THE EFFECT OF AMINOGLYCOSIDE ANTIBIOTIC THERAPY ON THE RENAL EXCRETION OF DIVALENT CATIONS, RETINOL-BINDING PROTEIN AND MURAMIDASE

MARK EASTER

Master of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

January 1990

The University of Aston In Birmingham

The effect of Aminoglycoside therapy on the Renal Excretion of Divalent Cations, Retinol-Binding Protein, and Muramidase

Mark Easter MPhil (1990)

There is currently no satisfactory method for predicting nephrotoxicity in patients receiving aminoglycoside antibiotics. Serum creatinine measurements, which estimate glomerular function, are an inappropriate index to use when the site of toxicity is the proximal tubular cells. Disturbance of electrolyte homeostasis is a frequent consequence of treatment with aminoglycosides, but this has seldom been prospectively investigated.

The aims of this study were to see if renal excretion of retinol-binding protein and muramidase could be used as early markers of aminoglycoside nephrotoxicity, and to examine the effects of aminoglycoside administration on the renal excretion of calcium and magnesium.

Ten patients receiving treatment with intravenous aminoglycosides for sub-acute bacterial endocarditis and infections related to neutropenia and cystic fibrosis were studied. A control group consisted of 12 patients with similar clinical conditions but intravenous treatment with antibiotics other than aminoglycosides. 2hr and 24 hr urine collections were made each day prior to, during, and where possible, following treatment with antibiotics. Daily serum samples were also taken.

The excretion of retinol-binding protein was elevated in most aminoglycoside-treated patients regardless of any change in serum creatinine. Muramidase excretion was less sensitive to aminoglycoside administration but this may have been secondary to low serum levels.

Fractional excretion of calcium was raised in most patients in both the aminoglycoside-treated group and the control group although total excretion was unchanged. Fractional excretion of magnesium was elevated only in the gentamicin treated patients, in those patients who received the longest courses of treatments or the highest total doses. There was no apparent relationship between the development of magnesium and calcium wasting. Renal wasting of electrolytes appeared to be related to damage to the proximal tubular cells, as judged by low molecular weight protein excretion.

Key words : Aminoglycoside-nephrotoxicity, retinol-binding protein, muramidase, calcium, magnesium.

Dedicated, with love to Wendy and Tim, and with respect and affection, to my parents.

Acknowledgements

This study was supported by a research grant from the Research Committee of the West Midlands Regional Health Authority.

I would like to thank the pharmacy staff, nurses, and clinical chemistry staff of East Birmingham Hospital not only for their assistance in obtaining study material but for their encouragement and friendship. I would also like to thank for their advice and help: Professor A M Geddes, department of communicable diseases, East Birmingham Hospital; Mr E Legg, clinical chemistry, East Birmingham Hospital, Dr M J Leyland, East Birmingham Hospital; Dr R JanMohammed, East Birmingham Hospital and Dr D E Stapleforth, East Birmingham Hospital.

I would especially like to thank Dr E S Harpur for his constant support, enthusiasm and guidance throughout my research project.

Many thanks go to Dr P A Lambert for his invaluable help and support throughout the latter stages of this project, and the pharmacy staff at the Birmingham General Hospital for their friendship, tolerance and encouragement.

Finally, this project would not have been possible without the generosity of my patients.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Ethical approval for this study

A protocol of the study was approved by the ethical committee at East Birmingham Hospital, prior to the commencement of this study. The decision to start gentamicin therapy was made by the physician responsible for the patient and was in no way influenced by this study. Before asking for their consent to take part in the study, all patients in the aminoglycoside-treated and control groups were informed that daily urine and blood collections were required. It was also explained to the patients that the information obtained was for research purposes only and would not affect their treatment.

List of abbreviations used in this study

AAP	Alanine aminopeptidase
AG	Aminoglycoside
BSA	Bovine serum albumin
B_2M	Beta$_2$-microglobulin
EBH	East Birmingham Hospital
ELISA	Enzyme-linked immunosorbent assay
EXca	Calcium excretion
EXmg	Magnesium excretion
FEca	Fractional excretion of calcium
FEmg	Fractional excretion of magnesium
GFR	Glomerular filtration rate
hr	Hour
iPTH	Immunoreactive parathyroid hormone
LMW	Low molecular weight
MUR	Muramidase
NAG	N-acetyl-beta-D-glucosaminidase
OPD	O-phenylendiamine
PBS	Phosphate buffered saline
RBP	Retinol-binding protein
SABE	Sub-acute bacterial endocarditis
Sca	Serum calcium
Scr	Serum creatinine
Smg	Serum magnesium
S_{RBP}	Serum retinol-binding protein
S_{MUR}	Serum muramidase
SD	Standard deviation
TMB	3,3',5,5'Tetramethylbenzidine
USCR	Urine to serum creatinine ratio

INTRODUCTION.

The aminoglycoside antibiotics are potent bactericidal drugs which have a wide spectrum of activity against many Coliform - bacilli, Pseudomonas and Serratia species and Staphylococci. For this reason the AG are indicated for life-threatening sepsis or sepsis of unknown origin.

The group is characterised by a chemical structure consisting of one or more amino sugars joined by a glycosidic linkage. They are extremely polar cations with the number of amino groups per molecule governing the degree of cationic character [Cojocel and Hook, 1983]. This polarity means that they are extremely water soluble but they do not penetrate cells readily. In order to have a bactericidal action they must be actively taken up by the bacterial cell. The uptake mechanism is linked to respiratory processes and so these drugs are not active against anaerobic bacteria [Davis, 1987].

Aminoglycosides excert their bacterial toxicity by inhibition of protein synthesis. They interfere with translation by binding to the S12 protein of the 30S ribosome .This causes misreading of the mRNA and blocks protein synthesis totally [Davis, 1987]. Mammalian cells do not have the 30S subunit so the action is a

selective one.

Following intravenous administration, the aminoglycosides are almost exclusively cleared by the kidney. Biliary clearance is measurable but accounts for only a tiny fraction (1 - 2 %) of plasma clearance. They are freely filtered at the glomerulus and 15 to 20% is reabsorbed, via an active mechanism, by the proximal tubular cells. This uptake leads to renal cortical aminoglycoside concentrations of 5 to 20 times the serum level [Seale and Rennert, 1982]. The uptake of aminoglycosides involves binding to anionic sites on the brush border [Silverblatt and Kuehn 1979]. This binding is competitive [Just and Habermann 1977] and it is thought to initiate a series of steps culminating in pinocytosis.

1) Nephrotoxicity of the aminoglycoside antibiotics

Toxicity of the aminoglycoside antibiotics is pricipally seen as ototoxicity and/or nephrotoxicity although neuromuscular blockade and hypersensitivity have been reported [Dukes, 1975].

The aminoglycoside antibiotics have been associated with nephrotoxicity since the late 1960s, but the pathogenesis of this renal damage is still uncertain. They are toxic to the proximal tubular cells and with

continued administration acute renal failure can be precipitated. The reported incidence of nephrotoxicity varies from 4 to 24% [Kahlmeter and Dahlager, 1984]

The structural damage caused by the aminoglycoside antibiotics is associated with functional changes. Clinical observations and experimental findings include proteinuria, enzymuria, haematuria, cyclindruria, elevated blood urea nitrogen, reduced glomerular filtration rate (GFR), and impaired urinary concentrating ability [Seale and Rennert, 1982]. These abnormalities are usually associated with polyuria. Histological observations are typically proximal tubular damage progressing to acute tubular necrosis [Kosek et al, 1974]. However, even after severe damage with cell necrosis, tubular cells can regenerate even if aminoglycoside treatment is continued [Luft et al, 1978; Elliott et al, 1982].

This specificity of the nephrotoxic effects of the aminoglycosides may be related to the ability of the proximal tubular cells to concentrate them. Several studies have demonstrated the accumulation of aminoglycoside antibiotics in the renal cortex [Luft and Kleit 1974; Luft et al, 1975; Whelton, 1979] and the ratio of cortex-bound to serum aminoglycoside concentration ranges from 5 to 20, depending upon experimental conditions. The cortical uptake can be

blocked by amino acid infusion which presumably competes for proximal tubular reabsorption [Whelton et al, 1981]. The distribution of aminoglycosides throughout the proximal tubule is not homogeneous which suggests that the uptake is also dependent upon aminoglycoside concentration in the tubular fluid [Morin et al,1980].

The mechanism by which aminoglycoside antibiotics cause damage after they have entered the cell is unclear. Disturbance of polyphosphoinositide metabolism and inhibition of protein synthesis have been suggested [Seale and Rennert, 1982].

2) Clinical markers of aminoglycoside-induced nephrotoxicity

A) Serum creatinine

In clinical practice, and indeed in many clinical trials, nephrotoxicity is assessed by measurement of serum creatinine (Scr). However, the relationship between the plasma creatinine level and GFR is hyperbolic [Whitworth, 1982; Bauer et al, 1982] which results in a rapid rise in Scr being seen late in renal damage whereas in the early stages there may be considerable loss in renal function with very little change in Scr. It has been demonstrated that appreciable increases in plasma creatinine do not occur until the GFR is less than 50ml min^{-1} 1.73m^{-2} [Bauer et al, 1982].

Scr is determined by three factors : the rate of creatinine production, the rate of creatinine clearance and the volume of distribution of creatinine [Bauer et al, 1982; Bjornsson 1979]. However, it is generally recognised that Scr concentrations in patients with normal renal function are directly related to body weight or muscle mass and are lower in females than males. Scr concentrations also show a definite increase with age in subjects with normal renal function. Therefore, the clinical use of Scr measurements is limited since the same Scr concentrations may reflect different levels of renal function in different subjects [Bjornsson, 1979].

Scr is also an inappropriate index to use when extensive tubular damage may precede any changes in GFR or when renal function is unstable. Glomerular filtration does not decrease until the renal tubule toxin inflicts sufficient damage to activate the protective tubuloglomerular feedback loop and inhibit filtration [Schnermann and Levine, 1975; Thurau et al, 1972; Thurau et al, 1976]. This lag time may be small with highly nephrotoxic compounds or with aminoglycosides used in large doses in experimental models, but in the clinical situation there may be a considerable lag time between tubular insult and production of sufficient tubular damage to inhibit GFR. This lag time may be

between 3 and 10 days [Schentag 1983].

What is needed is a simple diagnostic test which is more specific to proximal tubular damage and can be used to monitor proximal tubular damage independent of GFR.

B) Protein excretion

One of the early manifestations of aminoglycoside-induced nephrotoxicity is an enzymuria and a low molecular weight (LMW) proteinuria [Merle el al, 1981]. Many urinary enzymes have been investigated, including N-acetyl-beta-D-glucosaminidase (NAG) and alanine aminopeptidase (AAP), most of which were shown to lack specificity for drug-induced damage or were too sensitive ie they were unable to distinguish between those patients who subsequently developed clinical nephrotoxicity, as indicated by a rise in Scr, and those that did not.

i) Low molecular weight proteins

LMW proteins (molecular weight < 45,000 daltons) are normally filtered at the glomerulus and then almost completely reabsorbed by the proximal tubule. They are hydrolysed to aminoacids within the lysosomes of the proximal tubular cells and the catabolic products are then returned to the circulation. In healthy people

with normal renal function, very small amounts of LMW proteins are found in the urine [Johansson and Ranvskov, 1972]. Damage to the proximal tubular cells by drugs or other means may result in decreased absorbtion of LMW proteins leading to increased urinary excretion. This was first demonstrated in battery factory workers with chronic cadmium poisoning [Friberg 1950] but a failure to reabsorb LMW proteins has also been demonstrated in normal human volunteers [Walenkamp et al, 1983; Mondorf and Shoeppe, 1984] and in patients [Donta and Lembke, 1985; Schentag, 1983; Davey et al, 1984; Davey et al, 1983] after a single dose of gentamicin.

The most commonly studied LMW protein as a marker for proximal tubular damage is Beta$_2$-microglobulin (B_2M). Recent studies have shown that B_2M is unstable in urine at a pH of 5.5 - 6.0 [Bernard et al, 1982; Davey and Gosling, 1982]. Furthermore, B_2M is cationic and the cationic aminoglycosides have been shown to competitively inhibit its proximal tubular absorption [Viau et al, 1984]. A more recently studied LMW protein is retinol binding protein (RBP). RBP is stable at the normal urinary pH [Bernard et al, 1982] and is anionic [Viau et al, 1984], and hence should not compete with the aminoglycosides for proximal tubular uptake.

Muramidase (MUR), also known as lysozyme, has been used as a marker for proximal tubular toxicity. A study in rats demonstrated that gentamicin impaired filtration, tubular reabsorption and lysosomal catabolism of MUR, due to an interaction between the two cations [Kuo and Hook, 1979].

a) RBP

RBP has a molecular weight of about 21,000. It is synthesised in the liver and its function is to transport retinol to its target tissues. In the serum, RBP is present in two forms; 95 % is bound in a 1:1 molar complex with thyroxine-binding prealbumin [Fex et al, 1979], and the remaining 5 % is free. Of this free RBP, 10-20% is bound to retinol (holo RBP), and the remaining 80-90% is not bound to retinol (apo RBP). Both forms of the free RBP are found in the urine. The prealbumin bound RBP has a molecular weight of 55,000 and hence is not found in the urine under normal circumstances. The filtered load of unbound RBP is approximately 240mg [Fex et al, 1979] but since much of the filtered RBP is reabsorbed by the proximal tubular cells, only about 0.11mg of RBP is excreted per day [Peterson and Berggard, 1971] under normal circumstances. Little work has been done to assess the usefulness of RBP as a marker of aminoglycoside-induced nephrotoxicity. Dawson [1986], found that elevated

excretion of RBP was seen in all aminoglycoside-treated patients whether or not they developed nephrotoxicity. Merle et al [1981] studied RBP as a marker of proximal tubular damage induced by gentamicin and sisomicin. They found that RBP excretion was elevated in patients treated with gentamicin but not those treated with sisomicin.

b) MUR

MUR is a bacteriolytic enzyme of molecular weight 15,000 - 16,000. It is produced by granulocytes and is probably concerned with defence against bacterial infection [Harrison et al, 1973]. It is cationic and has been shown to compete with gentamicin for uptake by the proximal tubular cells [Kuo and Hook, 1979]. An increased excretion of MUR in the urine may therefore be secondary to competition with aminoglycosides for proximal tubular uptake, or necrosis of the proximal tubular cells so that proteins cannot be reabsorbed. Several studies have looked at MUR as a marker of proximal tubular damage in patients secondary to aminoglycosides and other drugs [Prockop and Davidson, 1964; Harrison et al, 1973; Merle et al, 1981; Reed et al, 1981; Davey et al, 1984; Dawson, 1986]. MUR appears to be a good marker of proximal tubular damage although the results from these studies do not confirm whether MUR measurement is a useful predictor of

aminoglycoside-induced nephrotoxicity.

3) Electrolyte disturbance.

Although the nephrotoxic potential of aminoglycosides is well known, their ability to cause disturbance of electrolyte homeostasis is less well appreciated. The resulting electrolyte abnormalities have potentially serious, and even life-threatening, implications. It appears that these effects result from a failure of the kidney to conserve divalent cations following aminoglycoside-induced injury.

A) Renal handling of divalent cations.

i) Calcium.

The kidney has a role in maintaining calcium homeostasis, although bone metabolism is the major regulator of serum calcium (Sca) levels [Agus et al, 1982].

In plasma, calcium exists in three forms - as free ions, bound to protein and complexed with a variety of anions. Only the free and complexed forms, amounting to 55 to 60% of the total, are ultrafiltrable and available for glomerular filtration [Massry and Coburn, 1973; Agus et al, 1982].

Of the filtered calcium, 0.5 to 1% is excreted, the remaining 99% being reabsorbed [Massry and Coburn, 1973] . In mammals, calcium reabsorption occurs throughout the nephron. Approximately 50 to 55% of the filtered load is reabsorbed at the proximal tubule, 20 to 30% at the loop of Henle, 10 to 15% at the distal tubule, and the remaining 2 to 8% at the terminal nephron [Goldberg et al, 1976].

Calcium reabsorption is influenced by a number of factors, both hormonal and non-hormonal. The hormones which influence renal calcium handling include parathyroid hormone, vitamin D, mineralocorticoids and glucocorticoids [Massry and Coburn, 1973, Agus et al, 1982, Goldberg et al, 1976]. Other factors include volume expansion, the use of loop diuretics, and the reabsorption of sodium [Massry and Coburn, 1973; Rude and Singer, 1981].

ii) Magnesium.

The kidney plays a very important role in the maintenance of magnesium homeostasis [Rude and Singer, 1981]. If the magnesium intake is high then the kidney is able to excrete any excess magnesium absorbed from the intestine or mobilised from bone stores [Dirks, 1983]. Conversely, if the magnesium intake is low,

negative balance can be prevented by almost total renal conservation of the cation [Shils, 1969; Dirks, 1983]. Shils [1969] demonstrated that in experimentally induced magnesium depletion, the renal magnesium output was reduced to less than 1mmol per day within a few days.

Since approximately 30 to 35% of magnesium in the plasma is bound to protein only 65 to 70% is filterable in the glomerulus [Quamme, 1986, Dirks, 1983]. Of this ultrafilterable fraction, 80% is the free cation whilst the remainder is complexed with various salts [Dirks, 1983].

Approximately 3 to 5% of the filtered magnesium is excreted in the urine [Watson et al, 1983; Massry and Coburn, 1973; Ebel and Gunther, 1980; Dirks, 1983]. The remainder of the filtered load is absorbed, the major site being the loop of Henle [Rude and Singer, 1981; Dirks, 1983]. Approximately 15 to 20% is absorbed at the proximal tubule, and 5 to 10% is absorbed at the distal tubule. The remaining 65 to 70% is absorbed by the ascending limb of the loop of Henle.

The mechanism of renal magnesium transport is unclear although the tubular reabsorption is known to be influenced by a number of factors including volume expansion, alcohol intake, glucose administration, serum

magnesium level, and the use of loop diuretics [Massry and Coburn, 1973]. Hormones influencing renal magnesium handling include parathyroid hormone, calcitonin and mineralocorticoids [Massry and Coburn, 1973; Quamme, 1986].

B) Effect of Aminoglycosides on calcium and magnesium homeostasis

Electrolyte disturbance has been seen during and following treatment with aminoglycoside antibiotics. A syndrome of hypomagnesaemic hypocalcaemia and hypokalaemia has been reported [Bar et al, 1975]. The primary defect is thought to involve renal wasting of magnesium [Bar et al, 1975; Gozzard et al, 1986; Patel and Savage, 1979; Wilkinson et al, 1986] since the urinary magnesium output is inappropriately high for the serum magnesium [Wilkinson et al, 1986; Finton et al, 1983] and the hypocalcaemia and hypokalaemia can be reversed by magnesium replacement therapy [Rude and Singer, 1981; Wilkinson et al, 1986; Davies and Murray, 1986].

This syndrome has been reported following prolonged courses of high dose aminoglycoside therapy or with multiple short courses [Kelnar et al, 1978]. Many cases have been seen in patients with acute leukaemia following treatment with anthracycline antibiotics and

subsequent aminoglycoside use [Gozzard et al, 1986; Freedman et al 1982, Bar et al, 1975; Keating et al 1977]. This may be due to an interaction between the cytotoxic drug and the aminoglycoside or an effect of the disease process on renal function [Gozzard et al, 1986]. Moreover, many such patients receive multiple courses of aminoglycoside therapy and are likely to have a dietary magnesium deficiency [Freedman et al, 1982]. The onset of electrolyte disturbance sometimes occurs after discontinuation of antibiotic therapy, even five weeks after cessation of therapy [Kelnar et al, 1986]. Renal magnesium wasting has been reported to persist for three months after aminoglycoside therapy was stopped [Wilkinson et al, 1986].

The mechanism of the renal magnesium wasting is unclear although gentamicin-induced secondary hyperaldosteronism has been suggested [Holmes , 1970]. In this study, patients receiving long term gentamicin therapy developed hypomagnesaemia and hypokalaemia associated with high aldosterone levels. This has been supported by another group [Patel and Savage, 1979] where elevated levels of plasma renin and aldosterone were observed in a patient thought to be suffering from gentamicin-induced magnesium depletion. Another study however, found normal plasma aldosterone in two patients who developed severe hypomagnesaemic hypocalcaemia following treatment with large doses of gentamicin [Bar

et al, 1975].

Alternatively, renal wasting of magnesium may be due to an underlying disturbance of tubular magnesium transport induced by gentamicin [Patel and Savage, 1979]. Gentamicin has been shown to cause dose related damage to proximal tubular cells although it is not known whether the drug will interfere with the normal transport mechanisms. It has been shown, however, that at normal or depressed serum magnesium levels, the ascending arm of the loop of Henle is able to increase its magnesium reabsorptive capacity with increased delivery [Quamme and Dirks, 1980 and 1983]. Any decrease in proximal tubule reabsorption could therefore be compensated for by increased reabsorption in the loop of Henle.

The cause of the hypocalcaemia and the hypokalaemia is also unclear. It has been suggested that the hypomagnesaemia may cause the hypocalcaemia by an effect on parathyroid secretion and action. Rude et al [1978] showed that magnesium injection could raise immunoreactive parathyroid hormone (iPTH) levels in hypomagnesaemic hypocalcaemic patients with inappropriately low iPTH levels. The rapidity of this response indicated that the secretion of this hormone was stimulated by restoration of the serum magnesium level. It was postulated that the low serum magnesium

inhibited iPTH secretion by preventing activation of the magnesium-dependant cyclic AMP which mediates parathyroid hormone secretion. This has been supported by a number of groups who have demonstrated inappropriately low iPTH levels in patients who developed hypomagnesaemic hypocalcaemia following aminoglycoside therapy [Watson et al 1984; Watson et al, 1983; Freedman et al, 1982; Bar et al, 1975; Kelnar et al, 1978].

In contrast to this, it has been reported that parathyroid hormone extract failed to increase serum calcium levels in hypomagnesaemic alcoholics [Estep et al, 1969]. This resistance was reversed by magnesium repletion. In two other cases of hypomagnesaemic hypocalcaemia [Connor et al, 1972; Sherwood, 1970] elevated levels of iPTH have been reported which is consistant with end-organ resistance to PTH. It is postulated that this end-organ resistance is due to an alteration of magnesium dependant cyclic AMP activity in target organs [Rude et al, 1978].

It has also been suggested that hypomagnesaemia causes hypocalcaemia by a directed action on bone resorption which is independant of any effect on parathyroid hormone [Graber and Schulman, 1986].

Work in this laboratory has shown different responses to

chronic and acute administration of gentamicin in rats. During gentamicin infusion, calcium excretion increased immediately but rapidly returned to normal when the infusion ended [Foster and Harpur, 1986]. Magnesium excretion rose in a less well defined manner. Toxic damage in this study was unlikely, so this rapid effect implies a pharmacological action of gentamicin. A second study demonstrated that chronic administration of gentamicin in rats produced a chronic elevation of calcium and magnesium excretion which was maintained for several days after gentamicin was withdrawn. Toxic markers (NAG and lactate dehydrogenase) were seen so this chronic elevation of cation excretion appears to be a toxic effect of the drug [Harpur, E.S., personal communication].

In summary, it can be seen that the aminoglycosides have a profound effect on the metabolism of both calcium and magnesium. The primary event is thought to be renal magnesium wasting although the mechanism by which this is produced has yet to be elucidated.

AIMS AND OBJECTIVES

The primary aims of this study were to assess the usefulness of urinary excretion of RBP and MUR as clinical markers of aminoglycoside-induced nephrotoxicity and to study the effects of aminoglycosides on the renal handling of divalent cations in man. LMW proteinuria is an early manifestation of aminoglycoside-induced nephrotoxicity. An earlier study [Dawson, 1986] examined the excretion of RBP amd MUR as potential markers for aminoglycoside-induced nephrotoxicity. Excretion was measured over a 2 hr collection only, and many of the patients had received surgical treatment, a factor known to influence LMW protein excretion [Harrison et al, 1973; Wide and Thoren, 1972]. Many courses were of less than 7 days duration and so nephrotoxicity was less likely to be seen. The current study examined the excretion of LMW proteins over both a 2 hr and 24 hr period and in medical patients receiving longer durations of therapy. The control group was such that the clinical condition of these patients was very similar to the patients in the aminoglycoside-treated group.

Hypomagnesaemia, hypocalcaemia and hypokalaemia are seen during or following long courses, or frequent short courses, of aminoglycoside therapy [Kelnar, 1986]. These serum electrolyte abnormalities are thought to be

due to the renal wasting of magnesium [Bar et al, 1975; Gozzard et al, 1986; Patel and Savage, 1979; Wilkinson et al, 1986]. There has been only one previous attempt to prospectively study electrolyte excretion in aminoglycoside-treated patients [Dawson, 1986]. This was a small study, and many of the patients received only short courses of aminoglycoside therapy. Therefore, a study was made of the serum levels and renal excretion of calcium and magnesium in patients likely to receive long courses of aminoglycoside therapy, or who received frequent short courses. The renal wasting of cations is thought to be secondary to impaired tubular reabsorption [Zaloga et al, 1984] and so it was decided to measure the fractional excretion of calcium and magnesium which is a better marker of tubular reabsorption. It has also been suggested from animal studies [Foster and Harpur, 1986] that the renal cation wasting is a pharmacological effect of aminoglycoside administration. To examine this in man, it was decided to study cation excretion in a 2 hr urine collection immediately post-dose and in a 24 hr sample. If the aminoglycosides were having a pharmacological effect, then the enhanced excretion would be more pronounced during the 2 hr collection period. Moreover, a pharmacological effect of the aminoglycoside antibiotics on the renal excretion of electrolyte would be seen without changes of LMW protein excretion. Measurement of LMW protein excretion may, therefore,

also help interpret any abnormalities in electrolyte excretion.

MATERIALS AND METHODS

Patient selection and method of sample collection

Patients selected for inclusion in this study were likely to receive intravenous treatment with antibiotics at East Birmingham Hospital between April and December 1987. The study group consisted of patients that were likely to receive aminoglycoside therapy for greater than 1 week. Patients likely to receive shorter courses were also included where such treatment was frequently repeated. These criteria were chosen because electrolyte disturbance is more likely to occur in patients receiving long courses of aminoglycosides or frequent short courses [Kelnar et al, 1978]. The control group consisted of patients with similar clinical conditions to the study group, but likely to receive intravenous treatment with antibiotics other than aminoglycosides. Many of the patients recruited for this study were part of a trial comparing the use of gentamicin and piperacillin versus thienamycin as empiric therapy of bacterial infection in granulocytopenic cancer patients.

A 2 hr and, where possible, a 24 hr urine collection was made prior to the start of antibiotic therapy. A 5ml blood sample was taken at the mid-point of the 2hr collection. A 24 hr urine collection was then made each day from the start of therapy for the duration of the

patient's stay in hospital. The first dose of antibiotic each day was administered at 8am and the urine passed during the 2 hr period following this dose was kept for separate analysis. A 5ml blood sample was taken each day, at the mid-point of the 2 hr collection.

All samples were taken to Aston University for analysis. Urine samples were centrifuged at 2,500 rev min^{-1} for 10 minutes, divided into 20ml aliquots and stored at -18°C to await analysis for RBP, MUR and creatinine. Samples were also diluted in 0.1% acidified lanthanum chloride for the analysis of calcium and magnesium. These samples were stored at -18°C. Aliquots of serum were stored at -18°C to await analysis for RBP and MUR. Serum samples were also diluted in 0.1% acidified lanthanum chloride for the analysis of calcium and magnesium. These samples were stored at -18°C. Serum samples were routinely assayed for albumin by the clinical chemistry department at EBH.

Aminoglycoside blood levels were measured routinely in all patients. Initial measurements were made within 48 hours of the start of therapy and further measurements were made at intervals of three or four days during therapy. Dose adjustments were prescribed as necessary by the patient's physician in order to achieve peak plasma levels in the range of 6 - 10mg L^{-1} and trough levels of less than 2 mg L^{-1}.

MATERIALS

1) ELISA Assay

i) RBP standard preparations

(a) Protein standard plasma for NOR partigen (Behring Diagnostics, Hoechst U.K. Ltd., Hounslow , Middlesex) , was used as the standard preparation for the ELISA assay of RBP in serum. This was reported to contain 90mg/1 of RBP.

