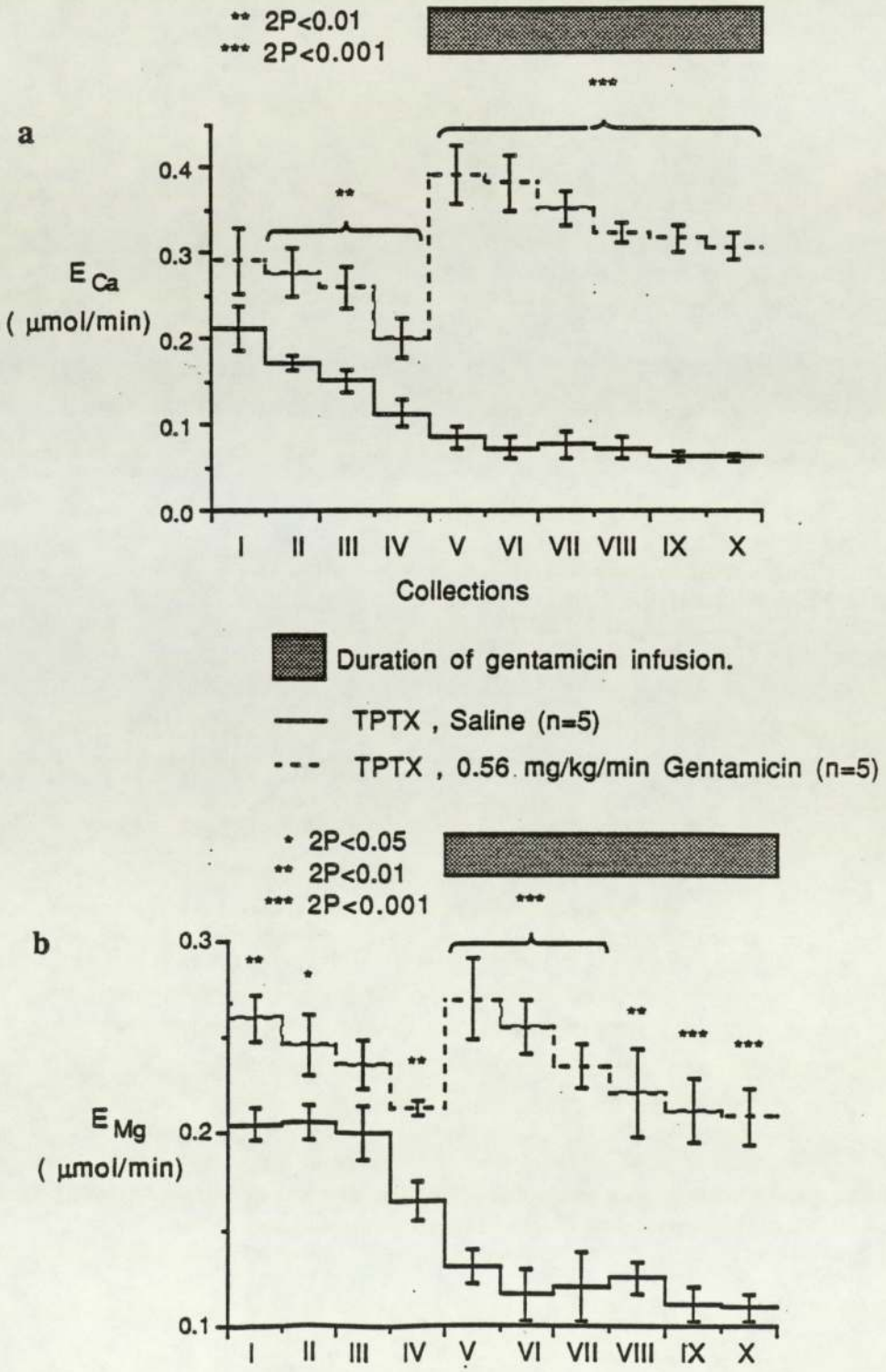
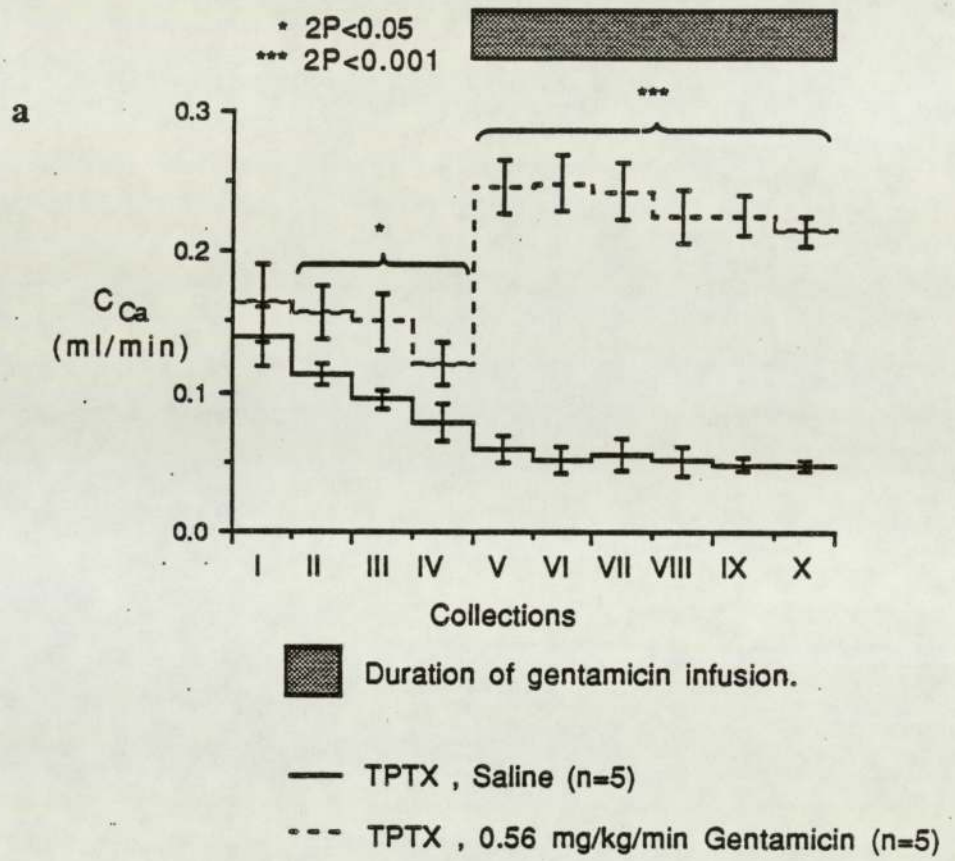


Figure 69. Comparison of clearances of cations between TPTX rats infused with saline and TPTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



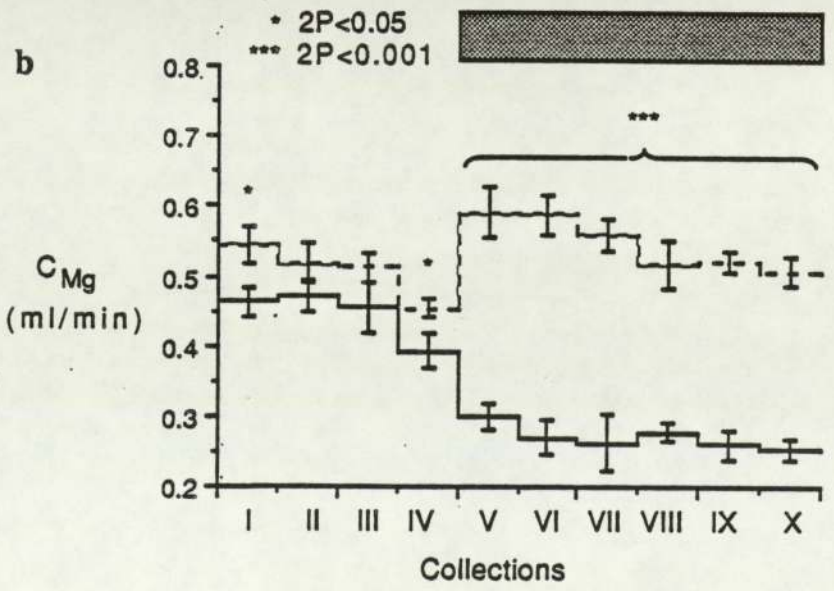


Figure 70. Comparison of fractional excretions of cations between TPTX rats infused with saline and TPTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

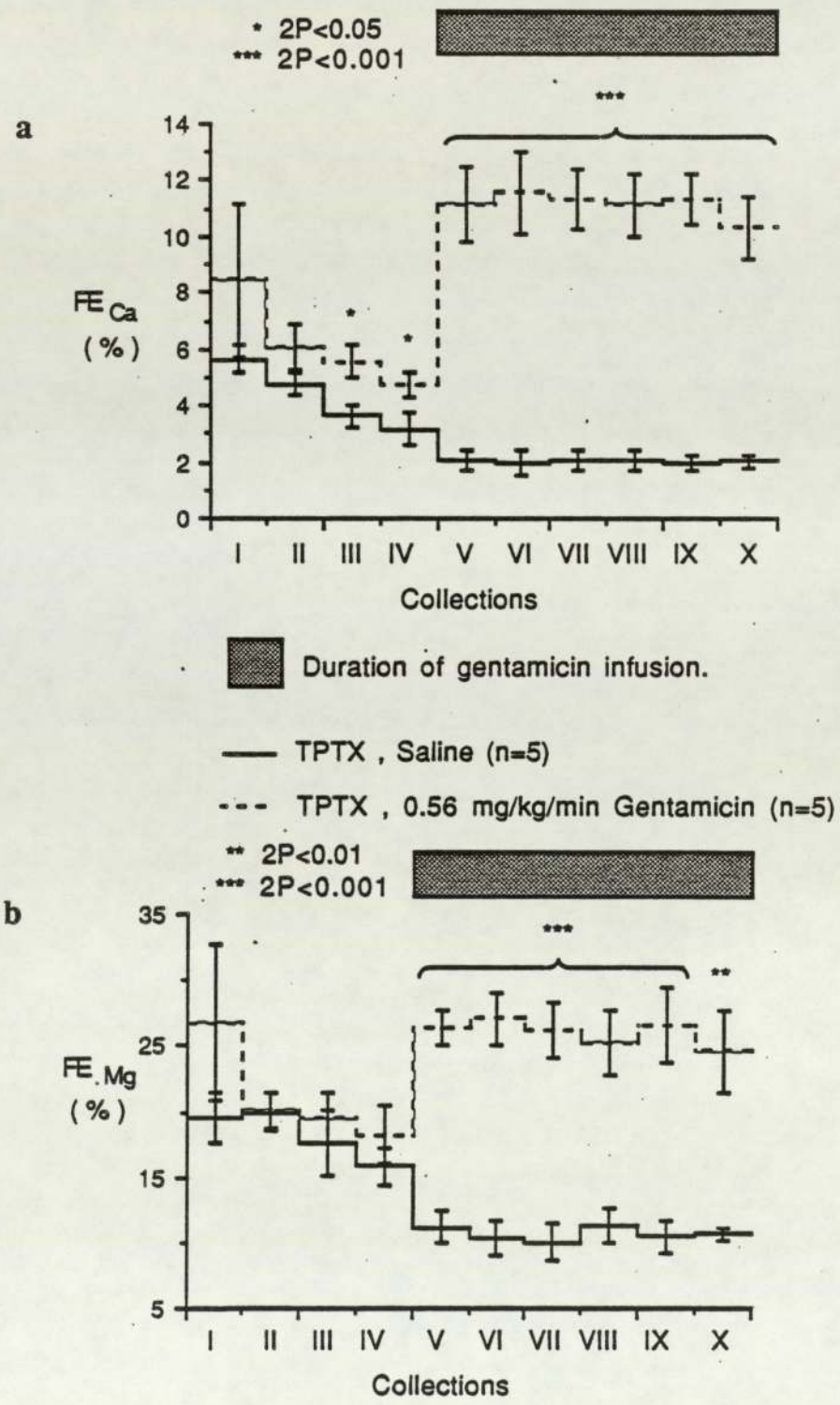
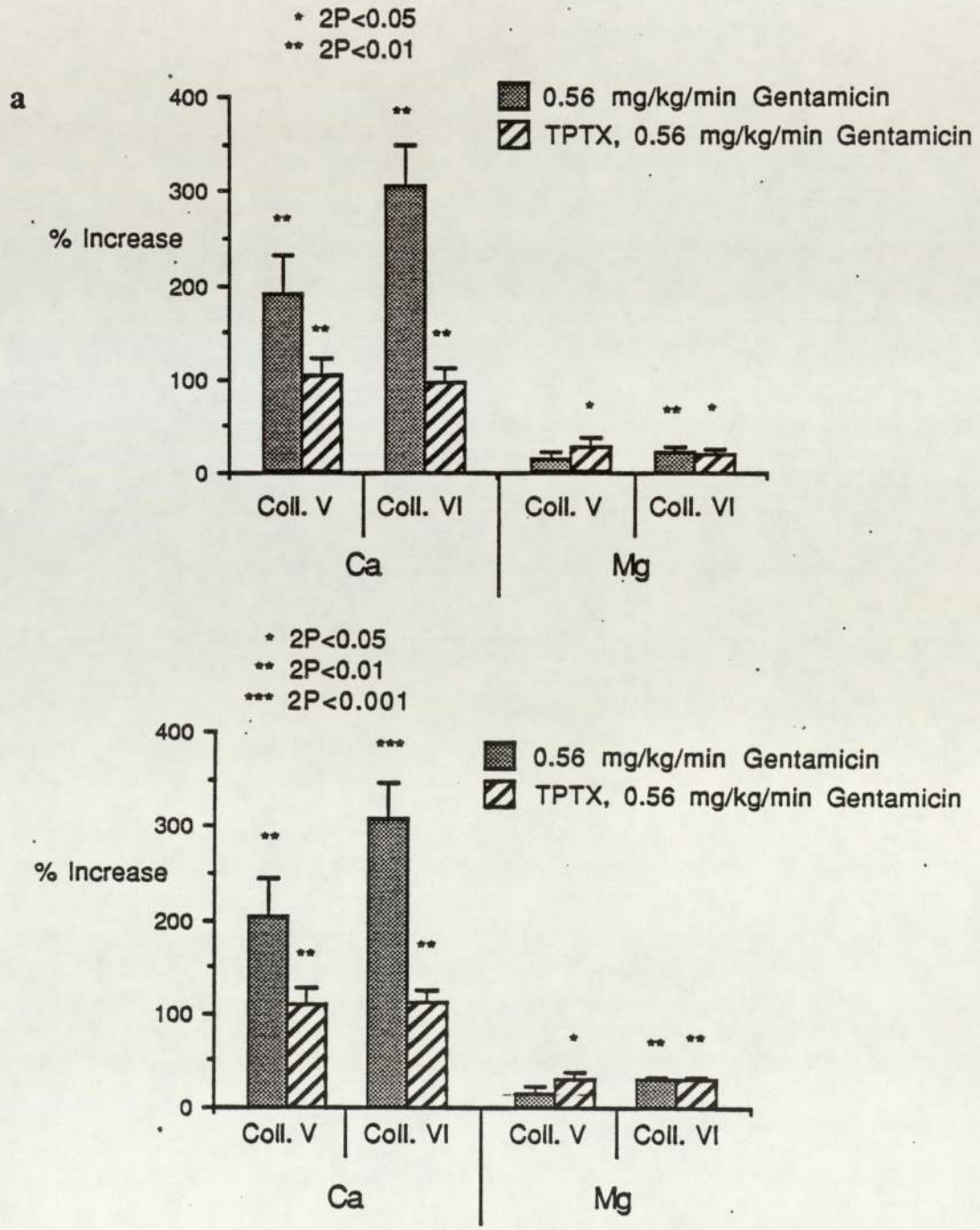


Figure 71. Histograms illustrating the effects of gentamicin infusion in TPTX and 'intact' rats on the excretion rates and clearances of calcium and magnesium. Each column represents the mean of the individual percentage increases in excretion rate or clearance at collection V or VI over values at collection IV from the same individuals ± s.e.m. Statistical comparisons were made using Student's 't' test for paired data. **a.** Excretion rates. **b.** Clearances.



4.7 The effect of selective parathyroidectomy on the renal handling of calcium and magnesium in the presence and absence of infused gentamicin

a) Introduction:

The validity of acute thyroparathyroidectomy as a manoeuvre to eliminate the influence of PTH on renal handling of divalent cations is in some doubt for the following reasons: Although the plasma half-life of PTH in the TPTX rat is less than 30 minutes its biological half-life at renal sites is unknown; therefore a renal response to PTH depletion may not occur immediately. Also, the procedure entails removal of the entire thyroid axis; thus tissue responsible for secretion of calcitonin is removed in addition to the parathyroid glands. Stimulation of calcitonin secretion may be involved in the hypocalcaemic response to streptomycin administration (Galante, 1970) and such an action may conceivably be shared with other aminoglycosides, including gentamicin. Finally, thyroxine may ameliorate the nephrotoxic effects of gentamicin (Cronin and Newman, 1985; Cronin et al, 1986) and may possibly be involved in other renal responses to the drug.

Therefore, in a second series of experiments the parathyroid glands were selectively destroyed. Also, the appearance of hypocalcaemia was confirmed before clearance experiments were carried out. Thus, only PTH-secreting tissue was removed and confirmation of a physiological effect of PTH depletion was obtained in these experiments. The objective of this study was identical to that of the previous study, to determine the involvement of PTH in the hypercalciuric and hypermagnesuric responses to gentamicin.

b) Methods:

Selective parathyroidectomy was carried out by cautery under

ether anaesthesia. These parathyroidectomised (PTX) rats were allowed to recover from anaesthesia and were returned to their cages. After approximately 48 hours these rats were used in clearance experiments; the surgical procedure described in 3.2 was carried out unmodified but for an additional arterial blood sample obtained immediately after arterial cannulation (Sample A). A value for plasma calcium concentration was determined from this sample. Clearance experiments were only completed if this value was more than two standard deviations below the mean plasma calcium concentration in rats with intact parathyroid glands undergoing identical surgery (Figure 73). The experimental protocol is outlined in Figure 72. Three rats received an infusion of saline only, of these two rats received 0.17 U/min of bovine PTH during the period indicated. Three further rats received an infusion containing 0.56 mg/kg/min gentamicin during the experimental period, of these two rats also received 0.17 U/min PTH during the final 90 minutes of infusion. Statistical comparisons were made with control group B and with those intact rats infused with 0.56 mg/kg/min gentamicin described in section 4.2.

c) Results:

i) Saline infusion:

Mean urine flow rate (Figure 74a), GFR (Figure 74b) and mean plasma magnesium concentration (Figure 75b) were not consistently significantly different between intact and PTX rats receiving a saline infusion. Mean plasma calcium concentration was consistently lower in PTX rats (Figure 75a), although the differences between these and intact rats were rarely statistically significant.

Mean excretion rate (Figure 76a) and clearance (Figure 77a) of calcium were not significantly different between saline-infused intact and PTX rats during collections I-VII. Mean fractional

excretion of calcium was similarly unaffected by PTX indicating that the tubular reabsorption of calcium did not differ significantly between these two groups (Figure 78a). The single animal which continued to receive a saline infusion during collections VIII-X showed a small decrease in calciuria, mean calcium excretion rate (Figure 89a) and clearance (Figure 92a) fell to approximately 75-80% of values during collections V-VII. Mean fractional excretion of calcium fell to a similar extent indicating that these decreases were the result of changes in tubular calcium reabsorption (Figure 95a). In contrast, the two rats which received PTH during collections VIII-X showed much more marked decreases in calciuria during this period (Figures 90a, 91a, 93a, 94a, 96a and 97a), in one mean calcium excretion rate (Figure 91a) and clearance (Figure 94a) fell to approximately 50-60% of values during collections V-VII, these changes were again reflected by parallel changes in the fractional excretion of calcium, indicating that the response to PTH was mediated by an increase in the tubular reabsorption of calcium (Figure 97a).

Mean magnesium excretion rate (Figure 76b) and clearance (Figure 77b) were not significantly different between intact and PTX rats receiving saline infusion during collections I-VII. The mean fractional excretion of magnesium was also unaffected by PTX indicating that the tubular reabsorption of magnesium did not differ significantly between these groups (Figure 78b) In the animal which received saline alone during collections VIII-X magnesuria remained essentially stable (Figures 89b, 92b and 95b). In contrast, the two rats which received PTH during collections VIII-X showed a marked reduction in magnesuria (Figures 90b, 91b, 93b, 94b, 96b and 97b), in one case mean magnesium excretion rate (Figure 91b) and clearance (Figure 94b) fell to approximately 60-70% of values during collections V-VII. In each case these changes could be explained by

parallel changes in the mean fractional excretion of magnesium, indicating that they were brought about by changes in tubular magnesium reabsorption (Figure 97b).

Mean phosphate excretion rate was consistently depressed in all PTX rats with respect to intact rats receiving saline infusion during collections I-VII (Figure 88b). The animal which continued to receive saline infusion during collections VIII-X continued to show depressed phosphate excretion rate (Figure 89c). In contrast, in both animals which received PTH infusion during collections VIII-X a dramatic increase in phosphate excretion rate to values approximately double (Figure 91c) or over five times (Figure 90c) those seen in intact rats was seen.

ii) Gentamicin infusion:

Mean urine flow rate (Figure 79) and mean plasma magnesium concentration (Figure 80b) was not consistently significantly different between intact and PTX rats which received 0.56 mg/kg/min gentamicin. GFR was stable in these PTX rats (Figure 83b). The mean plasma calcium concentration in PTX rats was consistently lower than in intact rats (Figure 80a) although, again, the differences were rarely statistically significant. Mean urine flow rate (Figure 83a), GFR (Figure 83b) and the concentrations of calcium and magnesium in plasma (Figures 84a and 84b) were not consistently, significantly different between PTX rats infused with saline and PTX rats infused with gentamicin.

Mean calcium excretion rate (Figure 81a) and clearance (Figure 82a) were not consistently significantly different between gentamicin-infused PTX and intact rats during collections I-VII. Both increased to 300-350% of control values in response to gentamicin infusion in PTX rats. In the animal which did not receive PTH during collections VIII-X a small decrease in calciuria was observed, calcium excretion rate (Figure 89a) and clearance (Figure 92a) fell to approximately

90% of values during collections V-VII. In one animal which received PTH during collections VIII-X calciuria increased slightly (Figures 90a and 93a). In the third rat a marked reduction of calciuria was stimulated by PTH, values of both parameters during collections VIII-X approximated to 65% of values during collections V-VII (Figures 91a and 94a). Mean fractional excretion of calcium changed in parallel with excretion rate and clearance in each case indicating that the opposing responses to gentamicin and PTH were due to changes in tubular calcium reabsorption (Figures 95a, 96a and 97a). Mean calcium excretion rate (Figure 85a) and clearance (Figure 86a) were not significantly different between PTX rats infused with saline and PTX rats infused with gentamicin during the control period. Introduction of infused gentamicin caused a significant increase in both parameters ($2P<0.01$ or 0.05) in the PTX rats receiving the drug in comparison with PTX controls. These changes were explicable by a similar rise in the fractional excretion of calcium in gentamicin-infused PTX rats (Figure 87a).

Mean magnesium excretion rate (Figure 81b) and clearance (Figure 82b) were not significantly different between intact and PTX rats receiving a gentamicin infusion. Both increased to 140-150% of control values after introduction of infused gentamicin in PTX rats. Excretion rate (Figure 89b) and clearance (Figure 92b) were essentially stable for the final collections in that animal which did not receive PTH. In one animal, which did receive PTH during collections VIII-X, a slight increase in magnesiuria, as well as calciuria, was observed (Figures 90b and 93b). In the final animal a marked reduction in magnesiuria was produced by PTH, mean magnesium excretion rate (Figure 91b) and clearance (Figure 94b) during collections VIII-X fell to approximately 70% of values during collections V-VII. All of these changes could be explained by alterations in fractional excretion of magnesium, indicating that

gentamicin and PTH both produced their actions via effects on tubular magnesium reabsorption (Figures 95b, 96b and 97b). Mean magnesium excretion rate (Figure 85b) and clearance (Figure 86b) were not consistently, significantly different between PTX rats infused with saline and PTX rats infused with gentamicin during the control period. Gentamicin infusion produced a significant ($2P<0.05$ or 0.01) rise in both parameters during collection V only, following this gentamicin-infused PTX rats were not significantly different from saline-infused PTX rats. Mean fractional excretion of magnesium was also elevated during gentamicin infusion but remained at significantly raised levels during collections VI and VII, indicating a sustained impairment of magnesium reabsorption (Figure 87b).

Mean phosphate excretion rate was not as dramatically depressed in this group of PTX rats as those which received an infusion of saline alone (Figure 88a). The rat which did not receive an infusion of PTH showed a depressed phosphate excretion rate for the full duration of the collection period (Figure 89c). The animal which did not show any reduction in calcium or magnesium output in response to PTH did not show a depressed phosphate output at any time and this parameter was also unaffected by infusion of PTH (Figure 90c). The final animal had a depressed phosphate excretion rate during collections I-VII, which increased markedly after infusion of PTH (Figure 91c). infusion of gentamicin did not appear to affect phosphate output in any of these animals (Figure 88a).

d) Discussion:

PTX in saline-infused rats did not affect urine flow rate, GFR, plasma magnesium concentration or the renal handling of calcium or magnesium with respect to intact saline-infused rats. Plasma calcium concentration, in PTX rats, was consistently lower than in intact rats.

PTX rats infused with gentamicin were not significantly different to intact rats infused with gentamicin in any of these parameters.

Plasma calcium concentration was not as consistently depressed in this group of PTX rats when compared with intact controls but, as noted earlier, the group of intact gentamicin-infused rats used in these comparisons appeared to be slightly hypocalcaemic themselves.

The hypercalciuric and hypermagnesuric responses to gentamicin infusion were retained in parathyroidectomised (PTX) rats (Figure 98). This conclusion seems to support the notion that parathyroid hormone (PTH) is not involved in the mechanism of these responses. Unlike rats which underwent acute thyroparathyroidectomy, confirmation of a biological response to PTH depletion was provided by the appearance of hypocalcaemia in all of the animals used in this series of experiments. Furthermore, phosphate excretion rate was depressed in five out of six of these animals, a consistent feature of PTH depletion.

PTX per se did not appear to affect the renal handling of calcium or Mg, again this is probably due to masking of any subtle changes by the hypercalciuria and hypermagnesuria of saline diuresis.

Infusion of bovine PTH reduced both calciuria and magnesiuria in three out of four animals and increased phosphate output in the same animals. therefore, gentamicin did not in any way interfere with the actions of PTH at renal sites.

Broadly, these results support the conclusions reached after the acute TPTX study, the only response not shared with this study was that no reduction in magnesuria was produced by selective parathyroidectomy.

Figure 72. Protocol used in clearance experiments in which parathyroidectomised (PTX) rats were used.

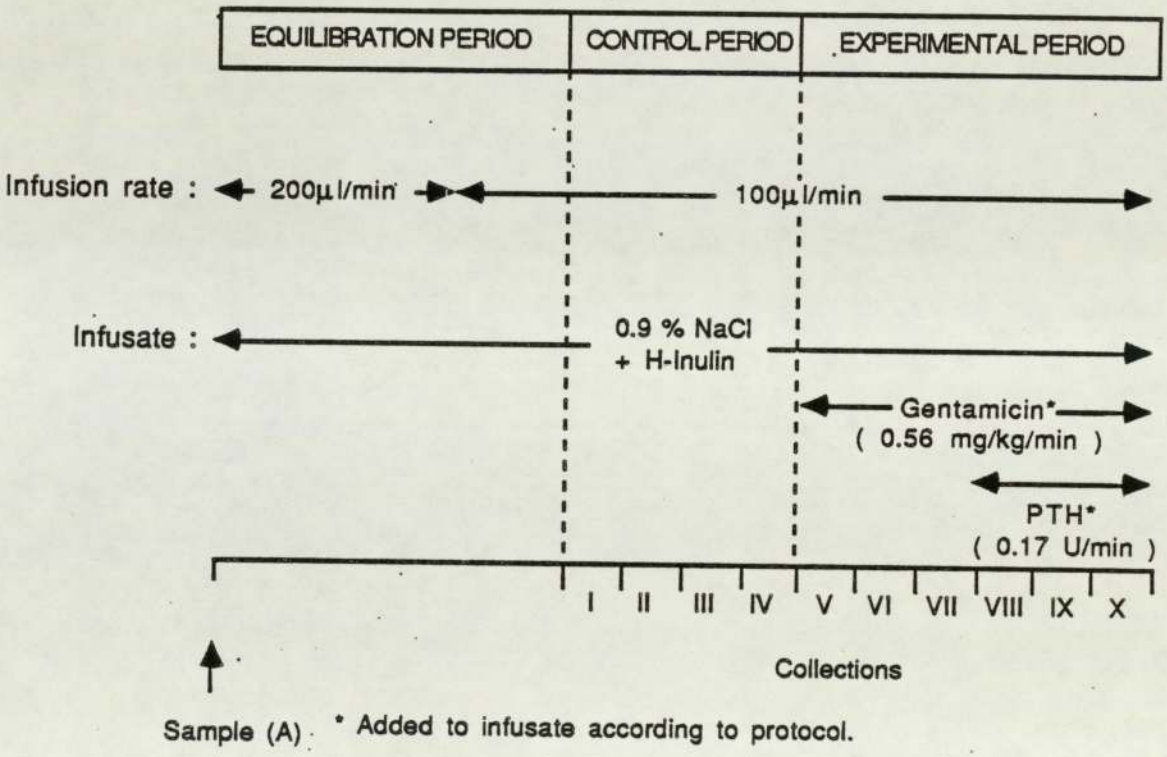


Figure 73. Concentrations of calcium in plasma at the beginning of clearance experiments (sample A) from PTX and non-PTX (intact) rats, expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

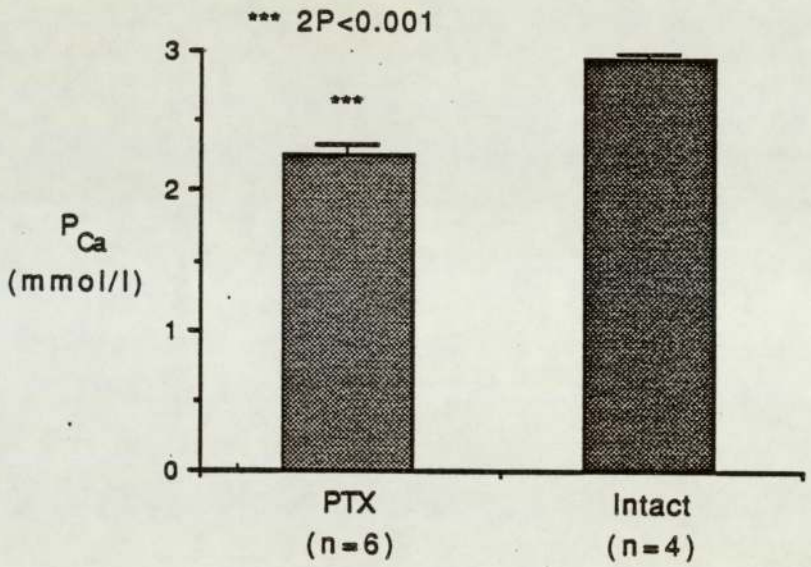


Figure 74. Comparison of **a.** urine flow rate and **b.** GFR between PTX rats infused with saline and 'intact' rats infused with saline. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

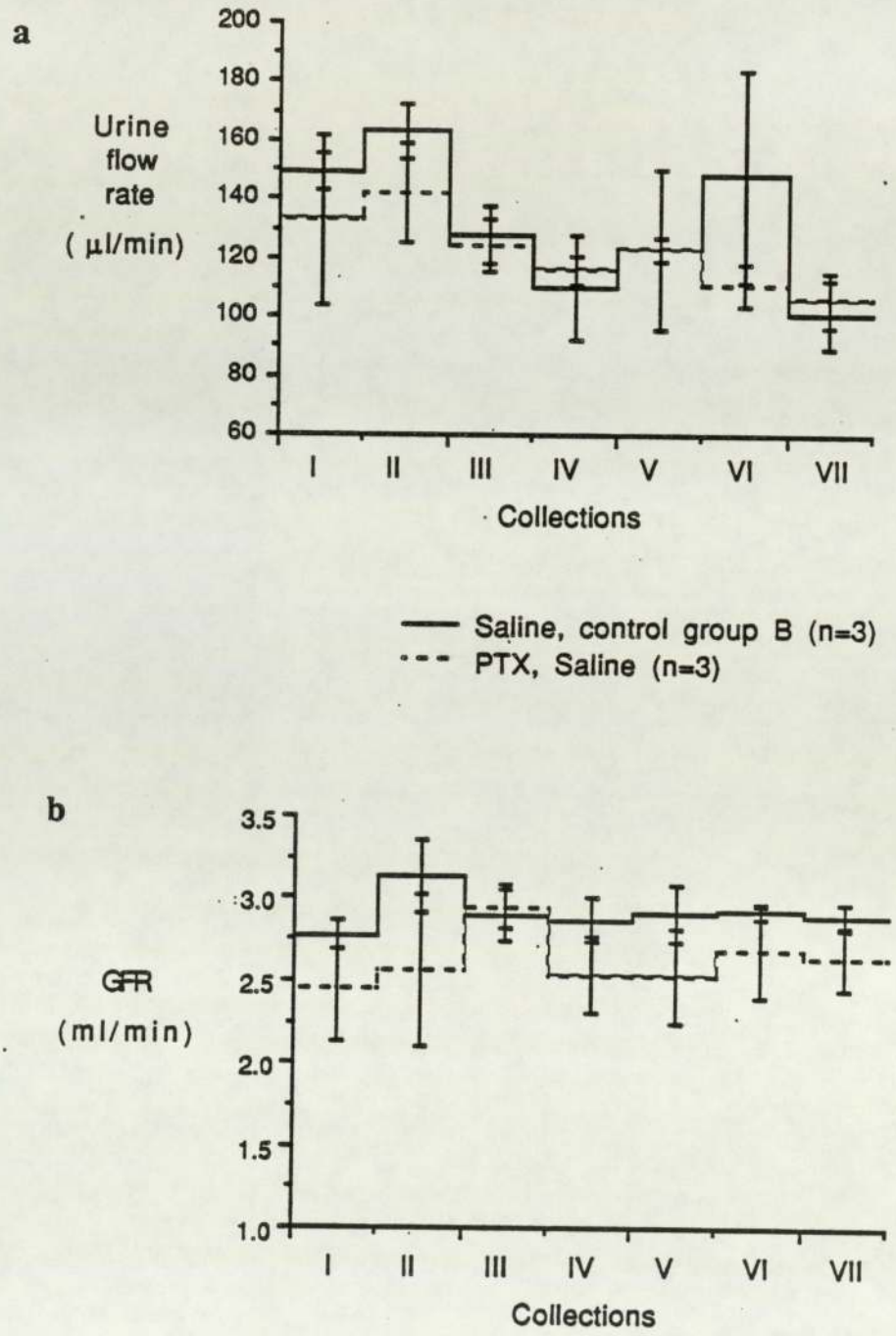


Figure 75. Comparison of concentrations of cations in plasma between PTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