(b) Purified RBP standard (courtesy of Dr. A. Bernard, Unite de Toxicologie Industrielle et medicine du travail , Brussels) , was used as the standard preparation for the ELISA assay of RBP in urine. This was reported to contain 864mg/1 of RBP.

ii) Antibody to RBP

Rabbit immunoglobulins to human RBP (Dako Ltd., High Wycombe, Bucks) were used. The concentration of this antibody preparation was quoted as 150mg/1.

iii) Antibody to RBP / enzyme conjugate

Horse-radish peroxidase conjugated rabbit immunoglobulins to human RBP (Dako Ltd) were used as the enzyme-conjugate preparation.

iv) Colour substrate

The colour substrate used for the assay was 3,5',5,5' Tetramethylbenzidine (TMB), (Sigma Chemical Company Ltd., Poole, Dorset). This produced a blue colour when broken down by the horse-radish peroxidase .

v) Microtitre plates

96-well , flat-bottomed,cobalt irradiated immulon plates , type 129b (Dynatech Laboratories Ltd, Billinghurst ,Sussex), were used for the assay.

vi) Plate reader

A Titertek Multiskan (Flow Laboratories, Rickmansworth, Herts) was used to read the absorbance of the developed plate.

vii) Buffers

All chemicals were of analytical grade.

BUFFER A Antibody coating buffer

Carbonate-bicarbonate buffer pH 9.6

Na_2CO_3 1.59 g

$NaHCO_3$ 2.93 g

made up to 1 Litre with distilled water.

This buffer was stored at 4°C for up to three weeks.

BUFFER B 'Blocking' buffer

Phosphate buffered saline with Bovine serum albumin (PBS / BSA) , pH 7.4 :

NaCl 8.0 g

KH_2PO_4 0.2 g

$Na_2HPO_4.12H_2O$ 2.9 g

KCl 0.2 g

BSA 1.0 g

made up to 1 Litre with distilled water.

This buffer was freshly made before each assay.

BUFFER C Washing buffer

Phosphate buffered saline with Tween (PBS / T) , pH 7.4 :

Formula as above except 0.5ml Tween 20 was added instead of BSA.

This buffer was freshly made before each assay.

BUFFER D Substrate buffer

Sodium acetate citrate buffer , pH 6.0-6.1 :

Sodium acetate 4.10 g to 500ml with distilled water

Citric acid 1.94 g to 100ml with distilled water

Citric acid solution was added dropwise to the sodium acetate solution to produce the correct pH. To 100ml of this buffer 40µl of 30% hydrogen peroxide and 10mg of TMB were added to produce the

substrate solution.This buffer was freshly made directly before use.

(viii)3,3',5,5' Tetramethylbenzidine solution

A solution of TMB 10 mg/ml in dimethyl - sulphoxide was prepared and stored at -18°C. This was then used in the preparation of the substrate solution.

2) Muramidase assay

i) Muramidase standard preparation

Chicken egg white muramidase , grade 1 (Sigma Chemical Company Ltd.) , was used as the standard for the assay.

ii) Substrate preparation

Micrococcus lysodeikticus dried cells (Sigma Chemical Company Ltd.) were used as the enzyme substrate.

iii) BUFFER E Phosphate buffer

The buffer was a sodium phosphate buffer 67 mM , pH 6.2.

iv) Spectrophotometer

A Cecil 5095 High Performance scanning Spectrophotometer was used for the experiment.

3) Determination of Calcium and Magnesium

i) Atomic Absorption Spectrophotometer

A Perkin Elmer 560 atomic absorption spectrophotometer.

ii) 0.1% acidified lanthanum chloride

0.1% aqueous solution $LaCl_3$ containing 50 mM HCl.

4) Determination of creatinine in serum and urine

i) Kit for determination of creatinine in serum and urine (Sigma Chemical Company Ltd.).

ii) Spectrophotometer.

A Cecil 5095 High Performance scanning spectrophotometer.

EXPERIMENTAL METHODS

1) RBP assay

RBP was measured using a modified version of a double antibody enzyme linked immunosorbant assay (ELISA) which was developed in this laboratory [Dawson, 1986].

A) Procedure.

1. Coating the wells with antibody.

100µl of anti-RBP antibody, diluted 1 in 100 with carbonate-bicarbonate buffer, was added to each well. The plate was then covered with a plastic film and incubated overnight at 4 °C.

2. Washing.

The plate was emptied by inversion. The inverted plate was then held against some layers of soft paper tissue and tapped lightly to remove all residual liquid. The plate was washed by adding 200µl of Buffer B to each well and then leaving for three minutes. The plate was then emptied as above and the washing procedure repeated twice more. The third washing solution was left for one hour at room temperature.

3. Incubation with test sample.

Following the one hour incubation with the washing solution the plate was shaken dry as in step 2. Doubling dilutions (1 in 2000 to 1 in 64000) of RBP standard were prepared in buffer C and 100µl were added to the first two columns. 100µl of sample were added to each of the other wells. Blood samples were diluted 1 in 2000 to 1 in 64000 and urine samples were diluted 1 in 20 to 1 in 2500. All dilutions were made in buffer C. The bottom two wells of each column were controls and were filled with 100µl of buffer C only.

The plate was then covered with plastic film and incubated at room temperature for two hours.

4. The plate was then washed as described in section 2, using buffer C. The plate was left empty following the third wash.

5. Incubation with peroxidase-conjugated antibody.

100µl of peroxidase-conjugated antibody diluted 1 in 300 in buffer C were added to each well. The plate was covered and incubated at room temperature for two hours.

6. The plate was washed five times with buffer C as described in section 2. Immediately after discarding the last wash, the colour reaction was initiated.

7.Enzymatic colour reaction.

100µl of substrate solution were then added to each well. The plate was covered with plastic film and left at room temperature for 10 minutes.

8. Stopping the enzymatic reaction.

The reaction was stopped by adding 100µl of 2.5M H_2SO_4 to each well. Care was taken to ensure that each well had been incubated for exactly the same length of time.

9. Reading the result.

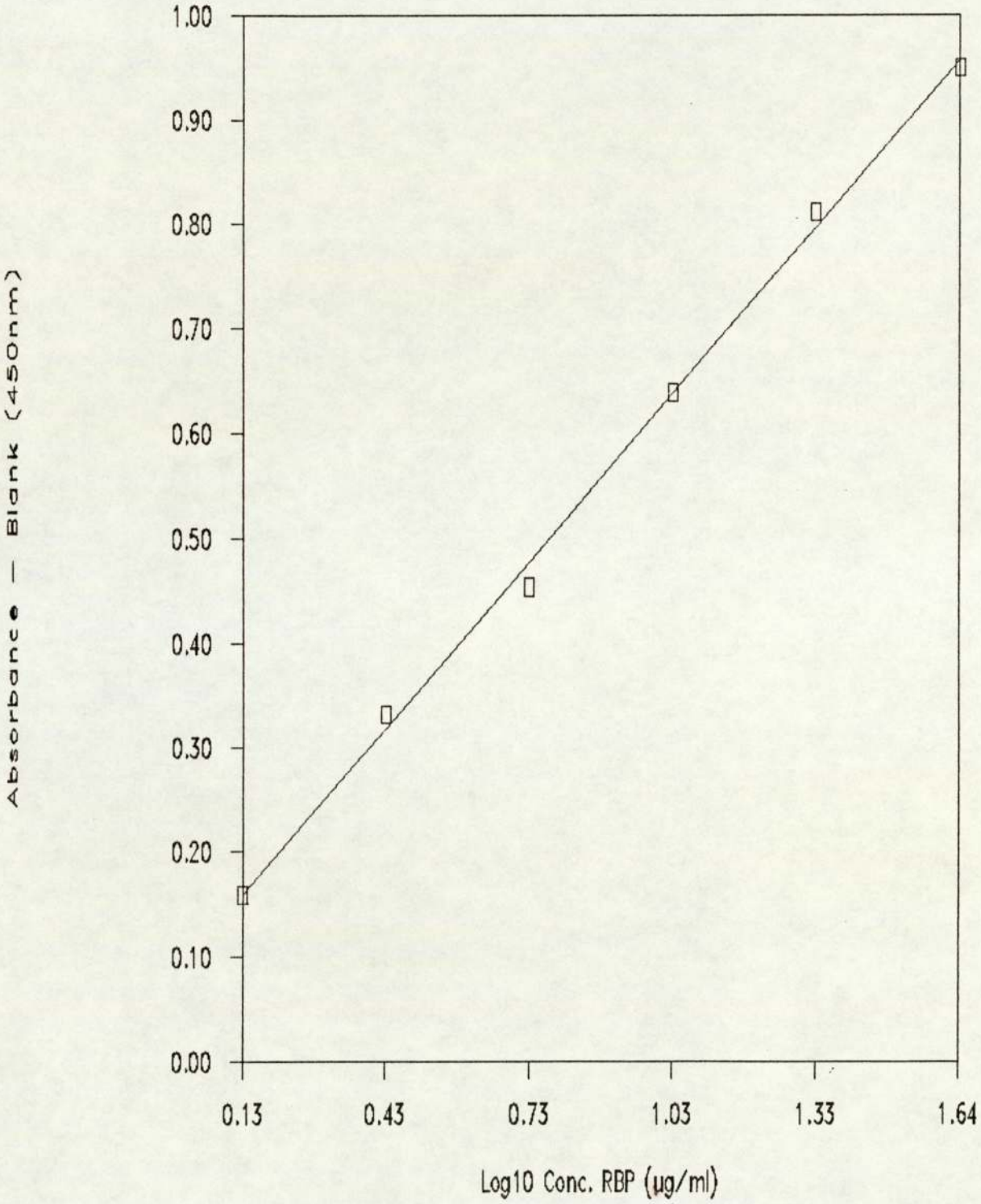
The results were read at 450nm using a Titertek plate reader.

10. A standard curve was plotted on semilogarithmic paper with A_{450nm} as ordinate and $\log_{10}$ concentration as absissa. The results were calculated using linear regression analysis on a Casio fx-3600P calculator. A typical calibration curve is shown in Fig 1.

B) Development of the RBP assay

The only problem with the original assay from which this was developed was the high base line absorbance. This resulted in a decreased accuracy of the assay. In an attempt to reduce this background absorbance it was decided to :

Fig 1 : Calibration curve for RBP



1) Alter the substrate incubation time

2) change the substrate.

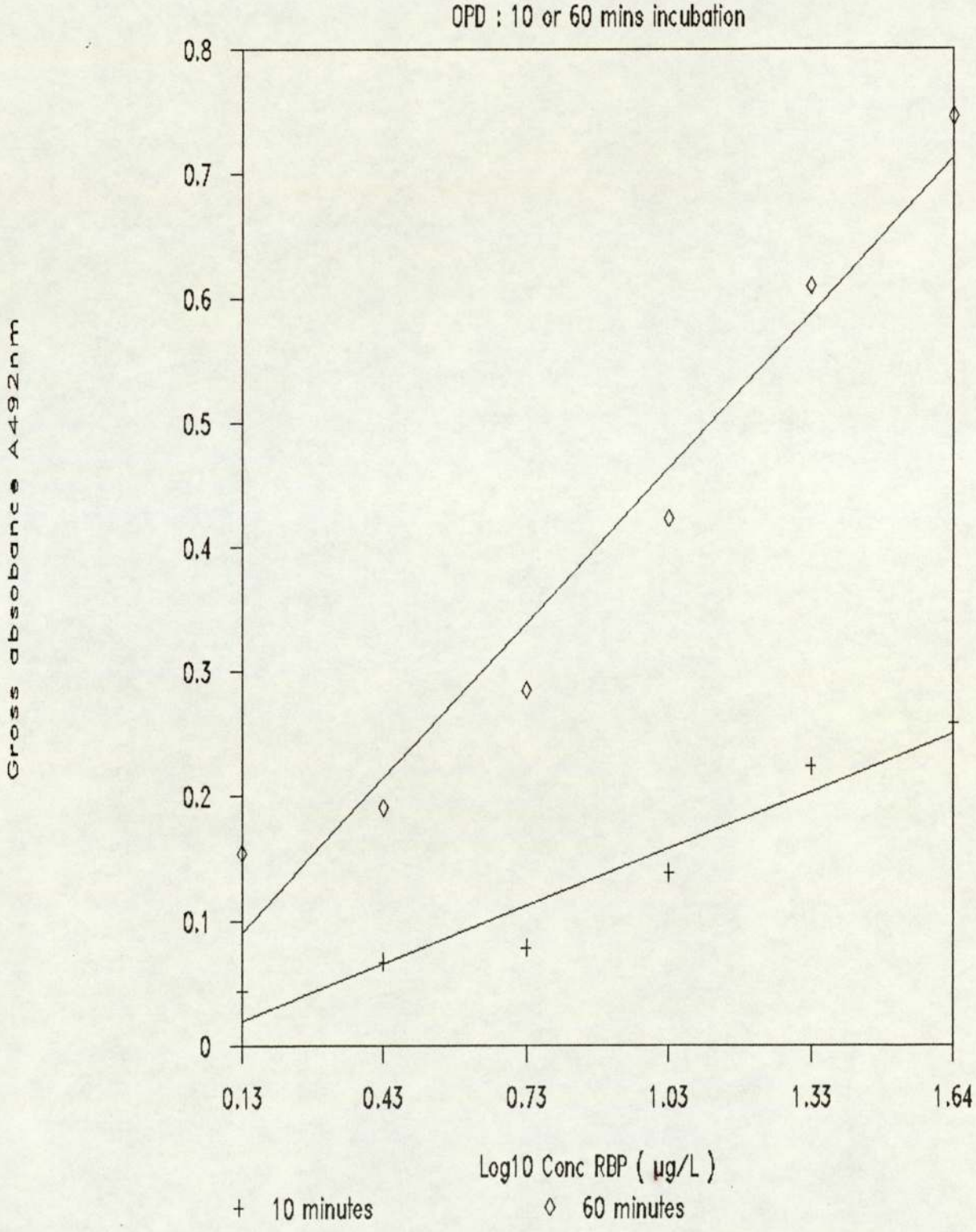
i) Effect of shortening the substrate incubation time.

The initial substrate used as described in the original assay [Dawson, 1986] was O-phenylendiamine (OPD). However, as OPD is degraded in the presence of light it was decided to use a shorter substrate incubation time in an attempt to reduce the influence of light on OPD peroxidation in the control wells. An incubation time of 10 minutes was tried as this had been used by other workers (personal communication, G. Beards, W.H.O. labs, East Birmingham Hospital) and had been found to allow full colour development whilst reducing spontaneous OPD breakdown.

The effect of substrate incubation time on the ELISA was determined by performing comparative titrations using the origional assay [Dawson, 1986] and plotting curves of optical density (A_{492nm}) versus RBP concentration for each of the two incubation times. For this determination the antigen was pure RBP.

The resulting calibration curves are shown in Fig 2. Although the control value was reduced using the shorter incubation time, the gradient of the

Fig 2 : RBP calibration curve



calibration curve was also greatly reduced. This results in a much smaller difference in the optical density (A_{492nm}) between low and high concentrations of RBP and hence a decrease in accuracy.

ii) Effect of changing the substrate.

OPD has two major disadvantages for use in a routine quantitative assay. It breaks down rapidly in light and it is a carcinogen. Another substrate for the peroxidase reaction, 3,3',5,5'-tetramethylbenzidine (TMB) is light stable and is thought to be less carcinogenic. It was thought that this greater light stability would result in a decrease of the optical density (A_{492nm}) of the control wells and so therefore improve the accuracy of the assay. On this basis it was decided to assess the suitability of this compound as a substrate for the assay.

The assay was performed as above using a substrate incubation time of 10 minutes [Beards et al, 1984] but the slope of the calibration curve was very shallow. To determine the optimum time for substrate incubation the assay was performed as described above using eight sets of duplicate doubling dilutions with a control column not containing RBP. The difference in absorbance

with time was assessed by stopping the colour development in each set of dilutions at 10 minute intervals. Optical density (A_{492nm}) versus RBP concentration was plotted for each incubation time and the results are shown in Fig 3a and Fig 3b.

It can be seen that an incubation time of 10 minutes gave a calibration curve with a gradient substantially lower than for other incubation times. An incubation time of 30 or 40 minutes gave a calibration curve with the greatest gradient and hence the greatest difference in optical density between low and high concentrations of RBP. For all of these incubation times the absorbance of the control wells was not significantly different to the absorbance seen using OPD as the substrate.

The intraassay coefficient of variation was determined for the ELISA assay, by measuring the concentration of RBP in ten samples at four different dilutions of pure RBP standard. The mean intraassay coefficient of variation was 16%.

The interassay coefficient of variation was determined for the ELISA method, by measuring the concentration of RBP in four different dilutions of pure RBP standard in 5 ELISA assays (performed on different days). The mean interassay coefficient of variation was 12%.

Fig 3a : RBP calibration curve

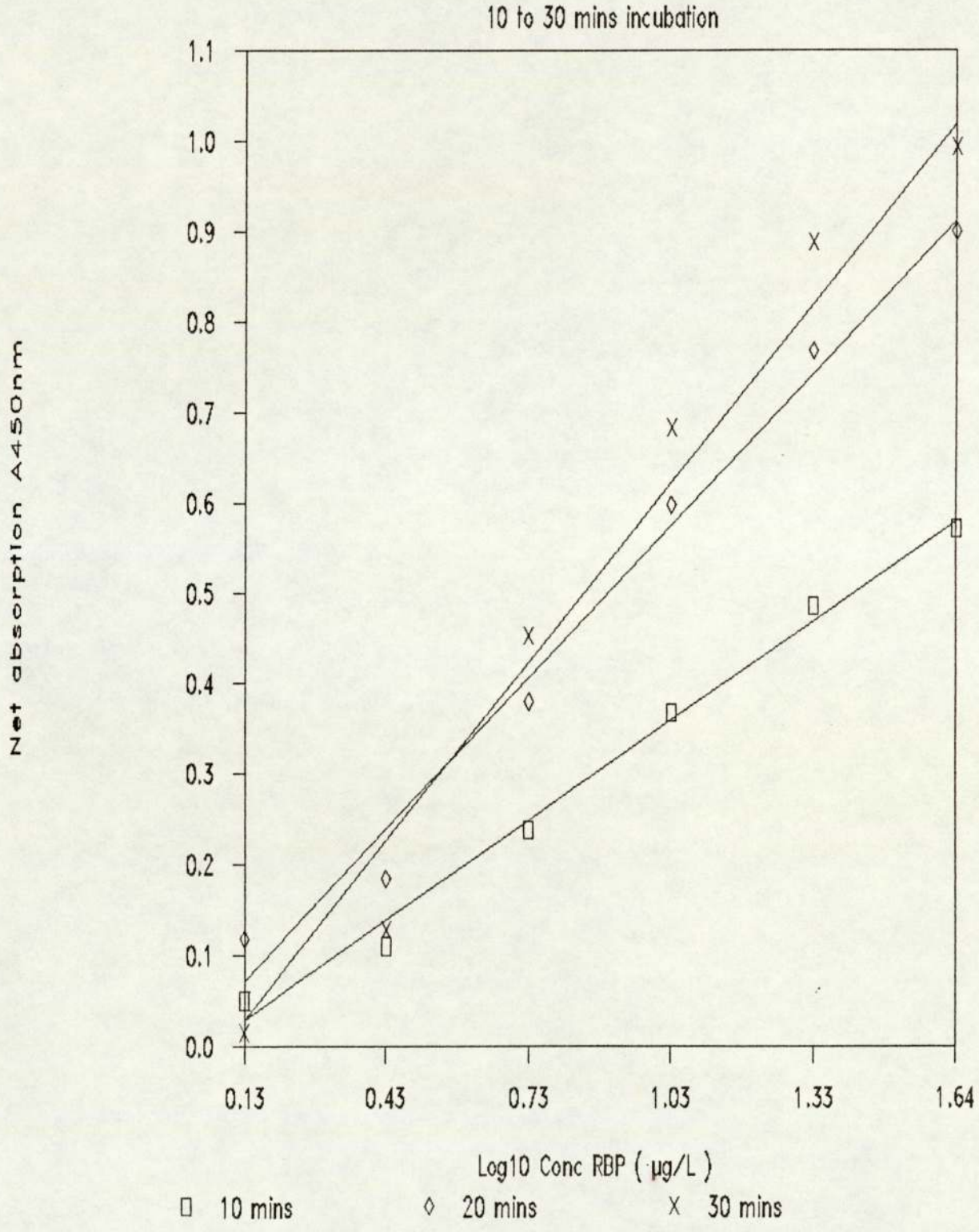


Fig 3b : RBP calibration curve

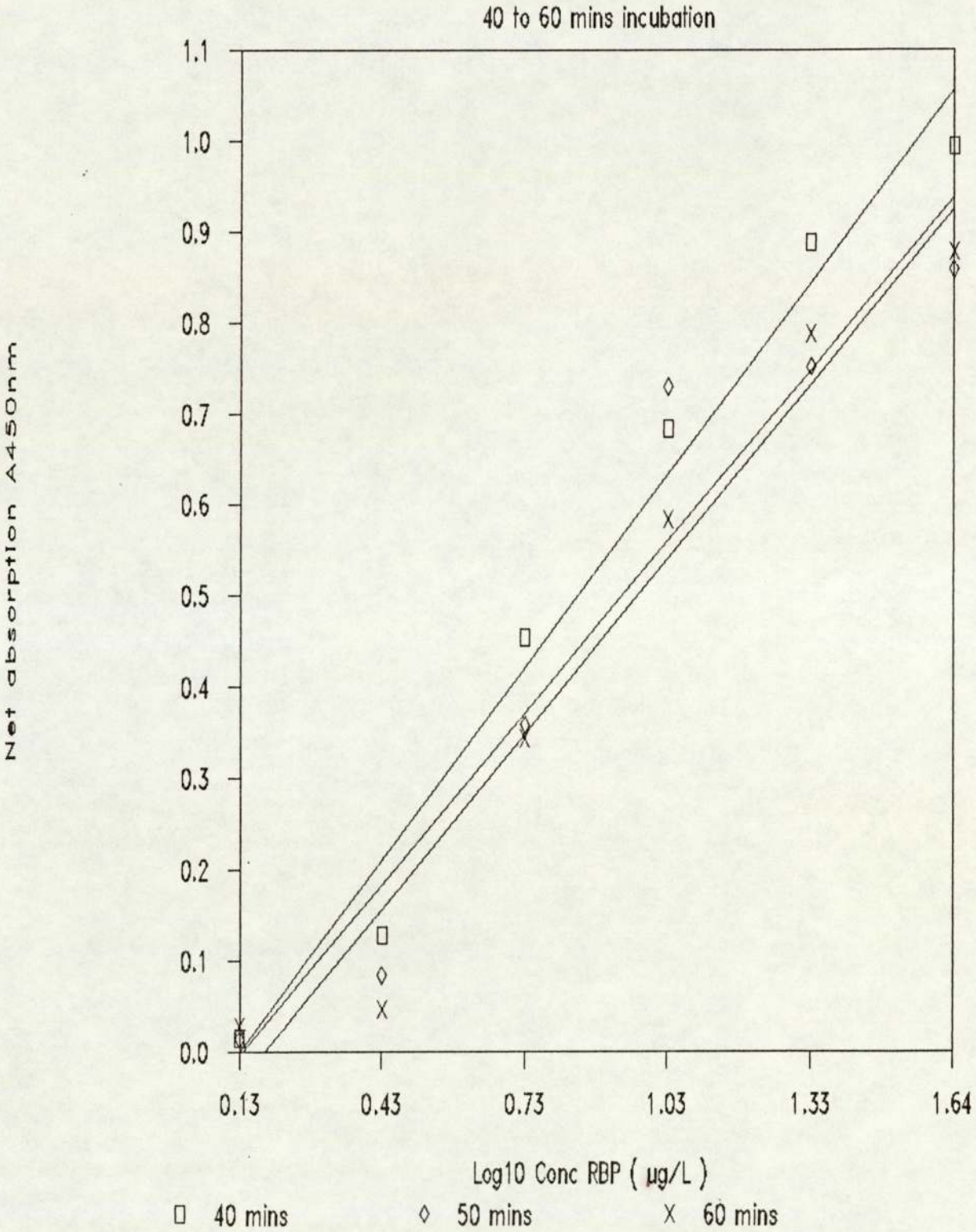
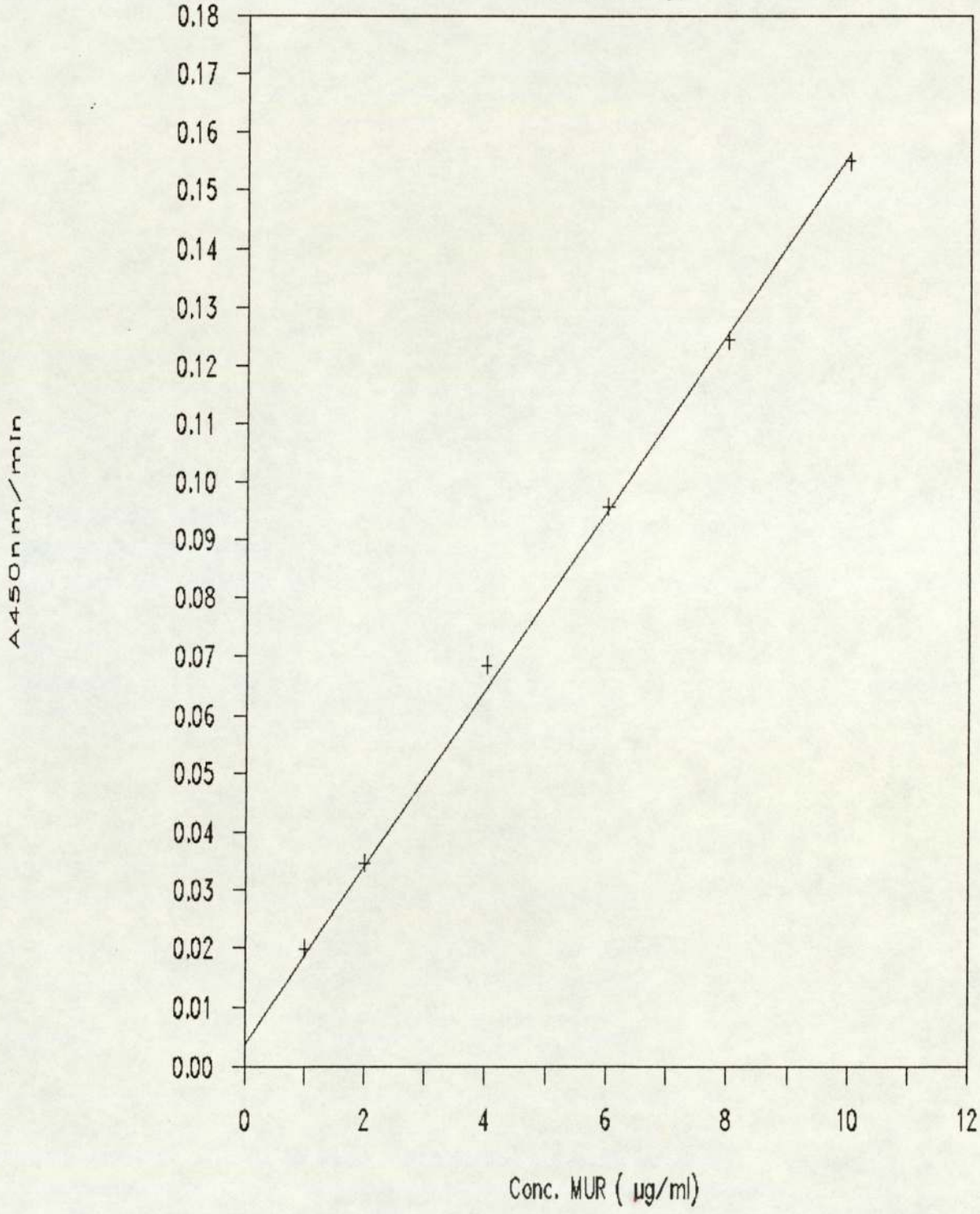


Fig 4 : Typical calibration curve for the MUR assay.



From these studies it can be seen that TMB offered no advantages over OPD regarding the accuracy of the assay. It was however decided to use TMB as the substrate for the assay since it was possible to reduce the incubation time and simpilify the conduct of the assay due to the light stability of the compound. The reduced potential for carcinogenicity of TMB would also minimise any risk to personnel performing the assay.

2) The muramidase assay

Muramidase was assayed by the turbidometric method of Harrison et al [1973]. This is a modified version of the assay originally developed by Boasson [1938] and is based on the optical clearing resulting from lysis of the sensitive organism Micrococcus lysodeikticus.

A) Assay procedure

A suspension of Micrococcus lysodeikticus (250 ug ml^{-1}) was freshly prepared in buffer E and kept on ice. Standard solutions of lysozyme in sodium phosphate buffer within the range 1.0 to 10ugml^{-1} were also made freshly. Serum samples were diluted 1 in 8 to avoid the inhibitory effect of macromolecules in serum. Urine samples were diluted as required. All dilutions were made immediately before use in buffer E. All solutions were kept on ice.

The assay was performed in duplicate. Samples of 100ul were added to 1ml of substrate suspension which had been incubated at 37°C for 2 minutes and the reaction was allowed to proceed at this temperature. Optical densities at 450nm were measured at 0.5 minutes and 5 minutes. The difference in the optical density at these time periods was referred to a standard curve constructed from the standards containing egg white lysozyme in buffer.