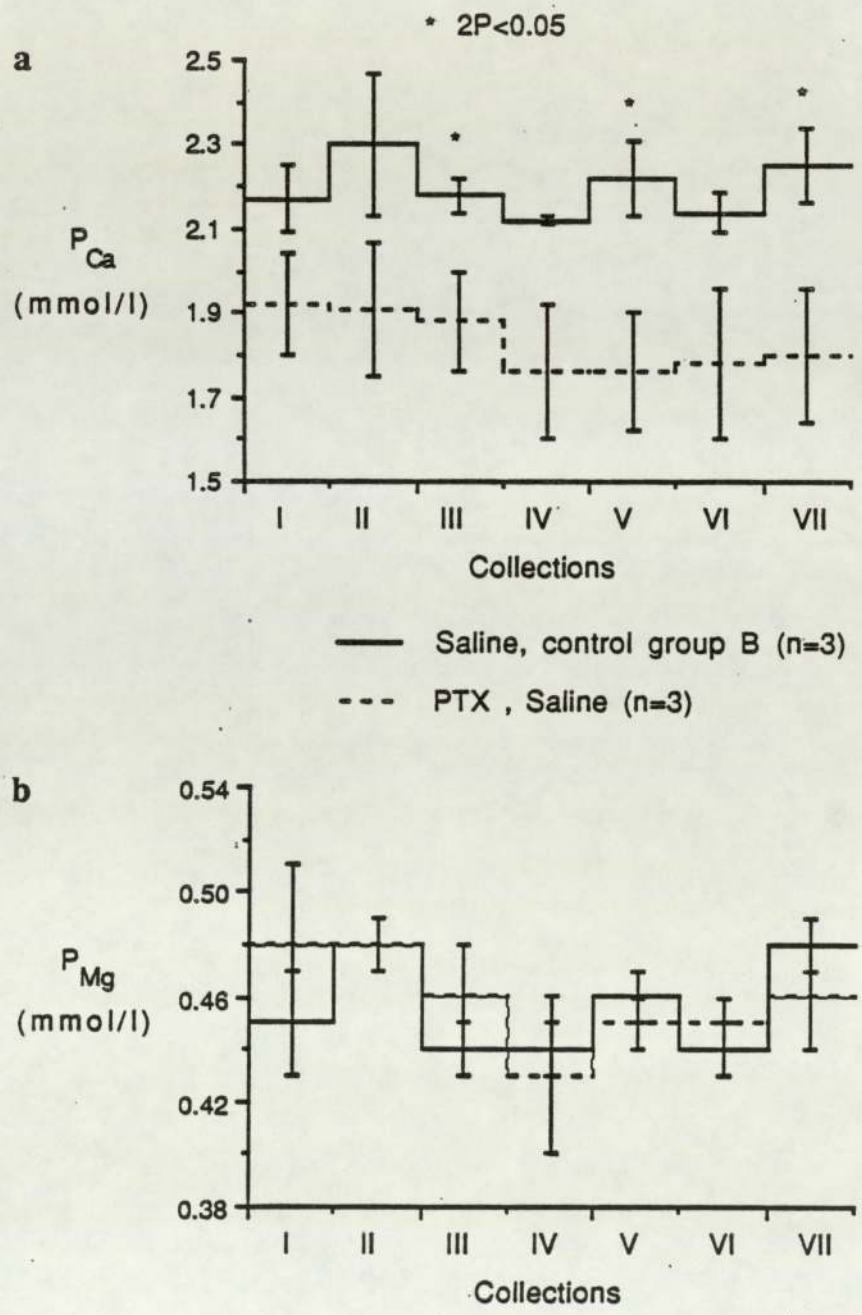


Figure 76. Comparison of excretion rates of cations between PTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

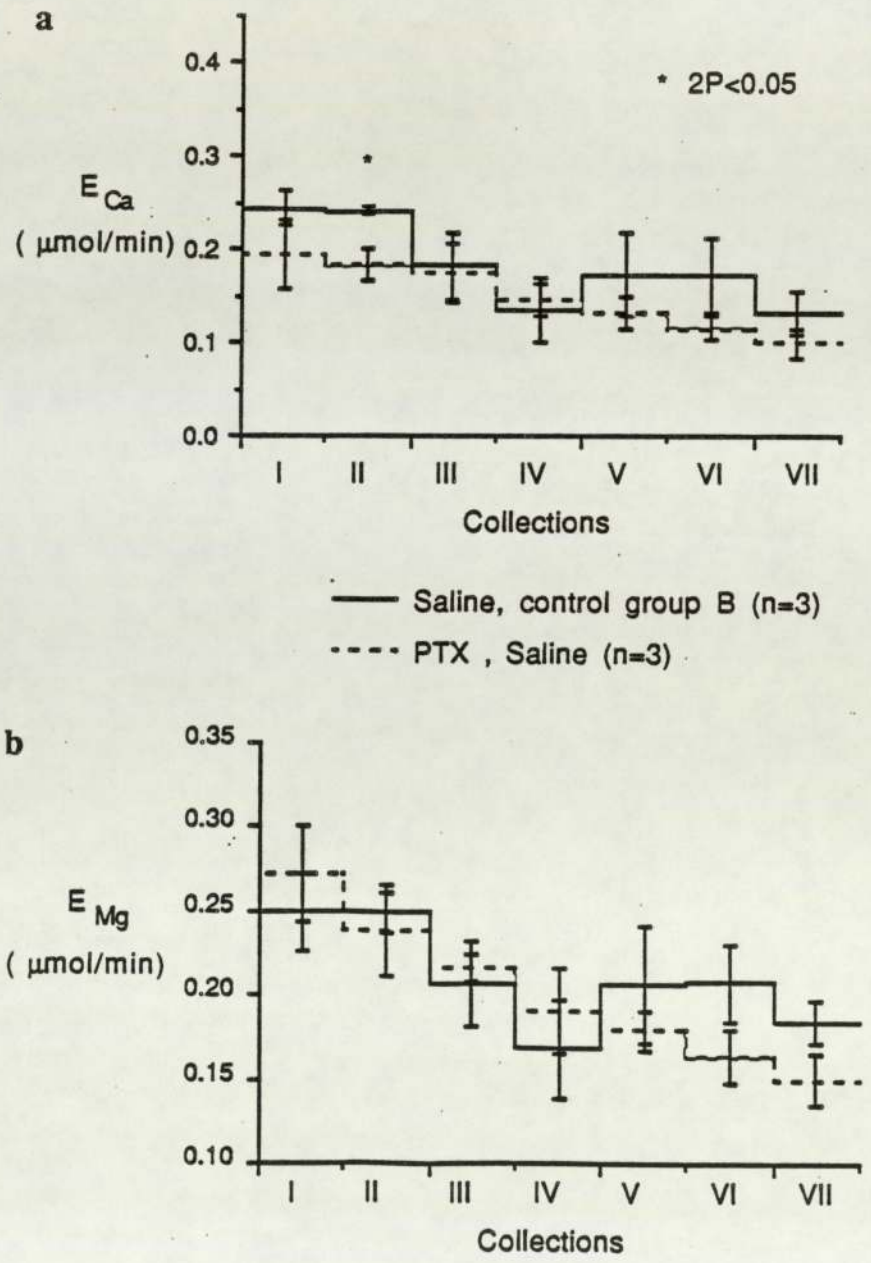


Figure 77. Comparison of clearances of cations between PTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

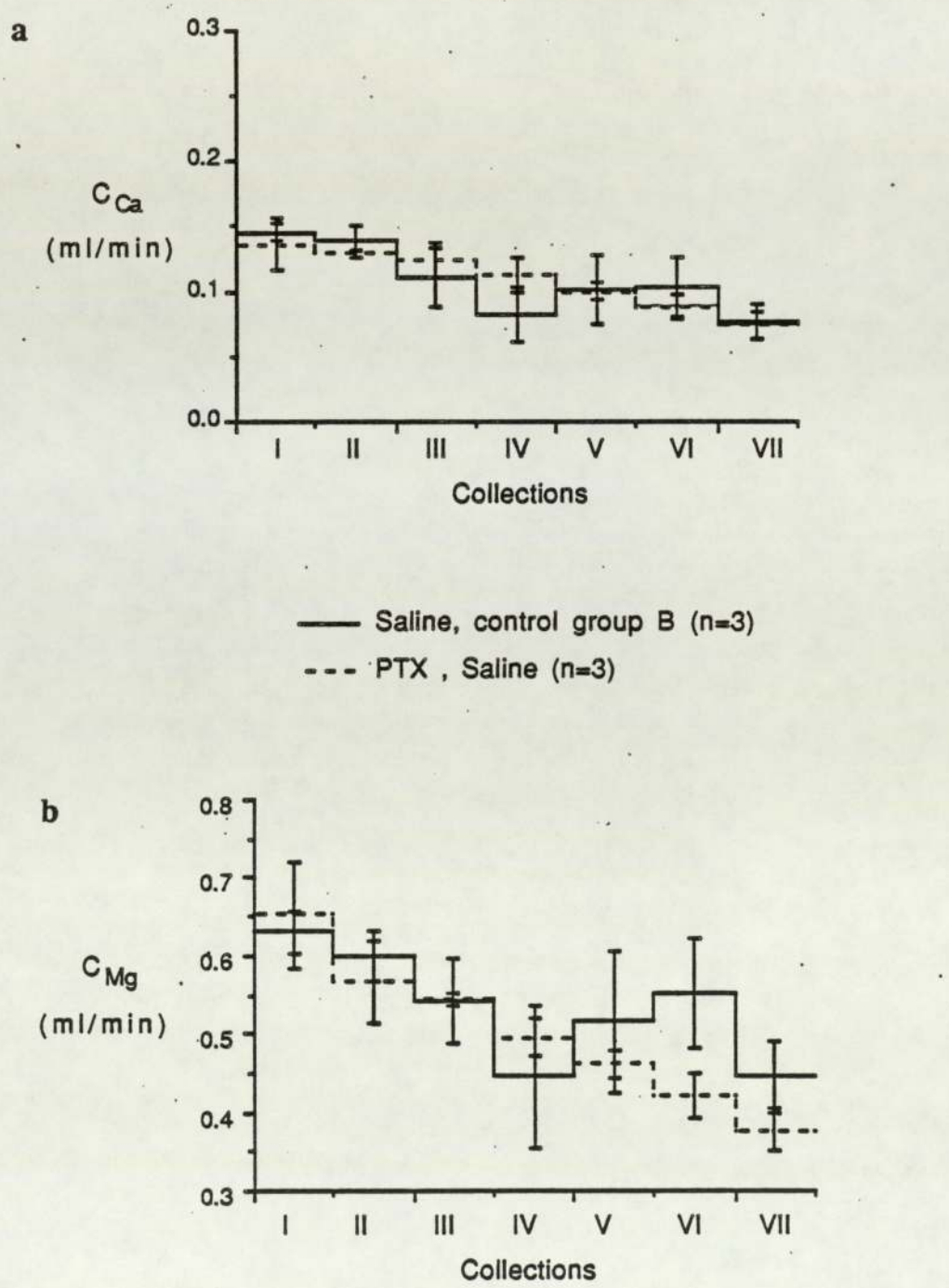
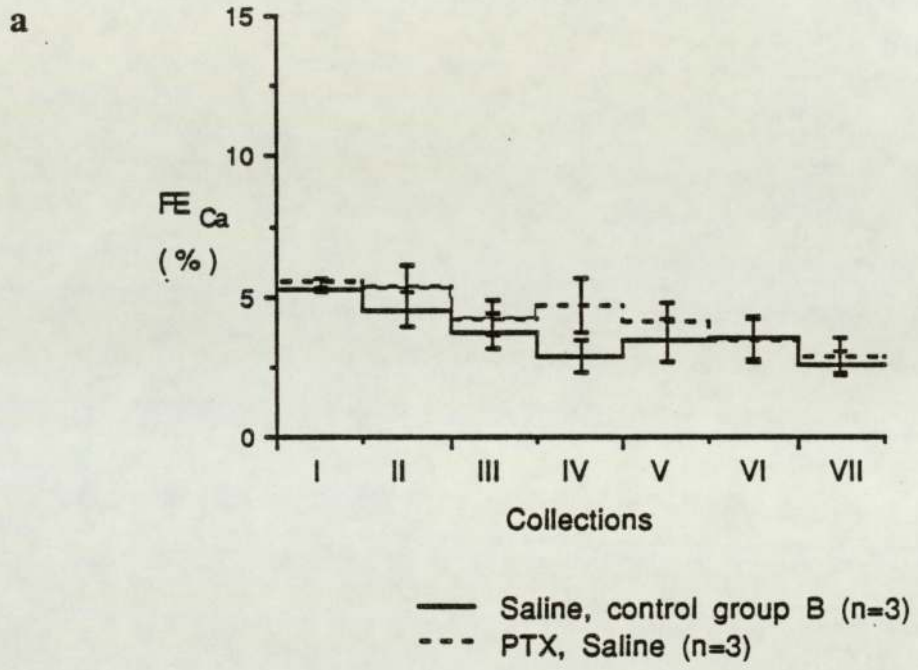


Figure 78. Comparison of fractional excretions of cations between PTX rats infused with saline and 'intact' rats infused with saline, both expressed as mean + s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



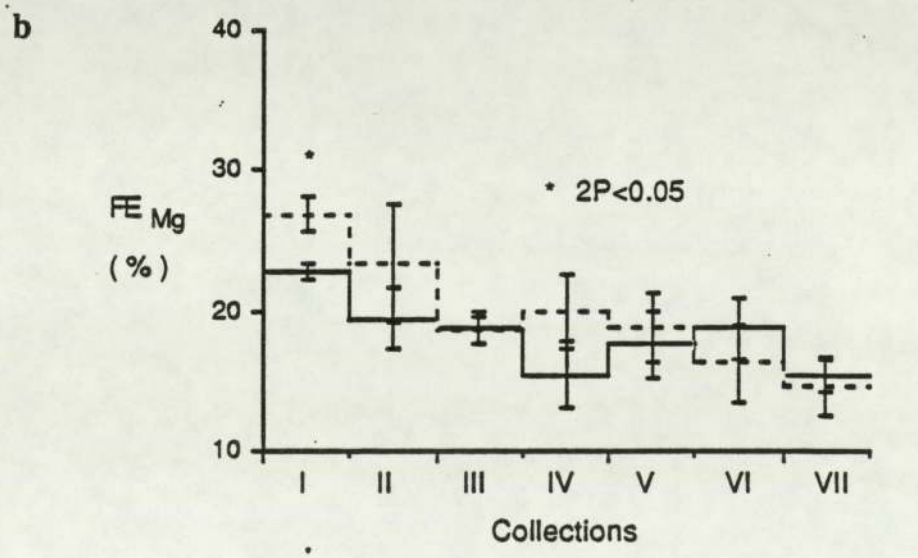


Figure 79. Comparison of urine flow rate between PTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

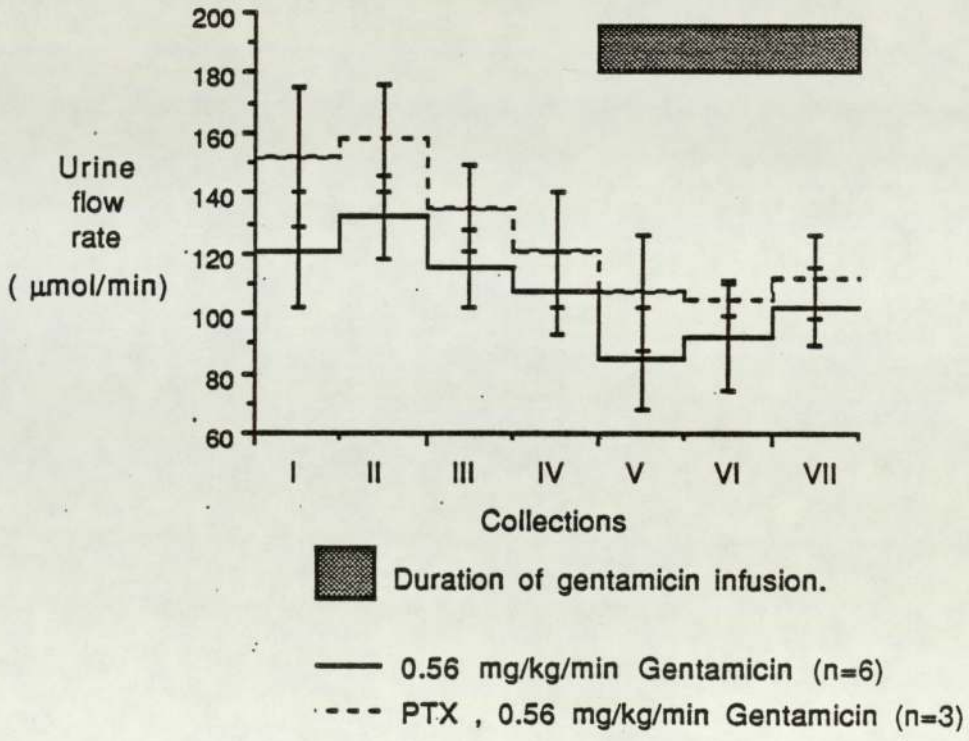
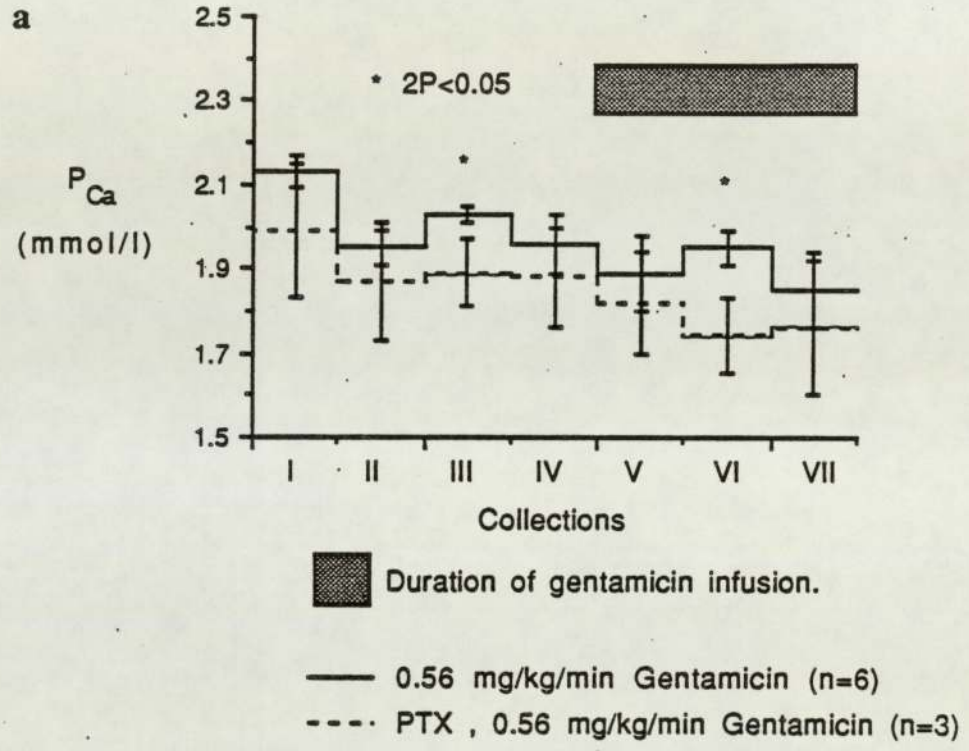


Figure 80. Comparison of concentrations of cations in plasma between PTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



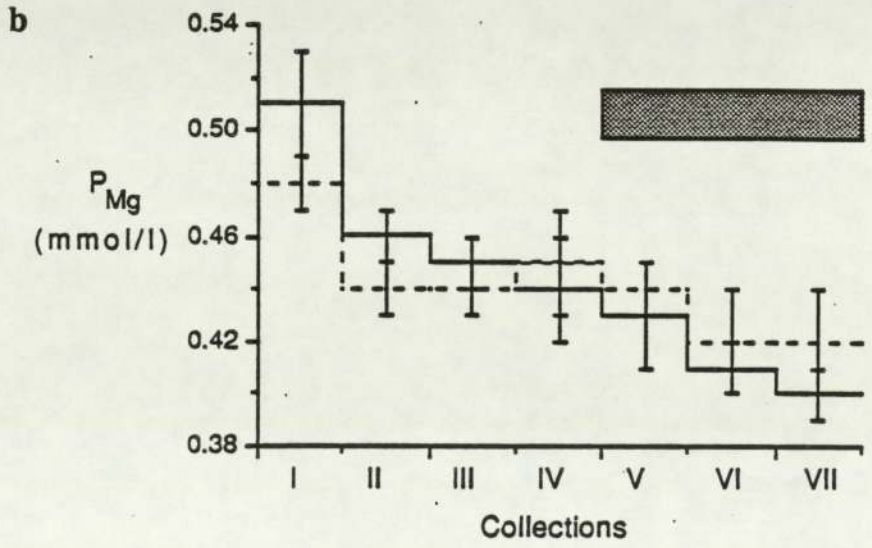
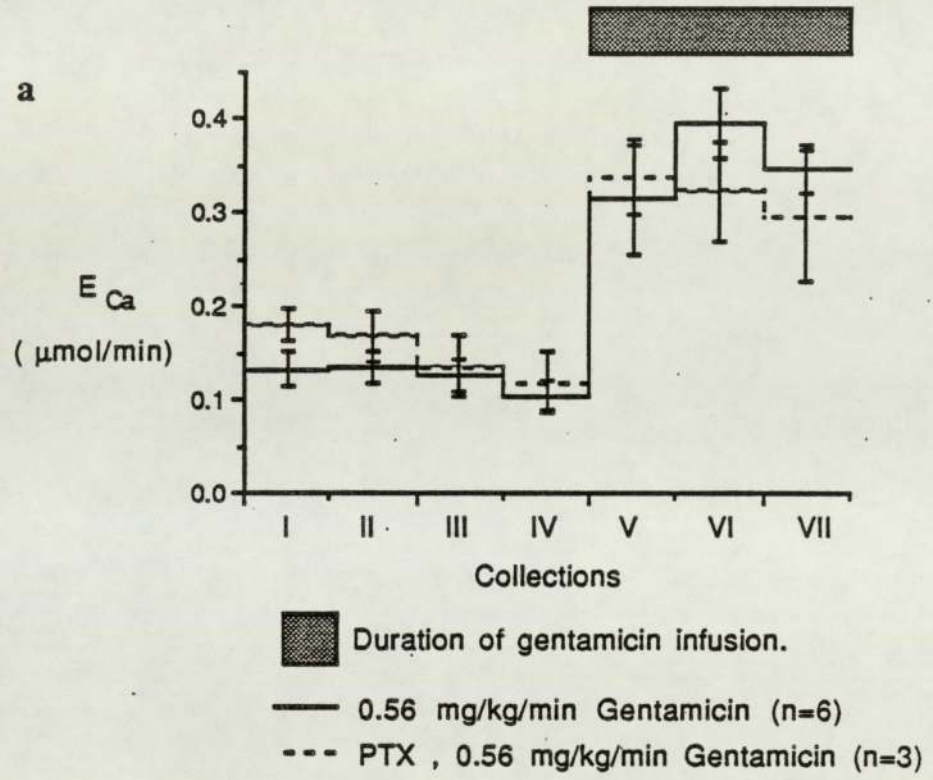


Figure 81. Comparison of excretion rates of cations between PTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



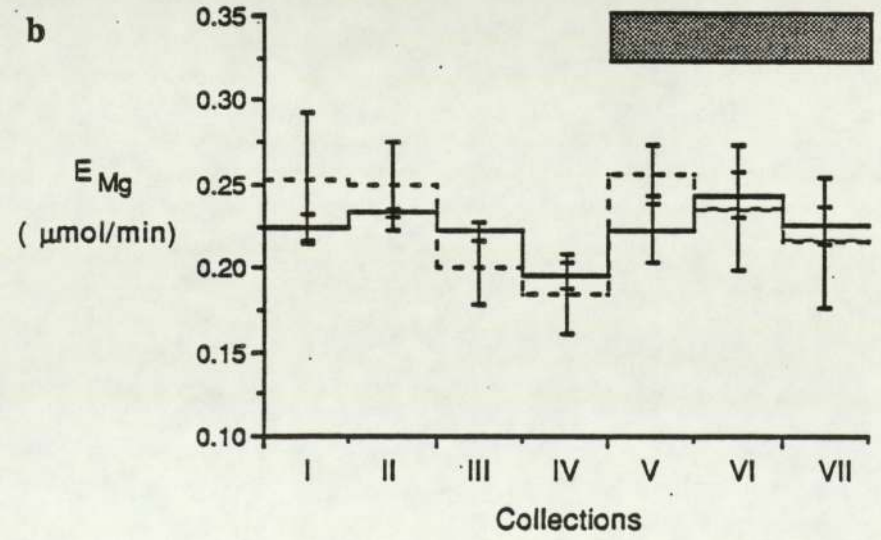


Figure 82. Comparison of clearances of cations between PTX rats infused with gentamicin and 'intact' rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

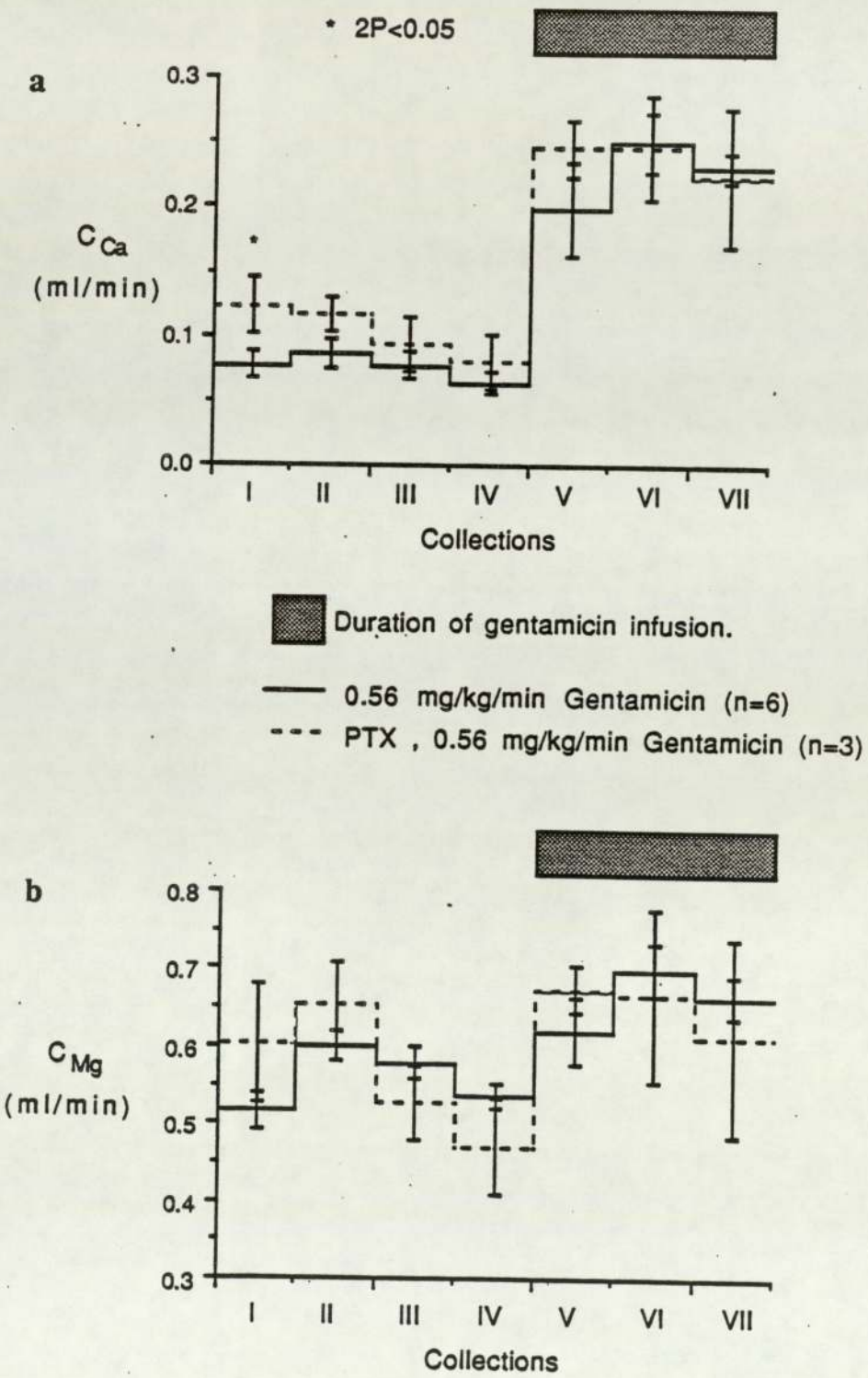


Figure 83. Comparison of **a.** urine flow rate and **b.** GFR between PTX rats infused with saline and PTX rats infused with gentamicin. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