The results were calculated using linear regression analysis and were expressed as $\mu g ml^{-1}$ egg white lysozyme. A typical calibration curve is shown in Fig 4. Since human lysozyme has approximately 1.5 times the activity of egg white lysozyme, the figures do not represent true concentrations.

3) The creatinine assay.

Creatinine was determined using a kit bought from the Sigma Chemical Company Ltd.

The assay is based on the Jaffe reaction, where a yellow/orange colour forms when creatinine is treated with alkaline picrate.

4) Determination of calcium and magnesium.

A) Calcium

For calcium determination all serum samples were diluted 1 in 100 and urine samples were diluted 1 in 500. The atomic absorption instrument was adjusted to a zero reading using 0.1% acidified lanthanum chloride in distilled water and all dilutions were prepared in this solution. An air/acetylene flame was used and determination made at a wavelenght of 422.7nm. The sensitivity was 0.08ug/ml and the calibration curve was linear up to a calcium concentration of 5ug/ml. A one point calibration of 5ug/ml was used. The standard used to give this calibration was made up from a combined calcium/magnesium stock solution containing $Ca(NO_3)_2$ and $Mg(NO_3)_2$ atomic absorption standards (1mg/ml and 10mg/ml respectively) in 0.1% acidified lanthanum chloride. All glassware was washed, rinsed in distilled water, and dried thoroughly before use.

B) Magnesium

For magnesium determination, all samples were diluted as for calcium. An air/acetylene flame was used and determinations were made at a wavelength of 222.3nm. The sensitivity of the determination was 0.007ug/ml and the absorbance was linear with respect to concentration

up to a magnesium concentration of 0.5ug/ml. The atomic absorption instrument was sdjusted to zero using 0.1% acidified lanthanum chloride in distilled water and a one point calibration curve of 0.5ug/ml was used. The standard used to provide this calibration was made up from same combined calcium/magnesium stock solution used to calibrate the calcium determination.

CLINICAL RESULTS.

1) Introduction.

Twenty seven patients were originally entered into the study. Eleven of these were subsequently excluded for the reasons shown in Table 1 :

Table 1 : Reason for exclusion from study

Reason for exclusion	Number of patients
No iv antibiotic therapy given	5
Inability to comply with protocol	2
Insufficient data	4

This left 16 patients who received a total of twenty two courses of antibiotic treatment. There were 10 courses of aminoglycoside therapy and 12 courses of control therapy. Patients were allocated a code number consecutively on entry to the study but patients who did not fit the criteria for the study were later excluded. This resulted in some numbers not appearing in the list. Where patients received more than one antibiotic course, the course is indicated by .1, .2 etc. Tables 2a and 2b show the clinical details for the aminoglycoside treated patients and the control group respectively. In tables 3a and 3b are listed the reasons why antibiotic therapy was prescibed. They also give a summary of any potentially nephrotoxic therapy given prior to or during antibiotic treatment.

Table 2a : Clinical details for the patients who received treatment with aminoglycoside antibiotics.

Patient course	Age	Sex	Diagnosis	Antibiotic therapy	Duration (days)
1.1	19	M	ALL	Gentamicin	7
1.2	20	M	ALL	Gentamicin	9
3	67	F	SABE	Gentamicin	16
4.1	55	M	AML	Gentamicin	6
5.2	28	F	AML	Gentamicin	10
5.3	28	F	AML	Gentamicin	9
8	27	M	CF	Tobramycin	10
14.1	34	F	ALL	Gentamicin	10
20	45	M	SABE	Gentamicin	27
29	22	F	CF	Gentamicin	12

M = Male
F = Female
ALL = Acute lymphoblastic leukaemia
AML = Acute myeloid leukaemia
SABE = Sub-acute bacterial endocarditis
CF = Cystic fibrosis

Table 2b : Clinical details for the patients who received treatment with antibiotics other than aminoglycosides.

Patient course	Age	Sex	Diagnosis	Antibiotic therapy	Duration (days)
4.2	55	M	AML	Thienamycin	8
5.1	28	F	AML	Thienamycin	7
6	49	F	NHL	Thienamycin	11
9	63	M	AML	Thienamycin	15
14.2	34	F	ALL	Thienamycin	8
18	53	M	MYELO	Penicillin G	6
21	33	M	AA	Cipro/Vanc	8
22	23	F	Hodgkins	Cipro/Vanc	7
23	27	M	CF	Ceftazidime	17
24.1	57	M	AML	Thienamycin	12
24.2	57	M	AML	Cipro/Vanc	8
27	62	M	AML	Thienamycin	12

M = Male
F = Female
ALL = Acute lymphoblastic leukaemia
AML = Acute myeloid leukaemia
CF = Cystic fibrosis
NHL = Non hodgkins lymphoma
Hodgkins = Hodgkin's disease
Cipro/Vanc = A combination of ciprofloxacin and vancomycin.
MYELO= myelodysplastic syndrome

Table 3a : Aminoglycoside-treated patients - Reason for antibiotic treatment, and summary of any potentially nephrotoxic therapy given.

Patient course	Reason for antibiotic therapy	Other potentially nephrotoxic therapy	Day(s)$ given
1.1	Neutropenia	TBI	-10
1.2	UTI	None	
3	Septicaemia	Frusemide	1 to 40
4.1	Neutropenia	DAT 2 + 7	-14,-13
5.2	Neutropenia	DAT 3 + 10	-21,-20,-19
5.2		DAT 2 + 7*	13,14
5.3	Neutropenia	DAT 2 + 7*	-15,-14
8	Chest infection	None	
14.1	Neutropenia	MOPP	3
20	Septicaemia	Frusemide	1
29	Chest infection	None	

$ - indicates days of administration relative to first day of aminoglycoside therapy (Day 1).
* - Same course of treatment.
UTI - Urinary tract infection
TBI - Total body irradiation
MOPP - Combination of daunorubicin, vincristine, asparaginase and prednisolone.
DAT - Combination of daunorubicin, cytosine and 6-thioguanine. The first digit indicates the duration of daunorubicin treatment. The second digit indicates the duration of cytosine and 6-thioguanine treatment.

Table 3b : Control patients - Reason for antibiotic treatment, and summary of any potentially nephrotoxic therapy given.

Patient course	Reason for antibiotic therapy	Other potentially nephrotoxic therapy	Day(s)$ given
4.2	Neutropenia	DAT 2 + 7	-42,-41,-10,-11
5.1	Neutropenia	DAT 3 + 10	-11,-10.-9
6	Neutropenia	CHOP	-2
9	Septicaemic	DAT 1 + 5	-11
9		DAT 1 + 5	3
14.2	Wound infection	MOPP	-27
18	Neutropenia	TBI	-11
18		Cyclophosphamide	-17
21	UTI	None	
22	Neutropenia	BEAM	-19
23	Chest infection	None	
24.1	Neutropenia	DAT 1 + 5*	-11
24.1		DAT 1 + 5#	1
24.1		DAT 1 + 5!	7
24.2	Neutropenia	DAT 1 + 5*	-24
24.2		DAT 1 + 5#	-14
24.2		DAT 1 + 5!	-6
27	UTI	DAT 1 + 5	-4
27		DAT 1 + 5	10

Table 3b : continued

$ - indicates days of administration relative to first day of aminoglycoside therapy (Day 1).

*, #, ! - Indicates same course of treatment.

UTI - Urinary tract infection

TBI - Total body irradiation

CHOP - Combination of cyclophosphamide, doxorubicin, vincristine and prednisolone.

BEAM - Combination of carmustine, melphelan and cytosine.

DAT - Combination of daunorubicin, cytosine and 6-thioguanine. The first digit indicates the duration of daunorubicin treatment. The second digit indicates the duration of cytosine and 6-thioguanine treatment.

The day on which a patient started therapy is expressed as Day 1. All subsequent days are numbered consecutively from this. The days before therapy are indicated by negative signs with Day -1 being the day immediately before treatment. It was intented to collect samples from at least the day before therapy but this was not always possible because of the problems of identifying patients likely to enter the study. Where the term initial data is used in this study it relates to the closest day prior to commencement of antibiotic therapy when samples were taken or to the earliest day in therapy when samples were taken. Since some study patients were discharged immediately following antibiotic treatment, it was not always possible to collect post treatment data.

Table 4 : Demographic data for the aminoglycoside-treated patients and the control group.

Parameter	Aminoglycoside	Control
No of antibiotic courses	10	12
Males	5	8
Females	5	4
Age (years)*	34.5 ± 16.1	45.1 ± 14.9
Duration of therapy (days)*	11.4 ± 5.5	9.9 ± 3.5

* expressed as mean ± standard deviation.

There were no significant differences between patients given aminoglycosides and other intravenous antibiotics in age, sex or duration of therapy.

It was not possible to make daily serum measurements in the patients with cystic fibrosis as they were unwilling to give daily blood samples. Serum was obtained from blood samples that were drawn for routine measurements by medical staff. Serum data are therefore limited in these patients.

2) Serum creatinine.

The magnitude of the change in the Scr which was taken to indicate a nephrotoxic reaction in this study was defined by Smith et al [1982]. If the initial Scr was less than 265 micromol L^{-1} then nephrotoxicity was indicated by a rise of at least 44 micromol L^{-1}. If the initial Scr was greater than 265 micromol L^{-1} then nephrotoxicity was indicated by a rise of at least 88 micromol L^{-1}. The maximum rise in Scr was determined by subtracting the initial Scr value from the highest Scr measured during therapy or within 48 hours of termination of therapy.

A) Aminoglycoside-treated patients.

Table 5a shows the initial and maximum Scr values in the aminoglycoside-treated patients. No patient showed a toxic rise as defined by Smith et al. There were insufficient data for useful analysis in patient course 1.2. Few data were also available for the two cystic fibrosis patients (patient courses 8 and 29). Scr measurements were available for Days 3 and 5 only in patient course 8. Three Scr measurements were available for patient course 29, on Days 3, 6 and 12. There was no increase in Scr in this patient. The remaining seven patients all showed a rise in Scr.

In patient course 3 there was a Scr on Day 35 of 101.8 micromol L^{-1} indicating possible nephrotoxicity. This occurred 10 days after the gentamicin course had ended. To determine whether this rise was due to a renal cause, a urine to serum creatinine ratio (USCR) was calculated. A USCR of > 40 is diagnostic of a pre-renal cause, a USCR of < 10 is diagnostic of a renal cause, whilst a USCR of between 10 and 40 favours a renal cause for the rise in Scr [Espinal and Gregory, 1980]. In this patient the USCR was calculated to be > 40 so a pre-renal cause was probably responsible for this rise in Scr.

Patient 20 had an elevation in Scr (Table 5a) which

Table 5a : Serum creatine data for the aminoglycoside-treated patients.

Patient course	Initial Scr (micromol L^{-1})	Maximum Scr (micromol L^{-1})	n	Day of maximum
1.1	38.0	53.98	21	15
1.2	59.0	no data	1	
3	55.8	73.5	29	16
4.1	69.0	87.6	15	5
5.2	38.1	62.0	27	10
5.3	44.3	68.1	17	7
8	46.9	53.1	2	5
14.1	47.8	82.3	14	4/12
20	91.2	127.4	23	10
29	73.0	71.0	3	6

n = number of measurements

Table 5b : Serum creatine data for the control patients.

Patient course	Initial Scr (micromol L^{-1})	Maximum Scr (micromol L^{-1})	n	Day of maximum
4.2	60.2	107.1	14	7
5.1	38.9	54.9	10	4
6	53.1	58.4	9	11
9	105.3	132.7	22	11
14.2	52.2	58.4	9	9
18	57.5	69.0	24	9
21	104.4	111.5	5	3
22	57.5	58.4	15	3
23	76.0	75.0	2	5
24.1	84.1	91.2	16	3
24.2	72.6	80.5	10	5
27	66.4	68.1	14	1

n = number of measurements

was nearly toxic by the criteria of Smith et al [1982]. The initial Scr was not measured until Day 4 as the patient was transferred from another hospital. If pre-treatment Scr measurements had been available this patient may have shown a toxic rise. The USCR was 13 favouring a renal cause for this increase in Scr.

The USCR was 20 in patient 14 (course 1), therefore favouring a renal cause for the increase in Scr. In all other patients the USCR was > 40 indicating a pre-renal cause of the increased Scr.

B) Control group.

In the control group, patient 4 (course 2) developed a clinically significant nephrotoxicity by the criteria of Smith et al [1982]. The rise in Scr was transient however and overall there was little change in Scr.

Patient 21 showed a fall of Scr from Day -1 to Day 1 of 57 micromol L^{-1} followed by a rise from Day 1 to Day 2 of 44 micromol L^{-1}. This rise occurred too soon in therapy to be attributable to antibiotic therapy. Furthermore, the USCR of > 40 indicates a pre-renal causation.

The USCR in patients 9 and 14 (course 2) were between 10 and 40, thereby indicating a renal cause for this

increase in Scr. In all the other patients there was only a small rise in serum creatinine (Table 5b). The USCR indicated a pre-renal causation for these rises.

Table 6 shows the mean day of the peak Scr for each group and the mean percentage rise from the initial measurement that this peak represents. It appears from this that the aminoglycoside-treated group showed a larger rise in Scr than the control group.

Analysis of the daily trend in Scr measurements indicates that the use of peak Scr data is misleading.

Table 6 : Summary of the mean (+ S.D.) day of peak Scr and the mean (+ S.D.) percentage rise in Scr for the aminoglycoside-treated and the control group.

	Day of peak Scr measurement (Mean ± S.D.)	Maximum percentage rise in Scr measurement (Mean ± S.D.)
Aminoglycoside patients#	9.0 ± 4.3	36.9 ± 24.9
Control patients	5.8 ± 3.5	20.1 ± 23.0

Does not include patient course 1.2

In the aminoglycoside-treated group only patient 3 showed a steady rise in Scr and in the control group only patient 18 showed a steady rise in Scr. In both of these patients a fall in creatinine clearance was seen. All remaining patients showed either no change in the Scr during the observation period or only a slight elevation of the Scr.

3) Serum Retinol-Binding Protein concentrations.

The reference range for S_{RBP} has been defined in healthy adults as 30 to 60 mg L^{-1} [Bosin and Monji, 1983]. In the present study serum levels of RBP were measured in each patient as near to the peak urinary excretion of RBP as possible. Tables 7a and 7b show the results of the S_{RBP} assays for each study group.

In the aminoglycoside-treated group, only patient 3 had a S_{RBP} greater than 60 mg L^{-1}. In the control group patient 14 (course 2) and patient 22 exceeded this level. The renal threshold for RBP is unknown but earlier work [Dawson, 1986] indicates that it is unlikely to be less than 100 mg L^{-1}.

Four measurements in the aminoglycoside-treated group were below 30 mg L^{-1}. In the control group, 6 measurements were below this level.

Table 7a : Serum RBP levels for the aminoglycoside-treated patients.

Patient course	Serum RBP concentration (mg L^{-1})	Day of measurement
1.1	ND	
1.2	14.9	5
3	38.7	17
	79.3	34
4.1	44.6	5
5.2*	25.2	
5.3*	25.2	
8	22.4	5
14.1	11.2	4
20	35.3	26
29	ND	
Mean (± S.D.)	33.9 ± 21.6	

* data derived from patient course 5.1

ND = Not determined.

Table 7b : Serum RBP levels for the control patients.

Patient course	Serum RBP concentration (mg L^{-1})	Day of measurement
4.2	45.7	-6
5.1	25.2	6
6	59.3	2
	25.9	11
9	17.3	7
14.2	92.9	8
18	16.1	-3
21	32.3	1
22	70.3	-4
23	15.3	5
24.1	17.2	10
24.2	7.2	3
27	15.3	10
Mean ($\pm$ S.D.)	34.6 $\pm$ 26.8	

4) Serum Muramidase concentrations.

The reference range for S_{MUR} has been defined as 5.1 to 14 mg L^{-1} [Harrison et al, 1986]. Similarly to the S_{RBP} determinations, S_{MUR} was assayed as near to the peak urinary excretion as possible but not necessarily in the same samples. Tables 8a and 8b show the results of the S_{MUR} assays for each study group.

Only patient 3 had a S_{MUR} that exceeded 14mg L^{-1}. This occurred at the end of the study period and was only marginally above reference values (15.9mg L^{-1}). Two previous S_{MUR} measurements were normal. The renal threshold for muramidase is reported to be greater than 42 mg L^{-1} [Harrison et al, 1968; Hayslett et al, 1968; Prockop and Davidson, 1964] so this serum level of 15.9mg L^{-1} is unlikely to have affected urinary excretion of muramidase.

Many patients in either group had a S_{MUR} that was less than 5.1mg L^{-1}. In most cases a probable explanation for the low S_{MUR} was evident. Patient 1 (course 1) received bone marrow ablation therapy followed by an autologous bone marow transplant 15 days prior to S_{MUR} measurement. Patient 5 (course 3) and patient 14 (course 1) both received treatment with myelosuppressive chemotherapy (DAT and MOPP respectively) prior to measurement of the S_{MUR}

Table 8a : Serum muramidase levels for the aminoglycoside-treated patients.

Patient course	Serum MUR concentration (mg L^{-1})	Day of measurement
1.1	2.3	5
1.2	ND	
3	10.2	10
3	11.0	17
3	15.9	31
4.1	8.1	5
4.1	10.2	10
5.2	10.4	4
5.3	3.0	1
5.3	5.3	16
8	11.5	5
14.1	1.8	8
14.1	0.9	12
20	9.3	15
29	4.9	6

ND = Not determined

Table 8b : Serum muramidase levels for the control patients.

Patient course	Serum MUR concentration (mg L^{-1})	Day of measurement
4.2	3.0	14
5.1	ND	
6	7.3	11
9	16.4	-2
9	6.9	19
14.2	2.4	5
18	1.5	3
21	3.8	1
22	12.0	6
22	13.4	13
23	ND	
24.1*	0.5	10
24.2*	0.5	-4
27	2.6	6

ND = Not determined

* = Same serum sample

concentration.

In the control group, patients 4 (course 2), 24 (course 1) and 27 all received DAT prior to measurement of the S_{MUR} concentration. Patient 14 (course 2) received other myelosuppressive therapy and patient 18 had a course of bone marrow ablation followed by autologous bone marrow transplantation. In each case these factors could provide an explanation for the lowered S_{MUR}.

For patient courses 21 and 29 there is no apparent explanation for the depressed S_{MUR} levels.

5) Urinary excretion of Retinol Binding Protein.

Both 2 hr and 24 hr urinary collections were made. Although RBP excretion data are available in the literature [Lucertini et al, 1984; Bernard et al 1982], the data were obtained from studies in normal healthy adults. Therefore, for the purposes of this study, the reference range of urinary RBP excretion was calculated from the pre-dose collections in all patients. The results are shown in Tables 9a and 9b together with published normal data for comparison. Bernard et al [1982] measured RBP excretion in 150 healthy subjects by latex immuno assay. Lucertini et al

Table 9a : Table comparing the mean (+ S.D.) 24 hr RBP excretion obtained in this study with published data.

	Urinary RBP excretion * mg / g creatinine
This study	0.33 ± 0.51
Lucertini et al, 1984	0.10 ± 0.04
Bernard et al, 1982	0.05 ± 0.06

* mean ± standard deviation

Table 9b : Table comparing the mean (+ S.D.) 2 hr RBP excretion obtained in this study with published data.

	Urinary RBP excretion * mg / g creatinine
This study	0.25 ± 0.36
Dawson, 1986	0.33 ± 0.35

* mean ± standard deviation

[1984], using an ELISA, measured RBP excretion in 30 healthy subjects. In each case excretion was measured in untimed urine collections. The results in the current study are higher than these two published studies. This may reflect the difference in the method of sample collection or the fact that the patients in this study were ill and receiving nephrotoxic drugs.

The upper limit of normal excretion was calculated as the mean + 2 standard deviations. Hence, for the 2 hr collections the upper limit of normal RBP excretion was 1.0 mg/g creatinine. For the 24 hr collections the upper limit of normal was 1.3 mg/g creatinine. This compares very well with previous work [Dawson, 1986] where the upper limit of normal for a 2 hr excretion was found to be 1.0 mg/g creatinine in a series of relatively well patients awaiting surgery.

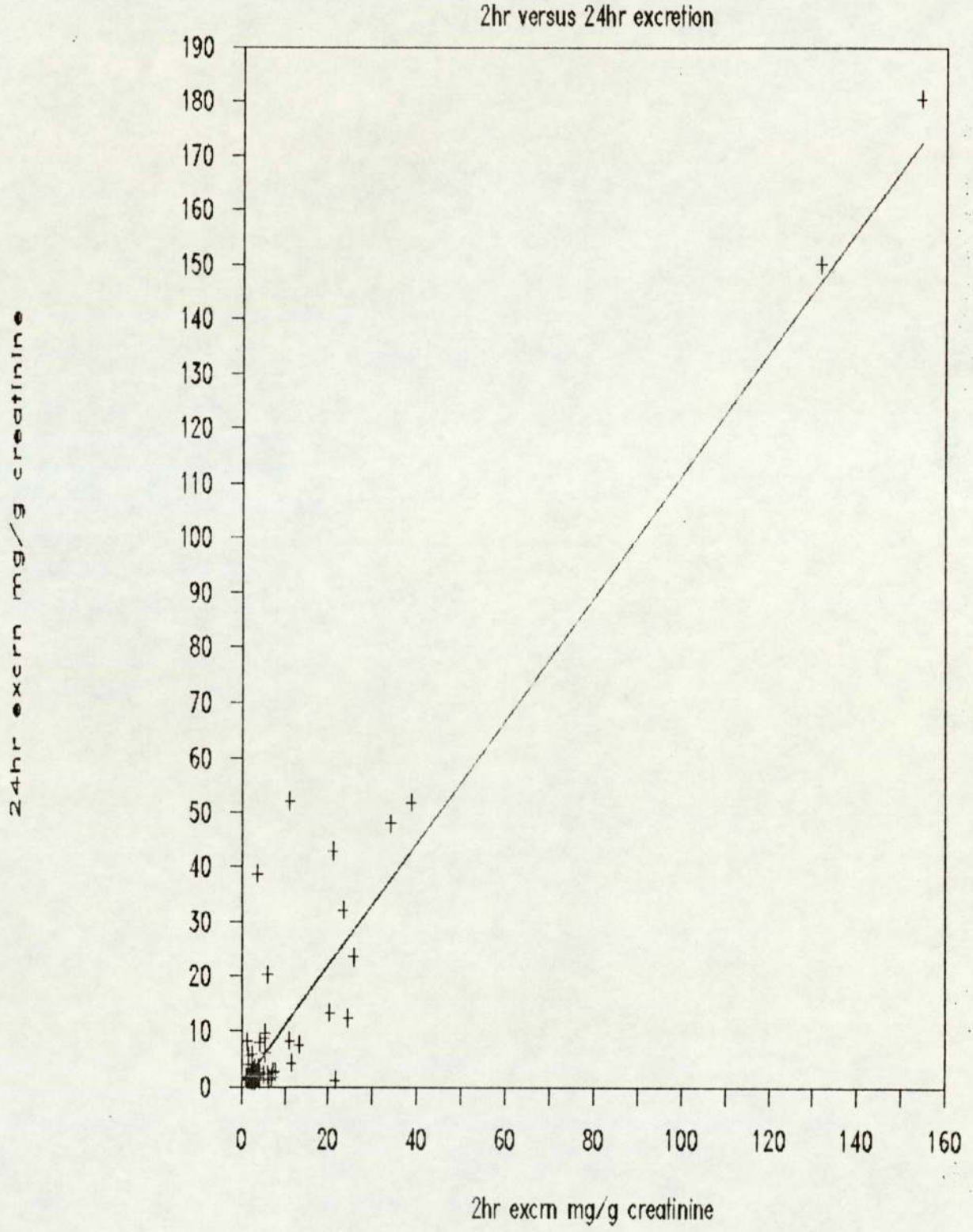
A) Comparison of the 2 hr RBP excretion with the 24 hr RBP excretion.

To see if the 2 hr RBP excretion could be used to predict the 24 hr RBP excretion, a regression analysis was performed. Data from all patients, aminoglycoside-treated and control patients, were used for this analysis and the data set was comprised of all values from those days where both 2 hr and 24 hr collections were available. A total of 316 data points

were compared. The resulting scatter diagram and regression line is shown in Fig 5. The correlation coefficient (r^2) was calculated to be 0.89 indicating an excellent relationship between RBP excretion in the two collection periods. It can be seen from Fig 5 however, that there are two data points which are separated from the main body of data. In order to asses the influence of these outlying data points on the coefficient of determination, the regression analysis was repeated without including them. The correlation coefficient for this analysis was 0.48, indicating a poor relationship between the 2 hr and 24 hr RBP excretion.

This lack of correlation is supported by the data (Tables 12 and 13, and Tables 14 and 15) which suggests that duration of aminoglycoside therapy has an influence on the timing and the magnitude of the 2 hr excretion but not the 24 hr excretion. It can also be illustrated by considering patient 20. Figs 9a and 9b show the 2 hr and 24 hr excretion profiles for RBP in patient 20. The 2 hr excretion shows a prolonged elevation which peaks on Day 11. The 24 hr plot shows no elevation of RBP excretion.

Fig 5 : Correlation curve for RBP



To further study the relationship between the 2 hr and 24 hr excretion periods, a regression analysis was performed on the data for the control patients and the aminoglycoside patients separately.

2 hr excretion data and 24 hr excretion data were compared for all control patients. The resulting scatter diagram and regression line are shown in Fig 6. A total of 143 data points were used for this analysis. The correlation coefficient (r^2) for all data points was 0.89. Similarly to the previous regression analysis there are two data points that differ greatly from the main body of results. The regression analysis was repeated without them. The correlation coefficient was calculated to be 0.47 indicating a poor relationship between the 2 hr and the 24 hr RBP excretion.

The resulting scatter diagram and regression line for the aminoglycoside treated patients is shown in fig 7. A total of 158 data points were used for the analysis and the correlation coefficient was calculated to be 0.50.

It was therefore considered invalid to use the 2 hr RBP excretion as a measure of the 24 hr RBP excretion.