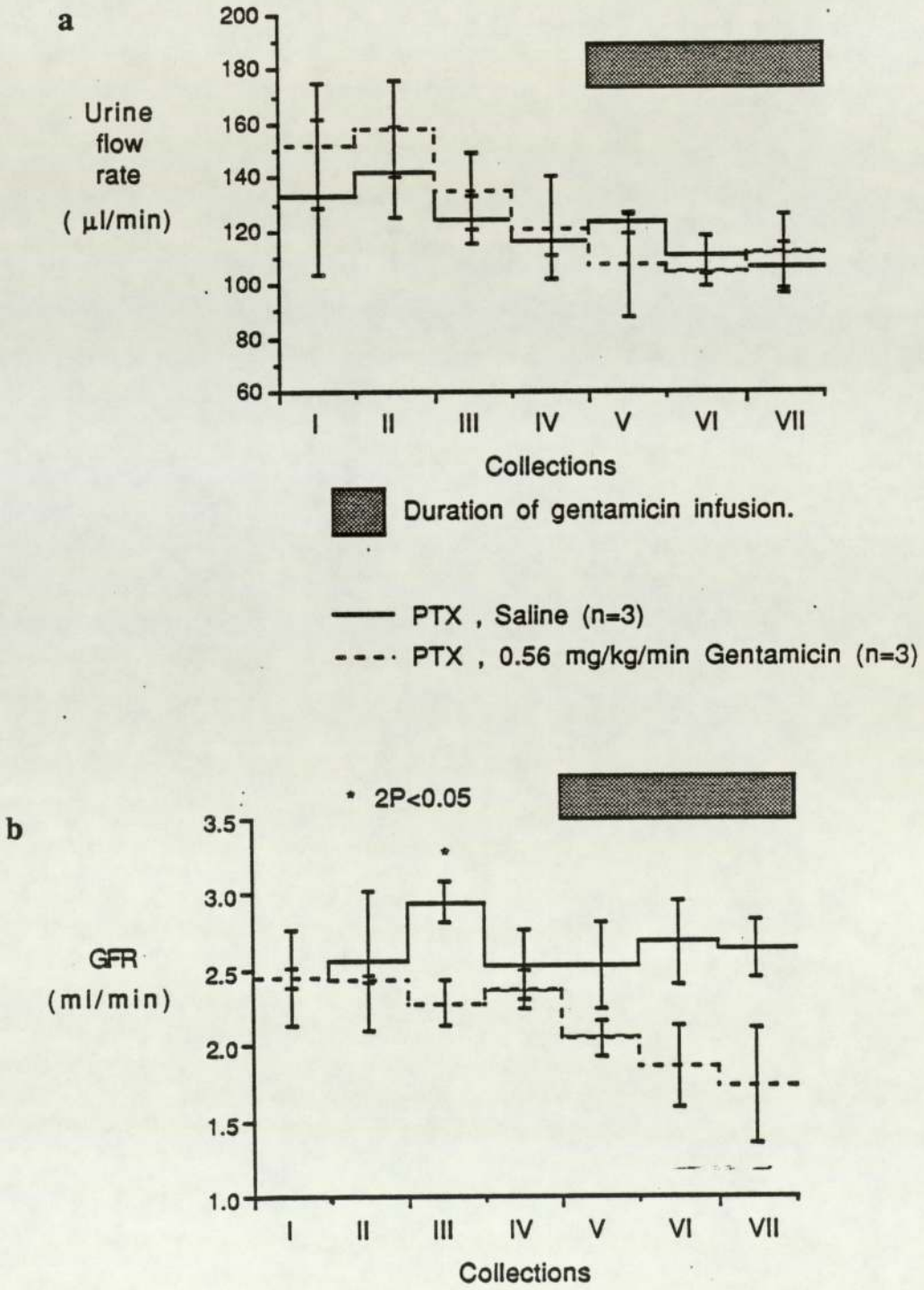
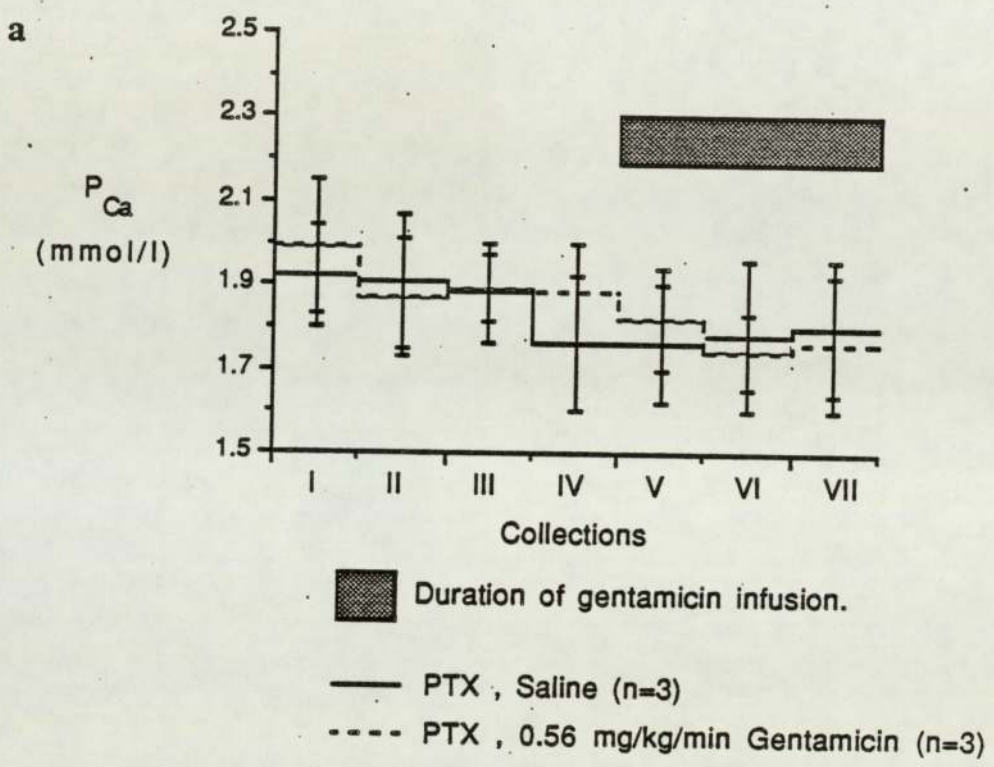


Figure 84. Comparison of concentrations of cations in plasma between PTX rats infused with saline and PTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



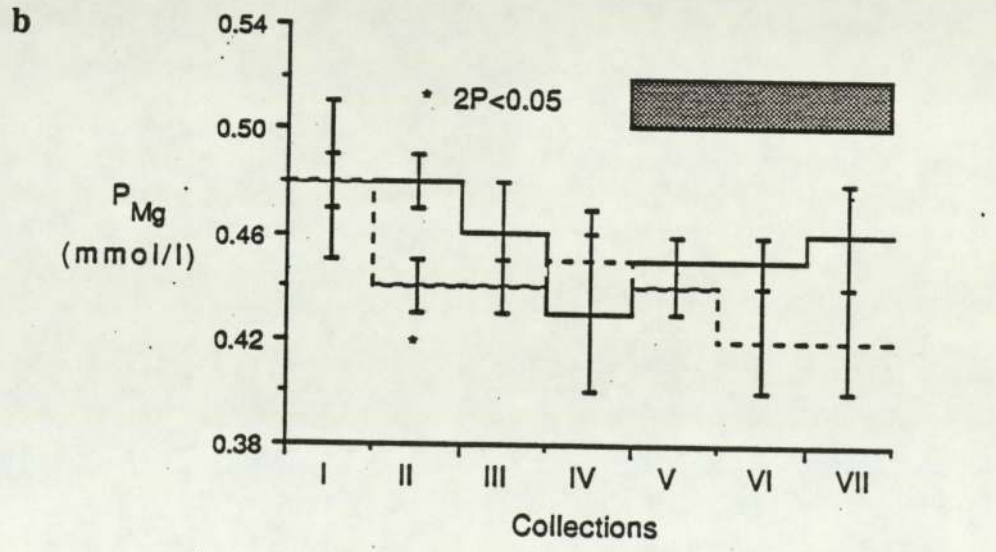
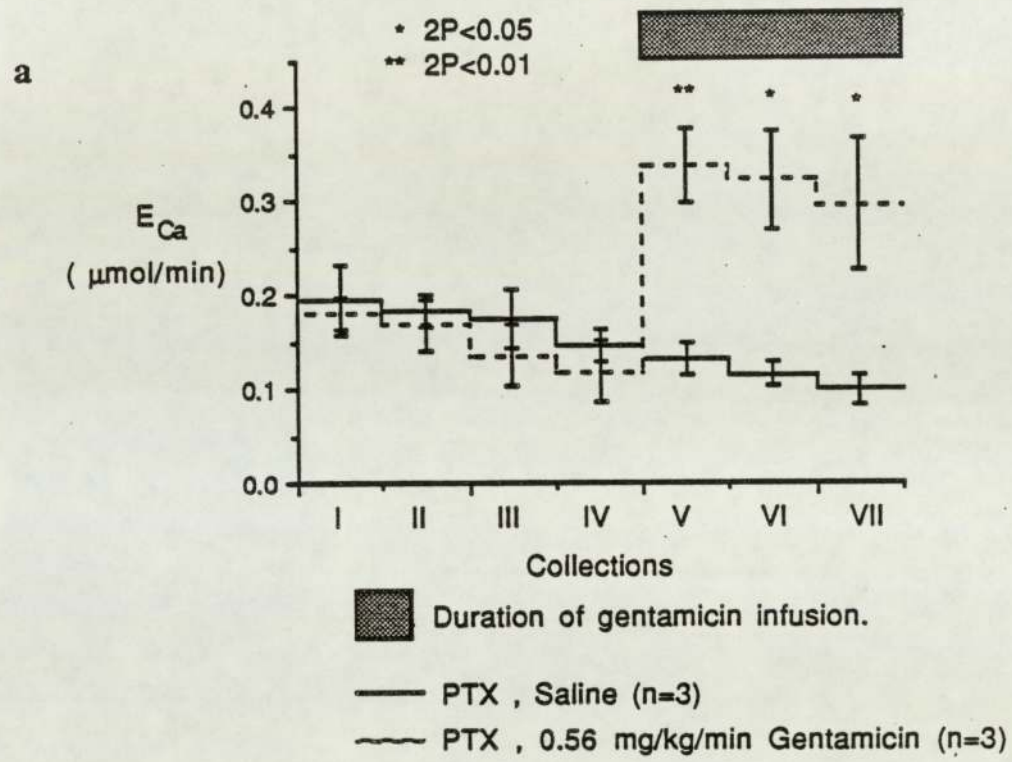


Figure 85. Comparison of excretion rates of cations between PTX rats infused with saline and PTX rats infused with gentamicin, both expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



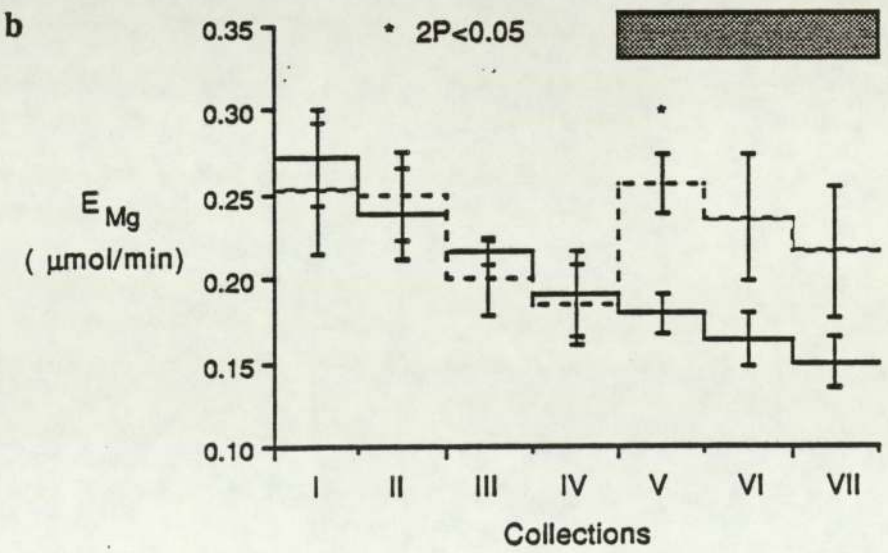
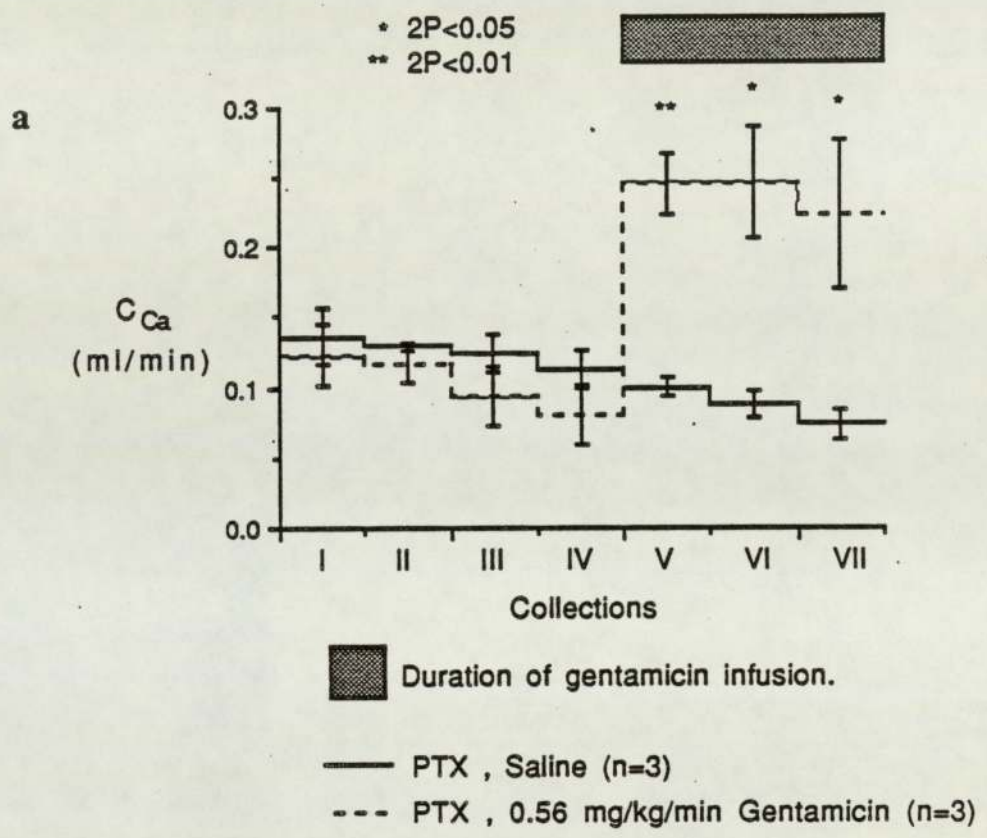


Figure 86. Comparison of clearances of cations between PTX rats infused with saline and PTX rats infused with gentamicin, both expressed as mean + s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



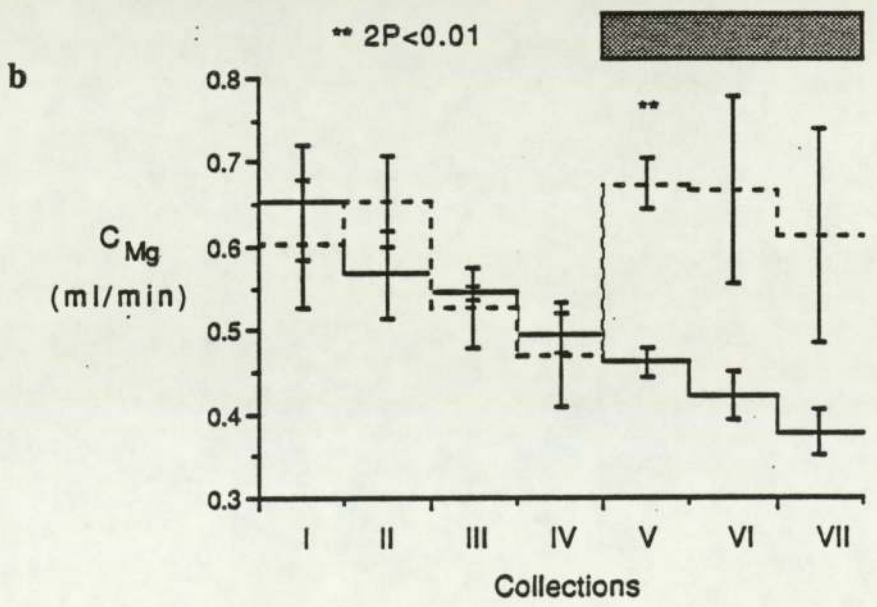


Figure 87. Comparison of fractional excretions of cations between PTX rats infused with saline and PTX rats infused with gentamicin, both expressed as mean + s.e.m. Statistical comparisons were made using Student's 't' test for small groups. a. Calcium. b. Magnesium.

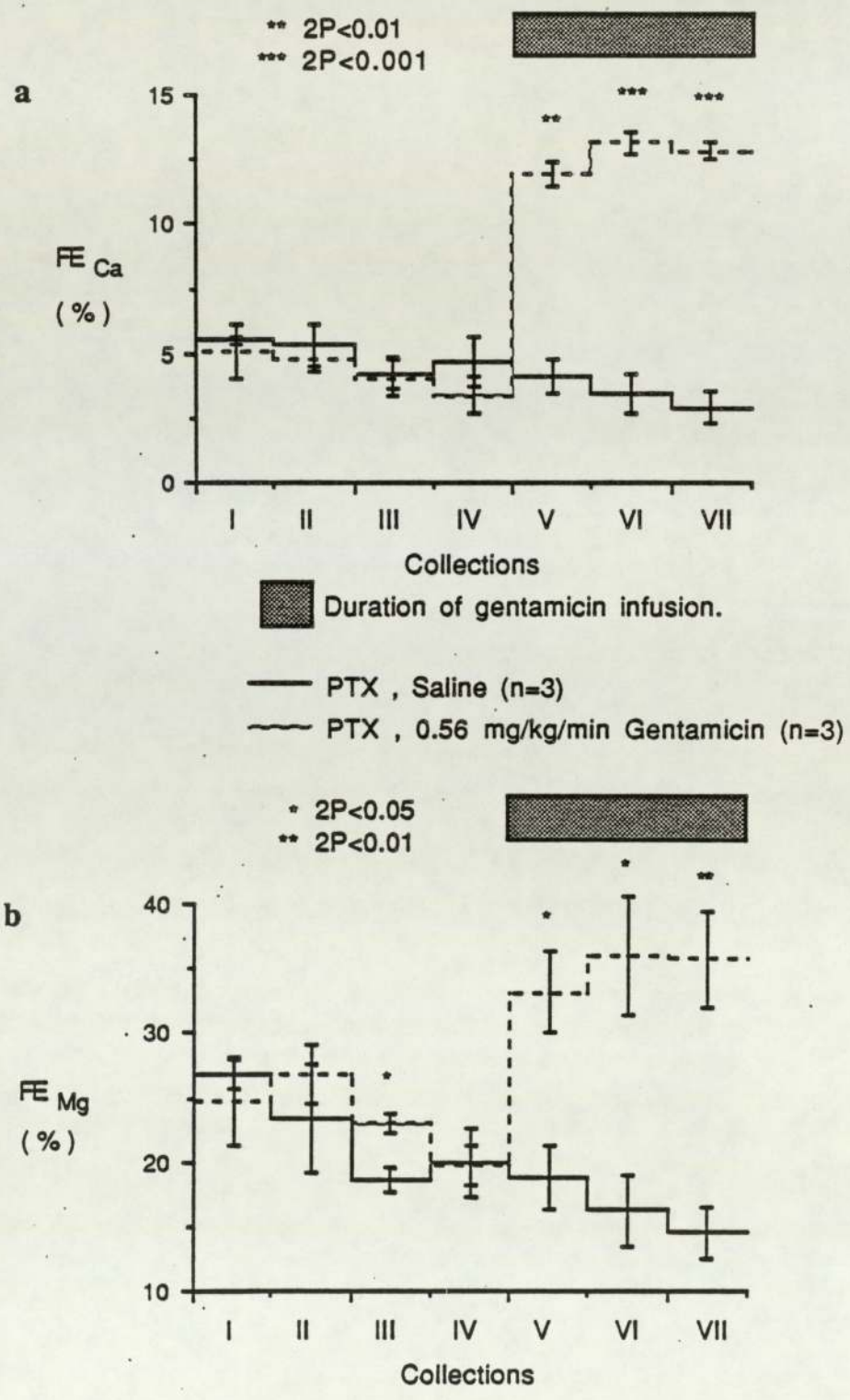


Figure 88. Excretion rates of phosphate expressed as mean ± s.e.m. (except 'intact' rats in panel **b** where n=2 and therefore no s.e.m. can be calculated). Statistical comparisons were made using Student's 't' test for small groups. **a.** Comparison between PTX rats infused with saline and PTX rats infused with gentamicin. **b.** Comparison between 'intact' rats infused with saline and all six PTX rats.

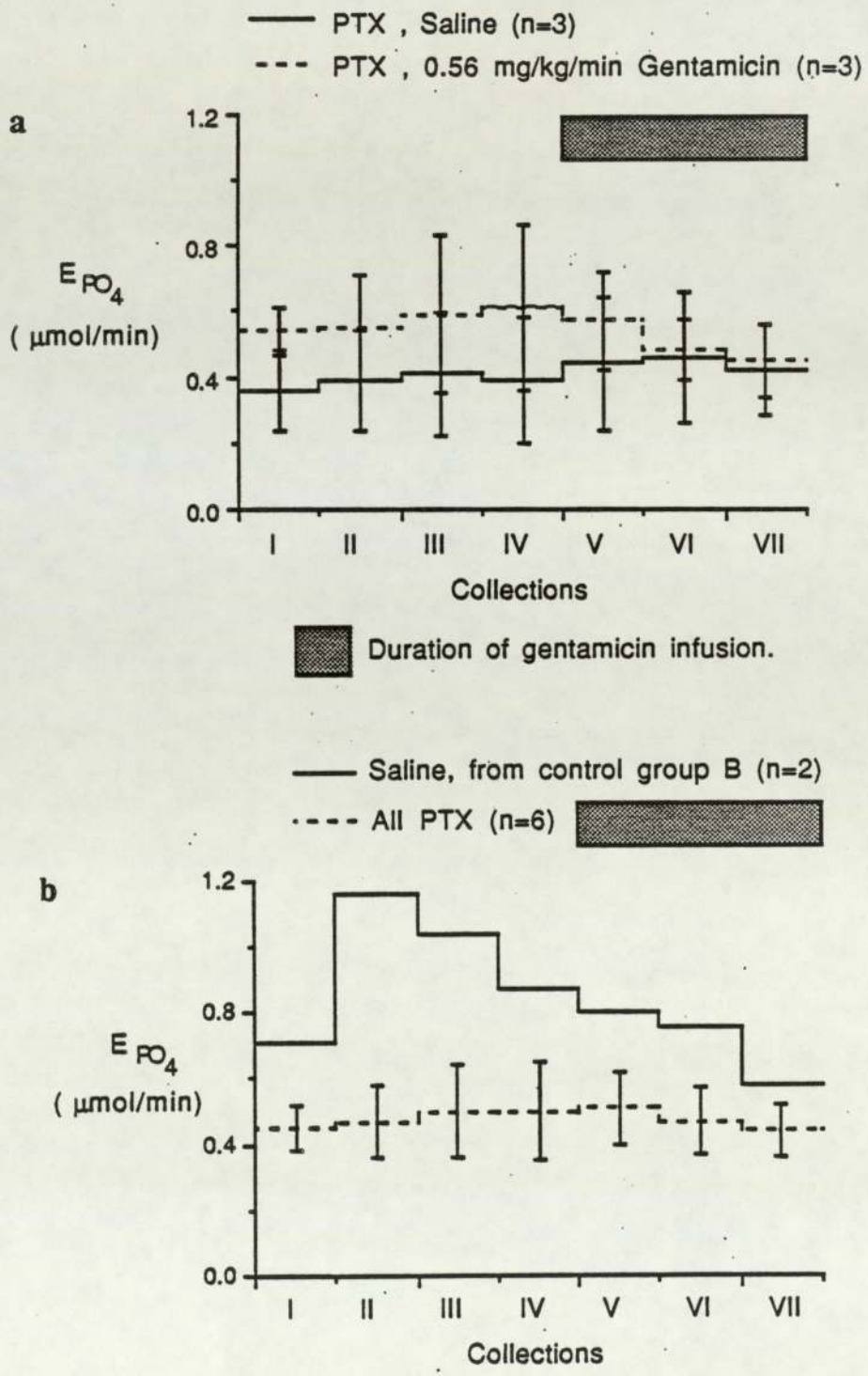


Figure 89. Comparison of excretion rates between one PTX rat infused with saline and one PTX rat infused with gentamicin. **a.** Calcium. **b.** Magnesium. **c.** Phosphate.

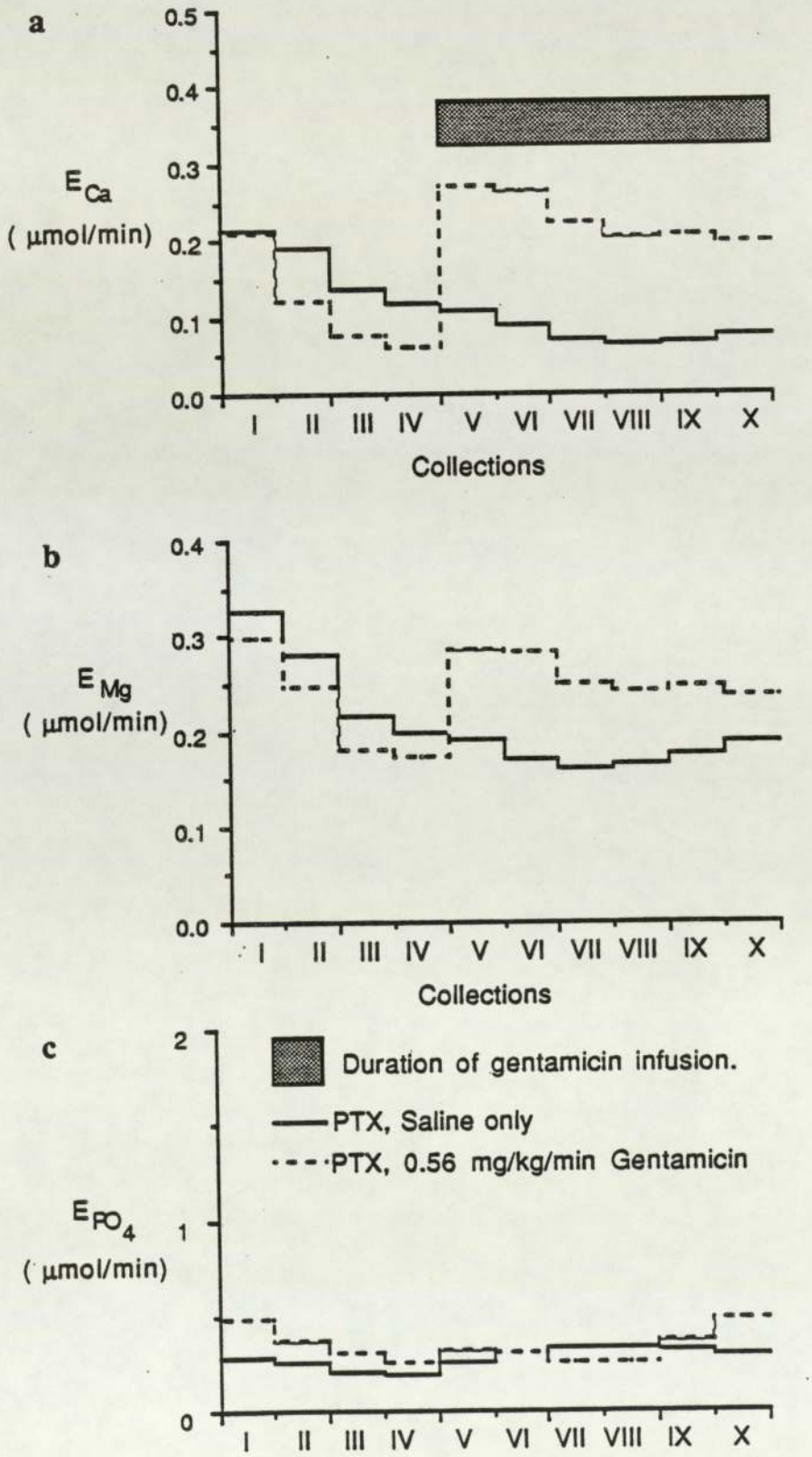


Figure 90. Comparison of excretion rates between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium. **c.** Phosphate.

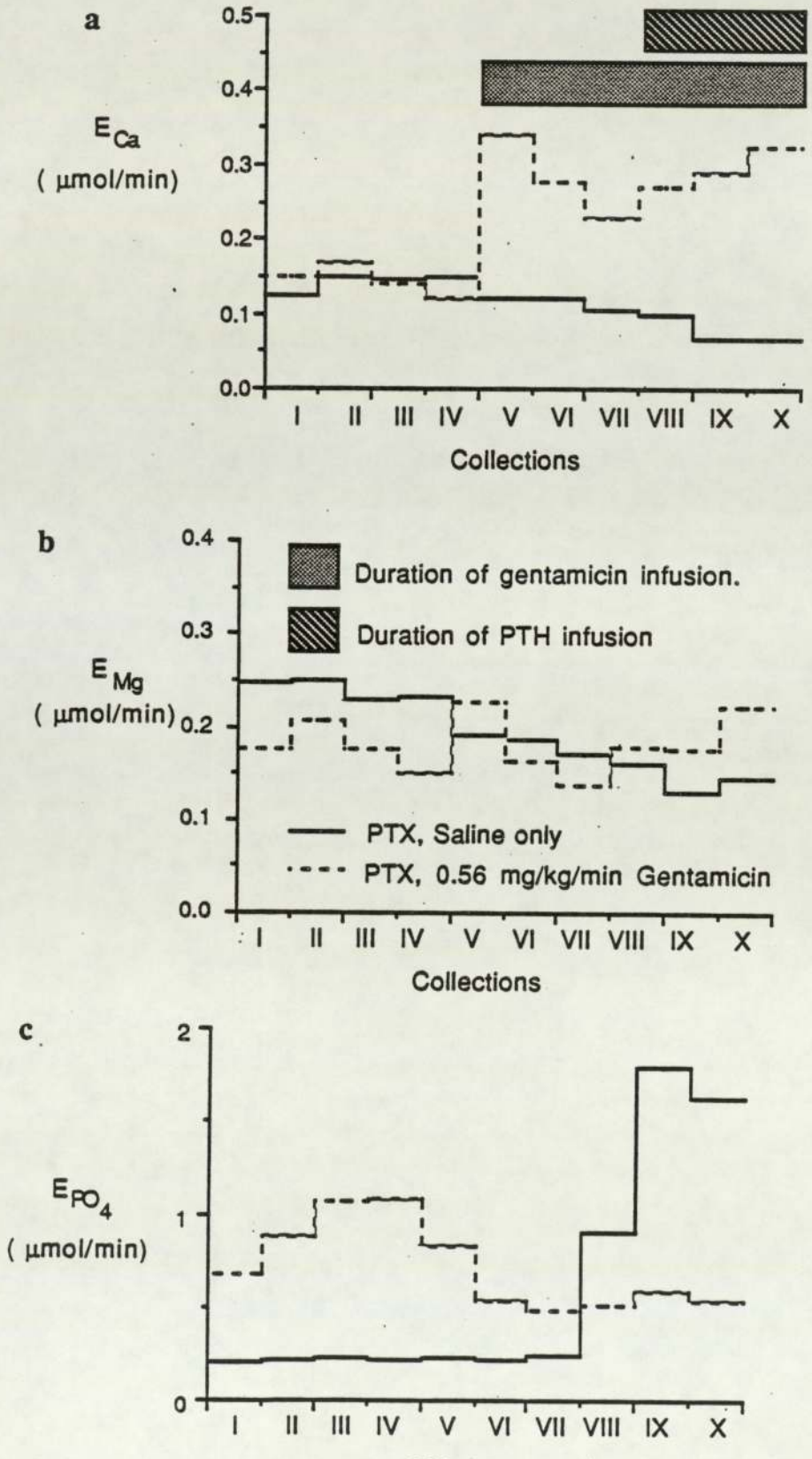


Figure 91. Comparison of excretion rates between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium. **c.** Phosphate.

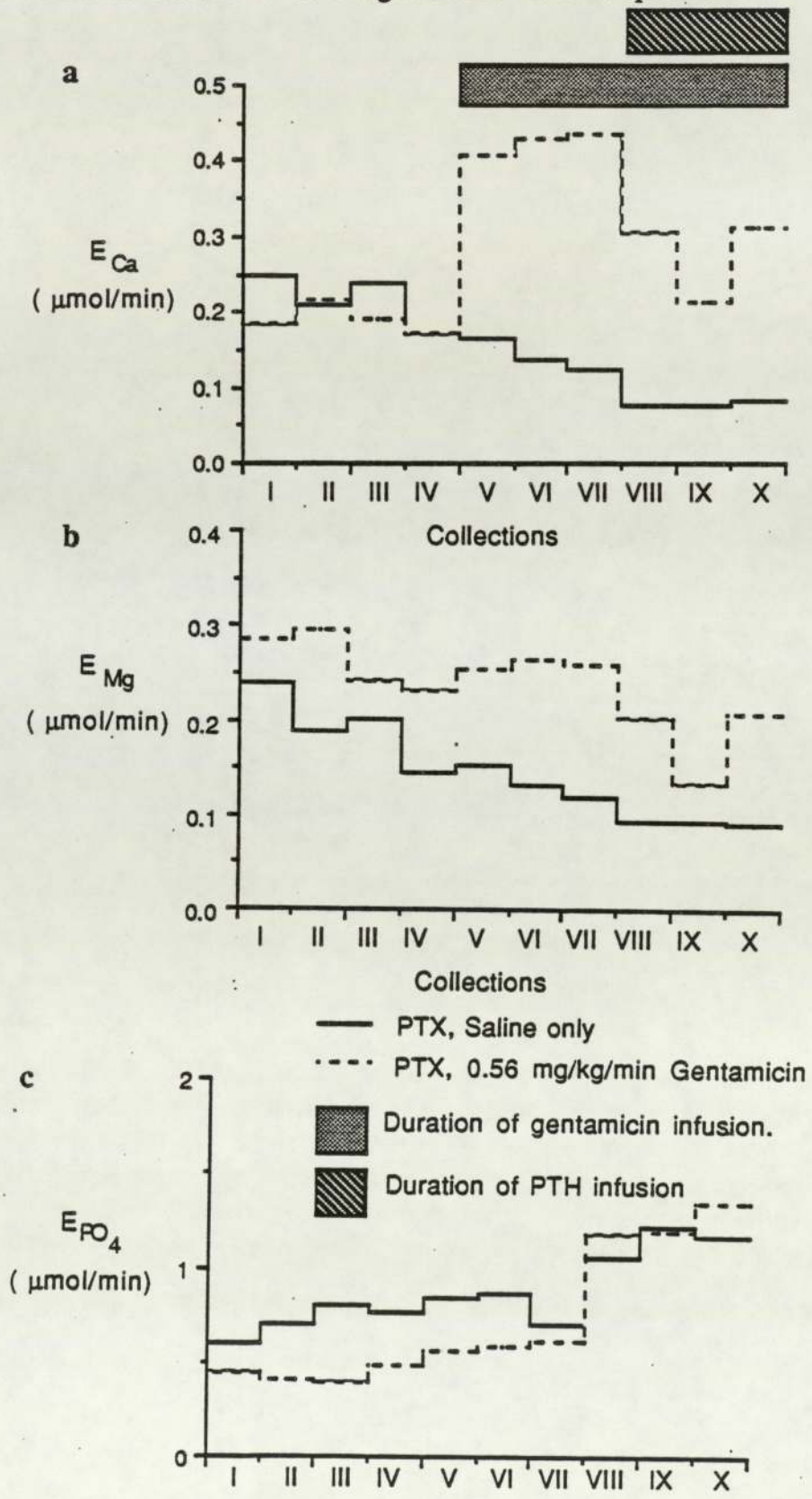


Figure 92. Comparison of clearances of cations between one PTX rat infused with saline and one PTX rat infused with gentamicin. **a.** Calcium. **b.** Magnesium.