Fig 6 : Correlation curve for RBP

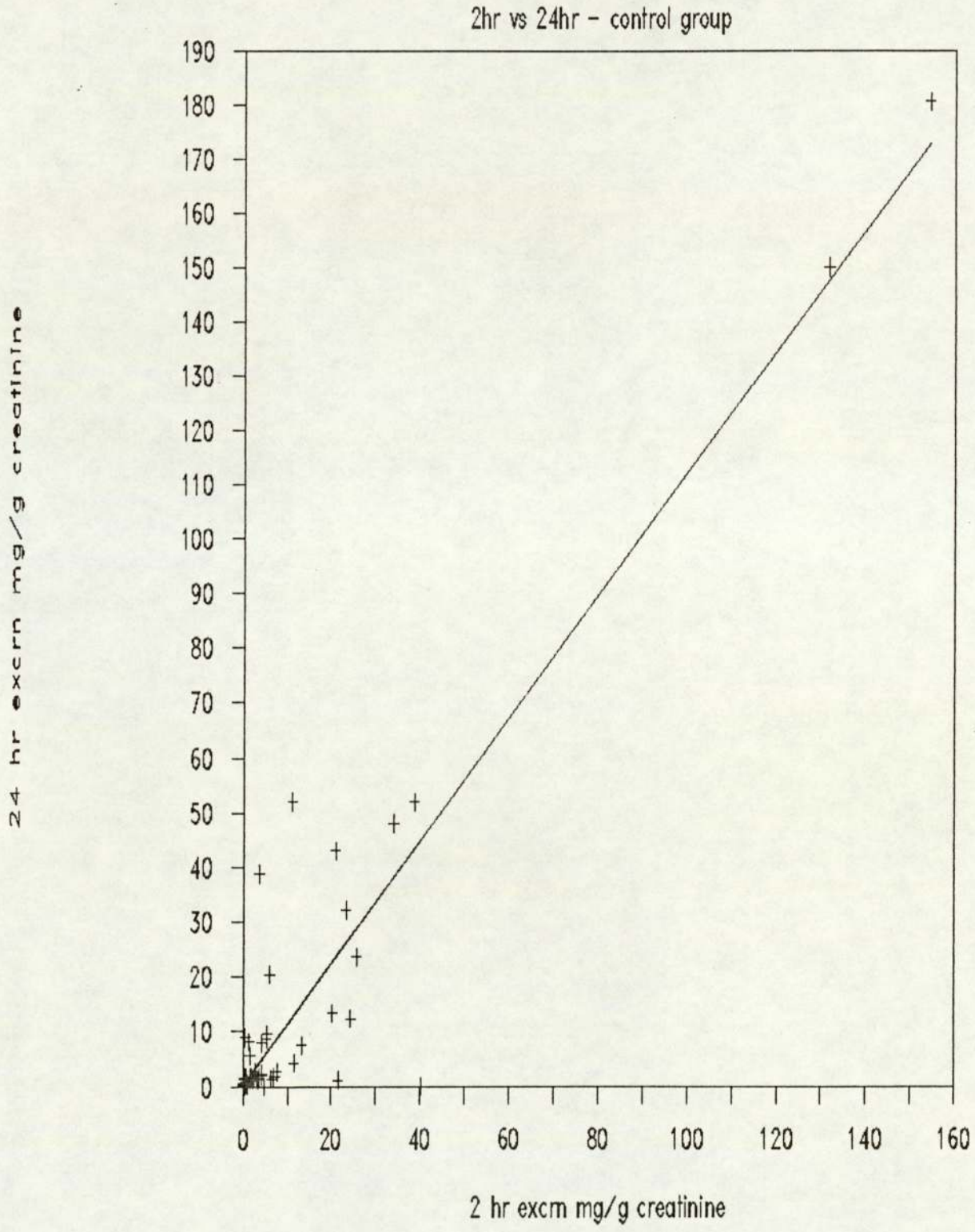
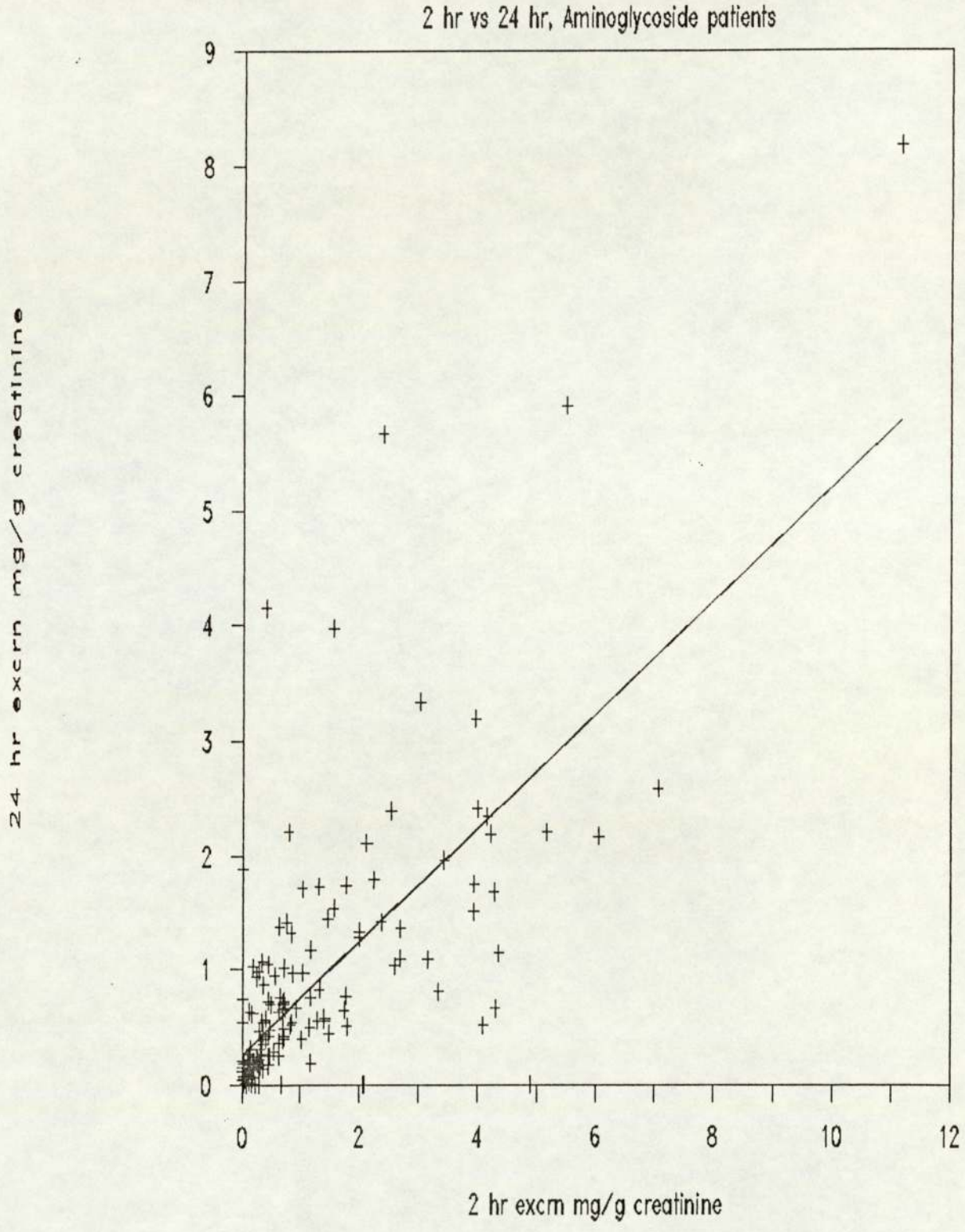


Fig 7 : Correlation curve for RBP



B) Aminoglycoside-treated patients.

i) 2 hr urinary excretion of RBP.

Table 10 shows the distribution of maximum RBP excretion in the aminoglycoside treated patients.

Table 10 : Distribution of maximum 2 hr urinary excretions of RBP in aminoglycoside-treated patients.

Maximum excretion mg/g cr	Number of patient courses	Patient course
=< 1.0	2	5.3, 8
>= 1.0	8	1.1, 1.2, 3, 4.1, 5.2, 14.1, 20, 29

Eight of the ten aminoglycoside-treated patients had a maximum 2 hr RBP excretion above the calculated maximum normal excretion of 1.0 mg/g creatinine. Of these 8, two were thought to be attributable to factors other than aminoglycoside administration. Table 11 shows the maximum 2 hr and 24 hr urinary RBP excretion together with the day on which it occurred. It also indicates where the elevated excretion was considered to be secondary to factors other than aminoglycoside therapy. Where no other causative factor could be identified it

Table 11 : Maximum 2 hr and 24 hr urinary excretion of RBP in aminoglycoside-treated patients.

Patient course	Max 2 hr RBP excrn (mg/g cr)	Day	True peak	Max 24 hr RBP excrn (mg/g cr)	Day	True peak
1.1	5.5	2	N$	5.9	2	N$
1.2	4.3	2	N$	4.0	6	Y
3	9.4	12	Y	4.1	12	Y
4.1#	>=1.2	>=6	Y	>=1.2	>=6	Y
5.2	6.1	6	Y	>=8.6	2	Y
5.3	0.9	2	N*	>=1.4	5	Y
8	0.1	3	N*	0.2	6	N*
14.1	11.2	4	Y	22.9	3	Y
20	4.1	11	Y	1.9	18	Y
29	1.3	7	N*	0.8	4	N*

True peak = Peak likely to be due to aminoglycoside therapy.
* No peak in excretion profile seen
$ Peak secondary to other factors.
# No data for days 7 to 11.

was considered likely that the increased RBP excretion was due to aminoglycoside therapy.

In patient course 1.1 the maximum 2 hr urinary RBP excretion occurred very early in therapy (Day 2). Moreover, the build up to this peak started during the pre-dose phase and returned rapidly to a normal value during aminoglycoside treatment. It was considered that another factor, possibly a pyrexia or the total body irradiation may have caused this peak. In patient 1 (course 2) the maximum excretion of RBP also occurred early in therapy (Table 11). No pre-dose excretion data are available, but all subsequent excretion levels were lower than this. It was considered that this early abnormally high RBP excretion was secondary to factors other than aminoglycoside administration.

Patients 5 (course 3), 8 and 29 did not show a peak in the excretion profile at all. The maximum excretion shown is the highest excretion seen in a series of normal, or in patient 29 slightly high, excretions.

Table 12 shows the relationship between the duration of therapy and the maximum excretion. Those excretions which were considered not to be secondary to aminoglycoside therapy were not included. It can be seen from this that the longer the duration of

Table 12 : Relationship between duration of aminoglycoside therapy and maximum 2 hr RBP excretion.

Duration of therapy (days)	n	Maximum 2 hr RBP excretion *	Patient course
< 10	2	1.06 ± 0.17	4, 5.3
10 - 15	4	4.66 ± 5.04	5.2, 8, 14, 29
> 15	2	6.75 ± 3.75	3, 20

n = number of patient courses

* expressed as mean (± S.D.) in mg/g creatinine

aminoglycoside therapy, the higher the maximum 2 hr excretion of RBP.

The relationship between the duration of aminoglycoside therapy and the day of the maximum 2 hr RBP excretion is shown in table 13. It appears that the longer the duration of therapy, the later the maximum excretion of RBP is seen.

Table 13 : Relationship between the day of the maximum 2 hr RBP excretion and the duration of aminoglycoside therapy.

Duration of therapy (days)	n	Day of max. excretion (mean ± S.D.)	Patient course
<10	2	4.0 ± 2.8	4, 5.3
10 - 15	4	5.0 ± 1.8	5.2, 8, 14, 29
> 15	2	11.5 ± 0.7	3, 20

n = number of patient courses

ii) 24 hr urinary excretion of RBP.

Table 14 shows the distribution of the maximum 24 hr RBP excretions in aminoglycoside-treated patients.

In seven of the 10 patient courses there was a maximum 24 hr RBP excretion above the maximum normal excretion for this study group (1.3 mg/g creatinine). Both of the cystic fibrosis patients (patients 8 and 29) showed no elevation in the 24 hr RBP excretion. Table 11 shows the maximum excretion measured for each patient

Table 14 : Distribution of the maximum 24 hr RBP excretion in aminoglycoside-treated patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 1.3	3	4, 8, 29
1.3 - 10	6	1.1, 1.2, 3, 5.2, 5.3, 20
> 10	1	14

and the day on which that excretion occurred. It also indicates where the maximum excretion was considered to be due to aminoglycoside therapy. It can be seen that there is generally a very good correspondence between the 2 hr and 24 hr maxima, although there is some variability on the day the maximum excretion is seen.

Patient 1 (course 1) had a very early peak in the excretion profile (Table 11). As with the 2 hr profile, the increase in RBP excretion began before aminoglycoside administration and returned rapidly to a normal value during therapy. Similarly, it was thought that this peak was secondary to a pyrexia or total body irradiation. The following analysis does not therefore

include this patient course.

There appears to be no relationship between the duration of therapy and the magnitude of the maximum 24 hr RBP excretion as shown in Table 15.

Table 15 : Relationship between duration of aminoglycoside-therapy and maximum 24 hr RBP excretion.

Duration of therapy (days)	n	Maximum 24 hr RBP excretion *	Patient course
< 10	3	2.2 ± 1.6	1.2, 4, 5.3
10 - 15	4	8.1 ± 10.6	5.2, 8, 14.1, 29
>15	2	3.7 ± 0.7	3, 20

* expressed as mean ± standard deviation

n = number of patient courses

Table 16 examines the relationship between duration of therapy and the time of maximum RBP excretion. There appears to be no relationship between these two parameters for the 24 hr RBP collection.

Table 16 : Relationship between the day of the maximum 24 hr RBP excretion and the duration of aminoglycoside therapy.

Duration of therapy (day)	n	Day of max. excretion (mean ± SD)	Patient course
< 10	3	4.3 ± 2.1	1.2, 4, 5.3
10 - 15	4	3.8 ± 1.7	5.2, 8, 14.1, 29
> 15	2	15.0 ± 4.2	3, 20

C) Control patients.

i) 2 hr urinary Retinol Binding Protein excretion.

Table 17 shows the distribution of maximum 2 hr RBP excretions in control patients.

Table 17 : Distribution of maximum 2 hr urinary excretions of RBP in control patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
< 1.0	3	4.2, 14.2, 23
1.0 - 10	5	18, 21, 22, 24.1, 27
> 10	4	5.1, 6, 9, 24.2

Nine of twelve patients had a maximum 2 hr RBP excretion above the reference range. In two of these, patients 18 and 22, the maxima were seen in the pre-dose phase. No elevations were subsequently seen during the treatment period in these patients. In patients 21 and 24 (course 2) the maximum excretions were seen early in the course of antibiotic therapy (Table 18) and the

increase in excretion had started during the pre-dose phase. Again, no subsequent elevations were seen in these patients. The peaks in excretion in these patients were considered secondary to other factors.

Table 18 : Maximum 2 hr and 24 hr urinary excretion of RBP in control patients.

Patient course	Max 2 hr RBP excrn (mg/g cr)	Day	True peak	Max 24 hr RBP excrn (mg/g cr)	Day	True peak
4.2	0.4	1	N	0.2	2	N
5.1	21.6	6	N	1.6	10	Y
6	154.2	10	Y	180.6	10	Y
9	41.1	8	Y	52.0	6	Y
14.2	0.3	2/9	N	0.3	7	N
18	1.1	2	N	0.2	15	N
21	2.5	1	N	1.6	1	N
22	1.9	1	N	1.3	1	N
23	0.2	16	N	0.1	15	N
24.1	7.7	10	Y	8.5	8	Y
24.2	11.5	3	N	4.2	3	N
27	6.0	12	Y	20.3	12	Y

True peak = Peak likely to be due to antibiotic therapy.

Patients 4 (course 2), 14 (course 2) and 23 had only very low excretions throughout the study period. No pre-dose data were available for patients 14 (course 2) and 23. However, data from patient 14 (course 1), in whom collections ended just 8 days prior to course 2, indicate that RBP excretion was normally very low.

In the control group, five patients had an abnormally high 2 hr RBP excretion not attributable to antibiotic therapy. Three patients showed no elevation in RBP excretion.

The mean ($\pm$ SD) pre-dose RBP excretion was 2.48 $\pm$ 5.69 mg/g creatinine and the mean ($\pm$ SD) maximum excretion was 17.78 $\pm$ 40.91. This occurred on Day (mean $\pm$ SD) 6.0 $\pm$ 4.7 of therapy. The pre-dose excretion for this group was therefore above the reference range, although much of this increase was because of the high initial excretion seen in patient 6, and the peaks in excretion seen during the pre-dose phase in patients 18, 21, 22, and 24 (course 2). If the data for these patients is not included in the analysis then the pre-dose excretion for the group (mean $\pm$ SD) is 0.26 $\pm$ 0.33 mg/g creatinine. The reason for the high initial excretion of RBP in patient course 6 is not known.

Table 19 relates the maximum 2 hr RBP excretion to the

duration of antibiotic therapy and Table 20 relates the day of this maximum excretion to the duration of therapy. Patients 18, 21, 22 and 24 (course 2) were not included in this analysis for the reasons given above.

Table 19 : Relationship between duration of antibiotic course and maximum 2 hr RBP excretion in control patients.

Duration of therapy (days)	n	Maximum 2 hr RBP excretion *	Patient course
< 10	3	7.4 ± 12.3	4.2, 5.1, 14.2
10 - 15	3	56.0 ± 85.1	6, 24.1, 27
>= 15	2	20.7 ± 28.9	9, 23

* Mean ± S.D.

n = number of patient courses

There is no relationship between the duration of the antibiotic course and the maximum 2 hr RBP excretion.

Table 20 : Relationship between the duration of the antibiotic course and the day of the maximum 2 hr RBP excretion in control patients.

Duration of therapy (days)	n	Day of max. excretion (mean ± SD)	Patient course
< 10	3	4.5 ± 3.7	4.2, 5.1, 14.2
10 - 15	3	10.7 ± 1.1	6, 24.1, 27
>= 15	2	12.0 ± 5.7	9, 23

n = number of patient courses

From this it appears that the longer the duration of antibiotic therapy, the later the maximum 2 hr excretion of RBP is seen.

ii) 24 hr retinol-binding protein excretion.

Table 21 shows the distribution of the 24 hr RBP excretions in the control group.

Table 21 : Distribution of maximum 24 hr RBP excretion in the control group.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 1.3	5	4.2, 14.2, 18, 22, 23
1.3 - 10	4	5.1, 21, 24.1, 24.2
> 10	3	6, 9, 27

Seven of the control group showed a RBP excretion above the reference range. Similarly to the 2 hr data, patients 18, 21, 22 and 24 (course 2) all showed maxima in the RBP excretion during the pre-dose phase and so could not be attributed to the antibiotic treatment. Subsequent excretion measurements in these patients were below 1.3 mg/g creatinine.

The mean pre-dose excretion was 1.7 ± 3.7 mg/g creatinine. If the pre-dose data for patients where pre-dose maxima in the RBP excretion were seen, and patient 6 are excluded then the mean (± SD) pre-dose excretion was 0.33 ± 0.41.

Tables 22 and 23 relate the duration of therapy to the maximum 24 hr RBP excretion and the day of that maximum excretion.

Table 22 : Relationship between duration of antibiotic therapy and maximum 24 hr RBP excretion in control patients.

Duration of therapy (days)	n	Maximum 24 hr RBP excretion *	Patient course
< 10	3	0.7 ± 0.7	4.2, 5.1, 14.2
10 - 15	3	69.8 ± 96.1	6, 24.1, 27
>= 15	2	26.1 ± 36.8	9, 23

n = number of patient courses
* = Mean ± SD as mg/g creatinine

There appears to be no relationship between the duration of the antibiotic course amd the level of the maximum RBP excretion.

Table 23 : Relationship between the day of the maximum 24 hr RBP excretion and the duration of control therapy.

Duration of therapy (days)	n	Day of max. excretion (mean ± SD)	Patient course
< 10	3	6.3 ± 4.0	4.2, 5.1, 14.2
10 - 15	3	10.0 ± 2.0	6, 24.1, 27
>= 15	2	10.8 ± 6.7	9, 23

n = number of patient courses

It appears from this, that the longer the duration of therapy, the later the maximum 24 hr RBP excretion is seen. The variance is however very large and it is difficult to draw any conclusions from these data.

D) Comparison of the aminoglycoside-treated group with the control group.

In the aminoglycoside-treated group there was a positive relationship between the duration of therapy and both the timing and magnitude of the peak 2 hr RBP excretion. This relationship was lost for the 24 hr RBP

excretion. In the control group there was no relationship for either excretion period between the duration of therapy and the magnitude of the RBP excretion. A positive relationship was seen between the duration of therapy and the timing of the maximum excretion.

The incidence of elevated RBP excretion was similar in the aminoglycoside-treated and the control group. In the aminoglycoside-treated group, 50% of the patient courses showed an elevated 2 hr and 24 hr excretion. In the control group, 42% of patient courses showed an elevated 2 hr and 24 hr RBP excretion. It is also of interest that the largest RBP excretions were seen in the control group.

In order to analyse the data more fully it was decided to examine RBP excretion in the different clinical groups.

i) RBP excretion in patients with sub-acute bacterial endocarditis.

Two patients with SABE were studied (patients 3 and 20). Both were treated with gentamicin. Patient 3 received 2.1g of gentamicin over a 16 day period and patient 20 received 4g of gentamicin over a 27 day period.

Fig 8a and Fig 8b show the 2 hr and 24 hr excretion profiles for patient 3. Definite peaks in both the 2 hr and 24 hr excretion were seen. The 2 hr RBP excretion profile is mirrored very closely by the 24 hr RBP excretion. The magnitute of the 24 hr excretion is about half that of the 2 hr excretion. The RBP excretion for both collection periods rapidly fell to a low level after the gentamicin administration was stopped on Day 16. The post-dose excretion levels show that the RBP excretion remained at normal levels.

Fig 9a and Fig 9b show the 2 hr and 24 hr RBP excretion profiles for patient 20. The 2 hr excretion shows a prolonged elevation which peaks on Day 11 or possibly on Day 12 (no samples available for this day). The 24 hr plot shows only a transient and slight elevation of RBP excretion. The elevated RBP excretion in the post-dose phase coincided with some dental treatment, involving intravenous vancomycin administration, received by this patient.

Thus, both patients receiving gentamicin for the treatment of SABE, showed definite peaks in the 2 hr excretion profile. The elevated RBP excretion in these patients was not associated with significant rises in Scr.

Plate 1

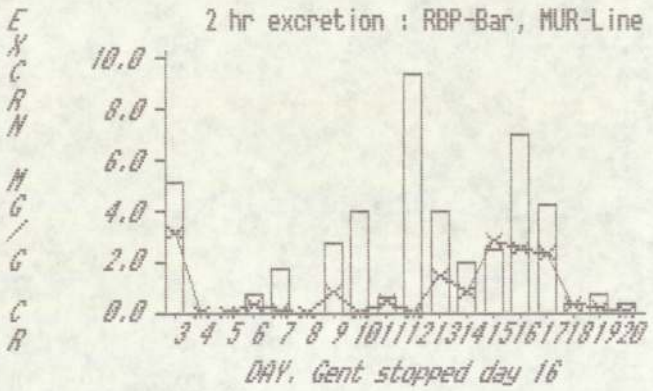


Plate 2

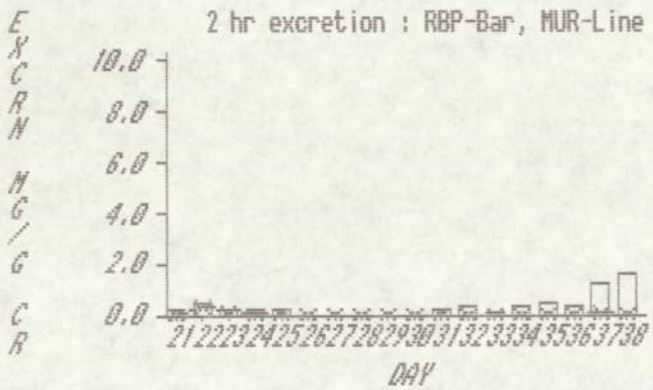


Fig 8a : 2 hr RBP and MUR excretion in patient 3.

Plate 2 is a continuation of Plate 1

Plate 1

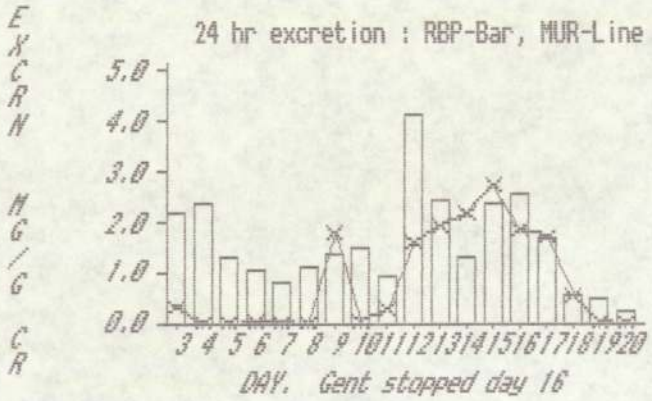


Plate 2

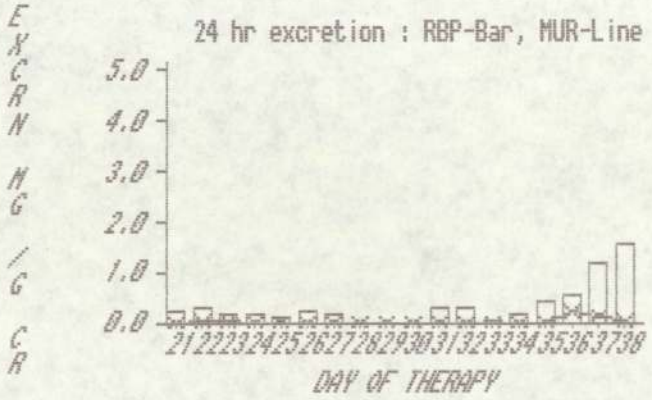


Fig 8b : 24 hr RBP and MUR excretion in patient 3.

Plate 2 is a continuation of Plate 1

Plate 1

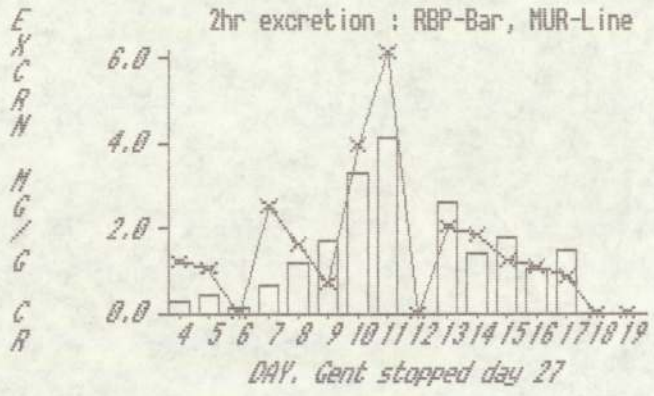


Plate 2

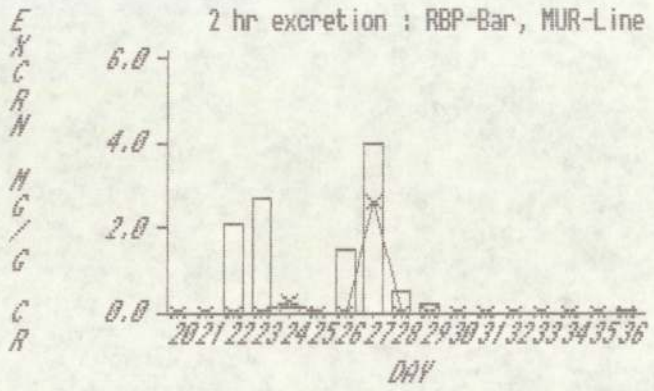


Fig 9a : 2 hr RBP and MUR excretion in patient 20

Plate 2 is a continuation of Plate 1

Plate 1

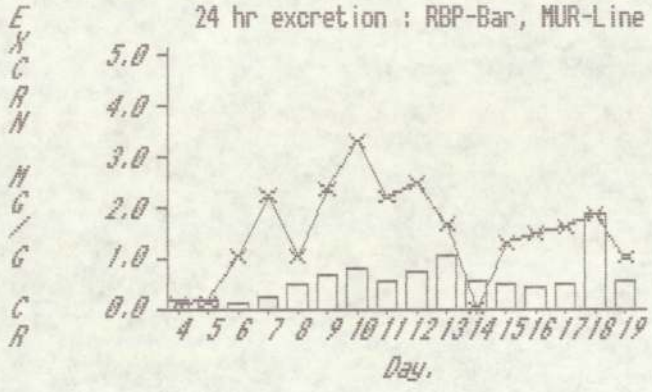


Plate 2

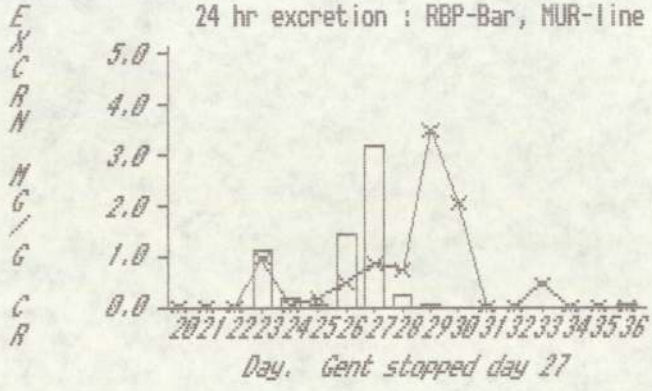


Fig 9b : 24 hr RBP and MUR excretion in patient 20

Plate 2 is a continuation of Plate 1

ii) RBP excretion in patients with cystic fibrosis.

Three patients with cystic fibrosis were studied; two in the aminoglycoside-treated group (patients 8 and 29) and one in the control group (patient 23). In each case the 2 hr and 24 hr excretion of RBP was either within the reference range or just above the reference range. No peaks in the RBP excretion were seen. Patient 8 received 3.38g of tobramycin during a 10 day course and patient 29 received 3g of gentamicin during a 12 day course. It is of interest that they showed little or no elevation in RBP excretion, whilst the patient treated for SABE with similar total doses of aminoglycoside, showed clear elevation of RBP excretion.

The RBP excretion level was lower in the patient treated with tobramycin than in the patient treated with gentamicin. Patient 23, who was treated with ceftazidime, showed the lowest RBP excretion of any of the patients with cystic fibrosis. All three patients had received aminoglycoside therapy previously. Patient 8 had received 22g of aminoglycosides previously, patient 23 had received 53g of aminoglycosides previously. The total lifetime aminoglycoside dose for patient 29 was not known but she received a total of 3.5g of gentamicin during two recent previous admissions. It is known that she had received many more courses of aminoglycoside therapy prior to these.

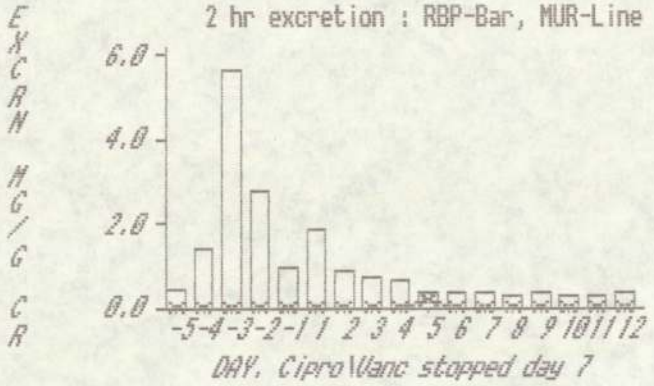
S_{RBP} data was only available in 2 of these patients (Tables 7a and 7b) and in each case the level was below the reference range.

iii) RBP excretion in patients with haematological disorders.

Most of the patients in the study were being treated for acute myeloid leukaemia or acute lymphoblastic leukaemia. The excretion profiles can be split into 3 general groups :

a) Patients showing an early RBP excretion above the reference range.

Patient 1 (courses 1 and 2) showed an initially abnormally high 2 hr RBP excretion with the maximum excretion on Day 2. This abnormally high excretion rapidly fell to normal levels during aminoglycoside therapy. A similar excretion profile was seen for the 24 hr collection in patient 1 (course 1) and in both collections for patients 18, 21, 22 and 24 (course 2) in the control group. The excretion profile for patient 22 illustrates this pattern (Fig 10). In patient 1 (course 2) a second, later peak in the excretion was seen during therapy although the level was lower that



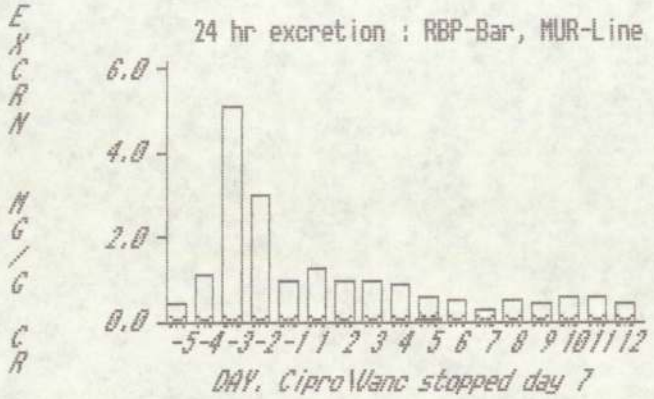


Fig 10 : 2 hr and 24 hr RBP and MUR excretion in patient 22.

the first peak. In all the other patient courses, RBP excretion had returned to normal levels by Day 3. RBP excretion then remained at this level throughout the study period. Extensive post-dose data in patients 1 (course 1) (9 days), 18 (9 days) and 22 (6 days), indicate that the RBP excretion in these patients was normally less than 1.0 mg/g creatinine.

b) Patients showing no elevation of the RBP excretion.

Patients 4 (course 2) and 14 (course 2) of the control group and patient 5 (course 3) of the aminoglycoside-treated group showed no elevation of the 2 hr or 24 hr urinary RBP excretion.

3) Patients showing later elevation in the 2 hr and 24 hr RBP excretion.

i) Aminoglycoside-treated patients.

Three patients in the aminoglycoside-treated group showed an elevation in both the 2 hr and 24 hr RBP excretion. Five days of pre-dose data in patient 4 (course 1) showed that RBP excretion was normally less than 1.0mg/g creatinine in this patient. For both collection periods the excretion gradually rose to a maximum. The exact day or size of the maximum excretion

is unclear as collections were not made for 5 days after the day on which the maximum excretion was measured. However, both the 2 hr and 24 hr maxima occurred at approximately the same time during therapy. Post-dose data collected over 14 days indicate that the RBP excretion returned to normal and stayed at a normal level for at least 2 weeks after gentamicin therapy was stopped.

Patient 5 (course 2) showed clear maxima in the RBP excretion for the 2 hr and 24 hr collections. The maximum excretions occurred on Days 6 and 2 respectively. Pre-dose data (3 days) and post-dose data (18 days) showed that the RBP excretion in this patient was usually within the reference range.

Patient 14 (course 1) showed a similar excretion profile to the above patients. The maximum excretion for the 2 hr and 24 hr collections were seen on Days 4 and 3 respectively.

For patients 5 (course 2) and 14 (course 1) the 24 hr maximum excretion was approximately double the 2 hr maximum excretion. In patient 4 (course 1) the exact relationship between the maximum excretions is unclear. The mean excretion maximum and day that maximum occurred are shown in Table 24.

Table 24 : Maximum 2 hr and 24 hr RBP excretions in patients 4.1, 5.2 and 14.1.

Collection period	Maximum excretion of RBP * mg/g creatinine	Day of maximum excretion *
2 hr	6.1 ± 5.0	5.7 ± 3.3
24 hr	10.9 ± 11.0	3.7 ± 2.1

* Mean ± SD

It is of interest that these patients had maximum 2 hr and 24 hr RBP excretions which were considerably greater than the maximum RBP excretions seen in the patients with SABE. It is also of interest that the 24 hr RBP excretion exceeded the 2 hr RBP excretion. In the patients with SABE, the 2 hr RBP excretion exceeded the 24 hr RBP excretion.

ii) Control group.

In the control group, 5 patients showed elevated 2 hr and 24 hr RBP excretions. In patient 6 very high RBP excretion levels were seen for the first 3 days of therapy (20 to 25 mg/g creatinine). A drop in excretion was then seen followed by a peak from Days 8 to 11. Maximum RBP excretions of 154 mg/g creatinine for the 2 hr excretion and 181 mg/g creatinine for the 24 hr excretion were seen on Day 10.

Patients 9, 24 (course 1) and 27 all had pre-dose data indicating normal excretion of RBP initially. Broad peaks in the excretion were seen during therapy with the 2 hr and 24 hr peaks being seen at approximately the same time. The 24 hr excretion was generally greater than the 2 hr excretion. Table 25 shows the mean ($\pm$ S.D.) maximum excretion and the mean ($\pm$ S.D.) day of the maximum excretion in these patients.

Patient 5 (course 1) showed a spike in the 2 hr RBP excretion. It was considered, because of the sharpness of this excretion maximum, not to be related to antibiotic administration.

Table 25 : Maximum 2 hr and 24 hr excretions in patients 6, 9, 24.1 and 27.

Collection period	Maximum excretion of RBP * mg/g creatinine	Day of maximum excretion *
2 hr	52.3 ± 69.9	10.0 ± 1.6
24 hr	65.4 ± 79.0	9.0 ± 2.6

* Mean ± S.D.

It can be seen that the 2 hr and 24 hr maxima occurred at approximately the same time and that the 24 hr excretion was generally higher than the 2 hr excretion. It is of interest that the RBP excretion, during both collection periods, is much higher in the control group than the aminoglycoside group.

d) Possible reasons for the high RBP excretions seen in the control patients with haematogical disorders.

Two patients in this sub group had RBP excretions greater than 50mg/g creatinine.

Patient 6 was being treated for non-Hodgkins lymphoma and had received a course of CHOP two days prior to antibiotic therapy. It is possible that the early elevation in RBP excretion was secondary to this CHOP therapy. The highest excretion was seen on Day 10 however, and followed a drop in the RBP excretion. This is therefore unlikely to be secondary to CHOP. A rapid fall in the RBP excretion was seen when antibiotic therapy was stopped.

The S_{RBP} in patient 6 was 59.3 mg L^{-1} on Day 2 and 25.9 mg L^{-1} on Day 11 (Table 7b). These peaks in RBP excretion were therefore not secondary to S_{RBP} levels exceeding the renal threshold. Table 4b shows the maximum rise in the Scr in this patient. It can be seen that there was only a very small change in the Scr level during antibiotic treatment. So the high RBP excretions seen in this patient were not associated with significant changes in renal function.

Patient 9 was newly diagnosed as having AML. He was given courses of DAT 1 + 5 on Days -11 to -6 and Days 3 to 8. Pre-dose urinary RBP excretion was normal for both 2 hr and 24 hr collections. For the first 6 days of antibiotic treatment thienamycin was given. This was then changed to ciprofloxacin and vancomycin because of a persistent pyrexia. Elevated RBP excretions were seen

from Day 1 although a broad peak was seen for each excretion period from Day 5 to 12. RBP excretion then returned to pre-dose levels for the duration of antibiotic treatment (to Day 16) and remained at this level throughout the post-dose collection period (5 days). A rise in the Scr level was seen in this patient but this was not clinically significant (Table 4b). On Day 7 the S_{RBP} level was 17.3mg L^{-1} indicating that the elevated RBP excretion was not secondary to an elevated S_{RBP} level.

Thus, the reasons for the large elevations in RBP excretions in these patients is unclear. It is possible that these elevations were due to infection, pyrexia, critical illness or cytotoxic administration.

6) Urinary muramidase excretion.

Since pre-dose urinary MUR excretion was generally below the sensitivity of the assay, it was not possible to calculate a baseline excretion level for the patients in this study. The normal urinary MUR excretion is reported to be 0.14 to 0.24 mg/g creatinine [Houser, 1983] based on untimed urine specimens obtained from 169 healthy subjects with an age range of 6 months to 61 years. MUR was assayed, as in the present study, using a turbidometric assay based on the lysis of Micrococcus lysodeikticus. The upper limit of normal MUR excretion

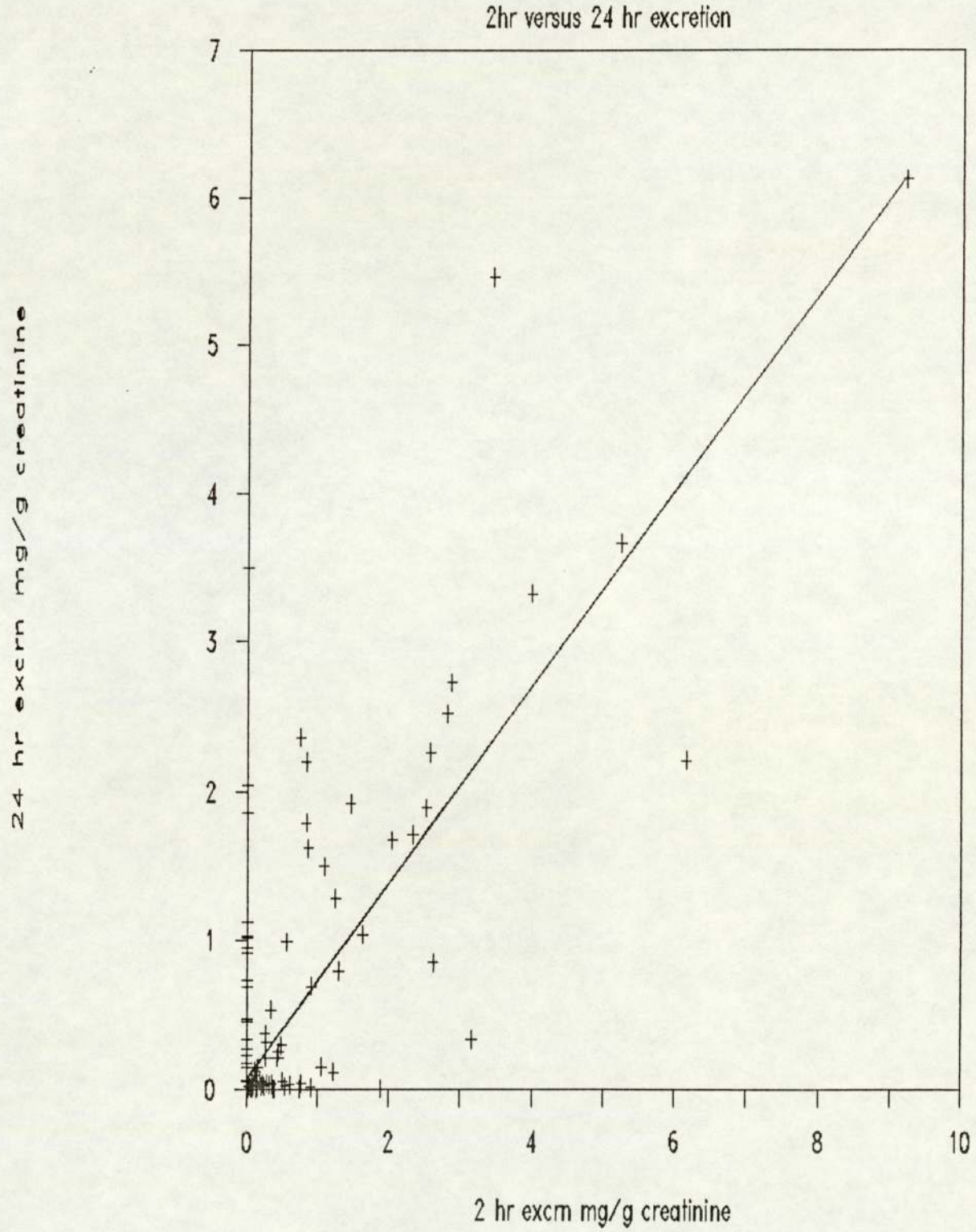
was calculated to be 0.58 mg/g creatinine, 95th percentile [Houser, 1983]. This was taken as the maximum normal excretion for the purposes of the present study.

A) Comparison of the 2 hr MUR excretion with the 24 hr MUR excretion.

To see if the 2 hr MUR excretion could be used to predict the 24 hr MUR excretion a regression analysis was performed. Data for all patients was used for this analysis and comprised those days where both 2 hr and 24 hr excretion was available. A total of 306 data points were compared. The resulting scatter diagram and regression line is shown in Fig 11. The correlation coefficient was calculated to be 0.63 indicating a moderate correlation.

It can be seen from Fig 11 however that there are many data points where the excretion is not detectable. Since this study is interested in elevated excretion of this protein, it was decided to look at the correlation between 2 hr and 24 hr MUR excretion at elevated excretion levels only. A total of 23 data points were compared and the correlation coefficient was calculated to be 0.56. This moderate correlation indicates that the 2 hr MUR excretion was not a good indicator of the 24 hr MUR excretion.

Fig 11 : Correlation curve for MUR



B) Aminoglycoside-treated group.

i) 2 hr urinary MUR excretion.

The distribution of maximum MUR excretion in the aminoglycoside-treated patients is shown in Table 26. Table 27 lists the maximum 2 hr and 24 hr MUR excretion seen in the aminoglycoside-treated patients.

Table 26 : Distribution of maximum 2 hr urinary excretions of MUR in aminoglycoside-treated patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 0.58	6	1.2, 4.1, 5.2, 5.3, 8,29
0.58 - 1.0	2	1.1, 14.1
> 1.0	2	3, 20

Table 27 : Maximum 2 hr and 24 hr urinary excretion of MUR in aminoglycoside-treated patients.

Patient course	Max 2 hr MUR excrn (mg/g cr)	Day	Max 24 hr MUR excrn (mg/g cr)	Day
1.1	0.7	2	0.2	16
1.2	NM		NM	
3	2.9	15	2.7	15
4.1	0.4	6	0.5	13
5.2	0.5	4	0.3	5
5.3	NM		NM	
8	0.2	5	NM	
14.1	0.8	8	NM	
20	4.0	10	3.3	10
29	NM		NM	

NM = Not measurable

Six of the 10 aminoglycoside-treated patients showed no elevation in the 2 hr urinary MUR excretion. The highest excretion levels were seen in the patients being treated for SABE (patients 3 and 20). Both patients had S_{MUR} levels well below the renal threshold (Table 7a). MUR excretion was undetectable for patients 1 (course 2), 5 (course 3) and 29 throughout the study period. The S_{MUR} was low in each of these patients (Table 8a).

ii) 24 hr urinary MUR excretion.

Table 28 shows the distribution of maximum 24 hr MUR excretions in the aminoglycoside-treated patients.

Table 28 : Distribution of the maximum 24 hr MUR excretion in aminoglycoside-treated patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 0.58	8	
> 0.58	2	3, 20

Only two patients had an elevation in the 24 hr urinary MUR excretion. Both patients were being treated for SABE and both had the highest maximum 2 hr MUR excretions. MUR excretion was not detectable in patients 1 (course 2), 5 (course 3), 8, and 29.

C) Control patients.

i) 2 hr urinary MUR excretion.

Table 29 shows the distribution of maximum 2 hr urinary MUR excretions seen in the control group. Table 31 lists the maximum 2 hr and 24 hr MUR excretions seen in the control group.

Table 29 : Distribution of maximum 2 hr urinary excretions of MUR in control patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 0.58	10	
0.58 - 1.0	1	9
> 1.0	1	6

In the control group, only 2 patients (patients 6 and 9) had an elevated 2 hr MUR excretion. It is of interest that patients 6 and patient 9 also showed the highest 2 hr RBP excretion. MUR excretion was not detectable in the remainder of the patient courses except for patient 22. The S_{MUR} level was low in all patients except for patients 6, 9, and 22. No measurements were made for patient 23 and patient 5 (course 1). The low excretion of MUR may be because of the low S_{MUR}.

ii) 24 hr urinary muramidase excretion.

The maximum 24 hr MUR excretions in the control group are shown in Table 31. The distribution of these excretions is shown in Table 30.

Table 30 : Distribution of maximum 24 hr MUR excretion in control patients.

Maximum excretion mg/g creatinine	Number of patients	Patient course
=< 0.58	9	
0.58 - 1.0	1	9
> 1.0	2	6, 14.2

Table 31 : Maximum 2 hr and 24 hr urinary excretion of MUR in control patients.

Patient course	Max 2 hr MUR excrn (mg/g cr)	Day	Max 24 hr MUR excrn (mg/g cr)	Day
4.2	NM		NM	
5.1	NM		NM	
6	3.4	10	5.5	10
9	0.9	2	1.0	6
14.2	NM		1.1	2
18	NM		NM	
21	NM		NM	
22	0.2	5	0.1	5
23	NM		NM	
24.1	NM		NM	
24.2	NM		NM	
27	NM		0.1	10

NM = not measurable

Three patients had 24 hr MUR excretions above the reference range. MUR excretion was only detectable in 4 patients. Patient 14.2 showed an abnormally high 24 hr MUR excretion but no 2 hr MUR excretion. Patients 6 and 9 both had elevated 2 hr MUR excretions.

D) Comparison of the aminoglycoside-treated group with the control group.

Elevated MUR excretion was seen in 4 patients in the aminoglycoside-treated group. Two patients (patients 3 and 20) showed elevations in both 2 hr and 24 hr MUR excretion. Patients 1 (course 1) and 14 (course 1) showed elevated MUR excretion during the 2 hr collection only.

Three patients in the control group had showed elevated MUR excretion. Two patients (patients 6 and 9) showed elevated 2 hr and 24 hr MUR excretion. Patient 14 (course 2) showed elevated 24 hr MUR excretion only. The maximum MUR excretions were of a similar magnitude in each group.

In order to analyse the data more fully, it was decided to examine MUR excretion in the different clinical groups.

Analysis of MUR excretion in the different clinical groups.

i) MUR excretion in patients with SABE.

Both patients (patients 3 and 20) who were treated with gentamicin for SABE showed elevated 2 hr and 24 hr MUR excretion.

The excretion profiles for patient 3 are shown in Fig 8a and Fig 8b. For each collection period a single broad peak in the excretion was seen. The magnitude of the MUR excretion was very similar for each collection period. Following cessation of gentamicin treatment the MUR excretion rapidly returned to normal levels. Extensive post-dose excretion measurements (32 days) showed that the MUR excretion in this patient was normally undetectable.

Fig 9a and Fig 9b show the MUR excretion profile for patient 20. No pre-dose data was available. A broad peak in the MUR excretion was seen for each collection period. Since some collections were missed the exact time that these peaks ended is unclear, although the MUR excretion appeared to return to normal levels during gentamicin therapy. The maximum 2 hr excretion was seen after the maximum 24 hr excretion, and was generally higher than the 24 hr excretion. A second peak in the

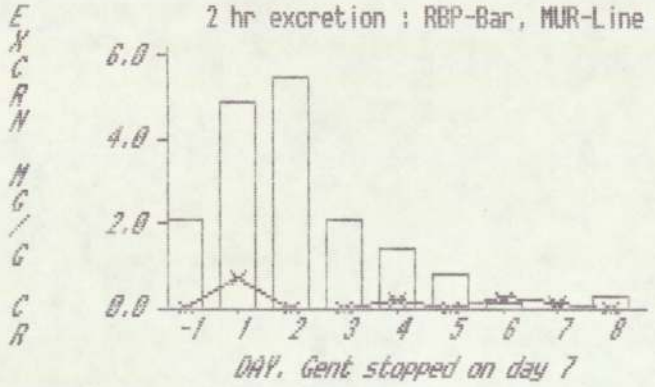
MUR excretion profile was seen on Days 25 to 30. This was considered to be secondary to dental surgery carried out on this patient.

ii) MUR excretion in patients with cystic fibrosis.

MUR excretion was not detectable in either the aminoglycoside treated patients (patients 8 and 29) or the control patient (patient 23) except for a single measurement (Day 5) during patient course 8. S_{MUR} levels were normal in patient 8 and slighly low in patient 29 (see Tables 7a and 7b). No measurements were made for patient 23. It is unlikely that the low S_{MUR} level in patient 29 contributed to the low level of excretion since patient 14 (course 2) had a S_{MUR} level of just 2.4mg L^{-1} and had an elevated 24 hr MUR excretion.

iii) MUR excretion in patients with haematological disorders.

Two patients treated with aminoglycosides [patients 1 (course 1) and 14 (course 1)] had elevated 2 hr urinary MUR excretions. Fig 12 shows the MUR excretion profile for patient 1 (course 1). Both patients showed a transient elevation of the 2 hr MUR excretion. The 24 hr MUR excretion was undetectable. The elevation



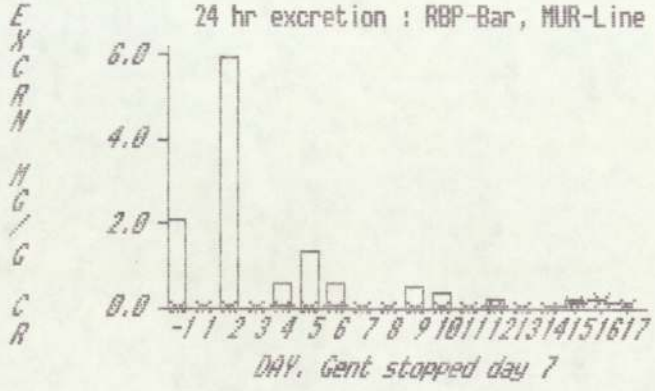


Fig 12 : 2 hr and 24 hr RBP and MUR excretion in patient 1 (course 1).

Plate 1

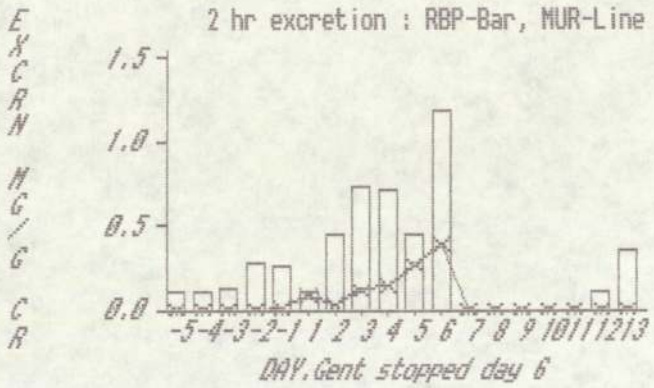


Plate 2

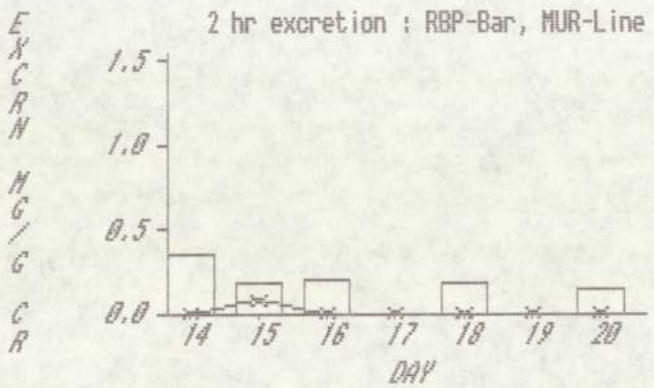


Fig 13a : 2 hr RBP and MUR excretion in patient 4 (course 1).

Plate 2 is a continuation of Plate 1

Plate 1

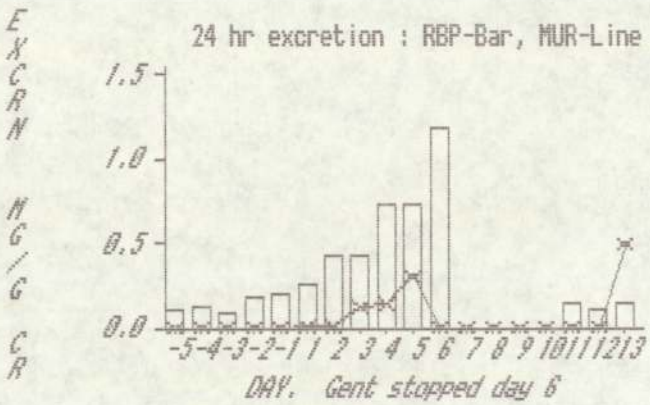


Plate 2

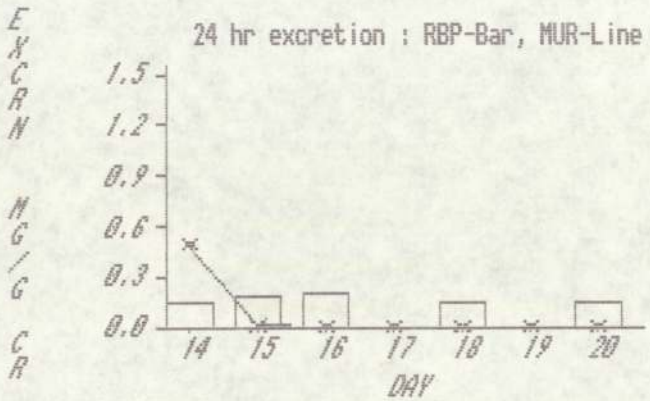


Fig 13b : 24 hr RBP and MUR excretion in patient 4 (course 1).

Plate 2 is a continuation of Plate 1

in 2 hr MUR excretion was very small and all other measurements were either very low or undetectable. It is likely that these elevations were random fluctuations in the MUR excretion.

In patient 4 (course 1) there was no abnormally high 2 hr or 24 hr MUR excretion. However Fig 13a and Fig 13b show that the excretion level gradually increased as treatment progressed. No collections were made from Days 7 to 11 and it is possible that a maximum excretion > 0.58mg/g creatinine may have been seen had these measurements been available.

Three patients in the control group [patients 6, 9 and 14 (course 2)] showed elevated MUR excretion. Patients 6 and 9 showed elevated 2 hr and 24 hr excretion. MUR excretion was high initially in patient 6 but a peak in the 2 hr and 24 hr excretion profile was seen from Days 8 to 12. MUR excretion fell rapidly when antibiotic therapy was stopped. The magnitude of the MUR excretion was similar to that seen in the patients with SABE.

Patient 9 had a pre-dose MUR excretion above the reference range. The abnormally high 2 hr and 24 hr MUR excretions seen during therapy were considerably lower than this pre-dose excretion and were considered to be part of a pre-dose peak.

Patient 14 (course 2) had a transient elevation of 24 hr MUR excretion only. The sharpness of this peak however suggests that is a random fluctuation in the MUR excretion.

The remainder of the patients with haematological disorders showed no elevation or significant increase in MUR excretion. Indeed it can be seen from Table 27 and Table 31 that many patients did not excrete measurable amounts of MUR.

7) Comparison of RBP and MUR excretion.

A) Aminoglycoside-treated patients.

Table 32 summarises the maximum 2 hr and 24 hr excretion of both LMW proteins and lists the day on which the maximum excretion was seen.

Four patients [patients 1 (course 1), 3, 14 (course 1) and 20] showed elevation of both the RBP and MUR excretion. In patient 3, elevated excretions of both proteins were seen for both collection periods. The day of the maximum RBP and MUR excretion was the same for each collection period. In patient 20, the 2 hr excretions of each LMW protein were very similar with the maximum excretion being seen the same day. During

Table 32 : Summary of the maximum 2 hr and 24 hr RBP and MUR excretions in aminoglycoside-treated patients, and the day on which they occurred.

Patient course	Maximum 2hr excretion*				Maximum 24hr excretion*			
	RBP	day	MUR	day	RBP	day	MUR	day
1.1	5.5	2	0.7	1	5.9	2	0.2	16
1.2	4.3	2	NM		4.0	6	NM	
3	9.4	12	2.9	15	4.2	12	2.7	15
4.1	1.2	6	0.4	6	1.2	6	0.5	13
5.2	6.1	6	0.5	4	8.6	2	0.3	5
5.3	0.9	2	NM		1.5	5	NM	
8	0.1	3	0.2	5	0.2	6	NM	
14.1	11.2	4	0.8	8	22.9	3	NM	
20	4.1	11	4.0	10	1.9	18	3.3	18
29	1.3	7	NM		0.8	4	NM	

* mg/g creatinine

NM = not measurable

the 24 hr collection, only the MUR excretion was elevated. Similarly, patients 1 and 14 showed elevated excretion of both proteins during the 2 hr collection period only. During the 24 hr collection, only the RBP excretion was elevated. It is likely that patient 4 (course 1) would have shown elevated excretion of both LMW proteins, for both the 2 hr and 24 hr collection periods, had all sample collections been made.