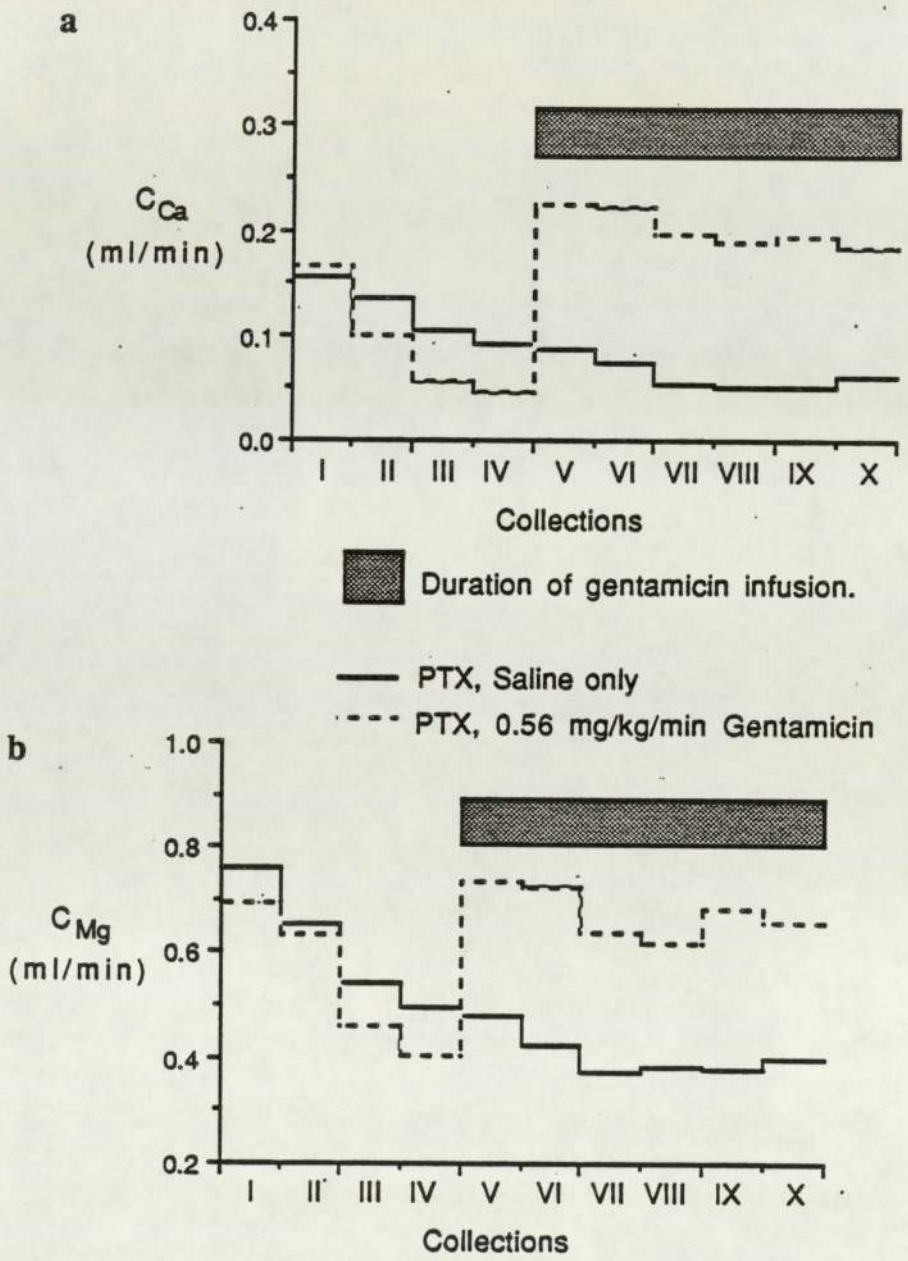


Figure 93. Comparison of clearances of cations between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium.

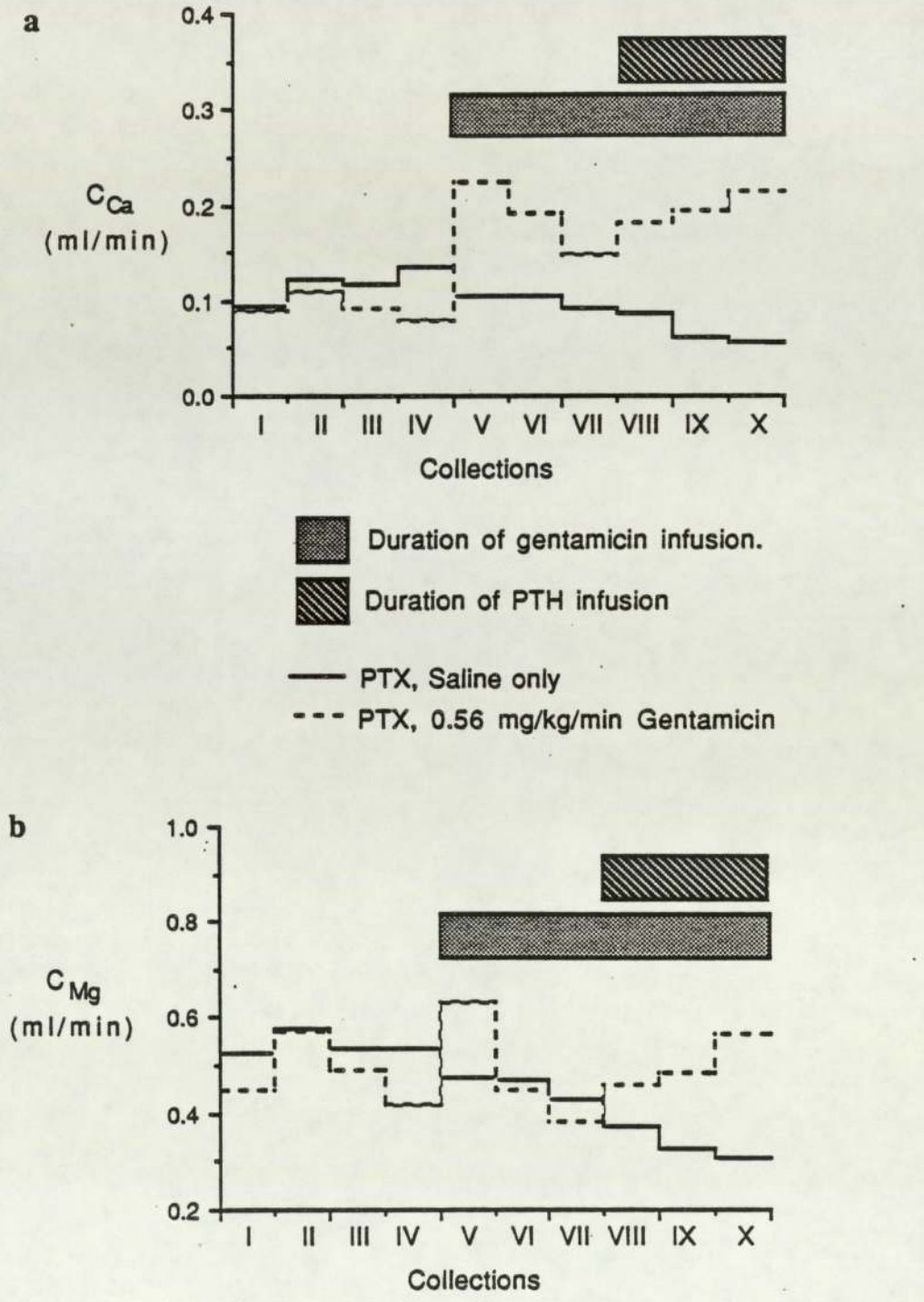


Figure 94. Comparison of clearances of cations between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium.

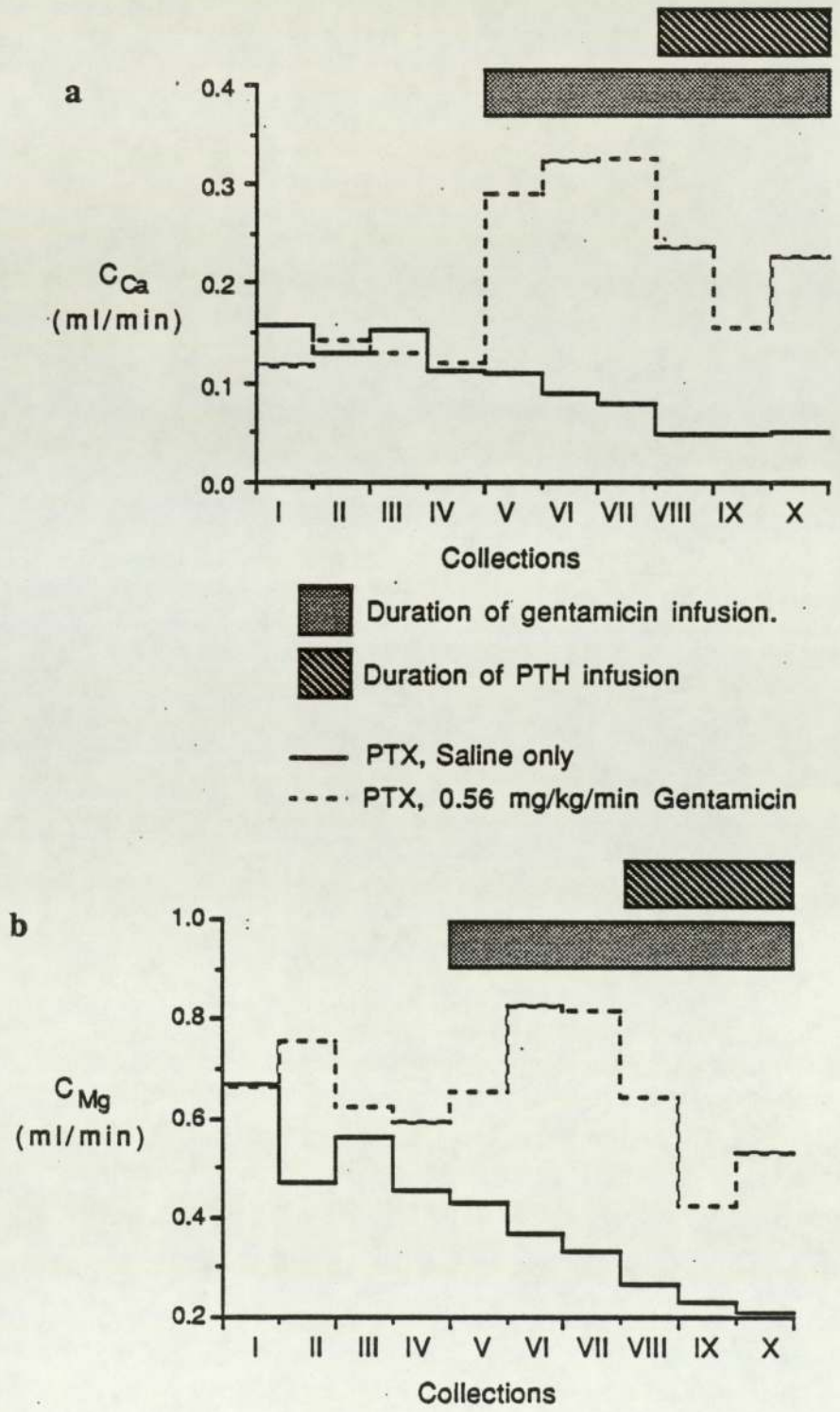
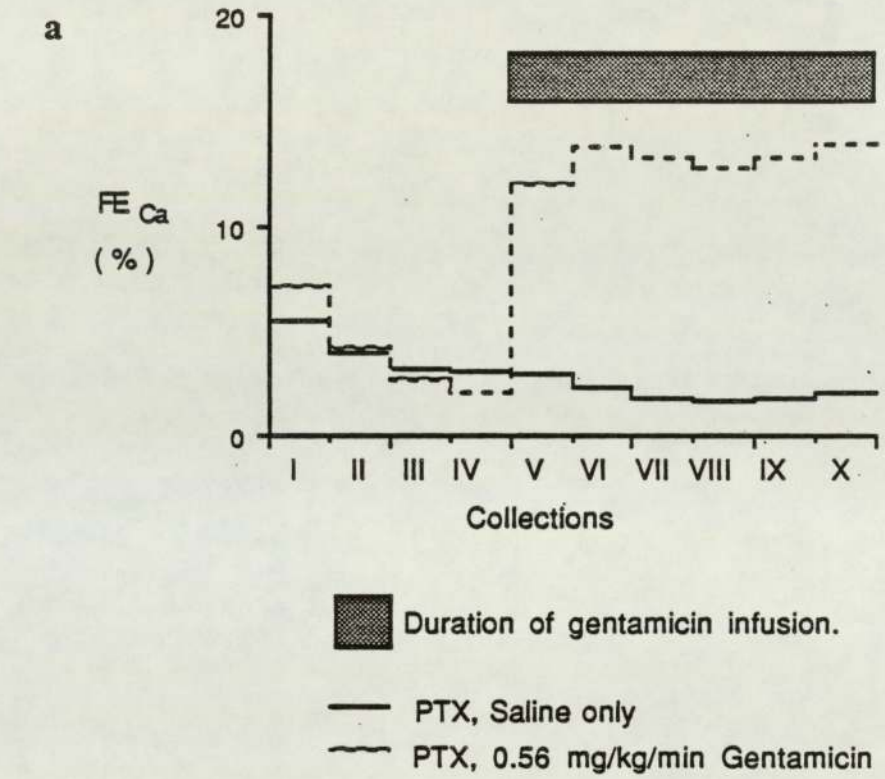


Figure 95. Comparison of fractional excretions of cations between one PTX rat infused with saline and one PTX rat infused with gentamicin. **a.** Calcium. **b.** Magnesium.



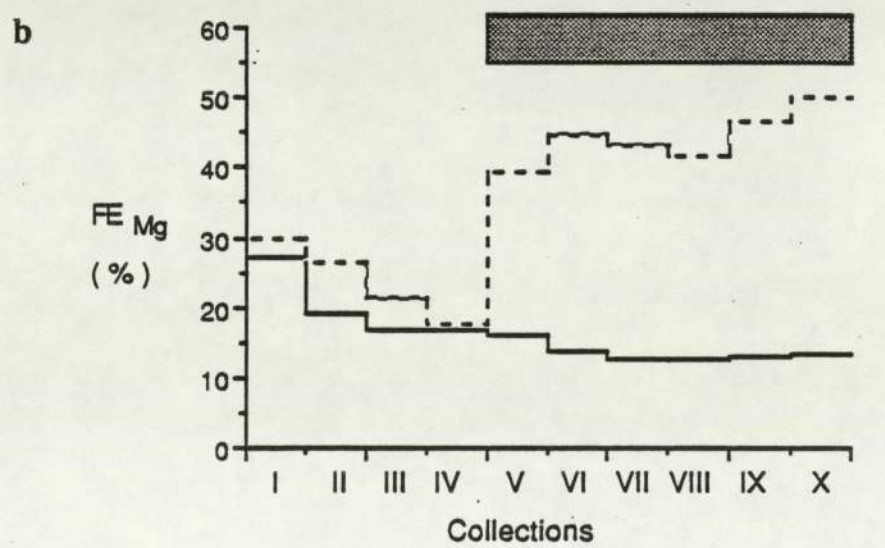


Figure 96. Comparison of fractional excretions of cations between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium.

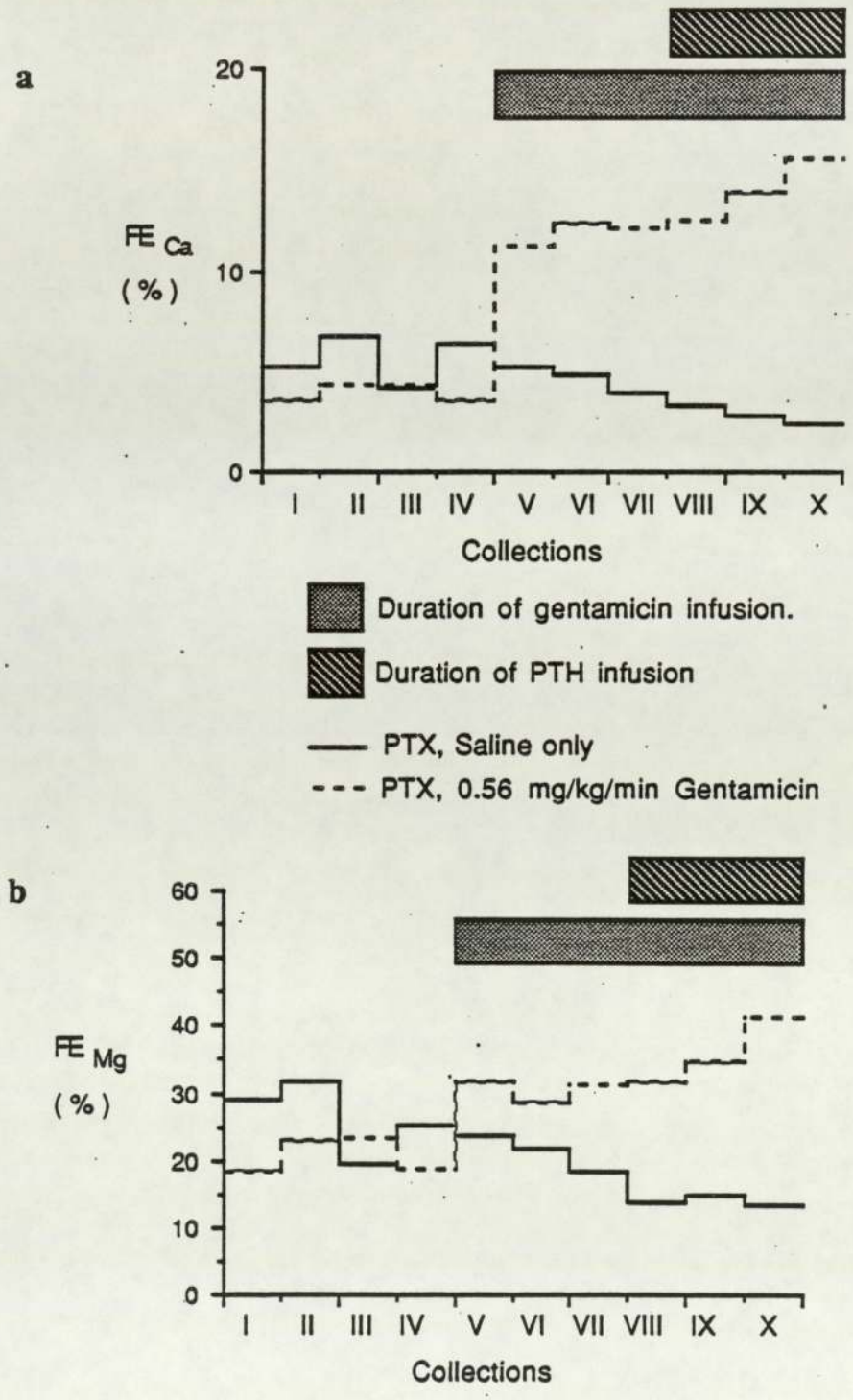


Figure 97. Comparison of fractional excretions of cations between one PTX rat infused with saline and PTH and one PTX rat infused with gentamicin and PTH. **a.** Calcium. **b.** Magnesium.

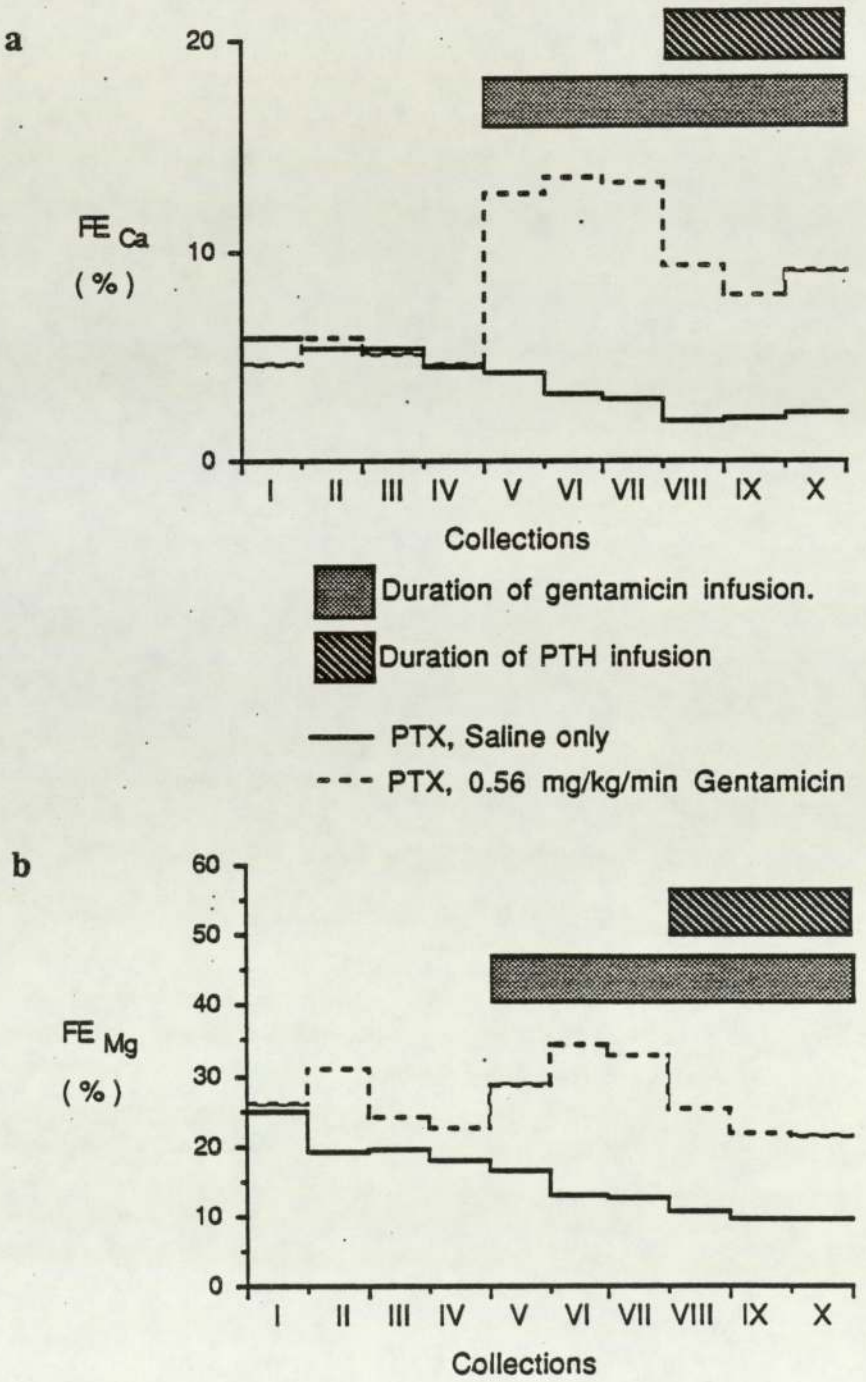
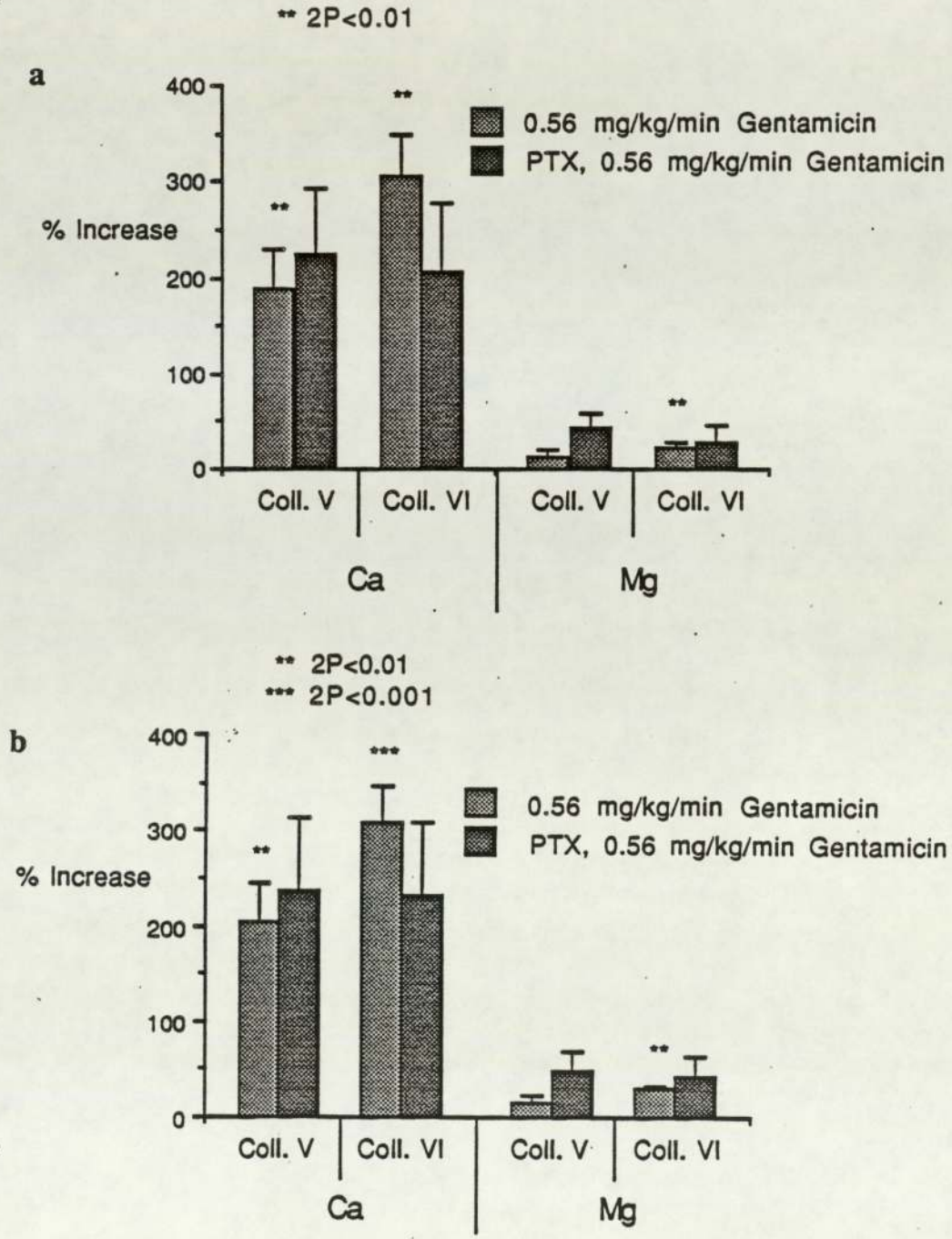


Figure 98. Histograms illustrating the effects of gentamicin infusion in PTX and 'intact' rats on the excretion rates and clearances of calcium and magnesium. Each column represents the mean of the individual percentage increases in excretion rate or clearance at collection V or VI over values at collection IV from the same individuals ± s.e.m. Statistical comparisons were made using Student's 't' test for paired data. **a.** Excretion rates. **b.** Clearances.



4.8 The effect of infused frusemide on the renal handling of calcium and magnesium in the presence and absence of infused gentamicin

a) Introduction:

The nephron site at which gentamicin and tobramycin interfere with calcium and magnesium reabsorption has not been accurately determined. ^{45}Ca microinjection experiments have demonstrated that gentamicin does not affect calcium reabsorption beyond the accessible distal convoluted tubule (Garland and Harpur, 1987). Gentamicin is reported to interact almost exclusively with proximal tubular cells (Silverblatt and Kuehn, 1979; Vandewalle et al, 1981) suggesting an action at this site, although there is no direct evidence to indicate that impairment of divalent cation reabsorption by gentamicin is a proximal effect. The site at which magnesium reabsorption is impaired has not been investigated.

Frusemide is known inhibit reabsorption of calcium and magnesium in the cortical thick ascending limb of the loop of Henle (Quamme, 1981; Bourdeau et al, 1982). Infusion of the drug therefore produces marked hypercalciuria and hypermagnesuria (Duarte, 1968; Edwards et al, 1973).

Therefore, if gentamicin produces its effects on calcium and magnesium reabsorption by a mechanism involving the proximal tubule only, concurrent administration with frusemide should produce entirely additive effects. If, alternatively, the responses to gentamicin are partly or wholly the result of actions involving the ascending limb, the combined effects of the two drugs should be less than additive.

b) Methods:

Two experiments were performed: In the first frusemide was

infused into four rats at a rate of 0.17 mg/kg/min (Figure 99). The rate of infusion of saline was adjusted to compensate for urinary losses by measuring urine volume immediately after each 30 minute collection and adjusting the infusion rate to deliver an approximately equal volume of saline during the following 30 minute collection. Statistical comparisons were made with control group B.

In the second experiment gentamicin was infused at a rate of 0.56 mg/kg/min into three rats, an infusion of 0.17 mg/kg/min frusemide was then introduced in the presence of a continuing gentamicin infusion (Figure 105). Saline infusion rate was again adjusted to compensate for urinary losses during infusion of both drugs. Staistical comparisons were made with six rats infused with 0.56 mg/kg/min gentamicin only (as described in section 4.2).

c) Results:

i) Frusemide infusion:

Mean plasma calcium concentration in frusemide-infused rats was significantly higher ($2P<0.01$) than controls at four points during the experimental period (Figure 101a). Mean plasma magnesium concentration was not consistently different from saline-infused controls (Figure 101b).

Mean urine flow rate in frusemide-infused rats was not significantly different from saline-infused controls during the control period, introduction of infused frusemide initiated a marked diuresis ($2P<0.01$) which fell off somewhat during collections VI-X but was still significantly ($2P<0.05$) higher than controls at the end of the experiment (Figure 100a).

GFR was not significantly different from saline-infused controls during the control period but was significantly depressed ($2P<0.01$ or $2P<0.05$) for the full duration of the experimental period (Figure 100b). However this is probably an artefactual result caused by disturbance of the input/output equilibrium of ^{3}H-Inulin created by

the rapid increase in infusion rate, and therefore ^{3}H-Inulin input, necessitated by frusemide infusion. A new equilibrium was not established before the end of the experiment. Consequently, fractional excretions, calculated as the ratios of calcium and magnesium clearances to ^{3}H-Inulin clearance, are probably similarly artefactual during the experimental period. The apparently sustained increases in the fractional excretions of calcium and magnesium are therefore unlikely to be an accurate reflection of true changes in these parameters (Figure 104).

Mean calcium excretion rate (Figure 102a) and clearance (Figure 103a) in frusemide-infused rats were not significantly different from saline-infused controls during the control period. Introduction of infused frusemide caused a significant ($2P<0.01$) increase in both parmeters. Excretion rate and clearance then declined rapidly to values which were indistinguishable from controls. Both parameters remained at these levels except for a transient, significant ($2P<0.05$) difference which coincided with a fall in control values. These responses could be explained by parallel changes in the fractional excretion of calcium (Figure 105a), although as noted above, measurement of fractional excretion was probably inaccurate.

Mean magnesium excretion rate (Figure 102b) and clearance (Figure 103b) in frusemide-infused rats were not significantly different from controls during the control period. Infusion of frusemide produced an immediate significant ($2P<0.05$) increase in both parameters, excretion rate and clearance then immediately declined to values which were indistiguishable from saline-infused controls. Both parameters remained at these levels for the remainder of the experimental period. These responses could be explained by parallel changes in the fractional excretion of magnesium (Figure 104b), but as noted previously these measurements are unlikely to be accurate.

ii) Frusemide and gentamicin infusion:

Mean plasma calcium concentration (Figure 107a) was significantly higher in rats receiving both drugs than in those receiving gentamicin only during both the control and experimental periods. As explained in previous sections these gentamicin-infused rats appear to be slightly hypocalcaemic, therefore this result is unlikely to indicate a hypercalcaemic response to frusemide. Mean plasma magnesium concentration (Figure 107b) was significantly higher ($2P<0.05$) in rats receiving frusemide and gentamicin at five points occurring during both the control and the experimental period. Mean urine flow rate was not significantly different between the two groups except during infusion of both drugs when a significant ($2P<0.001$) increase was produced in those rats receiving frusemide with gentamicin (Figure 106).

Mean calcium excretion rate (Figure 108a) and clearance (Figure 109a) were significantly ($2P<0.01$ or $2P<0.05$) higher in rats receiving both drugs than in those receiving gentamicin alone during the control period. Introduction of infused gentamicin increased both parameters to values which were not significantly different between the two groups. Introduction of infused frusemide caused a further increase in excretion rate and clearance in rats receiving both drugs to values significantly higher ($2P<0.001$) than in rats infused with gentamicin only. Mean calcium excretion rate recovered after the initial peak but was still significantly higher ($2P<0.05$) than in gentamicin-infused rats at the end of the experimental period. Mean calcium clearance, in contrast, fell immediately to values not significantly different from gentamicin-infused rats.

Mean magnesium excretion rate (Figure 108b) and clearance (Figure 109b) were occasionally significantly different between rats infused with gentamicin and rats infused with frusemide and

gentamicin during the control period. Introduction of infused gentamicin raised both parameters to values which were not significantly different between the two groups. Introduction of infused frusemide did produce any significant increases in excretion rate or clearance compared with rats infused with gentamicin only, although a small rise in both parameters was observed.

d) Discussion:

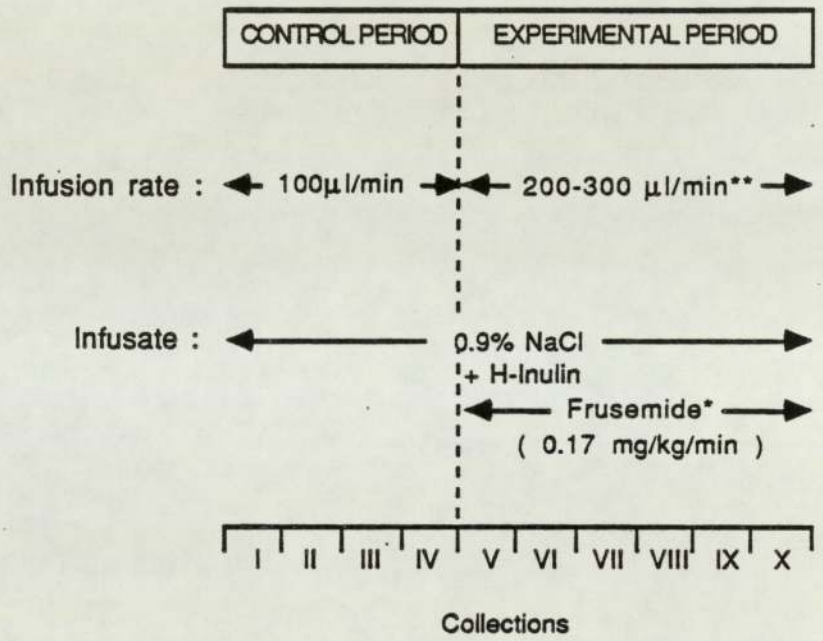
The hypercalciuric response to frusemide was only partially retained in the presence of gentamicin-induced hypercalciuria. Indeed, the peak calciuria produced by the two drugs combined was not significantly different to the peak calciuria produced by frusemide alone (Figure 110). The hypermagnesiuric response to frusemide was even more markedly diminished in the presence of gentamicin, producing no significant increase in magnesiuria above that seen when gentamicin was infused alone.

There are two possible explanations for the diminution of these responses in the presence of gentamicin: Most obviously it would suggest that the two drugs are inhibiting calcium and magnesium reabsorption in the ascending limb. The high infusion rate of frusemide would probably maximally inhibit reabsorption at this site when administered alone. Thus, if both gentamicin and frusemide are acting at this site, no greater inhibition of reabsorption could be produced by the combination than was produced by the diuretic alone.

Alternatively, frusemide and gentamicin could both inhibit calcium and magnesium reabsorption in the proximal tubule; although such an action of frusemide has been rejected (Edwards et al, 1973) the drug does have actions exerted in the proximal tubule, such as carbonic anhydrase inhibitory activity (Stein et al, 1968; Puschett and Goldberg, 1968). The former hypothesis is more likely due to the

lack of evidence for inhibition of proximal calcium and magnesium reabsorption by frusemide. Also the marked diminution of the hypermagnesiuric response to frusemide by gentamicin might suggest that both drugs are acting in the loop of Henle where the greatest reabsorption of magnesium takes place (Morel et al, 1969).

Figure 99. Protocol used in clearance experiments in which frusemide ('Lasix') was infused for three hours following a two hour saline infusion.



* Added to infusate according to protocol.

** Infusion rate adjusted to compensate for urinary losses.

Figure 100. a. Urine flow rate in rats infused with frusemide. **b.** GFR in rats infused with frusemide. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

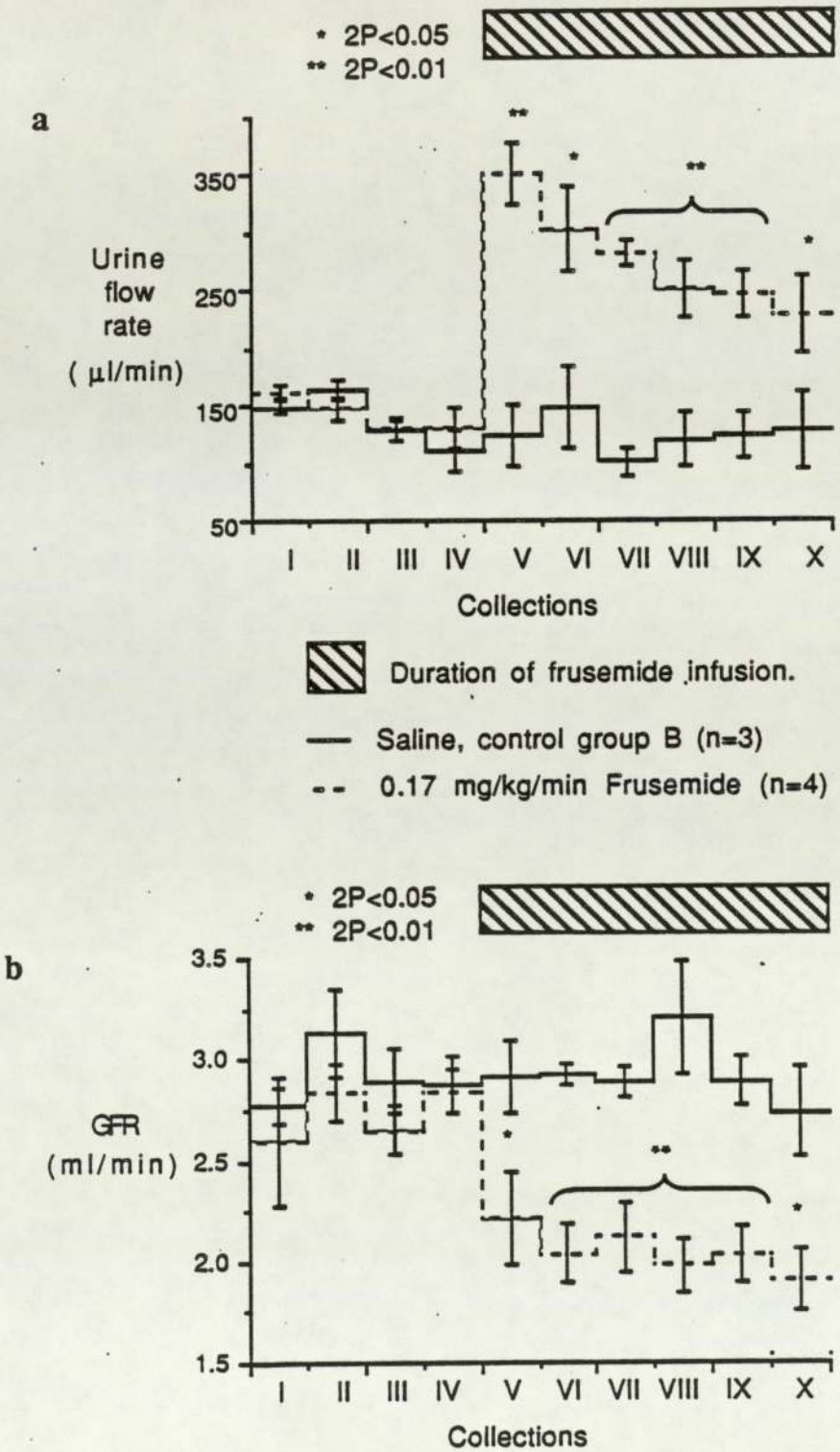
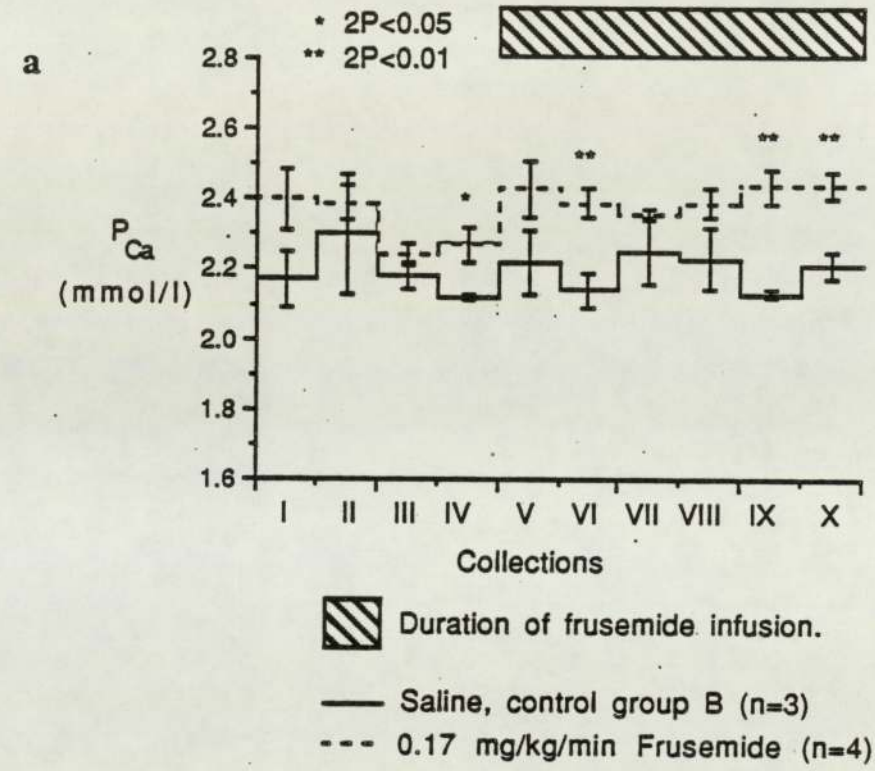


Figure 101. Concentrations of cations in plasma in rats infused with frusemide expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.



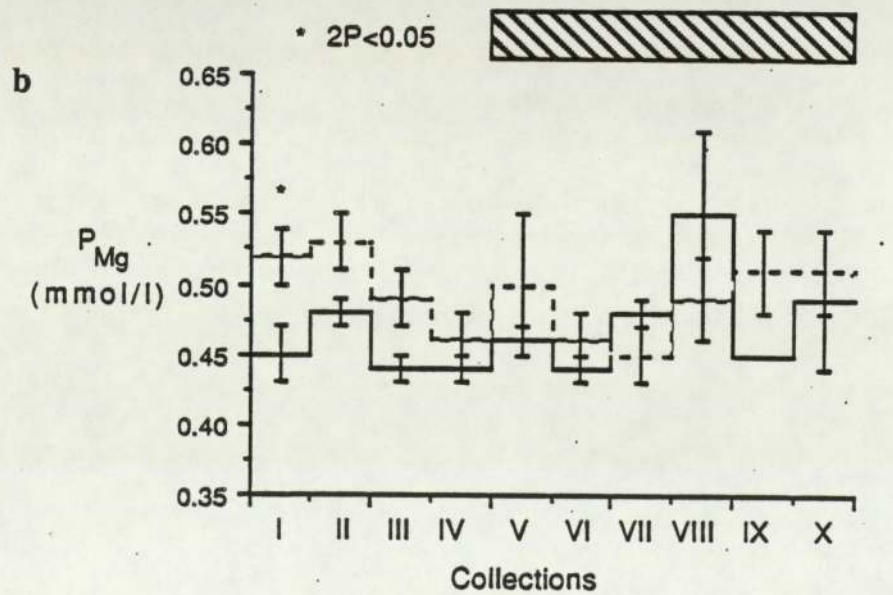
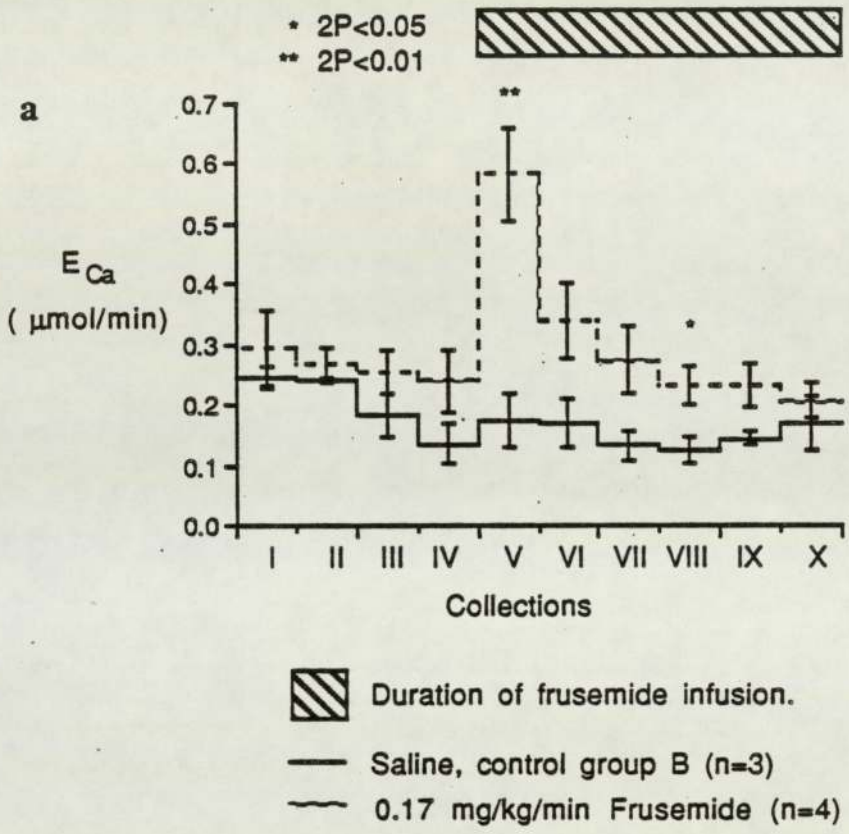


Figure 102. Excretion rates of cations in rats infused with frusemide expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. a. Calcium. b. Magnesium.



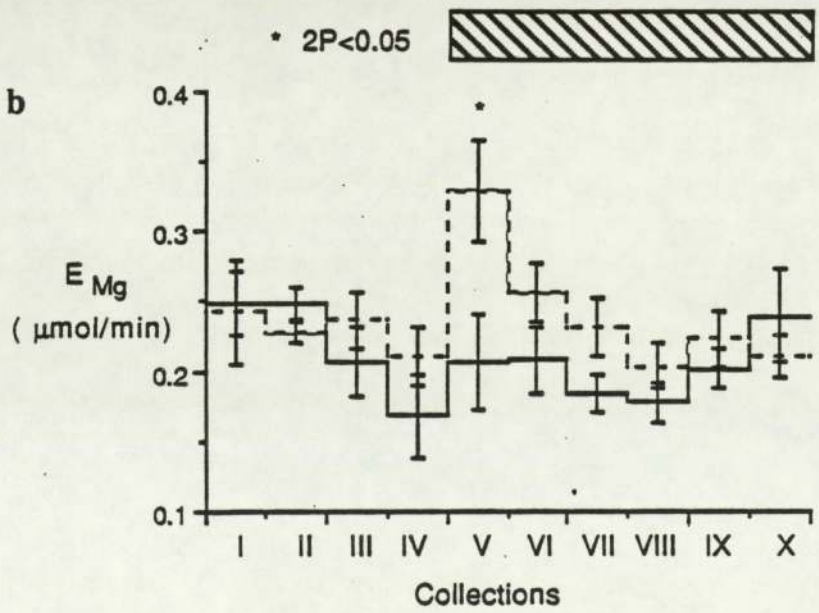


Figure 103. Clearances of cations in rats infused with frusemide expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

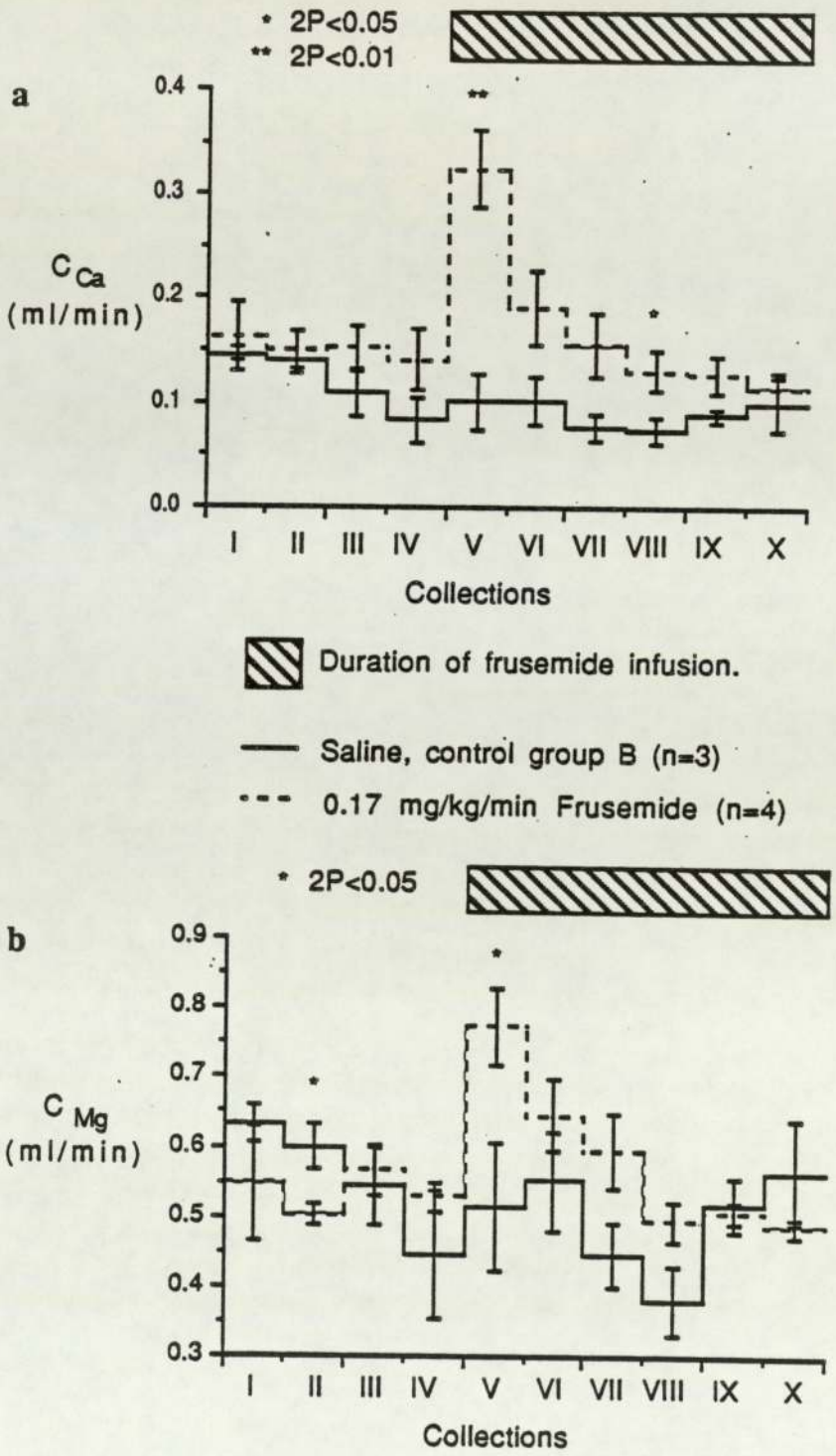


Figure 104. Fractional excretions of cations in rats infused with frusemide expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

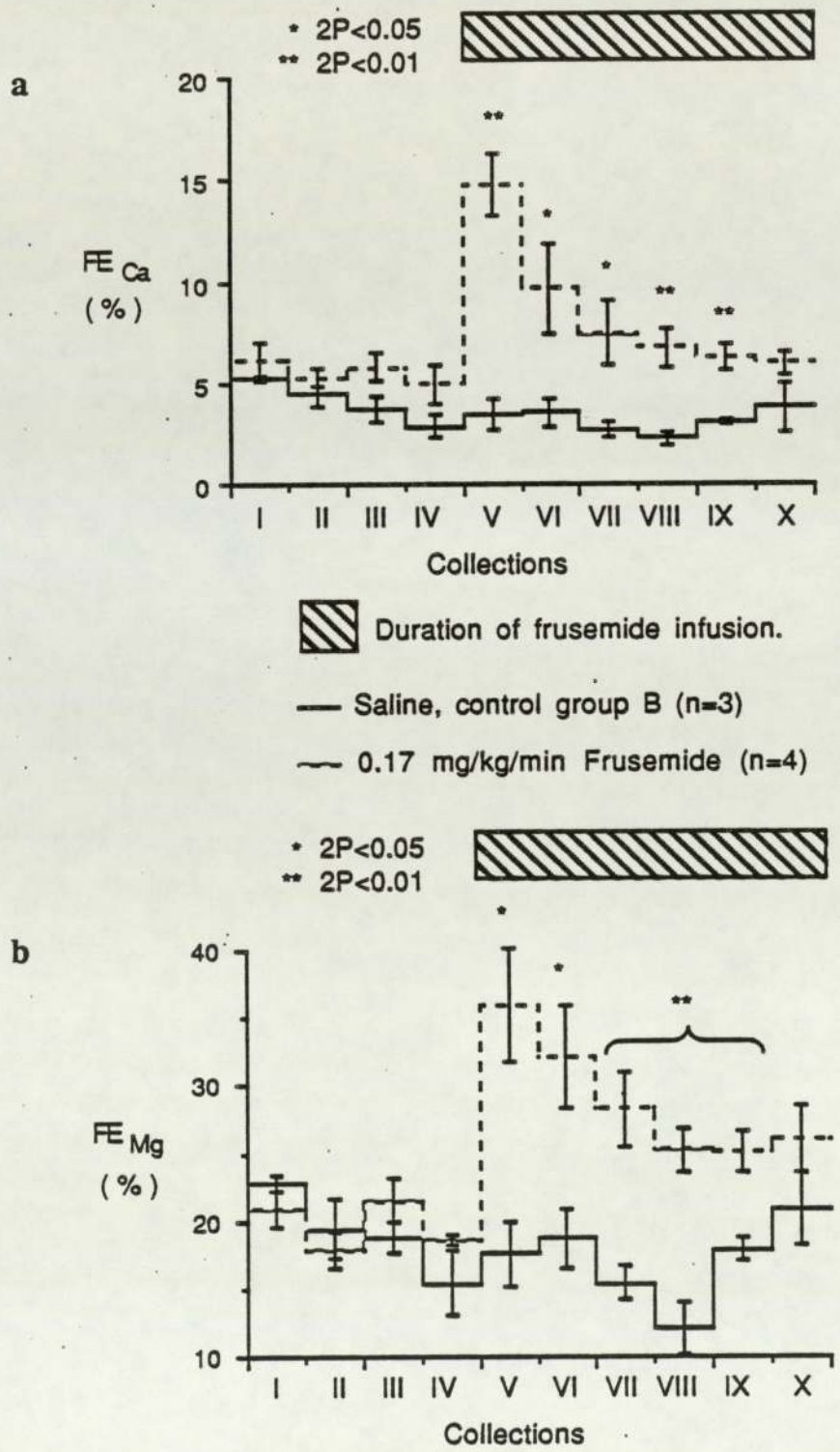
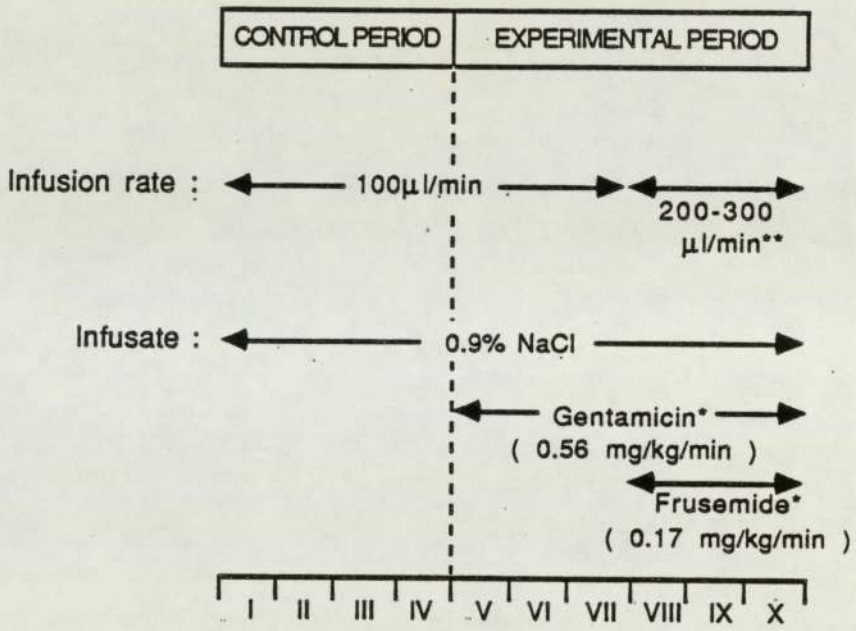


Figure 105. Protocol used in clearance experiments in which both gentamicin ('Cidomycin') and frusemide ('Lasix') were infused.



* Added to infusate according to protocol.

** Infusion rate adjusted to compensate for urinary losses.

Figure 106. Comparison of urine flow rate between rats infused with gentamicin alone and rats infused with gentamicin and frusemide. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups.

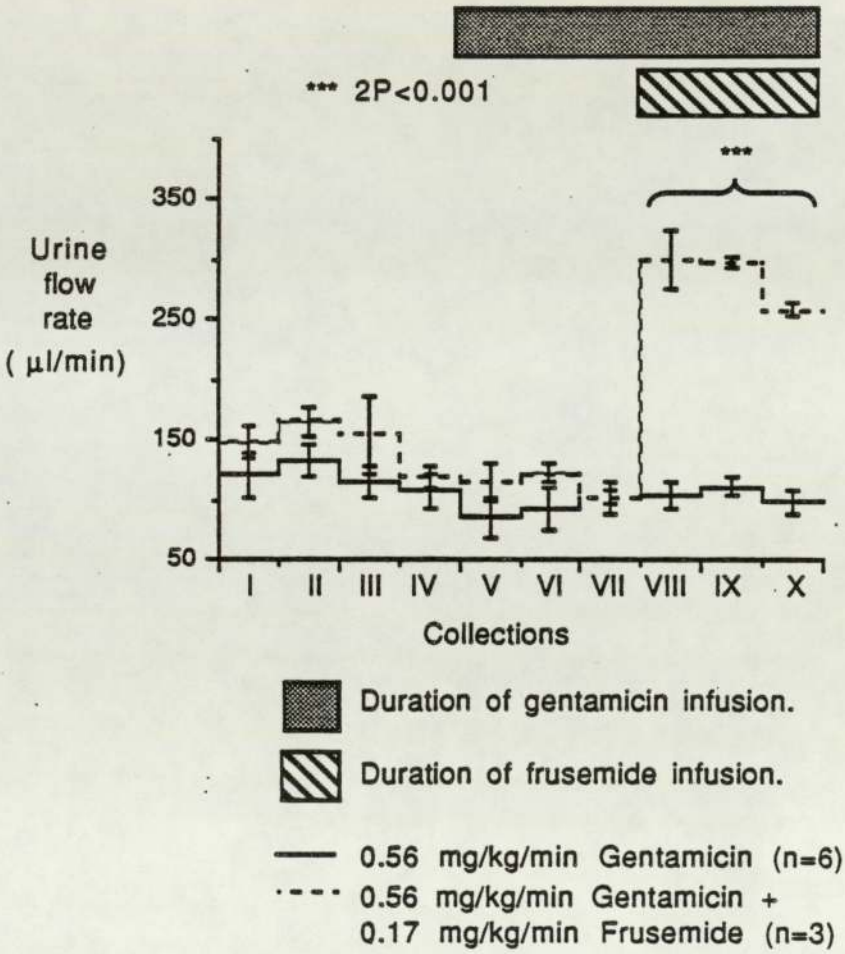


Figure 107. Comparison of concentrations of cations in plasma between rats infused with getamicin alone and rats infused with gentamicin and frusemide. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

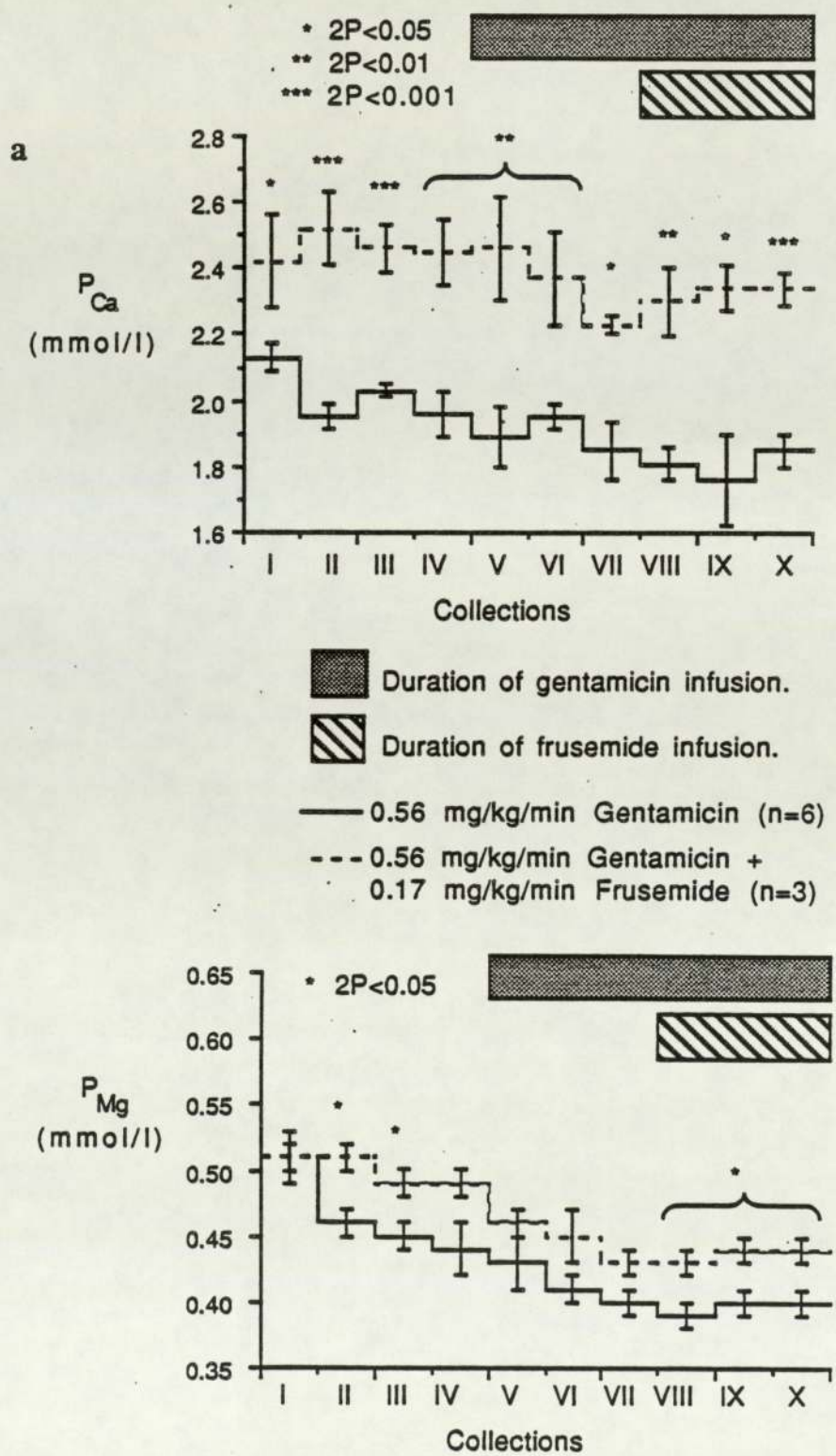


Figure 108. Comparison of excretion rates of cations between rats infused with getamicin alone and rats infused with gentamicin and frusemide. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

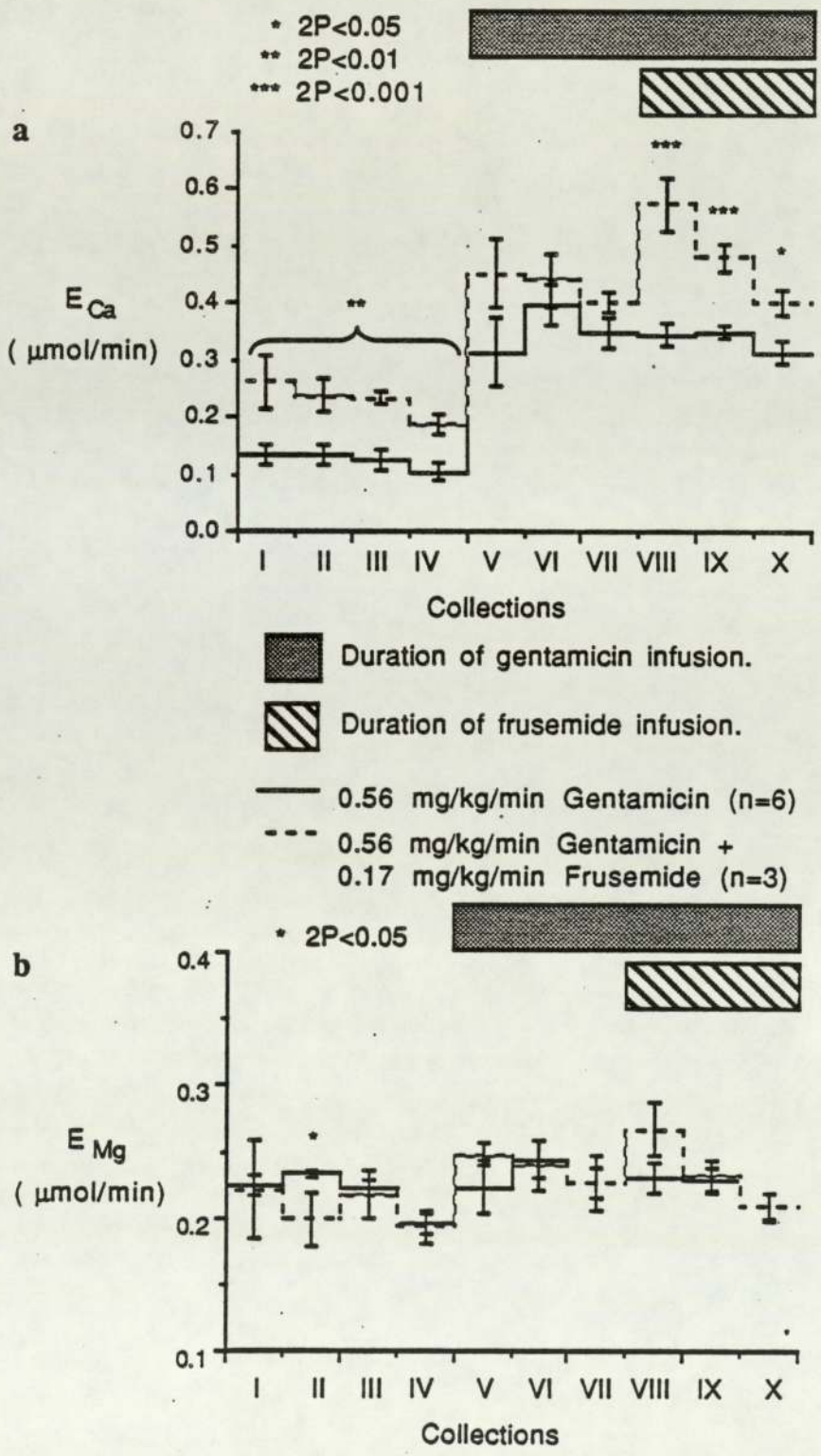


Figure 109. Comparison of clearances of cations between rats infused with getamicin alone and rats infused with gentamicin and frusemide. Both are expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Calcium. **b.** Magnesium.