No patients showed elevation of only MUR excretion whereas during 4 courses [patients 1 (course 2), 5 (course 2 and course 3) and 29] there was elevation of RBP excretion only.

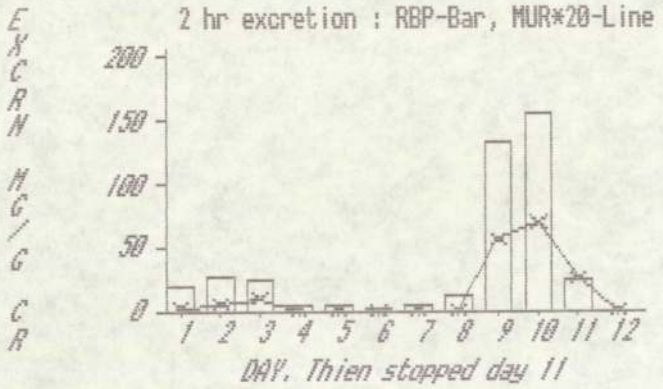
Patient 8 did not show elevated LMW protein excretion for either collection period.

It can be seen that MUR excretion was only elevated when the RBP excretion was elevated and that MUR excretion was most likely to be seen in the 2 hr collection period. 24 hr elevation of MUR excretion was only seen in the SABE patients. The maximum excretion of RBP and the maximum excretion of MUR were generally seen at the same time for a given collection period.

B) Control patients.

Table 33 summarises the 2 hr and 24 hr maximum LMW rotein excretion for the control patients and lists the day on which the maximum excretion was seen.

Two patients (patients 6 and 9) showed elevated RBP and MUR excretion for both collection periods. The elevated MUR excretion seen in patient 9 was considered to be secondary to factors other than antibiotic administration. The elevated RBP and MUR excretion in patient 6 followed a very similar profile (Fig 14) with the maxima occurring at the same time. In no other patients in the control group, including patients 5 (course 1) and 27 in whom the RBP excretion was greatly elevated, were both RBP and MUR excretion elevated.



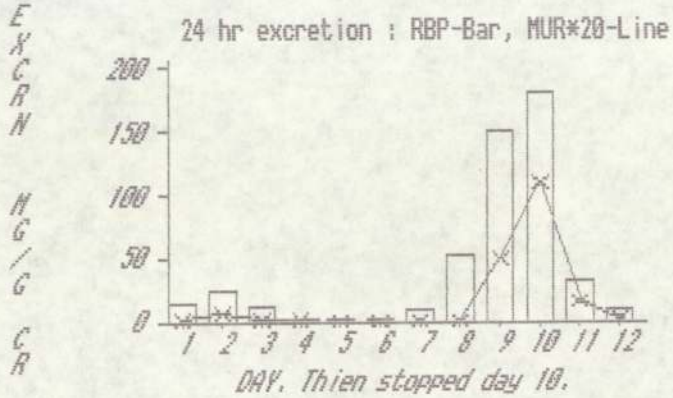


Fig 14 : 2 hr and 24 hr RBP and MUR excretion in patient 6.

Table 33 : Summary of the maximum 2 hr and 24 hr RBP and MUR excretions in control patients, and the day on which they occurred.

Patient course	Maximum 2hr excretion*				Maximum 24hr excretion*			
	RBP	day	MUR	day	RBP	day	MUR	day
4.2	0.4	1	NM		0.3	2	NM	
5.1	21.6	6	NM		1.6	10	NM	
6	154.2	10	3.4	10	180.6	10	5.5	10
9	42.1	8	0.9	2	52.0	6	1.0	6
14.2	0.3	9	NM		0.3	7	1.1	2
18	1.1	2	NM		0.2	15	NM	
21	2.5	1	NM		1.6	1	NM	
22	1.9	1	0.2	5	1.3	1	NM	
23	0.2	16	NM		0.1	15	NM	
24.1	7.7	10	NM		8.5	8	NM	
24.2	11.5	3	NM		4.2	3	NM	
27	6.0	12	NM		20.3	12	0.1	10

* mg/g creatinine

NM = not measurable

8) LMW protein excretion in patients who were treated with both aminoglycoside antibiotics and control therapy.

Three patients received courses of both aminoglycoside therapy and control therapy. It was decided to study these patients separately to further examine the effects of aminoglycoside antibiotics on LMW protein excretion.

A) Patient 4.

Patient 4 received a 6 day course of gentamicin (course 1) followed after 45 days by an 8 day course of thienamycin (course 2).

During course 1, small elevations in the excretion of RBP were seen for both collection periods. Five days of urine collection were missed so the excretion may have been higher. MUR excretion was not elevated although the maximum excretion may have been underestimated due to the missed urine collections. During course 2 there was no elevation or increase in excretion of either LMW protein for either collection period.

B) Patient 5.

Patient 5 received one 7 day course of thienamycin (course 1) followed by two courses of gentamicin

therapy (courses 2 and 3) of 10 and 9 days duration respectively. Course 2 followed course 1 by four days and course 3 followed course 2 by eighteen days. In each case the antibiotics were prescibed to treat a pyrexia secondary to neutropenia. During course 1, a sharp and transient elevation in the 2 hr RBP excretion was seen which was considered to be secondary to factors other than antibiotic therapy. A small elevation in the 24 hr RBP excretion was seen towards the end of therapy. There was no elevation in MUR excretion for any collection period.

During course 2, maxima in the excretion of RBP for both collection periods were seen. The magnitude of the maximum excretions was greater for this course than course 1. No elevation in MUR excretion was seen.

The RBP excretion in course 3 followed a similar pattern to course 1 with a slight elevation in RBP excretion being seen. Again there was no elevation in MUR excretion.

By comparing courses 1 and 2 it can be seen that gentamicin administration resulted in increased urinary RBP excretion for both collection periods. Administration of a second course of gentamicin (course 3) 18 days after the first (course 2) had no such effect on RBP excretion. MUR excretion was unaffected

in each case.

C) Patient 14.

Patient 14 was treated with a 10 day course of gentamicin (course 1) followed after 21 days by an 8 day course of thienamycin.

During course 1, RBP excretion was elevated for both collection periods. MUR excretion was not elevated. During course 2 the excretion of RBP was much lower than course 1, with no elevation in excretion seen. There was a transient rise in the 24 hr MUR excretion but this was considered unrelated to antibiotic administration.

In each of the three patients who were given both aminoglycoside and control antibiotics in separate courses, there was an increased level of RBP excretion during the aminoglycoside course. MUR excretion was affected only in patient 4. In this group, patient variation has been minimised, with the main variation being the timing and type of chemotherapy received before each course of antibiotic therapy. Patient 4 received DAT 2 + 7 fourteen days prior to course 1 and twelve days prior to course 2. Patient 5 received DAT 3 + 10 eleven days prior to course 1, and therefore 21 days prior to course 2. DAT 2 + 7 was given 16 days prior to course 3. Therefore there was little

difference in the chemotherapy administered to these patients. Patient 14 however, received MOPP on day 2 of course 1 and no chemotherapy before or during course 2.

9) Serum calcium concentrations.

The normal range for serum calcium (Sca) concentrations was defined as 2.1 to 2.6 mmol L^{-1} (clinical chemistry reference values, EBH). Hypocalcaemia was defined as a Sca concentration of < 2.1 mmol L^{-1}. Sca levels were corrected for low serum albumin where necessary.

Since it was not possible to obtain daily serum samples from the patients with cystic fibrosis these patients were excluded from this part of the study. Insufficient data were obtained from patient 1 (course 2) for meaningful analysis to be performed. The results from this patient have not therefore been presented.

A) Aminoglycoside-treated patients.

The initial Sca, the lowest Sca and the day of this nadir are listed in Table 34. The mean ($\pm$ S.D.) value for the initial and lowest Sca is also shown. It can be seen from Table 34 that a fall in the Sca level was seen in all patients except patient 1 (course 1). Furthermore, patient 1 (course 1) was initially hypocalcaemic whilst the remaining 6 patients showed an episode of hypocalcaemia during antibiotic treatment.

Table 34 : Initial Sca and lowest Sca measurements and the day on which the nadir occurred.

Patient course	Pre-dose Sca mmol L^{-1}	Lowest Sca mmol L^{-1}	Day of nadir	Last day of therapy
1.1	1.9	2.0	3/4	7
3	2.3 $	2.0	4	16
4.1	2.3	1.7	18	6
5.2	2.2	1.8	10	10
5.3	2.2	1.6	13	9
14.1	2.1	1.6	6	10
20	2.4 #	1.9	19	27
Mean	2.2	1.8		
S.D.	0.2	0.2		

$ serum sample Day 3 of therapy

serum sample Day 5 of therapy

However, analysis of the daily Sca measurements in each patient does not support the apparent development of hypocalcaemia in all patients.

In patient 1 (course 1) there was a gradual rise in the Sca level, as suggested by Table 34. From Day 9 the Sca level was normal. No serum albumin data were available for this patient.

In patients 3, 14 (course 1) and 20 the nadir for the Sca (Table 34) actually represents a transient fluctuation in a series of normal Sca determinations. These patients were therefore not considered to have developed hypocalcaemia.

In patient 4 (course 1) the hypocalcaemia began after the aminoglycoside therapy stopped. The hypocalcaemia began on or between Days 7 and 13 and lasted until at least Day 19 when the patient was discharged. From data obtained in this patient during a second course of treatment begun 5 days later, it is possible to say that the Sca had returned to normal by Day 24.

In patient 5 (courses 2 and 3) hypocalcaemia was seen during gentamicin administration. Patient 5 (course 2) was hypocalcaemic from Day 6 to Day 25. During course 3, patient 5 was hypocalcaemic from Day 1 to Day 15 when measurements were stopped. The lowest Sca level

of 1.57 mmol L^{-1} in this patient represented a transient sharp drop in the Sca concentration.

Of the aminoglycoside- treated patients, two (three courses) developed hypocalcaemia during antibiotic treatment. In each case the hypocalcaemia was persistent lasting at least 6 days. The hypocalcaemia was not severe enough in any case to require treatment. It was considered unlikely that the hypocalcaemia in patient 5 (course 3) was secondary to gentamicin due to its early onset.

B) Control patients.

The initial Sca and the lowest Sca and the day of this nadir are listed in Table 35. The mean ($\pm$ S.D.) value for the initial and lowest Sca is also shown.

From Table 35 it can be seen that 7 patients (8 courses) had a hypocalcaemic episode during antibiotic treatment. Patient 22 showed a rise in Sca and patients 4 (course 2) and 21 showed a fall in the Sca level although hypocalcaemia did not develop. In all cases the nadir was seen during antibiotic therapy.

Analysis of the daily Sca measurements does not support

Table 35 : Initial Sca and lowest Sca measurements and the day on which the nadir occurred.

Patient course	Pre-dose Sca mmol L^{-1}	Lowest Sca mmol L^{-1}	Day of nadir	Last day of therapy
4.2	2.5	2.1	2	8
5.1	2.5	1.9	6	7
6	2.2 $	2.0	8	11
9	1.8	1.6	8	15
14.2	2.2 $	2.0	2	8
18	2.3	1.8	6	6
21	2.2	2.1	6	8
22	2.1	2.4	7	7
24.1	2.5	1.8	4	12
24.2	2.4	1.8	4	8
27	2.0	1.5	11	12
Mean	2.2	1.9		
S.D.	0.2	0.3		

$ serum sample Day 1 of therapy.

the development of hypocalcaemia during each of these eight courses of treatment.

The lowest Sca levels in patients 6, 18 and 24 (course 1) were single measurements in a series of normal Sca levels. These patients were therefore considered not to have developed hypocalcaemia.

Patient 5 (course 1) developed hypocalcaemia on Day 5 of antibiotic treatment with the nadir occurring on Day 6. No Sca measurements were available for Days 8, 9 and 10 although the Sca was normal by Day 11 (2.22 mmol L^{-1}), so the hypocalcaemia was transient.

Patient 9 was hypocalcaemic initially. The Sca fell further however to its nadir on Day 8 (Table 35). The Sca level then rose and a normal level was reached by Day 15 (2.12 mmol L^{-1}).

A transient hypocalcaemia early in therapy (Days 2, 3 and 4 only) was seen in patient 14 (course 2). Subsequent measurements were within the normal range.

Patient 24 (course 2) also developed hypocalcaemia. The hypocalcaemia was seen on Days 4 and 5 and from Day 8 to Day 11.

Patient 27 was hypocalcaemic before and during

antibiotic administration. The Sca level fell early in treatment, rose to initial levels and then fell once more.

Five cases of hypocalcaemia were therefore seen in the control group. In each case the hypocalcaemia lasted for less than 7 days and in no case was treatment necessary. The hypocalcaemia seen in patient 14 (course 2) was considered to have occurred too early in therapy to be antibiotic related.

10) Serum magnesium concentrations.

The normal range for serum magnesium (Smg) concentrations was defined as 0.7 to 1.0 mmol L^{-1} (clinical chemistry reference values, EBH). Hypomagnesaemia was therefore defined as a Smg of < 0.7 mmol L^{-1}.

A) Aminoglycoside-treated patients.

The initial Smg, the lowest Smg and the day of this nadir are listed in Table 36. The mean ($\pm$ S.D.) value for the initial and lowest Smg is also shown.

The low Smg measurements for patients 4 (course 1), 5 (course 2), 14 (course 1) and 20 were transient

Table 36 : Initial Smg and lowest Smg measurements and the day on which the nadir occurred.

Patient course	Pre-dose Smg mmol L^{-1}	Lowest Smg mmol L^{-1}	Day of nadir	Last day of therapy
1.1	0.7	0.5	11	7
3	0.5 $	0.5	4	16
4.1	0.7	0.5	2	6
5.2	0.7	0.6	22	10
5.3	0.7	0.7	13	9
14.1	0.7	0.6	6	10
20	0.8 #	0.6	23	29
Mean	0.7	0.6		
S.D.	0.1	0.1		

$ serum sample Day 3 of therapy

serum sample Day 5 of therapy

falls in a series of normal measurements. These patients were considered therefore not to have developed hypomagnesaemia. Patient 5 (course 3) showed an increase in the Smg level during the antibiotic course. The nadir shown was a transient fall only.

Patient 1 (course 1) had a normal initial Smg level (Table 36). Hypomagnesaemia developed 3 days after gentamicin therapy was stopped (Day 10). This hypomagnesaemia persisted until at least Day 20 when the patient was discharged.

Patient 3 was mildly hypomagnesaemic from Day 3 to Day 19. Day 3 was the first available sample collection day and so it is not known if the patient had normal Smg levels prior to gentamicin therapy. The Smg remained within the normal range for the rest of the post-treatment period (21 days).

Two patients in the aminoglycoside-treated group developed hypomagnesaemia during or shortly after aminoglycoside therapy. Only in patient 1 was it thought likely to be attributable to aminoglycoside therapy. The hypomagnesaemia in patient 3 may have been secondary to aminoglycoside therapy but the lack of pre-dose data means more definite conclusions cannot be made.

B) Control patients.

The initial Smg, the lowest Smg and the day of this nadir are listed in Table 37. The mean (± S.D.) value for the initial and lowest Smg is also shown.

Patient 5 (course 1) showed a fall in the Smg without developing hypomagnesaemia. The Smg in patient 14 (course 2) rose during treatment, and the Smg for patient 24 (courses 1 and 2) showed little change. The Smg in patient 22 also increased during antibiotic therapy.

In patients 4 (course 2), 18, and 27 the nadir shown in Table 37 was a transient fall in a series of normomagnesaemic serum levels. These patients were therefore not considered to have developed hypomagnesaemia.

Patient 6 was hypomagnesaemic from the first serum sample (Day 1) until Day 9. The Smg level increased during therapy and this patient became normomagnesaemic.

Patient 9 was hypomagnesaemic initially (Table 37) but this worsened over Days 8 to 12 after which pre-dose levels were restored.

Table 37 : Initial Smg and lowest Smg measurements and the day on which the nadir occurred.

Patient course	Pre-dose Smg mmol L^{-1}	Lowest Smg mmol L^{-1}	Day of nadir	Last day of therapy
4.2	0.7	0.6	4	8
5.1	0.8	0.7	6	7
6	0.6 #	0.5	12	11
9	0.6	0.4	8	15
14.2	0.8 #	0.7	2	8
18	0.7	0.6	6	6
21	0.7	0.6	6	8
22	0.7	0.7	3	7
24.1	0.7	0.6	4	12
24.2	0.8	0.7	8	8
27	0.8	0.6	11	12
Mean	0.7	0.6		
S.D.	0.1	0.1		

serum sample Day 1 of therapy.

Only 2 serum levels were available for patient 21 although it appears that hypomagnesaemia occurred with therapy.

Thus, hypomagnesaemia developed in 3 of the control patients, in each case during the aminoglycoside course. The hypomagnesaemia in patient 6 occurred very early in therapy and was not considered to be related to antibiotic administration.

11) Urinary electrolyte excretion.

A) Aminoglycoside-treated patients.

i) 2 hr and 24 hr calcium excretion.

The normal 24 hr excretion of calcium (EXca) in hospitalised patients is 2.5 to 5.0 mmol/24 hour [clinical chemistry reference values, EBH]. The normal fractional excretion of calcium (FEca) is 0.5 to 1.0% [Massry and Cowburn, 1973]. Analysis of excretion data for any patient was based upon changes of excretion with time as well as on the absolute value. Tables 38 and 39 list the initial and maximum 2 hr FEca and 24 hr FEca in the aminoglycoside-treated group, and the day the FEca was measured. Table 40 lists the initial and maximum 24 EXca in the aminoglycoside-treated group, and the day of measurement. The Sca on

Table 38 : Summary of the initial 2 hr FEca and maximum 2hr FEca in aminoglycoside-treated patients, and the day the maximum FEca was seen.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
1.1	2.2	-1	2.5	7
3	3.8	3	19.2	16
4.1	1.0	-2	3.7	12
5.2	1.0	5	2.4	8/22
5.3	0.5	-1	1.4	13
14.1	1.6	-1	5.0	10
20	1.7	5	8.3	10
Mean	1.7		6.1	12.2
S.D.	1.1		6.2	4.9

* = normal range 0.5 to 1.0%

Table 39 : Summary of the initial 24 hr FEca and maximum 24 hr FEca in aminoglycoside-treated patients, and the the day the maximum was measured.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
1.1	1.3 (2.0)	2	3.6 (2.1)	19
3	0.9 (2.3)	3	7.5 (2.3)	26
4.1	1.7 (2.3)	-2	2.8 (2.0)	13
5.2	1.2 (2.2)	5	2.1 (1.8)	22
5.3	0.6 (2.2)	-1	1.5 (1.6)	13
14.1	2.3 (2.1)	1	5.7 (2.4)	9
20	2.1 (2.4)	5	4.5 (2.6)	10
Mean	1.4		4.0	16.0
± S.D.	0.6		2.1	6.4

* = normal range 0.5 to 1.0%
Sca (mmol L^{-1}) shown in brackets

Table 40 : Summary of the initial and maximum 24 hr EXca in aminoglycoside-treated patients and the day the maximum was measured.

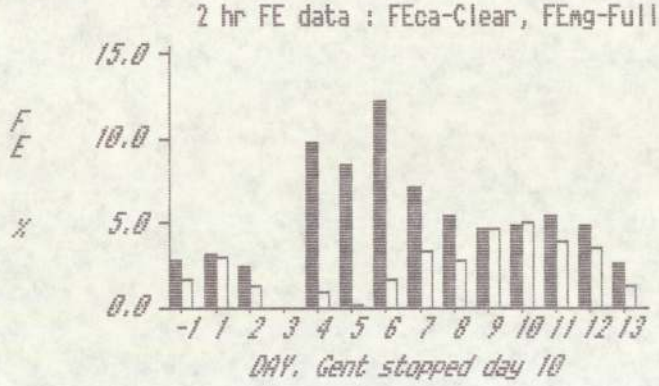
Patient course	Initial excrn*	Day of initial	Maximum excrn*	Day of maximum
1.1	3.4 (2.0)	2	4.2 (2.2)	14
3	0.6 (2.3)	3	6.0 (2.3)	26
4.1	7.0 (2.3)	-2	14.2 (1.9)	17
5.2	5.0 (2.2)	5	3.9 (1.9)	16
5.3	1.9 (2.2)	-1	3.1 (2.0)	7
14.1	4.2 (2.1)	1	7.2 (2.4)	8/13
20	5.9 (2.4)	5	5.2 (2.8)	15
Mean	4.0		6.3	
± S.D.	2.2		3.8	

* = normal range 2.5 to 5.0 mmol L^{-1}
Sca (mmol L^{-1}) shown in brackets

the day of the maximum FEca or EXca is shown, and the mean (± SD) FEca or EXca is also shown.

All patients had an abnormally high 2 hr and 24 hr FEca during the study period. The mean data (Table 38 and Table 39) suggests that the maximum 2 hr FEca was greater than the maximum 24 hr FEca and that the maximum 2 hr FEca was seen before the maximum 24 hr FEca.

Patients 3, 14 (course 1) and 20 all showed a definite elevation in the 2 hr and 24 hr FEca with aminoglycoside treatment. The excretion profiles were very similar for the 2 hr and 24 hr collection periods. This is illustrated by the fractional excretion profiles for patient 14 (course 1) shown in Fig 15. The FEca rose to the maximum value shown in Table 38 and Table 39 and then fell to a lower, but elevated level. In patients 3 and 20, where many days of post-dose collections were available (22 and 9 days respectively), this elevated FEca was maintained when aminoglycoside therapy was stopped. The excretion profile for patient 3 is shown in Fig 16a and Fig 16b. Only in patient 14 (course 1) was this elevation in FEca accompanied by an inappropriately high EXca. This was seen between Days 7 and 13 when the EXca was above the normal range and the Sca was normal. This may have been secondary to MOPP administration on Day 3. In patient 20 the 24 hr EXca fell during treatment. The days of the initial and



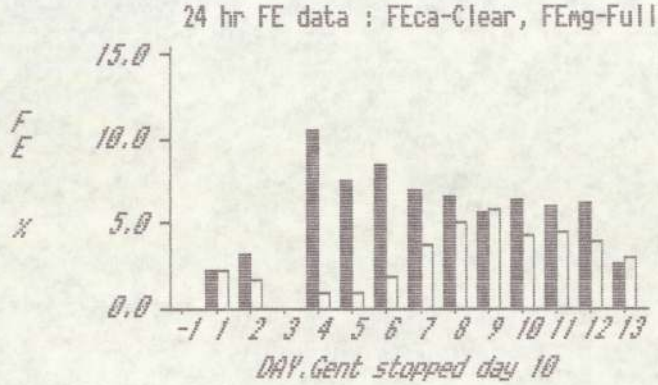


Fig 15 : 2 hr and 24 hr FEca and FEmg in patient 14 (course 1).

Plate 1

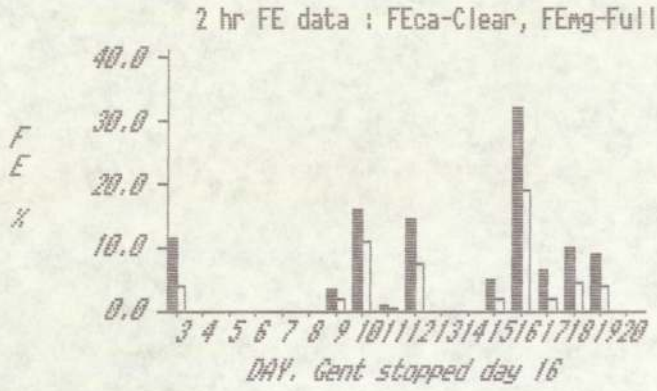


Plate 2

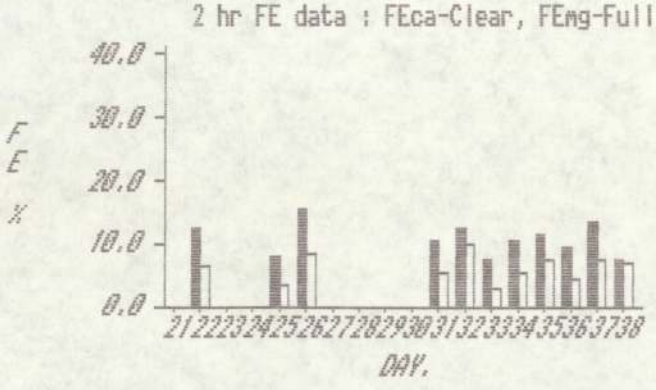


Fig 16a : 2 hr FEca and 2 hr FEmg in patient 3.

Plate 2 is a continuation of Plate 1

Plate 1

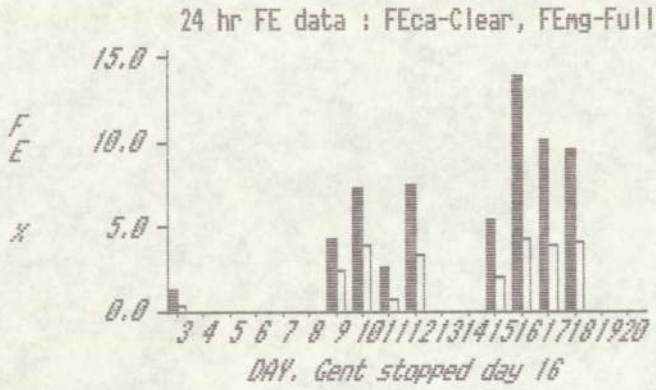


Plate 2

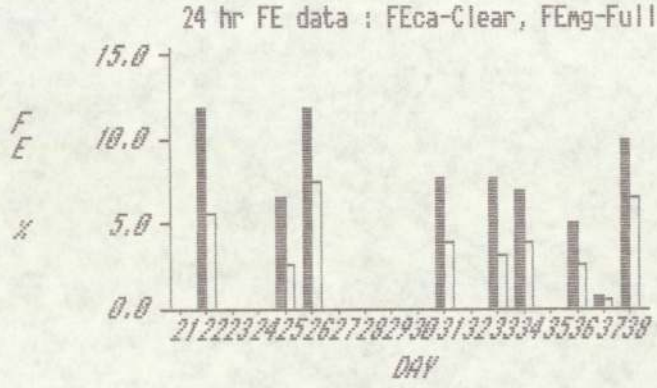


Fig 16b : 24 hr FEca and 24 hr FEmg in patient 3

Plate 2 is a continuation of Plate 1

the maximum excretion shown (Table 39) were the only days that the EXca was elevated in this patient. Patient 3 showed a rise in the 24 hr EXca although the maximum excretion shown was the only abnormally high excretion seen. In each case the EXca was considered appropriate for the Sca level.

The elevated 2 hr and 24 hr FEca in patient 5 (course 3) was very brief and was considered to represent a transient fluctuation in an otherwise normal FEca. The EXca and the Sca were low in this patient. Patient 5 (course 2) showed a definite elevation in the 2 hr FEca. No early or pre-dose 24 hr data were available in this patient although the elevated 24 hr FEca fell to normal levels for the last 5 days of collection. This suggests that an early rise in the 24hr FEca had occurred.

Patient 4 (course 1) had a 2 hr and 24 hr FEca above the normal range during the pre-dose period. No elevation in the FEca was seen during aminoglycoside therapy. The EXca was also above the normal range throughout the study period in this patient. Because of the high pre-dose data, the inappropriate EXca and the development of hypocalcaemia were considered to be secondary to factors other than aminoglycoside administration.

Patient 1 (course 1) had a 2 hr and 24 hr FEca which was initially above the normal range. This remained unchanged during aminoglycoside administration. No pre-dose data were available for the 24 hr FEca. The 24 hr FEca was abnormally high throughout aminoglycoside therapy although a small elevation was seen on days 15 to 17 of therapy. The EXca and the Sca were normal throughout the study period.

This high FEca was associated with hypocalcaemia in 3 patients [patients 1 (course 1), 4 (course 1) and 5 (courses 2 and 3)]. Those patients who showed definite rises in FEca [patients 3, 14 (course 1), and 20] remained normocalcaemic. In no patient was the elevated FEca associated with hypercalcaemia.

a) Relationship between hypocalcaemia and FEca.

In only one patient (patient 4) did the abnormally high 2 hr and 24 hr FEca precede the hypocalcaemia. The hypocalcaemia was seen from Day 7/13 (Table 34) and the FEca was above the normal range during the pre-dose period.

In the remaining three patients with hypocalcaemia there was no such relationship. In patient 5 (course 2) the hypocalcaemia preceded the elevation in 2hr FEca and followed the elevation in 24 hr FEca. All parameters

returned to normal at the same time (Day 25). Patient 5 (course 3) was hypocalcaemic from Day 1 to 15. As described above, the FEca in this patient was normal.