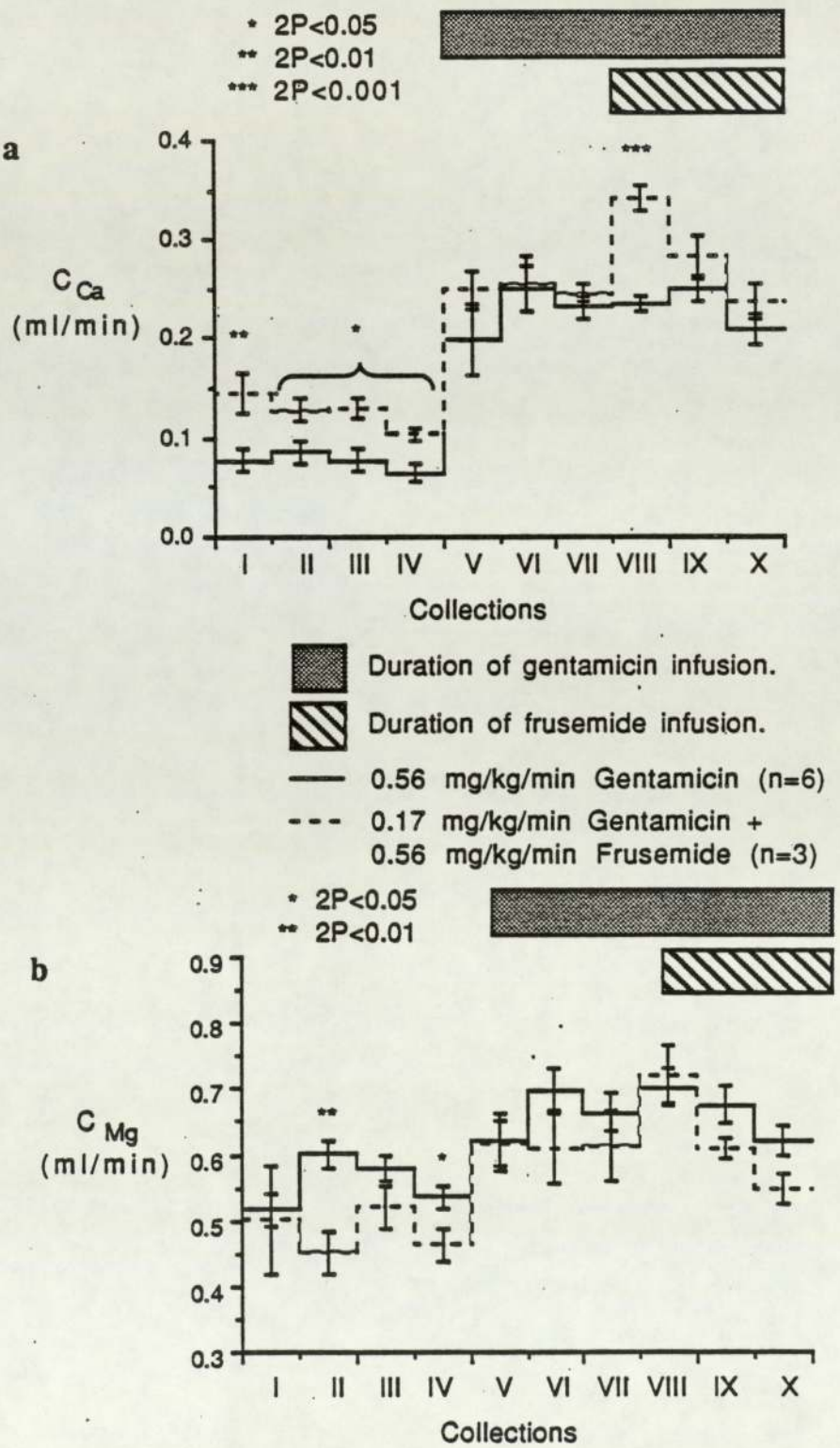


Figure 110. Histograms illustrating the similarity in the maximal responses to frusemide alone (F) and frusemide with gentamicin (F+G). Each column represents the peak value of each parameter in either experimental group, expressed as mean ± s.e.m. Statistical comparisons were made using Student's 't' test for small groups. **a.** Excretion rate of calcium. **b.** Excretion rate of magnesium. **c.** Clearance of calcium. **d.** Clearance of magnesium. **e.** Fractional excretion of calcium. **f.** Fractional excretion of magnesium.

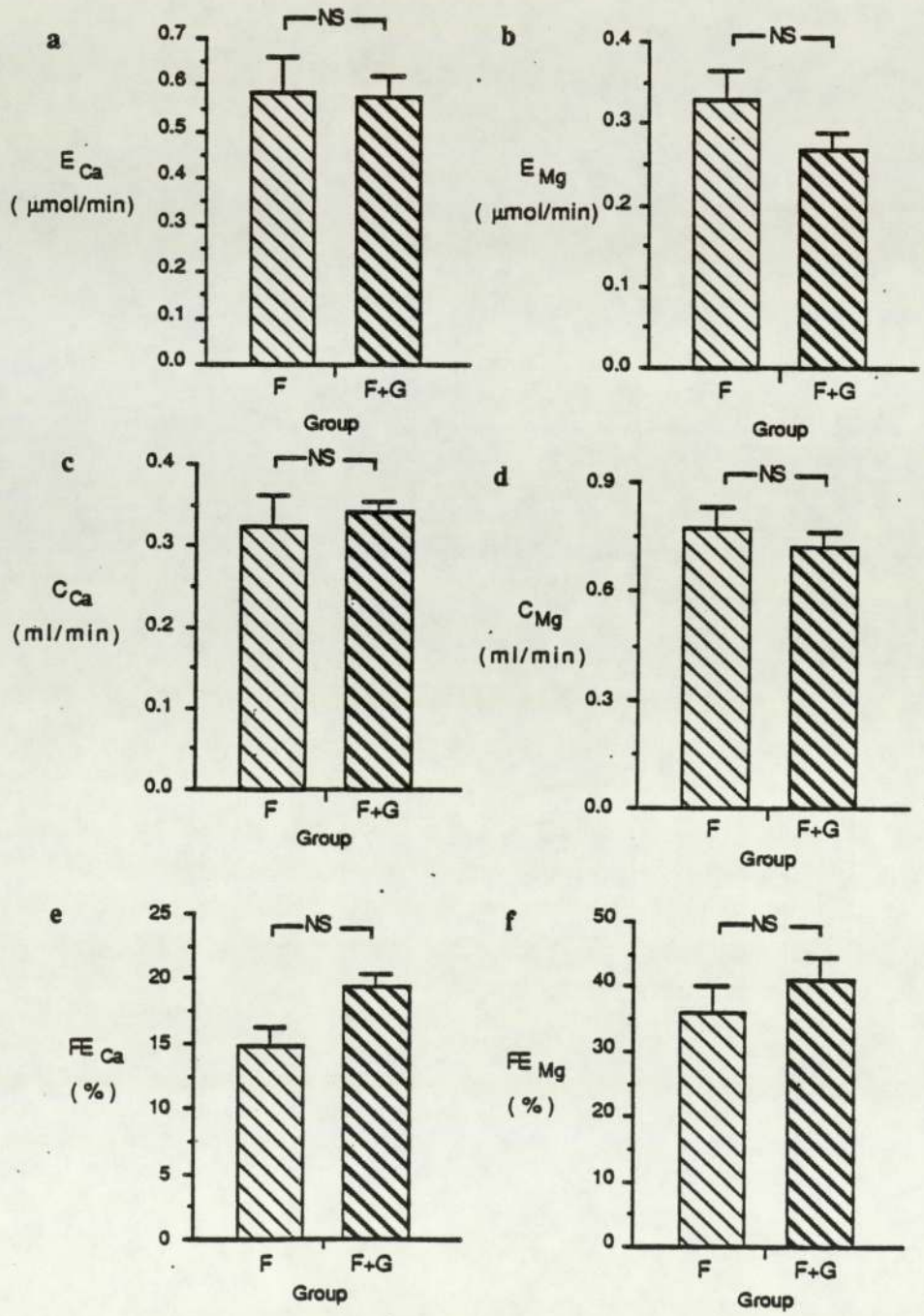


Table 1. The effect of various experimental manoeuvres on the fractions of ultrafilterable calcium and magnesium in plasma, expressed as percentages of total plasma calcium and magnesium ($\%UF_{Ca}$ and $\%UF_{Mg}$ respectively). **a.** Experimental groups compared with control group A. **b.** Experimental groups compared with control group B.

a Group	n	$\%UF_{Ca}$	$\%UF_{Mg}$
Saline (control group A)	4	82.38 ± 0.84	85.18 ± 1.40
0.56 mg/kg/min gentamicin (G)	4	81.08 ± 1.05^{NS}	84.50 ± 1.46^{NS}
Saline, TPTX	4	80.00 ± 3.22^{NS}	92.35 ± 1.94*
0.56 mg/kg/min gentamicin, TPTX	3	79.90 ± 2.30^{NS}	92.27 ± 2.80*

b Group	n	$\%UF_{Ca}$	$\%UF_{Mg}$
Saline (control group B)	3	77.00 ± 1.14	87.17 ± 1.15
0.17 mg/kg/min frusemide	5	74.08 ± 1.26^{NS}	86.20 ± 1.29^{NS}
0.56 mg/kg/min gentamicin + 0.17 mg/kg/min frusemide	4	73.13 ± 2.61*	86.48 ± 1.77^{NS}
Saline, PTX	3	74.00 ± 2.38^{NS}	87.63 ± 2.93^{NS}
0.56 mg/kg/min gentamicin, PTX	3	75.97 ± 1.84*	86.57 ± 5.54^{NS}

NS Not significantly different from appropriate control group.

* Significantly different ($2P<0.05$) from appropriate control group.

NS Not significantly different from 0.56 mg/kg/min (G).

* Significantly different ($2P<0.05$) from 0.56 mg/kg/min (G).

CHAPTER 5: DISCUSSION

5.1 General discussion

The acute hypercalciuric and hypermagnesiuric responses to gentamicin and tobramycin infusion in the anaesthetised rat were characterised by the following features:

a) Both responses were initiated within thirty minutes of the introduction of infused drug and were fully established within an hour.

b) The hypercalciuric response was sustained throughout drug infusion; the hypermagnesiuric response was not as persistent, declining after approximately two hours of drug infusion.

c) The hypercalciuric response was reversed within three hours of the end of gentamicin infusion; the hypermagnesiuric response was reversed within one hour of stopping gentamicin infusion.

d) No concurrent changes in sodium or potassium output were produced by infusion of gentamicin.

e) Both responses to gentamicin were unaffected by removal of the parathyroid glands.

f) The hypercalciuric and hypermagnesiuric responses to infused frusemide were indistinguishable from the responses to frusemide with gentamicin.

Considered individually these six elements of the renal responses to gentamicin reveal a number of important similarities with and differences from studies reported in the literature:

a) Effects on the renal handling of cations occurring within thirty minutes of aminoglycoside administration have not been previously demonstrated. This is unsurprising in view of the fact that no reported studies have attempted to measure urinary electrolytes such a short time after introducing an aminoglycoside.

Pastoriza et al (1983) demonstrated increased fractional excretion of calcium after four hours of netilmicin infusion in the rat, fractional excretion of magnesium increased within one hour in the same study, GFR was stable. These changes in fractional excretion indicate reduced tubular reabsorption of these cations, suggesting some similarity between gentamicin and netilmicin in perturbing divalent cation conservation. Although fractional excretions of potassium and magnesium were within expected limits (12.7-21.9% and 9.1-10.4% respectively) the fractional excretion of calcium in saline-infused control rats in this study was extremely low, 0.2%, such a value in animals receiving a saline infusion is puzzling, although fractional excretion of sodium in the same rats was also very low (0.1-0.4%). It seems that a very low infusion rate was used in these experiments or that the animals were subject to severe dietary restriction of sodium and calcium, in either case this study cannot be considered directly comparable with the work presently under discussion.

Neomycin administration to dogs at a dose of 11 mg/kg/day (Crawford and Teske, 1978) produced a rise in calcium excretion after only four hours; surprisingly, doubling the dose of aminoglycoside delayed the appearance of hypercalciuria by 24 hours. Other urinary electrolytes were not measured in this study. GFR was not estimated in these experiments and therefore fractional excretion of calcium cannot be calculated. There were no changes in total or unbound serum calcium attributable to neomycin and therefore, although the origin of of this calciuria cannot be accurately determined, it can be concluded that hypercalcaemia was not responsible for the hypercalciuric response to neomycin. The only other measurement made in this study was of urine flow rate which decreased slightly in drug-treated dogs. Since polyuria is a well-recognised feature of aminoglycoside nephrotoxicity this result probably indicates that no toxic damage was produced in these

experiments.

Administration of gentamicin to Fischer 344 rats, at doses as low as 10 mg/kg/day, prompted marked hypercalciuria within 24 hours of injection (Harpur et al, 1985a). Magnesium excretion, in contrast, was only elevated after three daily injections of gentamicin. Fractional excretions were not measured but these results appear to confirm the striking rapidity of the hypercalciuric response to gentamicin. There was some evidence of toxic renal damage in these rats, polyuria and enzymuria appeared at the higher doses of gentamicin (20 and 30 mg/kg/day).

Infusion of gentamicin in sheep produced a rise in magnesium excretion after one hour (Bennett et al, 1983) although this conclusion was drawn on the basis of data from only two animals, calcium excretion was not measured in this study. Plasma magnesium was stable in the two animals in which magnesium excretion was shown to rise, indicating that hypermagnesaemia was not responsible for the hypermagnesiuria, GFR and RBF were stable.

In other studies in which hypercalciuria or hypermagnesiuria have been produced by aminoglycosides a delay of over 24 hours between drug administration and response was seen (Smith et al, 1981; Chahwala and Harpur, 1983; Elliott et al, 1987). These latter were all chronic studies in which manifestations of renal damage were often evident, therefore the appearance of urinary wasting of electrolytes in such studies may simply represent generalised disturbance of renal reabsorptive mechanisms. Although no histological data has been presented for the acute experiments in this thesis substantial changes in tubular integrity would be unlikely; over such a short time-course only subtle changes, such as myeloid body formation, would be expected (Williams et al, 1981).

b) In general, when an increase in calcium output caused by an

aminoglycoside has been demonstrated the response persists during continued drug administration once established. This is true in the dog (Crawford and Teske, 1978) and in the rat (Chahwala and Harpur, 1983; Harpur et al, 1985a; Harpur et al, 1985b; Elliott et al, 1987).

Persistent hypermagnesiuria, however, is a less consistent finding. In the rat gentamicin-induced changes in magnesium output have been obscured by instability in the magnesium output of controls (Chahwala and Harpur, 1983) or have been transient (Harpur et al, 1985a) although, Harpur et al (1985b) demonstrated persistent hypermagnesiuria during long term gentamicin dosing in the rat from the third day of injection until the end of the study.

Taken together these results may be seen as evidence which supports the conclusion from the present acute infusion experiments, that the hypermagnesiuric response to gentamicin is less persistent than the hypercalciuric response. However, such generalisations may not be valid and caution should be exercised in drawing comparisons between the chronic experiments cited above and acute infusion experiments. There appear to be important differences between the chronic and acute situations, such as the reversibility and the effect of parathyroidectomy on these responses (see below).

c) The issue of the reversibility of changes in divalent cation excretion induced by aminoglycosides is contentious. Rapid reversibility has been clearly demonstrated in some studies, the results of Crawford and Teske (1978) showed evidence of reversal of neomycin-induced hypercalciuria 28 hours after the second dose. Harpur et al (1985a) demonstrated complete reversal of the rise in calcium excretion prompted by 30 mg/kg/day gentamicin seven days after the last injection. In contrast, in the study of Chahwala and Harpur (1983) raised calcium output produced by gentamicin

persisted for five days after the last daily injection. In neither of these studies was magnesium excretion consistently elevated and therefore the reversibility of gentamicin-induced hypermagnesiuria could not be assessed. Harpur et al (1985b) produced increased calcium and magnesium excretion in rats during long term gentamicin administration. Following the last dose of the drug calcium excretion remained elevated for a further three weeks. Raised magnesium output also persisted after the last injection but a trend towards reversal of this response was discernable.

Reversibility has not been studied in previous acute studies and therefore direct comparisons cannot be made. As stated in section 4.4 the persistence of calciuria and (in Harpur et al, 1985b only) magnesiuria after the end of drug treatment in most chronic studies may indicate an important difference between acute and chronic changes caused by aminoglycosides in the renal handling of calcium and magnesium. It seems likely that in those experiments in which there is no evidence for toxic damage (Crawford and Teske, 1978) rapid reversibility of the hypercalciuric response occurs. In chronic studies in which extensive renal damage was probably produced (Chahwala and Harpur, 1983; Harpur et al, 1985b) persistence of this response was seen and may be assumed to be a consequence of the toxic insult. Marginal damage produced by gentamicin (Harpur et al, 1985a) may lead to an intermediate response in which reversal of hypercalciuria occurs over a time course measured in days rather than hours.

d) Alterations in the renal handling of sodium and potassium have been reported as consequences of aminoglycoside administration. The most frequent finding has been increased potassium output following various aminoglycosides. This has been reported for the rat (Chiu et al, 1977; Mitchell et al, 1977; Luft et al, 1978a; Smith et al,

1981; Pastoriza et al, 1983) the dog (Cronin et al, 1980; Brinker et al, 1981) and the sheep (Bennett et al, 1983). In two studies unchanged potassium excretion in rats (Chahwala and Harpur, 1983) and dogs (Chiu et al, 1976) has been observed following gentamicin. In one study (Cronin and Newman, 1985) a decrease in potassium excretion was seen after gentamicin administration to the rat.

Fractional excretion of potassium was measured in all of the above studies with the exceptions of Brinker et al (1981), Chahwala and Harpur (1983) and Cronin and Newman (1985) indicating that the drug effect in most cases was to reduce tubular potassium reabsorption. This evidence contrasts strongly with the results from the acute experiments presented herein. However, all the literature cited above, except Pastoriza et al (1983) involved chronic studies in which the characteristics and possibly the mechanism of the renal responses to aminoglycosides may differ substantially from the acute model, as stated previously. Indications of toxic renal damage were present in most of these studies suggesting that hyperkaliuria may simply be a consequence of generalised disruption of renal function. Evidence of renal damage was noticeably absent in those studies in which no changes in potassium excetion were produced, except for Chahwala and Harpur (1983) in which extensive renal damage was produced but potassium excretion was unaffected.

Effects of aminoglycosides on sodium excretion have been extremely inconsistent. Increased (Luft et al, 1978a; Smith et al, 1981; Pastoriza et al, 1983) decreased (Bennett et al, 1983; Elliott et al, 1987) and unaltered (Mitchell et al, 1977; Chiu et al, 1976; Chahwala and Harpur, 1983; Cronin and Newman, 1985; Bennett et al, 1985) sodium output have all been reported as responses to aminoglycoside administration.

In only three studies were aminoglycosides shown to increase the fractional excretion of sodium. In the study of Luft et al (1978a) the

drugs involved, gentamicin and kanamycin, only produced this response in tandem with a reduction in creatinine clearance, indicating toxic renal damage and severe impairment of whole kidney function, including the ability of the nephron to reabsorb electrolytes.

It is of interest that Smith et al (1981) demonstrated that raised sodium output was produced by gentamicin in sodium-depleted rats and that adrenalectomy prevented this increase, suggesting that aldosterone is necessary for the natriuretic response to gentamicin. Furthermore, in the study of Pastoriza et al (1983) in which netilmicin infusion was shown to increase fractional excretion of sodium, values in saline-infused control rats were surprisingly low, as stated above, possibly indicating that these rats were sodium-depleted in some manner. Therefore, it may be concluded that a natriuretic response to aminoglycosides is only evident in sodium-depleted animals, in which high circulating levels of aldosterone would be expected. Aminoglycosides may bring about increased sodium output by a mechanism involving interference with the action or secretion of aldosterone, therefore no such response would be produced in adrenalectomised animals (Smith et al, 1981). In addition, no such response to aminoglycosides would be expected in sodium-replete animals in which circulating aldosterone was minimal prior to drug administration. The rats used in the present acute clearance experiments were fed a sodium-replete diet and were infused with saline, sodium depletion in these animals was therefore extremely unlikely.

e) The present results indicating that parathyroidectomy does not affect the hypercalciuric and hypermagnesiuric effects of gentamicin are at variance with previous studies. Bennett et al (1985) and Elliott et al (1987) adminisered gentamicin to PTX and sham-operated rats:

In the first study urinary calcium increased fifty-fold in sham-operated rats after 10 days of 40 mg/kg/day gentamicin whereas calcium output only rose three-fold in PTX rats receiving the same drug regimen. Elliott et al (1987) administered 20 mg/kg/day gentamicin for 21 days, at the end of this period calcium output had increased eight-fold in sham-operated rats but had less than doubled in PTX rats. In contrast, in a similar study (Cronin and Newman, 1985) urinary calcium increased to similar values in sham-operated and PTX rats after 8 days of gentamicin injection (60 mg/kg/day) although pretreatment values were not given. The essential difference between this latter study and those of Bennett et al (1985) and Elliott et al (1987) is that in Cronin and Newman's experiments rats were fed a standard diet and were thus calcium-replete. In the former studies sham-operated rats were fed a calcium-deficient diet to stimulate PTH secretion. Therefore in the study of Cronin and Newman (1985) the difference in PTH status between sham-operated and PTX rats was probably minimal, PTH secretion being suppressed in the former group by the provision of an adequate dietary calcium intake. The diet fed to all rats used in the acute clearance experiments discussed herein was calcium-supplemented and therefore PTH secretion was probably suppressed in non-PTX rats. Thus, these intact animals may not provide appropriate control data for PTX rats and such work necessitates the use of calcium-depleted controls in which PTH secretion would be stimulated.

f) The peak elevation in divalent cation output produced by concurrent frusemide and gentamicin infusion was not significantly different from the peak elevation produced by frusemide alone. This result suggests that both agents inhibit calcium and magnesium reabsorption at the same site.

5.2 Discussion of the nephron site at which aminoglycosides may impair divalent cation reabsorption

If it is assumed that the effect of gentamicin on magnesium reabsorption is exerted at the same site as its effect on calcium reabsorption there are essentially three nephron segments at which this site may be located: The proximal convoluted tubule, the proximal straight tubule or the thick ascending limb of the loop of Henle.

The thin portions of the loop of Henle in the rabbit are reported to be essentially impermeable to calcium (Rocha et al, 1977) therefore a drug effect at this site would not produce any alterations in calcium excretion rate. There is no direct evidence for magnesium reabsorption in the thin descending or ascending limbs, although permeability of the papillary tip of the loop to magnesium would explain the observation of net magnesium secretion between late proximal and early distal tubular fluid in magnesium-loaded rats (Brunette et al, 1975; Brunette et al, 1978).

Gentamicin did not increase the recovery of ^{45}Ca following microinjection of the isotope at distal sites whereas ^{45}Ca recovery was significantly increased following proximal microinjection (Garland and Harpur, 1987). Therefore an effect of gentamicin on calcium, and probably magnesium, reabsorption in segments beyond the distal puncture site can be discounted.

Calcium reabsorption is closely associated with sodium reabsorption in the proximal convoluted tubule (Walser, 1961; Lassiter et al, 1963; Duarte and Watson, 1967), calcium fluxes are thought to be mostly passive in this segment, although there is evidence for active transport of calcium in the rat (Ullrich et al, 1976). Therefore, as stated in section 4.1, an effect of gentamicin on proximal calcium reabsorption without a similar effect on proximal sodium

stated in section 4.1, an effect of gentamicin on proximal calcium reabsorption without a similar effect on proximal sodium reabsorption is difficult to imagine. Possibly, calcium and sodium reabsorption are inhibited to an equal degree at a proximal site but the increased delivery of sodium is reabsorbed at more distal sites whereas distal calcium reabsorptive capacity is overwhelmed. This increase in distal sodium reabsorption would presumably involve aldosterone-dependent mechanisms, one of the few factors which dissociates calcium and magnesium reabsorption. Therefore, it would be expected that adrenalectomy would unmask a natriuretic effect of gentamicin by reducing distal sodium reabsorption; according to Smith et al (1981) the opposite is true.

Reabsorption of magnesium in the proximal convoluted tubule does not represent a major component of the total reabsorption of this cation, indeed permeability of the isolated rat proximal tubule is reported to be very low (Quamme and Dirks, 1980). Therefore, impairment of proximal magnesium reabsorption by gentamicin would presumably produce only a minimal increase in magnesium output, if any.

Calcium reabsorption in the rat proximal straight tubule is apparently active and possibly inhibited by frusemide (Jamison et al, 1974). The transport of sodium and calcium are dissociated at this site, TF/UF_{Ca} at the hairpin bend of the loop of Henle is consistently lower than TF/P_{Na} at this point in both rat and psammomys (Jamison et al, 1974; deRouffignac et al, 1973). This indicates greater reabsorption of calcium from or greater addition of sodium to the descending limb, the former presumably occurring in the proximal straight tubule. Therefore, calcium reabsorption could be impaired at this site without any effect on sodium reabsorption. The apparent reduction of the peak hypercalciuric response to frusemide in the

presence of gentamicin would be consistent with an effect of gentamicin at this site since, as stated above, frusemide appears to produce some inhibition of calcium reabsorption in the proximal straight tubule. Although, it should be stressed that there is no direct evidence that the hypercalciuric and hypermagnesiuric responses to gentamicin are a result of an action in this segment.

Experiments on isolated segments of rabbit nephron (Quamme and Smith, 1984) indicate that there is little magnesium transport in the proximal straight tubule, therefore it seems unlikely that inhibition of reabsorption by gentamicin at this site would produce a significant hypermagnesiuria.

There is passive transport of calcium in the thick ascending limb of the loop of Henle which is dependent on the lumen-positive potential difference which drives sodium reabsorption (Bourdeau and Burg, 1979; Shareghi and Stoner, 1978), in addition there is an active flux independent of this potential difference (Rocha et al, 1977; Imai, 1978; Suki et al, 1980) in the rabbit. However, there is a evidence that the thick ascending limb possesses profound functional heterogeneity (Suki et al, 1980): The cortical portion is reported to exhibit active calcium transport which is stimulated by PTH (Bourdeau and Burg, 1980) and which is insensitive to frusemide (Shareghi and Agus, 1982; Suki et al, 1980) whereas the medullary portion demonstrates passive calcium flux which is inhibited by frusemide but unaffected by PTH (Imai, 1978; Suki et al, 1980). Therefore, the effect of gentamicin on the responses to frusemide, if exerted in this segment, would presumably imply an action in the medullary portion. However, if this is correct, the mechanism implied would be an interference with the chloride flux which generates the potential difference driving passive cation reabsorption in this portion. Obviously, such a mechanism of action would produce marked increases in the distal delivery (and therefore, output) of all

the major cations, as is produced by frusemide, not a specific effect on calcium and magnesium. If, alternatively, gentamicin reduces the active, PTH-sensitive calcium reabsorption occurring in the cortical portion of the ascending limb no accompanying effects on other cations would be expected. But if this mechanism is correct no reduction in the response to frusemide would be expected when gentamicin is given simultaneously since the two drugs would be acting at different sites. Again, there is no unequivocal evidence for an effect of gentamicin in this segment and the functional heterogeneity mentioned above has only been demonstrated in one report.

The thick ascending limb of the loop of Henle is the major site of magnesium reabsorption (Morel et al, 1969) and reabsorption at this site appears to be essentially passive and secondary to sodium chloride movement (Shareghi and Agus, 1982; Shareghi et al, 1983). Most of the factors which modify magnesium reabsorption, such as parathyroid hormone (Shareghi and Agus, 1979), seem to act at this site. However, if magnesium reabsorption is entirely passive in the thick ascending limb an effect of gentamicin at this site would not be expected to be specific to the divalent cations, as argued above. Possibly a specific, active mechanism for magnesium reabsorption does exist at this site. Certainly, hypercalcaemia and hypermagnesaemia reduce magnesium reabsorption in the ascending limb without an effect on sodium chloride (Coburn et al, 1970; Quamme and Dirks, 1980; Quamme, 1980b; Shareghi and Agus, 1982; Shareghi et al, 1983) suggesting the existence of such a mechanism.

5.3 Discussion of the cellular site at which aminoglycosides may impair divalent cation transport

The cellular site at which gentamicin interferes with the transport of calcium and magnesium can only be conjectured. The precise mechanisms by which calcium and magnesium are transported from the tubular lumen to the blood are unknown. However, the substantial calcium backflux across the proximal tubular epithelium (Murayama et al, 1972) may indicate that a significant proportion of calcium reabsorption in this segment occurs via a paracellular route and therefore cellular mechanisms of calcium reabsorption are less relevant in the proximal tubule.

The movement of calcium across the renal brush border or luminal membrane is down a concentration gradient and is presumably a facilitated diffusion process. The carrier involved may be sodium-dependent (Borle, 1979) and may be a vitamin D-sensitive calcium-binding protein similar to that found in the intestinal mucosa (Taylor and Wasserman, 1972). Little is known about the mechanism of transfer of magnesium from the tubular lumen to the renal cell interior.

Intracellular calcium is controlled by calcium uptake by mitochondria and the endoplasmic reticulum (Studer and Borle, 1980; Moore et al, 1974) and also by proposed carriers situated on the basolateral membrane. These carriers consist of a Ca ATPase (Kinne-Saffran and Kinne, 1974) and a Na/Ca exchange (Blaustein, 1974). Gentamicin could presumably produce hypercalciuria by interfering with any of these systems or by interaction with a yet undiscovered component of renal cellular calcium metabolism.

The cellular aspects of renal magnesium transport have yet to be fully investigated, a basolateral Na/Mg exchange mechanism has been proposed but there is no experimental evidence for its

A large body of work has focussed on the initial interaction of gentamicin with the anionic phospholipids of the renal brush border (Sastrasinh et al, 1982a; Humes et al, 1984; Holohan et al, 1987) and the inhibition of this interaction by calcium (Humes et al, 1984). Furthermore, protection from the nephrotoxic effects of gentamicin is provided by oral loading with calcium in rats (Bennett et al, 1982b; Quarum et al, 1984; Humes et al, 1984). It was suggested that a possible mechanism for this protection could be a reduction in the binding of gentamicin to brush border phospholipid binding sites due to competition for these sites with calcium, the filtered load and luminal throughput of calcium presumably being increased by oral loading. An extension of this arguement provides a possible mechanism for gentamicin-induced hypercalciuria, luminal gentamicin could presumably compete with calcium for binding sites on the renal brush border thereby producing an increase in luminal calcium and consequent hypercalciuria. However, it is unlikely that the former theory is correct, for a number of reasons, and doubt is thus cast on the latter arguement. Firstly, hypercalciuria per se does not reduce gentamicin nephrotoxicity in rats (McCarron et al, 1984) therefore it was not the increase in the filtered load of calcium in calcium-loaded rats which was responsible for reducing toxic damage. Furthermore, in those experiments in which oral calcium loading was shown to reduce the functional impairment produced by gentamicin the renal cortical content of the drug was not lower in calcium-loaded rats when compared with calcium-depleted controls (Humes et al, 1984). This indicates that reduced gentamicin uptake, presumably subsequent to binding to the luminal membrane, was not the mechanism of protection from nephrotoxicity in calcium-loaded rats. However, this was not a universal finding as Bennett et al (1982) and Quarum et al (1984) did demonstrate a delay in the renal cortical accumulation of gentamicin in calcium-loaded animals.