In patient 1 (course 1), hypocalacaemia and abnormally high 2 hr FEca were seen from Day -1. No pre-dose 24 hr FEca was available but a 24 hr FEca above the normal range was seen from the first available day (Day 2). The abnormally high 2 hr and 24 hr FEca persisted when the Sca returned to normal on Day 8, and after aminoglycoside therapy was stopped on Day 7. So no apparent change in the FEca was seen in this patient during gentamicin therapy but on Day 15 there was a sharp rise in the 24 hr FEca (2hr data not measured). This continued until the patient was discharged on Day 20 . No change in the Sca level was seen during these 5 days.

ii) 2 hr and 24 hr magnesium excretion.

The normal 24 hr excretion of magnesium (EXmg) in hospitalised patients is 2.5 to 7.5mmol/24 hour [clinical chemistry reference values, EBH]. The normal fractional excretion of magnesium (FEmg) is 3.0 to 5.0% [Barton et al, 1984; Dirks, 1983; Ebel and Gunther, 1980]. For individual patients, changes in FEmg with time were studied as well as absolute values.

Table 41 lists the initial and maximum 2 hr FEmg in the aminoglycoside-treated patients and Table 42 lists the initial and maximum 24 hr FEmg in the aminoglycoside-treated patients. The initial and maximum 24 hr EXmg are shown in Table 43. The Smg on the day of the maximum FEmg or EXmg is shown and the day of the maximum excretion and the mean ($\pm$ S.D.) FEmg or EXmg is also shown.

The FEmg increased from initial levels in all of the aminoglycoside-treated patients. The mean data suggests that the maximum 2 hr FEmg is greater than the maximum 24 hr FEmg and that the maximum 2 hr and 24 hr FEmg are seen at the same time.

Patients 3, 5 (courses 2 and 3), and 14 (course 1) had an FEmg below the normal range initially. These patients were hypomagnesaemic and so this low FEmg was a normal response to the low Smg. All of these patients showed an elevation of the FEmg during aminoglycoside therapy without a change in the Smg, indicating a loss of this conservation. In patients 3 and 14 (course 1) the elevation in FEmg resulted in the 2 hr and 24 hr FEmg going above the normal range. An increase in the FEmg to abnormally high levels was also seen in patient 20 despite a Smg at the lower end of the normal range. Patients 14 (course 1) and 20 had normal EXmg and normal Smg. In patient 3 the initial EXmg was low as

Table 41 : Summary of the initial 2 hr FEmg and maximum 2 hr FEmg in aminoglycoside-treated patients and the day the maximum was measured.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
1.1	2.4	-1	7.9	6
3	11.4	3	32.0	16
4.1	1.6	-3	2.6	17
5.2	1.5	5	4.3	12
5.3	1.1	-1	4.0	13
14.1	2.7	-1	12.1	6
20	2.9	5	15.6	29
Mean	3.4		11.2	14.1
S.D.	3.6		10.3	7.9

* = normal range 3.0 to 5.0%

Table 42 : Summary of the initial 24 hr FEmg and maximum 24 hr FEmg in aminoglycoside-treated patients, and the day the maximum was measured.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
1.1	4.6 (0.7)	-1	4.7 (0.6)	19
3	1.2 (0.5)	3	14.0 (0.7)	16
4.1	4.0 (0.6)	-4	4.5 (0.7)	17
5.2	1.4 (0.7)	5	3.6 (0.8)	16
5.3	1.2 (0.7)	-1	3.7 (0.8)	11
14.1	2.2 (0.7)	1	10.5 (1.0)	4
20	5.0 (0.8)	5	13.0 (0.8)	10
Mean	2.8		7.7	13.3
S.D.	1.7		4.6	5.2

* = normal range 3.0 to 5.0%
Smg (mmol L^{-1}) in brackets

Table 43 : Summary of the initial 24 hr EXmg and maximum 24 hr EXmg in aminoglycoside-treated patients, and the day the maximum was measured.

Patient ¦ course	Initial EXmg*	Day of initial	Maximum EXmg *	Day of maximum
1.1	4.7 (0.7)	-1	2.6 (0.7)	5
3	0.4 (0.5)	3	4.0 (0.7)	16
4.1	2.7 (0.7)	-3	5.1 (0.7)	17
5.2	0.8 (0.7)	5	4.1 (0.9)	12
5.3	2.0 (0.7)	-1	4.8 (0.9)	6
14.1	1.7 (0.7)	1	7.8 (1.0)	4
20	4.9 (0.8)	5	4.4 (0.8)	11
Mean	2.5		4.7	
S.D.	1.8		1.6	

* = normal range 2.5 to 7.5 mmol/24 hr
Smg (mmol L^{-1}) shown in brackets

was the Smg. The peak EXmg was however normal in this patient whilst the Smg was borderline hypomagnesaemic, indicating loss of conservation.

In patient 5 (courses 2 and 3) a rise in the 2 hr and 24 hr FEmg was seen although the FEmg remained within the normal range. The EXmg was within the normal range in these courses.

Patient 1 (course 1) showed a rise in the 2hr FEmg to an elevated level and a fall followed by a rise in the 24 hr FEmg. The maximum EXmg was appropriate for the normal EXmg. Only patient 4 (course 1) showed no change in the 2 hr or 24 hr FEmg. The 2 hr FEmg was low, indicating magnesium conservation, whilst the 24 hr FEmg remained at a normal level. Both the 2 hr and 24 hr FEca were elevated in this patient.

In patient 3 the elevated FEmg was associated with a hypomagnesaemia from Day 3 to Day 19. Patient 1 (course 1) had a slighly elevated 2 hr FEmg towards the end of therapy when the Smg was normal and the 24 hr FEmg rose to normal levels on Days 14 to 20 when the Smg was low. Patients 14 (course 1) and 20 were normomagnesaemic (Table 36).

Of the seven courses of aminoglycoside treatment (six patients), two patients showed a FEmg above the

normal range which was associated with a hypomagnesaemia [patients 3 and 1 (course 1)], and two patients showed an FEmg above the normal range associated with normomagnesaemia [patients 14 (course 1) and 20].

a) Relationship between the hypomagnesaemia and the FEmg.

There was no clear relationship between the elevated FEmg and the development of hypomagnesaemia in aminoglycoside-treated patients. Patient 3 was hypomagnesaemic from Day =< 3. The elevation in 2 hr FEmg was also seen from Day =< 3 whilst the elevated 24 hr FEmg was not seen until Day 10. The FEmg on Day 9 was normal however (4.3%) which was inappropriately high for the low Smg.

Patient 1 (course 1) had an elevated 2hr FEmg prior to the hypomagnesaemia. An elevated 24 hr FEmg was not seen although the normal levels from Day 17 were inappropriate for the low Smg.

iii) Comparison of renal calcium and magnesium handling in aminoglycoside-treated patients.

a) Patients with calcium and magnesium wasting.

Renal wasting of cations occurs when the fractional

excretion is within the normal range and the serum level is below the normal range, or when the fractional excretion is above the normal range and the serum level is either within or below the normal range.

Four patients showed 2 hr and 24 hr wasting of both calcium and magnesium. Patients 3, 14 (course 1) and 20 had the largest FEmg and FEca . These patients also had the longest courses of aminoglycoside therapy (patient 3 and 20) or the highest total aminoglycoside dose (14 (course 1) and 20). Serum electrolyte abnormalities were only seen in patient 3 who developed a hypomagnesaemia on Day =< 3 of therapy.

Patient 1 (course 1) also showed renal wasting of both cations. Both a hypomagnesaemia and a hypocalcaemia were seen in this patient.

Tables 44 and 45 shows the relationship between the 2hr and 24 hr calcium and magnesium wasting. It can be seen from Table 44 that the 2 hr and 24 hr calcium wasting started during antibiotic therapy, except for patient 1 (course 1) where the 2 hr wasting was present prior to antibiotic therapy. There is no clear relationship between the 2 hr and 24 hr wasting. The wasting of calcium in patient 14 (course 1) stopped soon after the aminoglycoside therapy was stopped but in the remaining patients the wasting for both the 2 hr and

24 hr collection periods persisted after aminoglycoside therapy stopped. The EXca in these patients was generally normal giving no indication of this wasting. In patient 14 (course 1) the EXca was high however from Days 7 to 13 when elevated FEca was seen.

The wasting of magnesium (Table 45) followed a similar pattern to the calcium wasting. In each case the 2 hr wasting started during aminoglycoside administration. The 24 hr wasting tended to follow the 2 hr wasting and in patient 1 (course 1), 24 hr wasting started after aminoglycoside therapy had stopped. In patients 1 (course 1) and 3, the EXmg was low indicating apparent magnesium conservation and in patients 14 (course 1) and 20, the EXmg was at the bottom of the normal range, again not showing this wasting effect (Table 42).

It was not possible to determine if magnesium or calcium wasting were seen first. In general, the wasting of both cations was seen at the same time.

Table 44 : Relationship between the 2 hr calcium wasting, the 24 hr calcium wasting and the antibiotic course.

Patient course	2 hr wasting Begin	2 hr wasting End	24hr wasting Begin	24hr wasting End	Last day of aminoglycoside therapy
1.1	-1	>=8	=<2	>=20	7
3	3	>=40	=<9	>=40	16
14.1	6	13	6	13	10
20	5	36	5	32	23

Table 45 : Relationship between the 2 hr magnesium wasting, the 24 hr magnesium wasting and the antibiotic course.

Patient course	2 hr wasting Begin	2 hr wasting End	24hr wasting Begin	24hr wasting End	last day of aminoglycoside therapy
1.1	4	>=9	15	20	7
3	3	>=40	=<9	>=40	16
14	4	8	4	12	10
20	6	33	7	15#	23

wasting in patient 20 also seen between days 30 and 36

b) Patients with calcium wasting only.

Two patients (three courses) showed inappropriate levels of calcium excretion without any abnormality in magnesium excretion [Patients 4 (course 1) and 5 (courses 2 and 3)]. Each case was associated with hypocalcaemia. In patient 4 (course 1) elevated FEca was seen prior to gentamicin administration and was therefore unrelated to aminoglycoside administration. This wasting of calcium was followed by a persistent hypocalcaemia. Patient 5 (course 2) had an initially normal and then a slightly elevated 2 hr and 24 hr FEca. The hypocalcaemia followed the elevated 2 hr FEca and preceded the elevated 24 hr FEca. During course 3, patient 5 was hypocalcaemic from Day 1. The 2 hr FEca was low throughout therapy whilst the 24 hr FEca was low initially and normal on Days 6 to 15 indicating significant wasting during this period. The EXca was low throughout therapy.

c) Patients showing magnesium wasting only.

No patient in the aminoglycoside-treated group showed wasting of magnesium only.

B) Control patients.

i) 2 hr and 24 hr calcium excretion.

Table 46 lists the initial and maximum 2hr FEca in the control group, and the day that the FEca was measured. Table 47 lists the initial and maximum 24 hr FEca in the control group and the day of measurement. The initial and maximum 24 hr EXca are shown in Table 48. The Sca on the day of the EXca or FEca is shown and the mean ($\pm$ S.D.) FEca or EXca is also shown.

Six patients (7 courses) in the control group had an elevated EXca during or after antibiotic therapy. In four cases [14 (course 2), 18, 24 (course 1) and 27], antibiotic therapy may have been the cause for this elevated excretion. The EXca increased during antibiotic therapy (benzylpenicillin) in patient 18. Extensive pre-dose data (11 days) indicated that the EXca in this patient was within the normal range throughout this period. The pre-dose data in patient 27 were above the normal range. However the EXca was elevated further during antibiotic treatment (thienamycin) but the maximum excretion seen in Table 48 was a transient fluctuation. All other EXca in this patient were within the range 6.3 to 10.5 mmol/ 24hr. Patient 14 (course 2) had a high EXca from Day 1 and this persisted until therapy (thienamycin) stopped on

Table 46 : Summary of the initial 2 hr FEca and maximum 2hr FEca in control patients, and the day the maximum FEca was seen.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
4.2	1.8	1	5.2	4
5.1	0.3	1	10.9	1
6	0.9	1	4.0	8
9	0.2	-1	1.7	13
14.2	1.7	1	7.9	3
18	2.0	-1	6.4	17
21	0.6	-1	0.9	6
22	<0.1	-1	<0.1	9
24.1	0.5	-1	1.7	12
24.2	1.5	-1	1.4	11
27	3.4	-1	4.4	12
Mean	1.2		4.1	8.7
S.D.	1.0		3.4	4.9

* = normal range 0.5 to 1.0%

Table 47 : Summary of the initial 24 hr FEca and maximum 24 hr FEca in the control patients, and the day the maximum was measured.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
4.2	2.9 (2.5)	-1	4.2 (2.3)	4
5.1	0.9 (2.3)	1	7.9 (1.9)	2
6	0.8 (2.2)	1	2.5 (2.5)	7
9	2.3 (1.8)	-1	3.2 (1.6)	9
14.2	3.5 (2.2)	1	6.2 (2.0)	3
18	3.6 (2.3)	-1	9.1 (2.3)	14
21	0.1 (2.2)	1	0.4 (2.5)	3
22	<0.1 (1.8)	-2	0.1 (2.5)	9
24.1	1.6 (2.5)	-1	2.7 (2.2)	13
24.2	1.9 (2.4)	1	4.2 (2.1)	10
27	2.8 (2.0)	-1	7.9 (1.9)	2
Mean	1.9		4.4	6.9
S.D.	1.3		3.0	4.4

* = normal range 0.5 to 1.0%

Sca (mmol L^{-1}) shown in brackets

Table 48 : Summary of the initial 24 hr EXca and maximum 24 hr EXca in the control patients, and the day the maximum was measured.

Patient course	Initial EXca*	Day of initial	Maximum EXca*	Day of maximum
4.2	10.6 (2.5)	-1	10.2 (2.1)	2
5.1	3.9 (2.5)	-1	5.0 (1.9)	2
6	4.7 (2.2)	1	7.2 (2.5)	7
9	2.7 (1.8)	-1	2.8 (1.6)	9
14.2	8.2 (2.2)	1	7.3 (2.3)	8
18	7.0 (2.3)	-1	13.9 (2.3)	13
21	0.7 (2.2)	-1	2.5 (2.1)	5
22	<0.1 (2.1)	-1	2.0 (2.5)	8
24.1	4.6 (2.5)	-1	6.4 (2.2)	13
24.2	6.4 (2.4)	-1	6.2 (2.3)	7
27	6.0 (2.0)	-1	19.8 (1.9)	2
Mean	5.0		8.2	7.7
S.D.	3.1		5.1	5.2

* = normal range 2.5 to 5.0 mmol/24 hr

Sca (mmol L-1) shown in brackets

Day 9. No pre-dose data were available for this patient but the abnormally high excretion may have been due to antibiotic treatment. Five days of pre-dose data indicated that the EXca in patient 24 was initially within the normal range. During antibiotic course 1 (thienamycin) the EXca rose and reached a peak on the last day of treatment (Day 13). During antibiotic course 2 (ciprofloxacin and vancomycin), which immediately followed course 1, the EXca then fell to pre-dose levels. The elevation during course 1 was considered to be secondary to antibiotic therapy as the fall in excretion was seen with a change of antibiotic. The Sca remained within the normal range throughout the two courses of treatment in this patient.

Elevations in EXca not thought to be due to antibiotic therapy were seen in three patients [Patients 4 (course 2), 6 and 24 (course 2)]. Patient 4 (course 2) had a very high EXca during the extensive pre-dose period (26 days) which continued during and after the treatment period. The reason for this high calcium excretion was unclear but it may have been secondary to the chemotherapy this patient received. This patient also had a high EXca during a previous course of gentamicin therapy (Table 39). In patient 6 the elevated EXca was a transient fluctuation in a series of normal excretions. The elevated excretion was not therefore considered to be secondary to antibiotic

therapy. Patient 24 (course 2) had an initially high EXca but showed a fall in EXca as described above.

Patient 21 had normal 2 hr and 24 hr FEca as well as a normal EXca. The FEca showed no change with antibiotic treatment. In patient 22 there was no change in the 2 hr and 24 hr FEca with antibiotic treatment. Both the FEca and EXca were very low indicating renal conservation of calcium. Neither patient was hypocalcaemic. Patients 5 (course 1) and 9 had elevated FEca without an elevation in the EXca. Both patients were hypocalcaemic during some stage of the antibiotic treatment.

All of the control patients had an abnormally high 2 hr and\or 24 hr FEca at some stage of the study. Patients 24 (course 2) and 27 showed no change in the FEca with antibiotic therapy. The peak shown for patient 27 in the 24 hr FEca was a single sharp high FEca in a series of elevated but constant FEca measurements.

Seven courses of antibiotic therapy were associated with elevations of the FEca which were possibly attributable to antibiotic treatment [patients 4 (course 2), 5 (course 1), 6, 9, 14 (course 2), 18 and 24 (course 1)]. In 5 patients a rise in both the 2 hr and 24 hr FEca was seen with antibiotic therapy [5 (course 1), 6, 14 (course 2), 18, and 24 (course 1)]. In each

case an abnormally high maximum FEca was also seen. Two patients showed a rise in the 2 hr FEca but not the 24 hr FEca [patients 4 (course 2) and 9].

Hypocalcaemia was seen in three of these patients [5 (course 1), 9, 14 (course 2)]. The elevation in 2 hr and 24 hr FEca was seen at the same time as the hypocalcaemia in one patient (patient 14). In patient 5 (course 1) the elevation in FEca preceded the hypocalcaemia whilst in patient 9 the elevated FEca followed the hypocalcaemia.

ii) 2 hr and 24 hr magnesium excretion.

Table 49 lists the initial and maximum 2hr FEmg in the control group, and the day that the FEmg was measured. Table 50 lists the initial and maximum 24 hr FEmg in the control group and the day of measurement. The initial and maximum EXmg is shown in Table 51. The Smg on the day of the FEmg or EXmg is shown and the mean ($\pm$ SD) FEmg or EXmg is also shown.

No patient in the control group showed an abnormal EXmg or FEmg that was considered to be secondary to antibiotic administration. Two patients were initially hypomagnesaemic (patients 6 and 9). In patient 6, both the 2 hr and 24 hr FEmg were below the normal range

Table 49 : Summary of the initial 2 hr FEmg and maximum 2hr FEmg in control patients, and the day the maximum FEmg was seen.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
4.2	2.5	-1	7.6	4
5.1	0.7	-1	7.2	6
6	1.9	1	10.7	8
9	4.1	-1	8.6	13
14.2	1.6	1	4.7	2
18	1.4	-1	3.1	8
21	0.8	-1	3.0	6
22	0.3	-1	1.7	10
24.1	1.7	-1	2.2	1
24.2	1.0	-1	1.0	10
27	2.0	-1	2.2	2
Mean	1.6		4.7	6.4
S.D.	1.0		3.3	3.9

* = normal range 3.0 to 5.0%

Table 50 : Summary of the initial 24 hr FEmg and maximum 24 hr FEmg in control patients, and the day the maximum was measured.

Patient course	Initial FE (%)*	Day of initial	Maximum FE (%)*	Day of maximum
4.2	2.6 (0.7)	-1	11.8 (0.6)	4
5.1	1.4 (0.8)	1	4.3 (0.7)	2
6	1.2 (0.6)	1	7.4 (0.6)	8
9	10.3 (0.6)	-1	27.5 (0.5)	18
14.2	3.3 (0.8)	1	4.5 (0.8)	5
18	2.3 (0.7)	-1	3.7 (0.7)	14
21	1.7 (0.7)	2	2.0 (0.7)	3
22	0.2 (0.7)	-1	3.0 (0.9)	10
24.1	2.3 (0.7)	-1	2.3 (0.8)	12
24.2	1.7 (0.8)	-1	2.3 (0.8)	10
27	1.6 (0.8)	-1	3.0 (0.6)	12
Mean	2.6		6.5	8.9
S.D.	2.7		7.5	5.0

* = normal range 3.0 to 5.0%

Smg (mmol L^{-1}) shown in brackets

Table 51 : Summary of the initial 24 hr EXmg and maximum 24 hr EXmg in control patients, and the day the maximum was measured.

Patient course	Initial EXmg*	Day of initial	Maximum EXmg*	Day of maximum
4.2	3.0 (0.7)	-1	7.1 (0.6)	4
5.1	2.3 (0.8)	1	5.0 (0.7)	2
6	2.2 (0.6)	1	4.4 (0.6)	8
9	4.6 (0.6)	-1	9.9 (0.9)	21
14.2	3.2 (0.8)	1	5.1 (0.9)	8
18	1.7 (0.7)	-1	2.6 (0.7)	11
21	0.9 (0.7)	1	2.3 (0.6)	5
22	0.1 (0.7)	-1	2.9 (0.9)	11
24.1	2.4 (0.7)	-1	2.5 (0.8)	12
24.2	1.6 (0.8)	-1	1.6 (0.7)	3
27	1.6 (0.8)	-1	2.4 (0.7)	8
Mean	2.1		4.2	8.5
S.D.	1.2		2.5	5.4

* = normal range 2.5 to 7.5 mmol\24 hr

Smg (mmol L^{-1}) shown in brackets

initially, indicating conservation of the cation. The abnormally high FEmg seen on Day 8 was a transient increase in a series of low FEmg levels and was not considered to be a true elevation. Patient 9 had a 2 hr and 24 hr FEmg above the normal range during the pre-dose period that persisted during the treatment period. The patient was hypomagnesaemic throughout the study. This renal wasting was considered to be secondary to factors other than antibiotic administration.

The remainder of the patients in the control group were borderline hypomagnesaemic initially. The low FEmg seen initially in this group was appropriate for the low serum levels. The FEmg remained at this low level throughout antibiotic treatment in all patients except patients 6 and 9, and patient 4 (course 2). The abnormally high FEmg seen in patient 4 (course 2) was a transient increase and so this was not considered to be a true peak in the excretion profile.

Patient 9 was the only patient in the control group to have an EXmg above the normal range. This was seen on Day 21 of therapy but was the only high EXmg in a series of normal excretions. This rise was considered to be too sudden to represent a true peak in the excretion profile. Patients 4 (course 2), 5 (course 1), 6, 14 (course 2), 18, 24 (course 1) and 27 all

had normal or slightly low EXmg throughout the study period. The EXmg was very low throughout the study period in patients 21, 22 and 24 (course 2). Patient 21 had a mild hypomagnesaemia and patients 22 and 24 (course 2) were normomagnesaemic. The renal function in these patients was normal also. The reasons for this low EXmg are therefore unclear.

It is of interest that four patients [patients 5 (course 1), 14 (course 2), 18, 27] all had large elevations of the 2 hr and 24 hr FEca and had very low FEmg levels.

iii) Comparison of renal calcium and magnesium handling in control patients.

a) Patients with renal wasting of both calcium and magnesium.

In the control group, one patient (patient 9) showed wasting of both calcium and magnesium. Wasting was evident during both the 2 hr and 24 hr collection periods. The electrolyte wasting was very severe as elevated fractional excretion was seen with low serum electrolyte levels. The cause of the wasting is unclear as it was seen before antibiotic therapy was started and before chemotherapy was administered. The renal loss of both cations increased with treatment but this may have

been secondary to DAT administration on Day 3. The daily excretion of both electrolytes was within the normal range (Table 47 and Table 50).

b) Patients with renal wasting of calcium only.

Eight control patients had renal calcium wasting. In four patients [patients 5 (course 1), 6, 14 (course 2), 24 (course 2)] the wasting was considered to be related to antibiotic administration. The calcium wasting was seen early in therapy with the 2 hr and 24 hr wasting being seen at the same time.

In three of these patients, the renal calcium wasting was associated with hypocalcaemia [patients 5, 14 (course 2) and 24 (course 2)]. The hypocalcaemia was transient and followed the wasting of the cation.

c) Patients with renal wasting of magnesium only.

Magnesium wasting alone was not seen in any patient in the control group. Only one patient showed magnesium wasting (patient 9) and this patient also had calcium wasting. This wasting was considered to be unrelated to antibiotic administration.

C) Electrolyte excretion in patients with evidence of proximal tubular damage.

Proximal tubular damage, as indicated by elevated RBP excretion, was seen in 6 patients in the aminoglycoside-treated group and 9 patients in the control group.

i) Aminogylcoside-treated patients.

In patient 1, elevated RBP excretion was considered to be secondary to pyrexia and not aminoglycoside administration. FEca was elevated from Day -1 as was the RBP excretion. A small, transient elevation in the FEmg was seen from Day 4 to Day 6, after the RBP excretion returned to normal levels. In patients 3, 4 (course 1), 5 (course 2), 14 (course 1) and 20, the elevated RBP excretion was considered to be secondary to aminoglycoside administration.

Patients 3 and 20 showed very similar RBP and electrolyte excretion profiles. Maxima in the fractional excretion of both electrolytes occurred at the same time as maxima in the RBP excretion, although elevation in the FEmg and FEca appeared to follow elevation in the RBP excretion. In each case, the elevated fractional excretion of both electrolytes persisted after aminoglycoside therapy was stopped. In

contrast, RBP excretion fell to normal levels when aminoglycoside administration was stopped.

In patient 4 (course 1) there was no relationship between the elevation in RBP excretion and the fractional excretion of magnesium or calcium. FEca was elevated during the pre-dose phase and remained elevated during aminoglycoside treatment. FEmg was below the normal range throughout the treatment period.

In patient 5 (course 2), the maximum FEca and FEmg were seen after the maximum RBP excretion. The FEmg was not elevated at any stage although a rise in the FEmg was seen, whilst the FEca was slightly elevated. The elevation in FEca persisted for at least 18 days after aminoglycoside therapy was stopped. In contrast patient 5 (course 3) showed no elevation in the RBP excretion. The FEca was below the normal range and the FEmg was normal in this patient.

Patient 14 (course 1) had an elevated RBP excretion early in therapy. The maximum FEmg was seen after the maximum RBP excretion and the maximum FEca followed the maximum FEmg. In each case, the fractional excretion returned to normal levels when aminoglycoside therapy was stopped.

Thus, it can be seen in the aminoglycoside-treated

patients that elevation of the FEca or FEmg occurred at the same time or following elevation of the RBP excretion. In the one patient in this group where RBP excretion was not elevated, there was no elevation in the FEmg or FEca.

ii) Control patients.

Elevated RBP excretion, indicating proximal tubular damage, was seen in 9 patients in the control group. In 5 of these patients the tubular damage may have been secondary to antibiotic administration [patients 5 (course 1), 6, 9, 24 (course 1) and 27].

In patient 5 (course 1) the small elevation in RBP excretion was preceded by an elevation in the FEca and FEmg. The elevated FEca was greater than the elevated FEmg.

Patients 6 and 9 showed large excretions of RBP indicating massive tubular insult. In each patient however, only small and transient elevations in FEmg were seen. In patient 9 the FEca was below the normal range, whilst in patient 6 there was a small and transient elevation of FEca.

Patients 24 (course 1) and 27 also showed elevation of RBP excretion without an effect on the FEca or the FEmg.

During the second antibiotic course in patient 24 (24.2) there was an elevation in the RBP excretion early in therapy. The FEmg remained below the normal range, whilst the FEca became elevated. The elevation was maintained at least until Day 11 (3 days after antibiotic therapy stopped) when measurements stopped.

Patients 4 (course 2), 14 (course 2) and 18 had no elevation in the RBP excretion. FEmg was normal in patients 4 and 14 and below the normal range in patient 18. The FEca showed a transient elevation early in therapy in patients 14 and 18, whilst in patient 4 a pesistant, slightly elevated FEca was seen. Patients 21 and 22 also had no elevation in the RBP excretion. Furthermore the fractional excretion of both electrolyte in each patient was below the normal range.

Thus in the control group, patients with tubular damage as indicated by elevated RBP excretion, did not always develop electrolyte abnormalities and any increases in the FE were generally small. Electrolyte disturbance was also seen prior to tubular damage. Moreover, elevations in fractional excretion were seen in patients with no tubular damage. FEca was affected to a greater extent than FEmg.

DISCUSSION AND CONCLUSIONS

Discussion and conclusions of protein excretion results.

The incidence of nephrotoxicity in the aminoglycoside-treated group was comparable with other studies [Kahlmeter and Dahlager, 1984]. Two patients showed a mild rise in Scr which may have been due to a renal cause. A more serious rise in Scr was probably avoided by careful monitoring of aminoglycoside serum levels. Aminoglycoside serum levels were kept within the therapeutic range in all patients. A third patient, patient 3, showed a pre-renal rise in Scr. The fall in GFR accompanying this rise in Scr suggests however that there was a renal component to this increase in Scr. The incidence of nephrotoxicity in the control group was 17% suggesting that other factors, possibly cytotoxic chemotherapy, were responsible for the renal impairement in the aminoglycoside-patients.