It was also hypothesised that oral calcium loading could reduce gentamicin nephrotoxicity by suppression of PTH secretion. PTH has been shown to stimulate synthesis and turnover of those phospholipids involved in gentamicin binding in rabbit renal cortex (Farese et al, 1980; Bidot-Lopez et al, 1981). Therefore, suppression of PTH secretion would possibly lead to a reduction in the number of potential gentamicin binding sites, reduced uptake and reduced toxicity. Bearing in mind that reduced uptake of gentamicin does not necessarily correlate with reduced toxicity this line of investigation was pursued and parathyroidectomy was found to reduce gentamicin nephrotoxicity (Bennett et al, 1985; Elliot et al, 1987). The mechanism of this protection was unknown but did not appear to be a result of reduced cortical accumulation of the drug (Bennett et al, 1985). A further study (Holohan et al, 1987) confused the issue by demonstrating that parathyroidectomy did not reduce the number of gentamicin binding sites in rat renal membrane vesicles but did reduce renal cortical gentamicin content.

It seems likely that parathyroidectomy and parathyroid suppression following oral calcium loading protect against gentamicin nephrotoxicity by a mechanism which involves interactions subsequent to gentamicin binding to the renal brush border and, presumably, uptake into renal cells. This would explain the observations that protection from toxic injury occurs despite significant renal cortical concentration of gentamicin (Bennett et al, 1985; Humes et al, 1984; Elliott et al, 1987) and that the protection does not appear to be mediated by a reduction in the number of gentamicin binding sites on the plasma membrane (Holohan et al, 1987). Therefore, similar arguements can probably applied to the mechanism of gentamicin-induced hypercalciuria, that the impairment of calcium reabsorption produced by gentamicin is probably mediated at an intracellular location.

probably mediated at an intracellular location.

A mechanism by which gentamicin could interfere with intracellular calcium metabolism is a reduction in calcium uptake by mitochondria, which has been demonstrated in vitro (Sastrasinh et al, 1982). No direct effects on Ca-ATPase or Na/Ca exchange attributable to gentamicin have been reported except for an inhibition of Ca-ATPase in rat basolateral membrane vesicles which could not be reproduced in vivo (personal communication). However, inhibition of the basolateral Na/K-ATPase may reduce calcium reabsorption by the following mechanism: Inhibition of Na/K-ATPase activity will tend to reduce the concentration gradient for sodium across the basolateral membrane, Na/Ca exchange requires this gradient to drive calcium efflux, therefore inhibition of Na/K-ATPase would be expected to decrease calcium efflux and net calcium reabsorption. Indeed, the specific Na/K-ATPase inhibitor, ouabain, has been shown to impair calcium reabsorption in microperfused rat proximal tubule (Ullrich et al, 1976). Since gentamicin has been shown to inhibit renal Na/K-ATPase (Cronin et al, 1982; Williams et al, 1984) such a mechanism could explain gentamicin-induced hypercalciuria.

In summary, the acute hypercalciuric and hypermagnesiuric effects of gentamicin appear to differ from similar responses seen in chronic models in which renal wasting of electrolytes is probably a manifestation of the developing toxic insult. The nephron site at which these acute effects of gentamicin are exerted cannot be firmly deduced from these results but probably resides in the loop of Henle or possibly the proximal convoluted tubule. The cellular site at which gentamicin impairs calcium and magnesium transport is unknown but may be located intracellularly rather than at the plasma membrane.

REFERENCES

Agus, Z.S.; Puschett, J.B.; Senesky, D.; Goldberg, M. (1971)
Mode of action of parathyroid hormone and cyclic adenosine 3', 5'-monophosphate on renal tubular phosphate reabsorption in the dog. J. Clin. Invest. 50: 617-630.

Agus, Z.S.; Gardner, L.B.; Beck, L.H. (1973)
Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium and phosphate. Am. J. Physiol. 224: 1143-1148.

Agus, Z.S.; Chiu, P.J.S.; Goldberg, M. (1977)
Regulation of urinary calcium excretion in the rat. Am. J. Physiol. 232: F545-F549.

Aldred, J.P.; Kleszynski, R.R.; Bastian, J.W. (1970)
Effects of acute administration of porcine and salmon calcitonin on urine electrolyte excretion in rats. Proc. Soc. Exp. Biol. Med. 134: 1175-1180.

Almeida, A.L.J.; Kudo, L.H.; Rocha, A.S. (1978)
Calcium reabsorption in isolated perfused pars recta of proximal tubule. Abstracts and Proceedings of the VIIth International Congress of Nephrology, Karger, Montreal. p E16.

Alon, U.; Wellons, M.D.; Chan, J.C.M. (1983)
Reversal of vitamin-D_2-induced hypercalciuria by chlorothiazide. Pediatr. Res. 17: 117-119.

Anderson, R.J.; Linas, S.L.; Berns, A.S.; Henrich, W.L.; Miller, T.R.; Gabow, P.A.; Schrier, R.W. (1977)
Nonoliguric acute renal failure. N. Engl. J. Med. 296: 1134-1138.

Appel,G.B.; Neu, H.C. (1978)
Gentamicin in 1978. Ann. Intern. Med. 89: 528-538.

Aubert-Tulkens, G.; Van Hoof, F.; Tulkens, P (1979)
Gentamicin-induced phospholipidosis in cultured rat fibroblasts. Lab. Invest. 40: 481-491.

Bailly, C.; Amiel, C. (1982)
Effect of glucagon on magnesium renal reabsorption in the rat. Pflugers Arch. 392: 360-365.

Bailly, C.; Roinel, N.; Amiel, C. (1984)
PTH-like glucagon stimulation of Ca and Mg reabsorption in Henle's loop of the rat. Am. J. Physiol. 246: F205-F212.

Bailly, C.; Roinel, N.; Amiel, C.; Coureau, C.; Couetle, C.; Legendre, Y.; Malorey, P. (1985)
Stimulation by glucagon and PTH of Ca and Mg reabsorption in the superficial distal tubule of the rat kidney. Pflugers Arch. 403: 2-34.

Bamford, M.F.M.; Jones, L.F. (1978)
Deafness and biochemical imbalance after burns treatment with topical antibiotics in young children. Arch. Dis. Child. 53: 326-329.

Bank, N.; Aynedjian, H.S. (1976)
A micropuncture study of the effect of parathyroid hormone on renal bicarbonate reabsorption. J. Clin. Invest. 58: 336-344.

Bar, R.S.; Wilson, H.E.; Mazzaferri, A. (1975)
Hypomagnesaemic hypocalcaemia secondary to renal wasting of magnesium: A posible consequence of high-dose gentamicin therapy. Ann. Intern. Med. 82: 646-649.

Barger, A.C.; Berlin, R.D.; Tulenko, J.T. (1958)
Infusion of aldosterone, 9-α fluorohydrocortisone and antidiuretic hormone into the renal artery of normal and adrenalectomised, anaesthetised dogs: Effect on electrolyte and water excretion. Endocrinol. 62: 804-817.

Battilana, C.A.; Bhattacharya, J.; Lacy, F.B.; Dobyan, D.C.; Johnston, P.A.; Jamison, R.L. (1978)
Effect of chronic potassium loading on potassium secretion by pars recta or descending limb of the juxtamedullary nephron in the rat. J. Clin. Invest. 62: 1093-1103.

Baylis, C.; Rennke, H.R.; Brenner, R.M. (1977)
Mechanisms of the defect in glomerular ultrafiltration associated with gentamicin administration. Kid. Int. 12: 344-348.

Beck, L.H.; Goldberg, M. (1973)
Effects of acetazolamide and parathyroidectomy on renal transport of sodium, calcium and phosphate. Am. J. Physiol. 224: 1136-1142.

Beck, L.H.; Senesky, D.; Goldberg, M. (1973)
Sodium-independent active potassium reabsorption in proximal tubule of the dog. J. Clin. Invest. 52: 2641-2645.

Beck, J.C.; Sacktor, B. (1978)
Membrane potential-sensitive fluorescence changes during Na^+-dependent D-glucose transport in renal brush border membrane vesicles. J. Biol. Chem. 253: 7158-7163.

Beck, P.R.; Thompson, R.B.; Chaudhuri, A.K.R. (1977)
Aminoglycoside antibiotics and renal function: Changes in urinary γ-glutamyl transferase excretion. J. Clin. Med. 30: 432-437.

Bengele, H.H.; Alexander, E.A.; Lechene, C.P. (1981)
Calcium and magnesium transport along the inner medullary collecting duct of the rat. Am. J. Physiol. 239: 24-29.

Bennett, W.M.; Hartnett, M.N.; Gilbert, D.N.; Houghton, D.C.; Porter, G.A. (1976)
Effect of sodium intake on gentamicin nephrotoxicity in the rat. Proc. Soc. Exp. Biol. Med. 151: 736-738.

Bennett, W.M.; Plamp, C.E.; Gilbert, D.N.; Parker, R.A.; Porter, G.A. (1979)
The influence of dosage regimen on experimental gentamicin nephrotoxicity: Dissociation of peak serum levels from renal failure. J. Infect. Dis. 140: 576-579.

Bennett, W.M.; Parker, R.A.; Elliott, W.C.; Gilbert, D.N.; Houghton, D.C. (1982a)
Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. J. Infect. Dis. 145: 370-373.

Bennett, W.M.; Elliott, W.C.; Houghton, D.C.; Gilbert, D.N.; deFehr, J.; McCarron, D.A.; (1982b)
Reduction of experimental gentamicin nephrotoxicity in rats by dietary calcium loading. Antimicrob. Agents Chemother. 22: 508-512.

Bennett, W.M.; McDougall, J.; Potocnik, S.; Wright, R.D.; Whitworth, A. (1983)
Renal handling and acute urinary electrolyte effects of

aminoglycoside antibiotics: use of a solitary autotransplant in the sheep. Life Sci. 32: 205-212.

Bennett, W.M.; Pulliam, J.P.; Porter, G.A.; Houghton, D.C. (1985)
Modification of experimental gentamicin nephrotoxicity by selective parathyroidectomy. Am. J. Physiol. 249: F832-F835.

Berliner, R.W.; Kennedy, T.J.; Orloff, J. (1951)
Relationship between acidification of the urine and potassium metabolism. Am. J. Med. 162: 348-367.

Berliner, R.W. (1952)
Renal secretion of potassium and hydrogen ions. Fed. Proc. 11: 695-700.

Berliner, R.W. (1961) Renal mechanisms for potassium excretion. Harvey Lect. 55: 141-171.

Bidot-Lopez, P.; Farese, R.V.; Sabir, M.A. (1981)
Parathyroid hormone and adenosine-3',5-monophosphate acutely increase phospholipids of the phosphatidate-polyphosphoinositide pathway in rabbit kidney cortex tubules in vitro by a cycloheximide-sensitive process. Endocrinol. 108: 2078-2081.

Blacklock, N.J.; Green, R.; Shafik, I. (1985)
Effects of magnesium infusion on renal calcium excertion in rats. J. Physiol. (London) 358: 108P.

Blaustein, M.P. (1974)
The interrelationship between sodium and calcium fluxes across cell membranes. Rev. Physiol. Biochem. Pharmacol. 70: 33-82.

Bloomer, H.A.; Rector, F.C.; Seldin, D.W. (1963)
The mechanism of potassium reabsorption in the proximal tubule of the rat. J. Clin. Invest. 42: 277-285.

Blythe, W.B.; Gitelman, H.J.; Welt, L.G. (1968)
Effect of expansion of the extracellular space on the rate of urinary excretion of calcium. Am. J. Physiol. 214: 52-37.

Borle, A.B. (1979)
Na/Ca exchange and renal cell Ca homeostasis. Fed. Proc. 38: 1392.

Boulpaep, E.L. (1972)
Permeability changes of the proximal tubule of Necturus during saline loading. Am. J. Physiol. 222: 517-525.

Boulpaep, E.L. (1976)
Recent advances in electrophysiology of the nephron. Annu. Rev. Physiol. 38: 20-36.

Bourdeau, J.E.; Burg, M.B. (1979)
Voltage dependence of calcium transport in the thick ascending limb of Henle's loop. Am. J. Physiol. 236: F357-F364.

Bourdeau, J.E.; Burg, M.B. (1980)
Effect of PTH on calcium transport across the cortical thick ascending limb of Henle's loop. Am. J. Physiol. 239: F121-126.

Bourdeau, J.E.; Buss, S.L.; Vurek, G.G. (1982)
Inhibition of calcium absorption in the cortical thick ascending limb of Henle's loop by furosemide. J. Pharmacol. Exp. Ther. 221: 815-819.

Bricker, N.S.; Schmidt, R.W.; Favre, H.; Fine, L.; Bourgognie, J.J. (1975)
On the biology of sodium excretion: The search for a natriuretic hormone. Yale J. Biol. Med. 48: 293-401.

Brill, D.; Kaloyanides, G.J. (1982)
Gentamicin inhibits a phosphatidylinositol-specific phospholipase C present in rat renal cortex. Clin. Res. 30: 538A.

Brinker, K.R.; Bulger, R.E.; Dobyan, D.C.; Stacey, T.R.; Southern, P.M.; Henrich, W.L.; Cronin, R.E. (1981)
Effect of potassium depletion on gentamicin nephrotoxicity. J. Lab. Clin. Med. 98: 292-301.

Brunette, M.G.; Crochet, M.D. (1975)
Fluorometric method for the determination of magnesium in renal tubular fluid. Analyt. Biochem. 65: 79-84.

Brunette, M.G.; Wen, S.F.; Evanson, R.L.; Dirks, J.H. (1969)
Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. Am. J. Physiol. 216: 1510-1516.

Brunette, M.; Aras, M. (1971)

magnesium. Am. J. Physiol. 221: 1142-1148.

Brunette, M.G.; Vigneault, N.; Carriere, S. (1974)
Micropuncture study of magnesium transport along the nephron in the young rat. Am. J. Physiol. 227: 891-896.

Brunette, M.G.; Vigneault, N.; Carriere, S. (1975)
Micropuncture study of magnesium transport in magnesium-loaded rats. Am. J. Physiol. 229: 1695-1701.

Brunette, M.G.; Vigneault, N.; Carriere, S. (1978)
Magnesium handling by the papilla of the young rat. Pflugers Arch. 373: 229-235.

Brunette, M.G.; Chabardes, D.; Imbert-Teboul, M.; Clique, A.; Montegut, M.; Morel, F. (1979)
Hormone sensitive adenylate cyclase activity along the nephron of genetically hypophosphataemic mice. Kid. Int. 15: 357-369.

Buckle, R.M.; Care, A.D.; Cooper, C.W.; Gitelman, H.J. (1968)
The influence of plasma magnesium concentration on parathyroid hormone secretion. J. Endocrinol. 42: 529.

Buerkert, J.; Marcus, D.; Jamison, R.L. (1972)
Renal tubule calcium reabsorption after parathyroidectomy. J. Clin. Invest. 51: 17a.

Burg, M.B.; Bourdeau, J.E. (1978)
Function of the thick ascending limb of Henle's loop. In: New Aspects of Renal Function, editors: H.G. Vogel, K.J. Ullrich, Excerpta medica, Amsterdam/Oxford. pp91-102.

Burg, M.B.; Green, N. (1973a)
Function of the thick ascending limb of Henle's loop. Am. J. Physiol. 224: 659-668.

Burg, M.B.; Green, N. (1973b)
Effect of ethacrynic acid on the thick ascending limb of Henle's loop. Kid. Int. 4: 301-308.

Burg, M.B.; Orloff, J. (1970)
Electrical potential differences across proximal convoluted tubules. Am. J. Physiol. 219: 1714-1718.

Am. J. Physiol. 219: 1714-1718.

Burg, M.B.; Patlak, C.; Green, N.; Villey, D. (1976)
Organic solutes in fluid absorption by renal proximal convoluted tubules. Am. J. Physiol. 231: 627-637.

Burg, M.B.; Stoner, L.; Cardinal, J.; Green, N. (1973)
Furosemide effect on isolated perfused tubules. Am. J. Physiol. 225: 119-124.

Burnatowska, M.A.; Harris, C.A.; Sutton, R.A.L.; Dirks, J.H. (1976)
Effects of PTH and cAMP on renal handling of calcium, magnesium and phosphate in the hamster. Abstracts of the 9th Annual Meeting of the American Society of Nephrology. p 2A.

Carney, S.L.; Wong, N.L.M.; Quamme, G.A.; Dirks, J.H. (1980)
Effect of magnesium deficiency on renal magnesium and calcium transport in the rat. J. Clin. Invest. 65: 180-188.

Chabardes, D.; Imbert, M.; Clique, A.; Montegut, M.; Morel, F. (1975)
PTH-sensitive adenyl cyclase activity in different segments of the rabbit nephron. Pflugers Arch. 354: 229-239.

Chabardes, D.; Imbert-Teboul, M.; Montegut, M.; Clique, A.; Morel, F. (1976)
Distribution of calcitonin-sensitive adenylate cyclase activity along the rabbit kidney tubule. Proc. Natl. Acad. Sci. (USA) 73: 3608-3612.

Chahwala, S.B.; Harpur, E.S. (1982)
An investigation of the effects of aminoglycoside antibiotics on Na^+-K^+ ATPase as a possible mechanism of toxicity. Res. Comm. Chem. Pathol. Pharmacol. 35: 63-78.

Chahwala, S.B.; Harpur, E.S. (1983)
Gentamicin-induced hypercalciuria in the rat. Acta Pharmacol. et Toxicol. 53: 358-362.

Chan, W.Y. (1971)
Effects of prolonged administration of vasopressin on plasma sodium and on renal excretion of electrolytes and water. J. Pharmacol. Exp. Ther. 178: 141-153.

Chantrelle, B.; Rector, F.C.; (1980)
Active and passive components of of volume reabsorption in rat superficial proximal convoluted tubule. Clin. Res. 28: 441A.

Chiu, P.J.S.; Brown, A.; Miller, G.; Long, J.F. (1976)
Renal extraction of gentamicin in anaesthetised dogs. Antimicrob. Agents Chemother. 10: 277-282.

Chiu, P.J.S.; Miller, G.H.; Brown, A.D.; Long, J.F.; Waitz, J.A. (1977)
Renal pharmacology of netilmicin. Antimicrob. Agents Chemother. 11: 821-825.

Chonko, A.; Savin, V.; Stewart, R.; Karniski, L.; Cuppage, F.; Hodges, G. (1979)
The effects of gentamicin on renal function in the mature versus immature rabbit. Proc. Am. Soc. Nephrol. 12: 2A.

Clark, J.D.; Kenny, A.D. (1969)
Hog thyrocalcitonin in the dog: Urinary calcium, phosphorus, magnesium and sodium responses. Endocrinol. 84: 1199-1205.

Coburn, J.W.; Massry, S.G. (1970)
Changes in serum and urinary calcium during phosphate depletion: Studies on mechanisms. J. Clin. Invest. 49: 1073-1087.

Coburn, J.W.; Massry, S.G.; Kleeman, C.R. (1970)
The effect of calcium infusion on renal handling of magnesium with normal and reduced glomerular filtration rate. Nephron 7: 131-143.

Cohen, L.; Lapkin, R.; Kaloyanides, G.J. (1975)
Effect of gentamicin on renal function in the rat. J. Pharmacol. Exp. Ther. 193: 264-273.

Collier, V.U.; Lietman, P.S.; Mitch, W.E. (1979)
Evidence for luminal uptake in the perfused rat kidney. J. Pharmacol. Exp. Ther. 210: 247-251.

Cortney, M.A. (1969)
Renal tubular transfer of water and electrolytes in adrenalectomised rats. Am. J. Physiol. 216: 589-600.

Costanzo, L.S.; Windhager, E.E. (1978)
Calcium and sodium transport by the distal convoluted tubule of the

rat. Am. J. Physiol. 235: F492-F506.

Crawford, L.M.; Teske, R.H. (1978)
Urinary calcium excretion by neomycin-treated dogs. Toxicol. Appl. Pharmacol. 44: 567-570.

Cronin, R.E.; Bulger, R.E.; Southern, P.; Henrich, W.L. (1980)
Natural history of aminoglycoside nephrotoxicity in the dog. J. Lab. Clin. Med. 95: 463-474.

Cronin, R.E.; Nix, K.L.; Ferguson, E.R.; Southern, P.M.; Henrich, W.L. (1982)
Renal cortex ion composition and Na/K-ATPase activity in gentamicin nephrotoxicity. Am. J. Physiol. 242: F477-F483.

Cronin, R.E.; Newman, J.A. (1985)
Protective effect of thyroxine but not parathyroidectomy on gentamicin nephrotoxicity. Am. J. Physiol. 248: F332-F339.

Cronin, R.E.; Brown, D.M.; Simonsen, R. (1986)
Protection by thyroxine in nephrotoxic acute renal failure. Am. J. Physiol. 251: F408-F416.

Cuppage, F.E.; Setter, K.; Sullivan, L.P.; Reitzes, E.J.; Melnykovych, A.O. (1977)
Gentamicin nephrotoxicity II. Physiological, biochemical and morphological effects of prolonged administration to rats. Virchow's Arch. B Cell Pathol. 24: 121-138.

Davey, P.; Gozzard, D.; Goodall, M.; Leyland, M.J. (1985)
Hypomagnesaemia: An underdiagnosed interaction between genatmicin and cytotoxic chemotherapy for acute non-lymphoblastic leukaemia. J. Antimicrob. Chemother. 15: 623-628.

Davidson, D.G.; Levinsky, N.G.; Berliner, R.W. (1958)
Maintenance of potassium excretion despite reduction of glomerular filtration during sodium diuresis. J. Clin. Invest. 37: 548-555.

deRouffignac, C.; Morel, F. (1969)
Micropuncture study of water, electrolyte and urea movements along the loops of Henle in Psammomys. J. Clin. Invest. 48: 474-486.

deRouffignac, C.; Morel, F.; Moss, N.; Roinel, N. (1973)
Micropuncture study of water and electrolyte movements along the loop of Henle in Psammomys with special reference to magnesium, calcium and phosphorus. Pflugers Arch. 344: 309-326.

deRouffignac, C.; Corman, B.; Roinel, N. (1983)
Stimulation by antidiuretic hormone of electrolyte tubular reabsorption in rat kidney. Am. J. Physiol. 244: F156-F164.

deWardener, H.E. (1977)
Natriuretic hormone. Clin. Sci. Mol. Med. 53: 1-12.

Diamond, J.; Bossert, W. (1967)
Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. J. Gen. Physiol. 50: 2061-2072.

Diezi, J.; Michoud, P.; Aceves, J.; Giebisch, G. (1973)
Micropuncture study of electrolyte transport across papilary collecting duct of the rat. Am. J. Physiol. 224: 623-634.

Diezi, J.; Michoud, P.; Grandchamp, A.; Giebisch, G. (1976)
Effects of nephrectomy on renal salt and water transport in the remaining kidney. Kid. Int. 10: 450-462.

Dobyan, D.C.; Lacy, F.B.; Jamison, R.L. (1979)
Suppression of potassium-recycling in the renal medulla by short-term potassium deprivation. Kid. Int. 16: 704-709.

Duarte, C.G.; Watson, J.F. (1967)
Calcium reabsorption in proximal tubule of the dog nephron. Am. J. Physiol. 212: 1355-1360.

Duarte, C.G. (1968)
Effects of ethacrynic acid and furosemide on urinary calcium, phosphate and magnesium. Metabolism 17: 867-876.

Duarte, C.G.; Chomety, F.; Giebisch, G. (1971)
Effect of amiloride, ouabain and furosemide on distal tubular function in the rat. Am. J. Physiol. 221: 632-639.

Edelman, A.; Curci, S.; Samarzija, I.; Fromter, E. (1979)
Determination of intracellular K^+ activity in rat kidney proximal

tubular cells. Pflugers Arch. 378: 37-45.

Edwards, B.R.; Sutton, R.A.L.; Dirks, J.H. (1971)
Micropuncture study of calcium reabsorption in the dog kidney. Clin. Res. 19: 807.

Edwards, B.R.; Baer, P.G.; Sutton, R.A.L.; Dirks, J.H. (1972)
Effects of parathyroid hormone on renal tubular calcium reabsorption in the dog. Clin. Res. 20: 956.

Edwards, B.R.; Baer, P.G.; Sutton, R.A.L.; Dirks, J.H. (1973)
Micropuncture study of diuretic effects on sodium and calcium reabsorption in the dog nephron. J. Clin. Invest. 52: 2418-2427.

Edwards, B.R.; Sutton, R.A.L.; Dirks, J.H. (1974)
Effect of calcium infusion on renal tubular reabsorption in the dog. Am. J. Physiol. 227: 13-18.

Elalouf, J.M.; Roinel, N.; deRouffignac, C. (1983)
Stimulation by human calcitonin of electrolyte transport in distal tubules of rat kidney. Pflugers Arch. 399: 111-118.

Elalouf, J.M.; Roinel, N.; deRouffignac, C.; (1984a)
ADH-like effects of calcitonin on electrolyte transport by Henle's loop of rat kidney. Am. J. Physiol. 246: F213-F220.

Elalouf, J.M.; Roinel, N.; deRouffignac, C. (1984b)
Effects of antidiuretic hormone on electrolyte reabsorption and secretion in distal tubules of rat kidney. Pflugers Arch. 401: 167-173.

Elliott, W.C.; Parker, R.A.; Houghton, D.C.; Gilbert, D.N.; Porter, G.A.; deFehr, J.; Bennett, W.M. (1980)
Effect of sodium bicarbonate and ammonium chloride ingestion in experimental gentamicin nephrotoxicity in rats. Res. Comm. Chem. Pathol. Pharmacol. 28: 483-495.

Elliott, W.C.; Patchin, D.S.; Jones, D.B. (1987)
Effect of parathyroid hormone activity on gentamicin nephrotoxicity. J. Lab. Clin. Med. 109: 48-54.

Estep, H.; Shaw, W.A.; Watlington, C.; Hobe, R.; Holland, W.; Tucker, S.G. (1969)
Hypocalcaemia due to hypomagnesaemia and reversible parathyroid

hormone unresponsiveness. J. Clin. Endocrinol. Metab. 29: 842-848.

Fabre, J.; Rudhart, M.; Blanchard, P.; Regamey, C. (1976)
Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. Kid. Int. 10: 444-449.

Falco, F.G.; Smith, H.M.; Arcieri, C.M. (1969)
Nephrotoxicity of aminoglycosides and gentamicin. J. Infect. Dis. 119: 406-409.

Farber, J.L. (1981)
The role of calcium in cell death. Life Sci. 29: 1289-1296.

Farese, R.V.; Bidot-Lopez, P.; Sabir, M.A.; Smith, J.S.; Schinbeckler, B.; Larson, R. (1980)
Parathyroid hormone acutely increases polyphosphoinositides of the rabbit kidney cortex by a cycloheximide-sensitive processJ. Clin. Invest. 65: 1523-1526.

Feldman, S.; Wang, M.; Kaloyanides, G.J. (1982)
Aminoglycosides induce a phospholipidosis in the renal cortex of the rat: An early manifestation of nephrotoxicity. J. Pharmacol. Exp. Ther. 220: 514-520.

Finton, C.K.; Bjorkland, R.N.; Zaloga, G.P.; Uddin, D.E.; Chernow, B. (1983)
Gentamicin-induced hypomagnesaemia. Am. Surg. 49: 576-578.

Foss, S.J.; Hammerman, M.R.; Sacktor, B. (1977)
Transport of amino acids in renal brush border membrane vesicles. J. Biol. Chem. 252: 583-590.

Frame, P.T.; Phair, J.P.; Watanakunakorn, C.; Bannister, T.W.P. (1977)
J. Infect. Dis. 135: 852-856.

Freedman, D.B.; Shannon, M.; Dandona, P.; Prentice, H.G.; Hoffbrand, A.V. (1982)
Hypoparathyroidism and hypocalcaemia during treatment for acute leuaemia. Br. Med. J. 284: 700-702.

Frick, A.; Rumrich, G.; Ullrich, K.J.; Lassiter, W.F. (1965)
Microperfusion study of calcium transport in the proximal tubule of

the rat kidney. Pflugers Arch. 286: 109-117.

Fromter, E. (1979)
Solute transport across epithelia: What can we learn from micropuncture studies on kidney tubules. J. Physiol. (London) 288: 1-31.

Fromter, E.; Rumrich, G.; Ullrich, K.J. (1973)
Phenomenologic description of Na^+, Cl^- and HCO_3^- absorption from proximal tubules. Pflugers Arch. 343: 189-200.

Fujimoto, M.; Kubota, T.; Katera, K. (1977)
Electrochemical profile of K and Cl ions across the proximal tubule of bullfrog kidneys. Contr. Nephrol. 6: 114-123.

Galante, L.; Horton, R.; Wiggins, R.C.; Gudmundsson, T.V.; MacIntyre, I. (1970)
The calcitonin-like action of streptomycin. In: New concepts in calcitonin research, Heinemann, London. pp427-433.

Garcia-Filho, E.; Malnic, G.; Giebisch, G. (1980)
Effects of changes in electrical potential difference on tubular potassium transport. Am. J. Physiol. U: F235-F246.

Garland, H.O.; Harpur, E.S. (1987)
Micropuncture study of gentamicin-induced hypercalciuria. Program Abstracts, 3rd. International Symposium on Nephrotoxicity, Guildford, 1987.

Giebisch, G.; Windhager, E.E. (1973)
Electrolyte transport across renal tubular membranes. In: Handbook of Physiology, Section 8: Renal Physiology, editors: J. Orloff, R.W. Berliner, American Physiological Society, Washington D.C. pp 315-376.

Gilbert, D.N.; Houghton, D.C.; Bennett, W.M.; Plamp, C.E.; Reger, K.; Porter, G.A. (1979)
Reversibility of gentamicin nephrotoxicity in rats: Recovery during continuous drug administration. Proc. Soc. Exp. Biol. Med. 160: 99-103.

Ginsberg, D.S.; Quintanilla, A.P.; Levin, M. (1976)
Renal glycosuria due to genatmicin in rabbits. J. Infect. Dis. 134: 432-

437.

Gitelman, H.J.; Graham, J.B.; Welt, L.G.; (1966)
A familial disorder characterised by hypokalaemia and hypomagnesaemia. Trans. Ass. Am. Physic. 79: 221-235.

Gitelman, H.J.; Kukolj, S.; Welt, L.T.G. (1968)
Influence of parathyroid glands on hypercalcaemia of experimental magnesium depletion in rat. J. Clin. Invest. 47: 118-126.

Goldfarb, S.; Westby, G.R.; Goldberg, M.; Agus, Z.S. (1977)
Renal tubular effects of chronic phosphate depletion. J. Clin. Invest. 59: 770-779.

Good, D.W.; Wright, F.S. (1979)
Luminal influences on potassium secretion: sodium concentration and fluid flow rate. Am. J. Physiol. 236: F192.

Goodhart, G.L.; Handelsman, S. (1985)
Gentamicin and hypokalaemia. Ann. Intern. Med. 103: 645-646.

Grantham, J.J.; Burg, M.B. (1966)
Effect of vasopressin and Cyclic AMP on permeability of isolated collecting tubules. Am. J. Physiol. 211: 255-266.

Grantham, J.J.; Burg, M.B.; Orloff, J. (1970)
The nature of transtubular Na and K transport in isolated rabbit renal collecting tubules. J. Clin. Invest. 49: 1815-1826.

Green, C.G.; Doershuk, C.F.; Stern, R.C. (1985)
Symptomatic hypomagnesaemia in cystic fibrosis. J. Pediatr. 107: 425-428.

Greger, R. (1981)
Chloride reabsorption in the rabbit cortical thick ascending limb of the loop of Henle. Pflugers Arch. 390: 38-43.

Gyselynck, A.M.; Forrey, A.; Cutler, R. (1971)
Pharmacokinetics of gentamicin: Distribution and plasma and renal clearance. J. Infect. Dis. 124: 570-576.

Hall, D.A.; Varney, D.M. (1980)

Effect of vasopressin on electrical potential difference and chloride transport in mouse medullary ascending limb of Henle's loop. J. Clin. Invest. 53: 393-402.

Hansen, M.M.; Kaaber, K. (1977)
Nephrotoxicity in combined cephalothin and gentamicin therapy. Acta Med. Scand. 201: 463-467.

Harpur, E.S.; Fathalla, S.A.K.; Phipps, D.J. (1985a)
Increased urinary excertion of calcium and magnesium is an early manifestation of gentamicin-induced nephrotoxicity in the rat. In: Renal heterogeneity and target cell toxicity, editors: P.H. Bach, E.A. Lock, John Wiley and Sons, Chichester. pp 349-352.

Harpur, E.S.; Fathalla, S.A.K.; Phipps, D.J.; Ishmael, J. (1985b)
Functional impairment of the rat kidney induced by a moderate dose of gentamicin does not recover during long-term administration of the drug. Program Abstracts: International Symposium on Drug-induced Nephrotoxicity, Antwerp, Belgium, 1985.

Harris, C.A.; Baer, P.G.; Chirito, E.; Dirks, J.H. (1974)
Composition of mammalian glomerular filtrate. Am. J. Physiol. 227: 972-976.

Harris, C.A.; Burnatowska, M.A.; Seely, J.F.; Sutton, R.A.L.; Quamme, G.A.; Dirks, J.H. (1979)
Effects of parathyroid hormone onelectrolyte transport in the hamster nephron. Am. J. Physiol. 236: 342-348.

Hebert, S.C.; Culpepper, R.M.; Andreoli, T.E. (1981)
NaCl transport in mouse medullary thick ascending limbs I. Functional nephron heterogeneity and ADH-stimulated NaCl cotransport. Am. J. Physiol. 241: 412-419.

Hierholzer, K. (1961)
Secretion of potasium and acidification in collecting ducts of mammalian kidney. Am. J. Physiol. 201: 318-324.

Hierholzer, K.; Wiederholt, M.; Holzgreve, H.; Giebisch, G.; Klosd, R.M. Windhager, E.E. (1965)
Micropuncture study of renal transtubular concentration gradients of sodium and potasium in adrenalectomised rats. Pflugers Arch. 285: 193-210.

Hierholzer, K.; Lange, S. (1974)
The effects of adrenal steroids on renal function. In: International Review of Science, Kidney and Urinary Tract Physiology, editor: K. Thurau, Butterworth, London. pp 273-319.

Holmes, A.M.; Hesling, C.M.; Wilson, T.M. (1970)
Drug-induced secondary hyperaldosteronism in patients with pulmonary tuberculosis. Quart. J. Med. 39: 299-315.

Holohan, P.D.; Elliott, W.C.; Grace, E.; Ross, C.R. (1987)
Effect of parathyroid hormone on gentamicin plasma membrane binding and tissue accumulation. J. Pharmacol. Exp. Ther. 243: 893-896.

Houghton, D.C.; Hartnett, M.N.; Campbell-Boswell, M.; Porter, G.A.; Bennett, W.M. (1976)
A light and electron microscopic analysis of gentamicin nephrotoxicity in rats. Am. J. Pathol. 82: 589-600.

Houghton, D.C.; Plamp, C.E.; deFehr, J.M.; Bennett, W.M.; Porter, G.A.; Gilbert, D.N. (1978)
Gentamicin and tobramycin nephrotoxicity: A morphologic and functional comparison in the rat. Am. J. Pathol. 93: 137-147.

Howell, D.S.; Davis, J.O. (1954)
Relationship of sodium retention to potassium excretion by the kidney during the administration of deoxycorticosterone acetate to dogs. Am. J. Physiol. 179: 359-367.

Hruban, Z.; Slesers, A.; Hopkins, E. (1972)
Drug-induced and naturally occurring myeloid bodies. Lab. Invest. 27: 62-70.

Hsu, C.H.; Kurtz, T.W.; Easterling, R.E.; Weller, J.M. (1974)
Potentiation of gentamicin nephrotoxicity by metabolic acidosis. Proc. Soc. Exp. Biol. Med. 146: 894-897.

Humes, H.D.; Sastrasinh, M.; Weinberg, J.M. (1984)
Calcium is a competitive inhibitor of gentamicin-renal membrane binding interactions and dietary calcium supplementation protects against gentamicin nephrotoxicity. J. Clin. Invest. 73: 134-147.

Imai, M. (1977)
Function of the thin ascending limb of Henle of rats and hamsters perfused in vitro. Am. J. Physiol. 232: F201-F209.

Imai, M. (1978)
Calcium transport across the rabbit thick ascending limb of Henle's loop perfused in vitro. Pflugers Arch. 374: 255-163.

Imai, M. (1981)
Effects of parathyroid hormone and N^6, $O^{2'}$-dibutyryl cyclic AMP on Ca^{2+} transport across the rabbit distal nephron segments perfused in vitro. Pflugers Arch. 390: 145-151.

Imai, M.; Kokko, J.P. (1974)
Sodium chloride, urea and water transport in the descending limb of Henle. J. Clin. Invest. 53: 393-402.

Imbert-Teboul, M.; Chabardes, D.; Montegut, M.; Clique, A.; Morel, F. (1978)
Vasopressin-dependent adenylate cyclase activities in the rat kidney medulla: Evidence for two separate sites of action. Endocrinol. 102: 1254-1261.

Jaffe, N.; Kim, B.S.; Vawter, G.F. (1972)
Hypocalcaemia - a complication of childhood leukaemia. Cancer 29: 392-398.

Jamison, R.L.; Buerkert, J.; Lacy, F. (1973)
A micropuncture study of Henle's thin loop in Brattleboro rats. Am. J. Physiol. 224: 180-185.

Jamison, R.L.; Frey, N.R.; Lacy, F.B. (1974)
Calcium reabsorption in the thin loop of Henle. Am. J. Physiol. 227: 745-751.

Jamison, R.L.; Lacy, F.B.; Pennell, J.P.; Sanjana, V.M. (1976)
Potassium secretion by the descending limb of pars recta of the juxtamedullary nephron in vivo. Kid. Int. 9: 323-332.

Jamison, R.L.; Roinel, N.; deRouffignac, C. (1979)
Urinary concentrating mechanism in the desert rodent Psammomys

obesus. Am. J. Physiol. 236: 448-453.

Just, M.; Erdmann, G.; Habermann, E. (1977)
The renal handling of polybasic drugs 1. Gentamicin and aprotinin in intact animals. Naunyn-Schmiedeberg's Arch. Pharmacol. 300: 67-76.

Kaloyanides, G.J.; MacLaughlin, S.G.A. (1983)
Adsorption of gentamicin to liposomes containing anionic phospholipid. Fed. Proc. 42: 876.

Kaloyanides, G.J.; Pastoriza-Munoz, E. (1980)
Aminoglycoside nephroyoxicity. Kid. Int. 18: 571-582.

Keating, M.J.; Sethi, M.R.; Bodey, G.P.; Samaan, N.A. (1977)
Hypocalcaemia with hypoparathyroidism and renal tubular dysfunction associated with aminoglycoside therapy. Cancer 29: 1410-1414.

Kelnar, C.J.H.; Taor, W.S.; Reynolds, D.J.; Smith, D.R.; Slavin, B.M.; Brook, C.G.D. (1978)
Hypomagnesaemic hypocalcaemia with hypokalaemia caused by treatment with high dose gentamicin. Arch. Dis. Child. 53: 817-820.

Khuri, R.N.; Wiederholt, M.; Strieder, N.; Giebisch, G. (1975)
The effect of graded solute diuresis on renal tubular sodium transport in the rat. Am. J. Physiol. 228: 1262-1268.

Kinne-Saffran, E.; Kinne, R. (1974)
Localization of a calcium-stimulated ATPase in the basal-lateral plasma membranes of the proximal tubule of rat kidney cortex. J. Membr. Biol. 17: 263-274.

Kleeman, C.R.; Bernstein, D.; Rockney, R.; Dowling, J.T.; Maxwell, M.H. (1961)
Studies on the renal clearance of diffusible calcium and the role of the parathyroid glands in its regulation. In: The Parathyroids, editors: Greep, R.O.; Talmage, R.V., Charles C. Thomas, Springfield, Illinois.

Kluwe, W.M.; Hook, J.B. (1978a)
Analysis of gentamicin uptake by rat renal cortical slices. Toxicol. Appl. Pharmacol. 45: 531-539.

Kluwe, W.M.; Hook, J.B. (1978b)

Functional nephrotoxicity of gentamicin in the rat. Toxicol. Appl. Pharmacol. 45: 163-175.

Kokko, J.P. (1970)
Sodium, chloride and water transport in the thin descending limb of Henle. J. Clin. Invest. 49: 1838-1846.

Kosek, J.C.; Mazze, R.I.; Cousins, M.J. (1974)
Nephrotoxicity of gentamicin. Lab. Invest. 30: 48-57.

Kuntziger, H.; Amiel, C.; Roinel, N.; Morel, F. (1974)
Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium and magnesium. Am. J. Physiol. 227: 905-911.

Lassiter, W.C.; Gottschalk, C.W.; Mylle, M. (1963)
Micropuncture study of renal tubular reabsorption of calcium in normal rodents. Am. J. Physiol. 204: 771-775.

Lau, K.; Goldfarb, S.; Goldberg, M.; Agus, Z.S. (1982)
Effects of phosphate administration on tubular calcium transport. J. Lab. Clin. Med. 99: 317-324.

Laurent, G.; Carlier, M-B.; Rollman, B.; Van Hoof, F.; Tulkens, P (1982)
Mechanism of Aminoglycoside-induced phospholipidosis: In vitro and in vivo studies with gentamicin and amikacin. Biochem. Pharmacol. 31: 3861-3870.

leGrimellec, C.; Roinel, N.; Morel, F. (1973)
Simultaneous Mg, Ca, P, K, Na, and Cl analysis in rat tubular fluid I. During perfusion of either inulin or ferrocyanide. Pflugers Arch. 340: 181-196.

leGrimellec, C.; Roinel, N.; Morel, F. (1974)
Simultaneous Mg, Ca, P, K and Cl analysis in rat tubular fluid III. During acute Ca plasma loading. Pflugers Arch. 346: 171-188.

leGrimellec, C.; Poujeol, P.; deRouffignac, C. (1975)
^{3}H-Inulin and electrolyte concentrations in Bowman's capsule in rat kidney. Pflugers Archiv. 354: 117-131.

Levine, D.Z.; Roinel, N.; deRouffignac, C. (1982)
Flow correlation influx of K, Ca, P, and Mg during continuous

microperfusion of the loop of Henle in the rat. Kid. Int. 22: 634-639.

Lipsky, J.J.; Lietman, P.S. (1982)
Aminoglycoside inhibition of a renal phosphatidylinositol phospholipase C. J. Pharmacol. Exp. Ther. 220: 287-292.

Luft, F.C.; Kleit, S.A. (1976)
Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J. Infect. Dis. 130: 656-659.

Luft, F.C.; Bloch, R.; Sloan, R.C.; Yum, M.N.; Costello, R.; Maxwell, D.R. (1978a)
Comparative nephrotoxicity of aminoglycoside antibiotics in rats. J. Inf. Dis. 138: 541-545.

Luft, F.C.; Rankin, L.I.; Sloan, R.S.; Yum, M.N. (1978b)
Recovery from aminoglycoside nephrotoxicity with continued drug administration. Antimicrob. Agents Chemother. 14: 284-287.

Lynch, R.; Schneider, E.; Willis, L.; Knox, F. (1972)
Absence of mineralocorticoid-dependent sodium reabsorption in the dog proximal tubule. Am. J. Physiol. 223: 40-48.

Mahafee, D.D.; Cooper, C.W.; Ramp, W.K.; Ontjes, D.A. (1982)
Magnesium promotes both parathyroid hormone secretion and adenosine-3, 5-monophosphate production in rat parathyroid tissues and reverses the inhibitory effects of calcium on adenylate cyclase. Endocrinol. 110: 487-495.

Malnic, G.; Klose, R.M.; Gioebisch, G. (1964)
Micropuncture study of renal potassium excretion in the rat. Am. J. Physiol. 206: 674-686.

Malnic, G.; deMello-Aures, M.; Giebisch, G. (1971)
Potassium transport across renal distal tubules during acid-base disturbances. Am. J. Physiol. 221: 1192-1208.

Massry, S.G.; Coburn, J.W.; Chapman, L.W.; Kleeman, C.R. (1967a)
The acute effects of adrenal steroids on the interrelationship between the renal excretion of sodium, calcium and magnesium. J. Lab. Clin. Med. 70: 563-572.

Massry, S.G.; Coburn, J.W.; Chapman, L.W.; Kleeman, C.R. (1967b)

The effect of NaCl infusion on urinary Ca^{2+} and Mg^{2+} during reduction in their filtered loads. Am. J. Physiol. 213: 1218-1244.

Massry, S.G.; Coburn, J.W.; Chapman, L.W.; Kleeman, C.R. (1969)
Renal handling of magnesium in the dog. Am. J. Physiol. 216: 1460-1467.

Massry, S.G.; Ahumada, J.J.; Coburn, J.W.; Kleeman, C.R. (1970)
Effect of $MgCl_2$ infusion on urinary Ca and Na during reduction in their filtered loads. Am. J. Physiol. 219: 881-885.

Massry, S.G.; Kleeman, C.R. (1972)
Calcium and magnesium excretion during acute rise in glomerular filtration rate. J. Lab. Clin. Med. 80: 654-664.

Massry, S.G.; Coburn, J.W. (1973)
The hormonal and non-hormonal control of renal excertion of calcium and magnesium. Nephron 10: 66-112.

Mavichak, V.; Wong, N.L.M.; Quamme, G.A.; Magil, A.B.; Sutton, R.A.L.; Dirks, J.H. (1985)
Studies on the pathogenesis of cisplatin-induced hypomagnesaemia in rats. Kid. Int. 28: 914-921.

McCarron, D.A.; Gilbert, D.N.; Houghton, W.C.; Bennett, W.M. (1984)
Hypercalciuria per se does not protect from gentamicin nephrotoxicity. Kid. Int. 25: 234.

Meintzer, R.B.; Steenbock, H. (1955)
Vitamin D and magnesium absorption. J. Nutr. 56: 285-294.

Melick, R.A.; Aurbach, G.D.; Potts, J.T. (1965)
Distribution and half-life of ^{131}I-labeled parathyroid hormone in the rat. Endocrinol. 77: 198-202.

Mitchell, C.J.; Bullock, S.; Ross, B.D. (1977)
Renal handling of gentamicin and other antibiotics by the isolated, perfused rat kidney: mechanisms of nephrotoxicity. J. Antimicrob. Chemother. 3: 593-600.

Moore, L.; Fitzpatrick, D.F.; Chen, T.S.; London, E.J. (1974)
Calcium pump activity of the renal plasma membrane and renal

microsomes. Biochem. et Biophys. Acta 345: 405-418.

Morel, F.; Roinel, N.; leGrimellec, C. (1969)
Electron probe analysis of tubular fluid composition. Nephron 6: 350-364.

Morel, F.; Chabardes, D.; Imbert, M. (1976)
Functional segmentation of the rabbit distal tubule by microdetermination of hormone-dependent adenylate cyclase activity. Kid. Int. 9: 264-277.

Morel, F.; Chabardes, D.; Imbert-Teboul, M.; leBouffant, F.; Hus-Citharel, A.; Montegut, M. (1982)
Multiple hormonal control of adenylate cyclase in distal segments of the rat kidney. Kid. Int. 21: suppl. 11, pp 55-62.

Morin, J.P.; Viotte, G.; Vandewalle, A.; Van Hoof, F.; Tulkens, P.; Fillastre, J.P. (1980)
Gentamicin-induced nephrotoxicity: A cell biology approach. Kid. Int. 18: 583-590.

Murayama, Y.; Morel, F.; leGrimellec, C. (1972)
Phosphate, calcium and magnesium transfer in proximal tubules and loops of Henle, as measured by single nephron microperfusion experiments in the rat. Pflugers Archiv. 333: 1-16.

Murer, H.; Hopfer, U.; Kinne, R. (1976)
Sodium/proton antiport in brush border membrane vesicles isolated from rat small intestine and kidney. Biochem. J. 154: 597-605.

O'Neil, R.G.; Helman, S.I. (1977)
Transport characteristics of renal collecting tubules.: Influences of DOCA and diet. Am. J. Physiol. 233: F544-F558.

Orenstein, S.R.; Orenstein, D.M. (1983)
Magnesium deficiency in cystic fibrosis. South. Med. J. 76: 1586-1590.

Pastoriza-Munoz, E.; Bowman, R.L.; Kaloyanides, G.J. (1979)
Renal tubular transport of gentamicin in the rat. Kid. Int. 16: 440-450.

Pastoriza, E.; Timmerman, D.; Kaloyanides, G.J. (1983)
Tubular wasting of cations associated with netilmicin infusion. Clin. Res. 31: 438A.

Patel, V.; Luft, F.C.; Yum, M.N.; Patel, B.; Zeman, W.; Kleit, S.A. (1975)
Enzymuria in gentamicin-induced kidney damage. Antimicrob. Agents Chemother. 7: 364-369.

Patel, R.; Savage, A. (1979)
Symptomatic hypomagnesaemia associated with gentamicin therapy. Nephron 23: 50-52.

Poujeol, P.; Chabardes, D.; Roinel, N.; deRouffignac, C. (1976)
Influence of extracellular fluid volume expansion on magnesium, calcium and phosphate handling along the rat nephron. Pflugers Arch. 365: 203-211.

Poujeol, P.; Touvay, C.; Roinel, N.; deRouffignac, C. (1980)
Stimulation of renal magnesium reabsorption by calcitonin in the rat. Am. J. Physiol. 239: 524-532.

Powell, J.H.; Reidenberg, M.M. (1982)
In vitro response of rat and human kidney lysosomes to aminoglycosides. Biochem. Pharmacol. 31: 3447-3453.

Proverbio, F.; Condescu-Guidi, M.; Whittembury, G. (1975)
Ouabain-insensitive Na stimulation of Mg2+-dependent ATPase in kidney tissue. Biochem. Biophys. Acta 394: 281-286.

Puschett, J.B.; Goldberg, M. (1968)
The acute effects of furosemide on acid and electrolyte excertion in man. J. Lab. Clin. Med. 71: 666-677.

Puschett, J.B.; Moranz, J.; Kurnick, W.S. (1972)
Evidence for a direct action of cholecalciferol and 25-hydroxycholecalciferol on the renal transport phosphate, sodium and calcium. J. Clin. Invest. 51: 373-385.

Quamme, G.A.; O'Callaghan, T.O.; Wong, N.L.M.; Sutton, R.A.L.; Dirks, J.H. (1976)
Hypercalciuria in the phosphate-depleted dog. A micropuncture study. Abstracts of the 9th Annual Meeting of the American Society of Nephrology. p6A.

Quamme, G.A.; Wong, N.L.M.; Dirks, J.H.; Roinel, N.; deRouffignac, C.; Morel, F. (1978)
Magnesium handling in the dog kidney: A micropuncture study. Pflugers Arch. 377: 95-99.

Quamme, G.A. (1980a)
Effect of calcitonin on calcium and magnesium transport in rat nephron. Am. J. Physiol. 238: E573-E578.

Quamme, G.A. (1980b)
Effect of hypercalcaemia on renal tubular handling of calcium and magnesium. Can. J. Physiol. Pharmacol. 60: 1275-1280.

Quamme, G.A. (1981)
Effect of furosemide on calcium and magnesium transport in the rat nephron. Am. J. Physiol. 241: F340-F347.

Quamme, G.A. (1986)
Renal handling of magnesium: Drug and hormone interactions. Magnesium 5: 248-272.

Quamme, G.A.; Dirks, J.H. (1980)
Effect of intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. Am. J. Physiol. 238: 187-198.

Quamme, G.A.; Dirks, J.H (1983)
Renal magnesium transport. In: Review of physiology, biochemistry and pharmacology vol. 97, editor: K. Ullrich, Springer-Verlag, Berlin. pp 69-110.

Quamme, G.A.; Smith, C. (1984)
Magnesium transport in isolated proximal straight tubule of the rabbit. Am. J. Physiol. 246: F544-F550.

Quamme, G.A.; Carney, S.C.; Wong, N.L.M.; Dirks, J.H. (1980)
Effect of parathyroid hormone on renal calcium and magnesium reabsorption in magnesium deficient rats. Pflugers Arch. 58: 1-7.

Quarum, M.L.; Houghton, D.C.; Gilbert, D.N.; McCarron, D.A.; Bennett, W.M. (1984)

Increasing dietary calcium moderates experimental gentamicin nephrotoxicity. J. Lab. Clin. Med. 103: 104-114.

Rankin, L.I.; Krous, H.; Fryer, A.W.; Whang, R. (1984)
Enhancement of gentamicin nephrotoxicity by magnesium depletion in the rat. Miner. Elect. Metab. 10: 199-203.

Rasmussen, H.; Anast, C.; Arnaud, C.; (1967)
Thyrocalcitonin, EGTA and urinary electrolyte excretion. J. Clin. Invest. 46: 746-752.

Reineck, H.J.; Osgood, R.W.; Ferris, T.F.; Stein, J.H. (1975)
Potassium transport in the distal tubule and collecting duct of the rat. Am. J. Physiol. 229: 1403-1409.

Reineck, H.J.; Osgood, R.W.; Stein, J.H. (1978)
Net potassium addotion beyond the superficial distal tubule of the rat. Am. J. Physiol. 235: F104-F110.

Relman, A.S.; Schwartz, W.B. (1952)
Effect of DOCA on electrolyte balance in normal man and its relationship to sodium chloride intake. Yale J. Biol. Med. 24: 540-550.

Rizzoli, R.; Fleisch, H.; Bonjour, J-P. (1977)
Effect of thyroparathyroidectomy on calcium metabolism in rats: Role of 1, 25-dihydroxyvitamin D_3. Am. J. Physiol. 233: E160-E164.

Rocha, A.S.; Kokko, J.P. (1973)
Sodium chloride and water transport in the medullary thick ascending limb of Henle. Evidence for active chloride transport. J. Clin. Invest. 52: 612-623.

Rocha, A.S.; Magaldi, J.B.; Kokko, J.P. (1977)
Calcium and phosphate transport in isolated segments of rabbit Henle's loop. J.Clin. Invest. 59: 975-983.

Roediger, W.E.W.; Ludwin, D.; Hinder, R.A. (1975)
Hypocalcaemic response to streptomycin in malignant hypercalcaemia. Postgrad. Med. J. 51: 399-401.

Rouse, D.; Ng, R.C.K.; Suki, W.N. (1980)
Calcium transport in the pars recta and thin descending limb of Henle of the rabbit, perfused in vitro. J. Clin. Invest. 65: 37-42.

Roy, D.R. (1985)
Effect of calcitonin on fractional delivery of sodium and solutes to the juxtamedullary end-descending limb of the rat. Can. J. Physiol. Pharmacol. 63: 89-95.

Rude, R.K.; Oldham, S.B.; Singer, F.R. (1976)
Functional hypoparathyroidism and parathyroid hormone end-organ resistance in human magnesium deficiency. Clin. Endocrinol. 5: 209-224.

Rude, R.K.; Oldham, S.B.; Sharp, C.F.; Singer, F.R. (1978)
Parathyroid hormone secretion in magnesium deficiency. J. Clin. Endocrinol. Metab. 47: 800-806.

Sastrasinh, M.; Knauss, T.C.; Weinberg, J.M.; Humes, H.D. (1982a)
Identification of the aminoglycoside binding site in rat renal brush border membranes. J. Pharmacol. Exp. Ther. 222: 350-358.

Sastrasinh, M.; Weinberg, J.M.; Humes, H.D. (1982b)
The effect of gentamicin on calcium uptake by renal mitochondria. Life Sci. 30: 2309-2315.

Schafer, J.A.; Patlak, C.S.; Andreoli, T.E. (1977)
Fluid absorption and active and passive ion flows in the rabbit superficial pars recta. Am. J. Physiol. 233: F154-F167.

Schentag, J.J.; Jusko, W.J. (1977)
Renal clearance and tissue accumulation of gentamicin. Clin. Pharmacol. Therap. 22: 364-370.

Schilsky, R.L.; Barlock, A.; Ozols, R.F. (1980)
Persistent hypomagnesaemia following cisplatin chemotherapy for testicular cancer. Cancer Treat. Rep. 66: 1767-1769.

Schwartz, G.J.; Burg, M.B. (1978)
Mineralocorticoid effects on cation transport by cortical collecting tubules in vitro. Am. J. Physiol. 235: F576-F585.

Senekjian, H.O.; Knight, T.F.; Weinman, E.J. (1981)
Micropuncture study of the renal handling of gentamicin in the rat. Kid Int. 19: 416-423.

Sethi, M.R.; Bakdash, M.N.; Samaan, N.A. (1975)
Hypocalcaemic effect of glycosidic antibiotics. Clin. Res. 23: 331A.

Shareghi, G.R.; Stoner, C.C. (1978)
Calcium transport across segments of the rabbit distal nephron in vitro. Am. J. Physiol. 235: F367-F375.

Shareghi, G.R.; Agus, Z.S. (1979a)
Phosphate transport in the light segment of the rabbit cortical collecting tubule. Clin. Res. 27: 430A.

Shareghi, G.R.; Agus, Z.S. (1979b)
PTH-sensitive magnesium transport in the rabbit cortical thick ascending limb of Henle. Kid. Int. 16: 837A.

Shareghi, G.R.; Agus, Z.S.; (1982)
Magnesium transport in the cortical thick ascending limb of Henle's loop of the rabbit. J. Clin. Invest. 69: 759-769.

Shareghi, G.R.; Goldfarb, S.; Agus, Z.S. (1983)
Characterization of Mg transport in medullary thick ascending limb of Henle. Clin. Res. 31: 441A.

Shils, M.E. (1969)
Experimental human magnesium depletion. Medicine 48: 61-85.

Shirley, D.G.; Poujeol, P.; leGrimellec, C. (1976)
Phosphate, calcium and magnesium fluxes into the lumen of the rat proximal convoluted tubule. Pflugers Arch. 362: 247-254.

Silverblatt, F.J.; Kuehn, C. (1979)
Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kid. Int. 15: 335-345.

Simmons, C.F.; Bogusky, R.T.; Humes, H.D. (1980)
Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. J. Pharmacol. Exp. Ther. 214: 709-715.

Slatopolsky, E.; Mercado, A.; Morrison, A.; Yates, J.; Klahr, S. (1976)
Inhibitory effects of hypermagnesaemia on the renal action of parathyroid hormone. J. Clin. Invest. 58: 1273-1279.

Smith, P.; Guntupalli, J.; Eby, B.; Lau, K. (1981)
Evidence that gentamicin produces tubular wastage of K and Mg independent of reduced GFR and aldosterone. Clin. Res. 29: 475A.

Soberon, L.; Bowman, R.L.; Pastoriza-Munoz, E.; Kaloyanides, G.J. (1979)
Comparative nephrotoxicities of gentamicin, netilmicin amd tobramycin in the rat. J. Pharmacol. Exp. Ther. 210: 334-343.

Sonnenberg, H. (1974)
Medullary collecting duct function in antidiuretic and in salt- or water-diuretic rats. Am. J. Physiol. 266: 501-506.

Sonnenberg, H. (1978)
Effects of furosemide, acetazolamide and mannitol on medullary collecting duct function in the rat. Pflugers Arch. 373: 113-126.

Sorensen, O.H.; Hindberg, I. (1972)
The acute and prolonged effect of porcine calcitonin on urine electrolyte excretion in intact and parathyroidectomised rats. Acata Endocrinol. 70: 295-307.

Stein, J.H.; Wilson, C.B.; Kirkendall, W.M. (1968)
Differences in the acute effects of furosemide and ethacrynic acid in man. J. Lab. Clin. Med. 71: 654- 665.

Stokes, J.B.; Ingram, M.J.; Williams, A.D.; Ingram, D. (1981)
Heterogeneity along the rabbit collecting tubule. Localization of mineralocorticoid hormone action to the cortical portion. Kid. Int. 20: 340-347.

Stokes, J.B. (1982a)
Sodium and potassium transport across the cortical and outer medullary collecting tubule of the rabbit. Evidence for diffusion across the outer medullary portion. Am. J. Physiol. 242: F514-F520.

Stokes, J.B. (1982b)
Ion transport by the cortical and outer medullary collecting tubule. Kid. Int. 22: 473-484.

Stoner, C.C.; Burg, M.B.; Orloff, J. (1974)
Ion transport in cortical collecting tubule: Effect of amiloride. Am. J.

Physiol. 227: 453-459.

Studer, R.K.; Borle, A.B. (1980)
The effects of hydrogen ions on the kinetics of calcium transport by rat kidney mitochondria. Arch. Biochem. Biophys. 203: 707-718.

Suki, W.N.; Rouse, D.; Ng, R.C.K.; Kokko, J.P. (1980)
Calcium transport in the thick ascending limb of Henle. Heterogeneity of function in the medullary and cortical segments. J. Clin. Invest. 66: 1004-1009.

Suki, W.N.; Rouse, D. (1981)
Hormonal regulation of calcium transport in thick ascending limb of renal tubules. Am. J. Physiol. 241: F171-F174.

Sutton, R.A.L.; Wong, N.L.M.; Dirks, J.H. (1976)
Effects of parathyroid hormone on sodium and calcium transport in the dog nephron. Abstracts of the 12th Annual Meeting of the American Society of Nephrology, Boston. p 70A.

Sutton, R.A.L.; Wong, N.L.M.; Dirks, J.H. (1979)
Effects of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. Kid. Int. 15: 520-523.

Taylor, A.N.; Wasserman, R.H. (1972)
Vitamin D-induced calcium-binding protein: Comparative aspects in kidney and intestine. Am. J. Physiol. 223: 110-114.

Taylor, A.; Windhager, E.E. (1979)
Possible role of cytosolic calcium and Na/Ca exchange in regulation of transepithelial sodium transport. Am. J. Physiol. 236: F505-F512.

Teixeira, R.B.; Kelly, J.; Alpert, H.; Pardo, V.; Vaamonde, C.A. (1982)
Complete protection from gentamicin-induced acute renal failure in the diabetes mellitus rat. Kid. Int. 21: 600-612.

Torribara, T.Y.; Terepka, A.R.; Dewey, P.A. (1957)
The ultrafilterable calcium in human serum I. Ultrafiltration methods and normal values. J. Clin. Invest. 36: 738-748.

Ullrich, K.J. (1960)
Function of the collecting ducts. Circulation 21: 869-874.

Ullrich, K.J.; Schmidt-Nielsen, B.; O'Dell, G.; Pehling, G.; Gottschalk, C.W.; Lassiter, W.E.; Mylle, M. (1963)
Micropuncture study of composition of proximal and distal tubular fluid in rat kidney. Am. J. Physiol. 204: 527-531.

Ullrich, K.J.; Rumrich, G.; Kloss, C.R. (1976)
Active Ca^{++} reabsorption in the proximal tubule of the rat kidney. Pflugers Archiv. 364: 223-228.

Ullrich, K.J.; Papavassilou, F. (1979)
Sodium reabsorption in the papillary collecting ducts of rats. Pflugers Arch. 379: 49-52.

Vander, A.J. (1961)
Potassium secretion and reabsorption in distal nephron. Am. J. Physiol. 201: 505-512.

Vandewalle, A.; Farman, N.; Morin, J.P.; Fillastre, J.P.; Hyatt, P.Y.; Bonvalet, J.P. (1981)
Gentamicin incorporation along the nephron: Autoradiographic study on isolated tubules. Kid. Int. 19: 529-539.

Walser, M. (1961)
Calcium clearance as a function of sodium clearance in the dog. Am. J. Physiol. 200: 1099-1104.

Walser, M. (1971)
Calcium-sodium interdependence in renal transport. In: Renal Pharmacology, editor: Fisher, J.W., Appleton-Century-Crofts, New York. pp 21-41.

Walser, M. (1973)
Divalent cations: physiochemical state in glomerular filtrate and urine and renal extraction. In: Handbook of physiology, vol 8: Renal physiology, editors: J. Orloff, R.W. Berliner, Waverly Press, Baltimore, Maryland. pp555-586.

Weinberg, J.M.; Simmons, C.F.; Humes, H.D. (1980a)
Alterations of mitochondrial respiration induced by aminoglycoside antibiotics. Res. Comm. Chem. Pathol. Pharmacol. 27: 521-531.

Weinberg, J.M.; Harding, P.G.; Humes, H.D. (1980b)

Mechanisms of gentamicin-induced dysfunction of renal cortical mitochondria II. Effects on mitochondrial monovalent cation transport. Arch. Biochem. Biophys. 205: 232-239.

Weinberg, J.M.; Harding, P.G.; Humes, D. (1983)
Alterations in renal cortex cation homeostasis during mercuric chloride and gentamicin nephrotoxicity. Exp. Mol. Pathol. 39: 43-60.

Welwood, J.M.; Lovell, D.; Thompson, A.E.; Tighe, J.R. (1976)
Renal damage caused by gentamicin: A study of the effects on renal morphology and urinary enzyme excretion. J. Pathol. 118: 171-182.

Wiederholt, M.; Schoormans, W.; Fischer, F.; Behn, C. (1972)
Mechanism of action of aldosterone on potassium transfer in the rat kidney. Pflugers Arch. 345: 159-178.

Wiederholt, M. ;Agulian, S.; Khuri, R.N. (1974)
Intracellular potassium in the distal tubule of the adrenalectomised and aldosterone-treated rat. Pflugers Arch. 347: 17-31.

Whittembury, G.; Rawlins, F.A. (1971)
Evidence of a paracellular pathway for ion flow in the kidney proximal tubule: Electromicroscopic determination of lanthanum precipitate in the tight junction. Pflugers Arch. 330: 302-317.

Wilkinson, R.; Lucas, G.L.; Heath, D.A.; Franklin, I.M.; Boughton, B.J. (1986)
Hypomagnesaemic tetany associated with prolonged treatment aminoglycosides. Br. Med. J. 292: 818-819.

Williams, P.D.; Holohan, P.D.; Ross, C.R. (1981)
Gentamicin nephrotoxicity I. Acute biochemical correlates in rats. Toxicol. Appl. Pharmacol. 61: 234-242.

Williams, P.D.; Trimble, M.E.; Crespo, L.; Holohan, P.D.; Freedman, J.C.; Ross, C.R. (1984)
Inhibition of renal Na^+-K^+-adenosine triphosphatase by gentamicin. J. Pharmacol. Exp. Ther. 231: 248-253.

Windhager, E.E.; Giebisch, G. (1961)
Micropucture study of renal tubular transfer of sodium chloride in the rat. Am. J. Physiol. 200: 581-590.

Membrane Disorders, editors: T.E. Andreoli, J.F. Hoffman, D.D. Fanestil, Plenum, New York. pp 681-706.

Wong, N.L.M.; Quamme, G.A.; Sutton, R.A.L.; Dirks, J.H. (1979)
Effects of mannitol on water and electrolyte transport in the dog kidney. J. Lab. Clin. Med. 94: 683-692.

Wong, N.L.M.; Dirks, J.H.; Quamme, G.A. (1983)
Tubular maximum reabsorptive capacity for magnesium in the dog. Am. J. Physiol. 244: 78-83.

Woodhall, P.; Tisher, C. (1973)
Response of the distal tubule and cortical collecting duct to vasopressin in the rat. J. Clin. Invest. 53: 3095-3106.

Zaloga, G.P.; Chernow, B.; Pock, A.; Wood, B.; Zaritsky, A. (1984)
Hypomagnesaemia is a common complication of aminoglycoside therapy. Surg. Gynaec. Obs. 158: 561-565.