Similarly to earlier studies [Merle et al, 1981; Dawson, 1986] the urinary excretion of RBP was raised in most aminoglycoside-treated patients whether or not a significant rise in the Scr was seen. Certainly, monitoring of the urinary excretion of RBP failed to identify specifically those patients who developed significantly increased Scr during aminoglycoside therapy. Indeed, the almost universally elevated

excretion of RBP in the aminoglycoside-treated patients, together with the difference in RBP excretion in those patients who received both aminoglycoside treatment and control therapy, suggests that elevated RBP excretion may be a function of aminoglycoside administration. Earlier work in healthy volunteers [Smith et al, 1986], indicated that gentamicin and netilmicin cause damage to the proximal tubule and inhibit the tubular reabsorption of Beta$_2$-microglobulin (B_2M) in virtually all patients.

MUR excretion was generally within the normal range, and elevations in excretion appeared to be more specific to those patients in the aminoglycoside-treated group with elevations in the Scr. Elevated MUR excretion was seen in both aminoglycoside-treated patients who showed mild nephrotoxicity and in patients 1 (course 1) and 3. Since only 2 patients in the control group had an elevated MUR excretion, this suggests that the MUR excretion was more specific for aminoglycoside-induced nephrotoxicity. However, the lack of elevated MUR excretion in the majority of patients in this study may have been secondary to S_{MUR} levels that were below the normal range. It is possible that a reduced filtered load would make any reduction in reabsorption difficult to detect as an elevated excretion. However, patient 14 (course 1) had a S_{MUR} (Table 8a) below the normal range on the same day that an elevated excretion of MUR

was apparent (Table 25). Since only patients 18 and 24 had SMUR level lower than patient 14 (Tables 8a and 8b), and all patients, except patient 3, had a normal creatinine clearance, it is probable that any significant impairment of MUR reabsorption would have been detectable in all the other patients.

As reported previously [Dawson, 1986], the excretion of LMW proteins was very low in the patients with cystic fibrosis. In the present study, the magnitude and timing of the maximum RBP excretion in the aminoglycoside-treated group appeared to be related to the duration of the aminoglycoside course. A greater degree of proteinuria might have been expected in these patients considering the duration of treatment used and the relatively high aminoglycoside doses. The reason for the low excretion is unclear. Low serum levels of RBP have been reported previously in patients with cystic fibrosis [Dawson, 1986; Smith et al, 1972; Navarro and Desquilbet, 1981] and this was also apparent in the present study (Table 7a and Table 7b). It was unlikely that the low serum levels were responsible for the low RBP excretion since patient 14 (course 1), 1 (course 2), 24 (course 2), 27 and 9 all showed elevated RBP excretion despite having lower than normal serum levels. Low SMUR levels were not apparent in patients with cystic fibrosis in this study. It has been suggested that a greater degree of tubular

proteinuria is not seen in these patients because of altered renal handling of LMW protein in these patients [Dawson, 1986]. It was also considered possible that these patients developed resistance to the nephrotoxic effects of aminoglycoside antibiotics following repeated courses [Dawson, 1986]. Acquired resistance to the nephrotoxic effects of aminoglycoside antibiotics has been reported in rats [Luft et al, 1978; Gilbert et al, 1979; Elliott et al, 1982]. Luft et al [1978] and Gilbert et al [1979] demonstrated that recovery of renal structure and function begins after 2 weeks of gentamicin treatment, even if drug administration is continued. It has also been shown in rats that this acquired resistance is short lived and that six months following resistance developing, the kidney is sensitive once more to the toxic effects of aminoglycosides [Elliot et al, 1982]. The mechanism of this acquired resistance is unclear but it is thought to be a characteristic of regenerating tubular epithelium. Two patients in the present study received more than one course of aminoglycoside therapy (patient 1 and patient 5). In each case, urinary excretion of RBP and MUR was reduced during the second course of treatment. It is possible that this, and the low excretion of LMW proteins in patients with cystic fibrosis may be due to an acquired resistance to the tubular toxicity of aminoglycoside antibiotics. No evidence of acquired resistance developing during prolonged courses of

treatment was apparent. Examination of the LMW protein excretion in patient 3 (Fig 6a and Fig 6b) who received gentamicin treatment for 16 days gave no indication of resistance developing. In patient 20, who received 27 days of gentamicin treatment, urine collections were missed from Day 18 to Day 22. When collections were resumed, RBP excretion was still elevated although MUR excretion had returned to normal. The significance of this is unclear.

All patients entered into this study were pyrexial. It has been shown that pyrexia can cause proteinuria [Jensen and Henriksen, 1974; Hemmingsen and Skaarup, 1977; Ehrich et al, 1981]. Both glomerular proteinuria, the excretion of albumin and other large proteins, and tubular proteinuria have been described [Hemmingsen and Skaarup, 1977]. The tubular proteinuria is thought to be caused by the fever per se and is transient, disappearing within three days of the pyrexia settling. The increased urinary excretion of LMW proteins may be due to a transient impairment of reabsorption, or an increased plasma concentration of these proteins [Hemmingsen and Skaarup, 1977]. It has also been suggested that infection may result in immunological injury to the glomerular and\or tubular basement membrane [Jensen and Henriksen, 1974]. Generalised sepsis has also been shown to cause tubular proteinuria [Linton et al, 1984] with large elevations

in B_2M being seen in critically ill septic patients. The protein excretion was unrelated to aminoglycoside administration, and studies in sheep indicated that the proteinuria is associated with damage to proximal tubular cells. Many patients in the present study, especially those with haematological malignancies, showed elevations in RBP excretion during the days before therapy and which settled rapidly during aminoglycoside therapy. The excretion profiles for patients 3 (Fig 8a and Fig 8b) and 6 (Fig 14) illustrate this effect. It is possible that these early elevations in excretion were secondary to the pyrexia.

Cytotoxic administration is known to damage the kidney and can cause acute renal failure. Many patient in the present study received cytotoxic treatment prior to or concurrently with aminoglycoside therapy. It appeared that the DAT combination had little or no effect on RBP or MUR excretion. Flemming et al [1980] studied the excretion of B_2M, N-acetyl-beta-D-glucosaminidase (NAG), and total protein in patients receiving treatment with DAT or other combinations of cytotoxic drugs. They also studied patients with haematological malignancies who had previously been given a variety of cytotoxic drugs and who had a septicaemia for which they were given aminoglycosides. Surgical patients receiving gentamicin prophylactically were also studied. They found that the administration of DAT or other cytotoxic

drugs had little effect on B_2M excretion. The most elevated LMW protein and enzymuria was seen in patients treated with aminoglycosides that had previously received cytotoxic chemotherapy. Thus, many factors influence the excretion of LMW proteins in sick patients. Pyrexia, sepsis, previous cytotoxic administration and previous aminoglycoside exposure were all possible complicating factors in this study. Indeed, the highest LMW protein excretions were seen in the control group. One or more of these factors will probably be present in the majority of patients receiving aminoglycoside antibiotics. Indeed, the RBP excretion of both the control group and aminoglycoside-treated group was higher during the pre-dose phase than is normal for healthy subjects [Lucertini et al, 1984; Bernard et al, 1982], indicating that the disease process or other factors were affecting LMW protein excretion prior to antibiotic administration. The usefulness of these LMW proteins as a marker for impending nephrotoxicity is doubtful. However, MUR may be a more sensitive marker for aminoglycoside-induced nephroxicity.

Although a poor marker of impending nephrotoxicity, urinary RBP excretion is a good marker of proximal tubular damage. It may therefore have a role in studies looking at the mechanism of aminoglycoside-induced abnormalaties in electrolyte homeostasis.

Discussion and conclusions of the electrolyte results.

In the aminoglycoside-treated group, six of the seven patients were borderline hypomagnesaemic on entry to the study and one patient was hypomagnesaemic. However, all patients may have been borderline hypomagnesaemic prior to aminoglycoside administration. Two patients became hypomagnesaemic with aminoglycoside therapy being a possible cause. The hypomagnesaemia seen in this group was unrelated to either the duration of the aminoglycoside course or the dose of aminoglycoside received during a course: the two patients who received the highest aminoglycoside doses [14 (course 1) and 20] did not develop hypomagnesaemia, and patient 3, who had a 16 day course of aminoglycoside therapy, developed a hypomagnesaemia but this was seen very early in therapy. As expected both patients who were hypomagnesaemic also demonstrated renal wasting of magnesium. In contrast, the hypomagnesaemia seen in the control group was not always associated with renal wasting of magnesium. Three patients became hypomagnesaemic in the control group. Patient 21, who developed hypomagnesaemia during therapy, conserved magnesium. Patients 6 and 9 showed a wasting of magnesium associated with this hypomagnesaemia, although in patient 9 the wasting was present before the antibiotic course was started.

Three patients (43%) in the aminoglycoside-treated group developed hypocalcaemia. In only one patient was the hypocalcaemia associated with a hypomagnesaemia although aminoglycoside-induced hypocalcaemia is thought to occur secondarily to the hypomagnesaemia [Zaloga et al, 1984; Wilkinson et al, 1986; Keating et al, 1977; Kelnar et al, 1978; Patel and Savage, 1979; Bar et al, 1975; Holmes et al, 1970]. The development of the hypocalcaemia was not related to either the duration of aminoglycoside administration or the total dose of aminoglycoside administered. Renal calcium wasting was present in all patients in this group. A similar incidence of hypocalcaemia (45%) was seen in the control group. Renal conservation of calcium was seen in one patient (patient 9), whilst the four other patients with hypocalcaemia had renal calcium wasting.

The occurrence of hypomagnesaemia and of hypocalcaemia in the aminoglycoside-treated patients was lower than reported in other studies [Davey et al, 1985; Zaloga et al, 1984; Freedman et al, 1982]. Davey et al [1985] reported that hypomagnesaemia occurred in 6 of 11 (55%) consecutively monitored patients with leukaemia who received gentamicin. Four of the six also developed hypocalcaemia. The lower incidence in the present study may reflect the mixed aetiologies of the patients studied. However, Zaloga et al [1984] reported a 38% incidence of hypomagnesaemia in patients without

leukaemia treated with gentamicin. Hypomagnesaemia occurred a mean (± S.D.) of 3.6 ± 0.6 days after commencing aminoglycoside therapy, although electrolyte abnormalities may not be seen until some time after the end of aminoglycoside therapy [Holmes et al, 1970; Bar et al, 1975; Kelnar et al, 1978; Watson et al, 1984]. Kelnar et al [1978] reported electrolyte abnormalities 5 weeks after cessation of the aminoglycoside course. It is possible, therefore, that further follow up may have revealed lowered serum electrolyte levels in the patients in the present study.

A similar incidence of hypomagnesaemia was seen in the control group. The two patients who demonstrated wasting of magnesium (patients 6 and 9) also had very high RBP and MUR excretions indicating renal tubular insult. The renal wasting seen in these patients may therefore reflect the toxic effects of cytotoxic administration in these patients. Patients with leukaemia with renal wasting of magnesium and have never received aminoglycoside antibiotics have been reported previously [Freedman et al, 1982].

Elevated magnesium and calcium excretion has been shown to occur in patients treated with short-term gentamicin therapy [Mountokalakis et al, 1985]. The cause of elevated excretion is thought to be impaired reabsorption [Zaloga et al, 1984]. In the present

study, elevated excretion of both cations was not a common finding. In the aminoglycoside-treated group, no patient had an elevated EXmg and only 2 patients had an elevated EXca. The incidence of elevated EXca and EXmg was also low in the control group. The lack of hypercalciuria and hypermagnesuria in the present study may be a result of the low serum electrolyte levels resulting in a lowered filtered load. Impaired reabsorption, as indicated by the elevated fractional excretion, would therefore not be manifest as an elevated excretion unless the impairment was very severe.

However, an impaired ability of the kidney to conserve these cations was a prominent effect. In the aminoglycoside treated group most patients were initially borderline hypomagnesaemic. The initial FEmg in these patients was low indicating conservation of the cation. During aminoglycoside therapy however, there was an elevation of the FEmg, sometimes to an abnormally high level. The Smg level remained low. This indicated a loss of the ability to conserve magnesium. In the control group, however, a similar ability to conserve magnesium was seen initially. This conservation was however maintained during antibiotic therapy. This suggests that the aminoglycoside antibiotics reduced the ability of the kidney to conserve magnesium.

Elevated fractional excretion of calcium was seen in all aminoglycoside-treated patients and 8 of the control patients. Impaired reabsorption of calcium has not been reported in published clinical cases of gentamicin-induced hypomagnesaemic hypocalcaemia [Bar et al, 1975, Zaloga et al, 1984; Patel and Savage, 1979; Freedman et al, 1982], but this is probably because urinary excretion of calcium was not measured. In animal studies, hypercalciuria has been reported frequently [Chahwala and Harpur, 1983; Cronin and Newman, 1985; Harpur et al, 1985] and indeed is more frequently found than hypermagnesuria. This may however reflect the difficulties in measuring magnesium excretion [Chahwala and Harpur, 1983]. The significance of the effect of aminoglycoside antibiotics on the tubular reabsorption of calcium is unclear in the present study because of the high incidence of wasting in the control group.

No attempt was made to quantify the severity of the renal wasting of the cations because of the wide variety of serum electrolyte levels and fractional excretion levels seen in the patients in this study.

Animal studies have suggested that the site(s) of the impaired reabsorption of the cations may include the proximal tubule [Foster and Harpur, 1986; Chahwala and Harpur, 1983; Garland and Harpur, 1987]. Indirect evidence for this is provided in this study by the close

association between the elevated excretion of RBP, suggesting tubular toxicity, and elevated fractional excretion of electrolytes in the aminoglycoside-treated group. Moreover, the elevation of electrolytes persisted after RBP excretion had returned to normal levels and after aminoglycoside therapy had been stopped. In the control group there was no such association between the RBP excretion and the elevated fractional excretion. Similarly to other clinical studies [Bar et al ,1975; Davey et al, 1985; Holmes et al, 1970; Patel and Savage, 1979; Zaloga et al, 1984], the impaired renal handling of electrolytes was seen without significant increases in the serum creatinine level.

Serum electrolyte disturbance was not seen in patient 20 who demonstrated persistent renal wasting of both magnesium and calcium. This supports the thesis that aminoglycoside therapy may only induce serum electrolyte disturbance in patients with some other predisposing factor [Zaloga et al, 1984]. Predisposing factors include an initially low serum magnesium level [Zaloga et al, 1984], cytotoxic drug administration [Davey et al, 1985] or other causes of electrolyte loss or inadequate intake. The two patients in the aminoglycoside-treated group who developed hypomagnesaemia had one or more of these factors present: patient 3 may have had an initially low serum

magnesium level and was treated with intravenous frusemide, a loop diuretic known to induce magnesium loss [Rude and Singer, 1981], and patient 4 had an initially low serum magnesium level and was treated with daunorubicin, a cytotoxic agent implicated as a risk factor in the electrolyte disturbance caused by aminoglycosides [Gozzard et al, 1986; Freedman et al, 1982].

Aminoglycoside therapy appears to have altered the renal handling of magnesium in these patients. The effect on renal calcium handling is complicated by the high incidence of calcium wasting in the control group. An effect, if any, of thienamycin on the renal handling of divalent cations has not been reported in the literature, although this may have been a complicating factor. Electrolyte homeostasis is also affected by age, sex, exercise and dietary intake [Robertson, 1976]. The diet of the patients in this study was not controlled although no patient received electrolyte supplements or total parenteral nutrition.

REFERENCES

Agus, Z.S., Wasserstein, A. and Goldfarb, S. 1982. Disorders of calcium and magnesium homeostasis. Am. J. Med. 72 : 473-488.

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

Bauer, J. H., Brooks, C. S. and Burch, R. N. 1982. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. Am. J. Kid. Dis. 2 : 77-346.

Beards, G.M., Campbell, A.D., Cottrell, N.R., Peires, J.S.M., Rees, N.,Sanders, R.C., Shirley, J.A., Wood, H.C. and Flewett, T.H. 1984
Enzyme-linked immunosorbant assays based on polyclonal and monoclonal antibodies for rotavirus detection. J. Clin. Microbiol. 19 : 248-254

Bernard, A.M., Moreau, D. and Lawerys, R. 1982
Comparison of retinol binding protein and Beta2-microglobulin determination in urine for early detection of tubular proteinuria. Clin. Chim. Acta. 126 : 1-7.

Bjornsson, T. D. 1979.
Use of serum creatinine concentrations to determine renal function. Clin. Pharmacokin. 4 : 200-222.

Boasson, E.H. 1938.
On the bacteriolysis of lysozyme. J. Immunol. 34 : 281.

Bosin, E. and Monji, N. 1983.
Quantitation of serum retinol-binding protein by an enzyme-linked immunosorbent assay. Anal. Biochem. 133 : 283-287.

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

Connor , T. B., Toskes, P., Mahaffey, J. 1972.
Parathyroid function during chronic magnesium deficiency.
Johns Hopkins Med. J. 131 : 100-117.

Cojocel, C and Hook, J.B. 1983.
Aminoglycoside nephrotoxicity. Trends Pharmac. Sci. 4 : 174-179.

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

Davey,P.G., Geddes, A.M. and Cowley, D.M. 1983.
Study of alanine aminopeptidase excretion as a test of gentamicin nephrotoxicity. J. Antimicrob. Chemother. 11 : 455-464

Davey, P.G., Cowley, D.M., Geddes, A.M. and Terry, J. 1984
Clinical evaluation of Beta$_2$-microglobulin, muramidase, and alanine aminopeptidase as markers of gentamicin nephrotoxicity. Contrib. Nephrol. 42 : 100-106

Davey, P.G. and Gosling, P. 1982.
Beta$_2$-microglobulin instability in pathological urine. Clin. Chem. Acta. 126 : 1-7

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

Davis, B. D. 1987
Mechanisms of bactericidal action of aminoglycosides. Microbiol. Rev. 51 : 341-350.

Davies, S. V. and Murray, J. A. 1986.
Amphotericin B , aminoglycosides and hypomagnesaemic tetany. Br. Med. J. 292 : 1395-1396.

Dawson, P. 1986.
The effect of gentamicin therapy on urinary excretion of retinol-binding protein , lysozyme and electrolytes. M. Phil. Thesis , Aston University.

Dirks, J. H. 1983
The kidney and magnesium regulation. Kid. Int. 23 : 771-777.

Donta, S. T. and Lembke, L. A. 1985.
Comparative effect of gentamicin and tobramycin on the excretion of N-acetyl-glucosaminidase . Antimicrob. Agents Chemother. 28 : 500

Dukes, M. N. G. 1975
Meylers side effects of drugs. Volume 8. Excerpta Medica, Amsterdam.

Ebel, H. and Gunther, T. 1980
Magnesium metabolism - A review. J. Clin. Chem. Clin. Biochem. 18: 257-270.

Elliott, W.C, Houghton, D.C., Gilbert, D.N., Baines-Hunter, J. and Bennett, W.M. 1982.
Gentamicin nephrotoxicity 1. Degree and permanence of acquired insensitivity. J. Lab. Clin. Med. 100 : 501-512

Ehrich, J.H.H., Foellmer, H.G., Krull, F., Withycombe, C.. Horstmann, R.D., Dietrich, M. 1981.
Proteinuria in non-renal infectious diseases. Contrib. Nephrol. 24 : 122-133.

Espinal, C. H. and Gregory, A.W. 1980.
Differential diagnosis of acute renal failure. Clin. Nephrol. 13 : 73-77.

Estep, H., Shaw, W. A., Watlington, C. 1969.
Hypocalcaemia due to hypomagnesaemia and reversible parathyroid hormone unresponsiveness. J. Clin. Endocrinol. Metab. 29 : 842-848.

Fex, G., Albertsson, P.A. and Hansson, B. 1979
Interaction between prealbumin and retinol binding protein studied by affinity chromatography, gel filtration, and two-phase partition. Eur. J. Biochem. 99 : 353-360

Finton, C.K., Borkland, S., Zaloga, G.P, Uddin, D.E. and Chernow, B. 1983.
Gentamicin induced hypomagnesaemia . Am. Surgeon . 49 : 578-578.

Flemming, J.J., Child, J.A., Cooper, E.H., Hay, A.M., Morgan, D.B. and Parapia, L. 1980.
Renal tubular damage without glomerular damage after cytotoxic drugs and aminoglycosides. Biomedicine 33 : 251-254.

Foster, J. E. and Harpur, E. S. 1986.
Infusion of gentamicin increases renal clearance of calcium and magnesium in Fischer 344 rats. Br. J. Pharmac. 88 : 428P.

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

Friberg, L. 1950.
Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium

poisoning. Acta. Med. Scand. 138 Suppl 240 ,1.

Garland, H.O. and Harpur. E.S. 1987.
Micropuncture study of gentamicin induced hypercalciuria. Program. Abstr. Third Inter. Symp. Nephrotox. Guilford.

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

Goldberg, M., Agus, Z.S., and Goldfarb, S. 1976.
Renal handling of calcium and phosphate. In (Ed) Thurau, K. Kidney and urinary tract physiology, Volume 2. University Park Press, Baltimore. pp 211-247.

Gozzard, D. I., Davey, P., and Leyland, M. J. 1986 [Letter]. Br. Med. J. 292 : 1272.

Graber, M. L. and Schulman, G. 1986.
Hypomagnesaemic hypocalcaemia independant of parathyroid hormone. Ann. Intern. Med. 104 : 804-805.

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

Harrison, J.F., Lunt, G.S., Scott, P. and Blainey, J.D. 1968.
Urinary lysozyme , ribonuclease and low molecular weight protein in renal disease. Lancet 1 : 371-375.

Harrison, J.F., Parker, R,.W. and DeSilva, K.L. 1973.
Lysozymuria and acute disorders of renal function. M. Clin. Path. 26 : 278-284

Hayslett, J.P., Perillie, P.E., and Finch, S.C. 1968
Urinary muramidase and renal disease: correlation with renal histology and implication for the mechanism of enzymuria. New Eng. J. Med. 279 : 269-274.

Hemmingsen, L. and Skaarup, P. 1977.
Urinary excretion of ten plasma proteins in patients with febrile diseases. Acta. Med. Scand. 201 : 359-364.

Holmes, A. M. , 1970.
Drug induced secondary aldosteronism in patients with pulmonary tuberculosis. Q. J. Med. 39 : 299-315.

Houser, M. T. 1983.
Improved turbidometric assay for lysozyme in urine.
Clin. Chem. 29 : 1488.

Jensen, H. and Henriksen, K. 1974.
Proteinuria in non-renal infectious diseases. Acta.
Med. Scand. 196 : 75-82.

Johansson, B. G. and Ranvskov, V. 1972.
The serum level and urinary excretion alpha$_2$-
microglobulin, Beta$_2$-microglobulin and lysozyme in renal
disease. Scand. J. Urol. Nephrol. 6 : 249-256.

Just, M. and Habermann, E. 1977
The renal handling of polybasic drugs : 11. In vitro
studies with brush border and lysosomal preparations.
Naunyn-Schmiedberg's Arch Pharmacol 300 : 67-76.

Kahlmeter, G. and Dahlager, J. J. 1984.
Aminoglycoside toxicity. A review of clinical studies
published between 1975 and 1982. J. Amimicrob.
Chemother. 13 : 9-22.

Keating, M. J., Sethi,M R., Bodey, G. P., and 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., and Brook, C. G. 1978.
Hypomagnesaemic hypocalcaemia with hypokalaemia caused
by treatment with high dose gentamicin. Arch. Dis.
Child. 52 : 817-820

Kelnar. C. J. H. 1986. Hypomagnesaemic tetany
associated with prolonged treatment with
aminoglycosides. Br. Med. J. 292 : 1017.

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

Kuo, C.H. and Hook, J.B. 1979
Specificity of gentamicin accumulation by the rat renal
cortex. Life Sci. 24 : 873-878.

Linton, A.L., Richmond, J.M., Walker, J.F. and Sibbald,
W.J. 1984.
Proteinuria and renal pathology in generalised sepsis.
Contrib. Nephrol. 42 : 118-122.

Lucertini, S., Valcavi, P., Mutti, A. and Franchini, I.
1984.
Enzyme-linked immunosorbent assay of retinol-binding

protein in serum and urine. Clin. Chem. 30 : 149-151.

Luft, F.C., Patel, V., Yum, M.N., Patel, B. and Kleit, S. A. 1975.
Experimental aminoglycoside toxicity. J. Lab. Clin. Med. 86 : 213-220.

Luft, F. C. and Kleit, S. A. 1974
Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J. Infect. Dis. 130 : 656-665.

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

Massry, S. G. and Cowburn, J. W. 1973
The hormonal and non-hormonal control of renal excretion of calcium and magnesium. Nephron 10 : 66-112.

Merle, L. , Charmes, J. P. , Dalette, J. P., Tronchet, J., Lachatre , G. and Nicot, G. 1981.
Enzymuria and low molecular weight proteinuria as markers of aminoglycoside nephrotoxicity. In : (Eds) Brown S.S, and Davies, D.S. Organ Directed Toxicity , Chemical Indices and mechanisms. Pergamon Press, Oxford. pp 85-90.

Mondorf, A. W. and Schoeppe, W. 1984.
Is the potential nephrotoxicity of drugs predictable ? Experiences with a volunteer model. Contr. Nephrol. 42 : 93.

Morin, J. P., Viotte, G. Van Hoof, F., Tulkens, P. and Fillastre, J. P. 1980.
Early events related to gentamicin-induced nephrotoxicity. In : Aminoglycoside toxicity, pp 610 -612.

Mountokalakis, T., Tzouvelekis, E. and Mayopoulou-Symvoulidou, D. 1983.
Effects of gentamicin on renal magnesium excretion. In : (Eds) Halpern and Durlach. Magnesium deficiency : First European congress on magnesium, Karger and Basel, Lisbon 1983: pp172-174.

Navarro, J. and Desquilbet, N. 1981.
Depressed plasma vitamin A and retinol-binding protein in cystic fibrosis, correlations with zinc deficiency (Letter). Am. J. Clin. Nutr. 34 (7) : 1439-1440.

Patel, R. and Savage, A. 1979.
Symptomatic hypocalcaemia associated with gentamicin therapy. Nephron. 23 : 50-52.

Peterson, P.A. and Berggard, I. 1971
Isolation and properties of human retinol transporting protein. J. Biol. Chem. 246 : 25-33

Prockop, D.J. and Davidson, W.D. 1964
A study of urinary and serum lysozyme in patients with renal disease. New Eng. J. Med. 270 : 269-274.

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

Quamme, G. A. and 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. and Dirks, J. H. 1983
Renal magnesium transport. Rev. Physiol. Biochem. Pharmacol 97 : 69-110

Reed, M.D., Vermeulen, M.W., Stern, R.C., Cheng, P.W., Powell, S.H. and Boat, T.F. 1981
Are measurements of urinary enzymes useful during aminoglycoside therapy ? Pediatr. Res. 15 : 1234-1239.

Robertson, W.G. 1976.
Urinary excretion. In : (Ed) Robertson, W.G. Calcium. Phosphate and Magnesium Metabolism. Publ. Churchill Livingstone : pp113-161.

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

Rude, R. K. and Singer, F. R. 1981.
Magnesium deficiency and excess. Ann. Rev. Med. 32 :245-259.

Schentag, J. J. 1983.
Specificity of renal tubular damage for aminoglycosides in critically ill patients. J. Clin. Pharmacol. 23 :473.

Seale, T. W. and Rennert, O. M. 1982.
Mechanism of antibiotic induced nephrotoxicity. Ann. Clin. Lab. Sci. 12 : 1-10.

Sherwood, L. M. 1970.
Magnesium ion and parathyroid function. N. Engl. J. Med. 282 : 752

Schnermann, J. and Levine, D.Z. 1975.
Tubular control of glomerular filtration rate in singlr nephrons. Can. J. Phys. Pharmacol. 53 : 326.

Shils, M. E. 1969
Experimental human magnesium depletion. Med. 48 : 61-85.

Silverblatt, F. J. and Kuehn, C. 1979.
Autoradiography of gentamicin by the rat proximal tubular cell. Kidney Int. 15 : 335-345

Smith, C.R., Lipskey, J.J., Laskin, O.L., Hellman, D.B., Mellits, E.D., Longstreth, J. and Lietman, P.S. 1982.
Double-blind comparison of the nephrotoxicity and auditory toxicity of gentamicin and tobramicin. New Eng. J. Med. 302 : 1106-1109.

Smith, C.R., Moore, R.D. and Leitman, P.S. 1986.
Studies of risk factors for aminoglycoside nephrotoxicity. Am. J. Kid. Dis. 8 : 308-313.

Smith, F.R., Underwood, B.A., Denning, C.R., Varma, A. and Goodman De W.S. 1972.
Depressed plasma retinol-binding protein levels in cystic fibrosis. J. Lab. Clin. Med. 80 : 423.

Thurau, K. and Boylan, J.W. 1976.
Acute renal success. Am. J. Med. 61 : 308.

Thurau, K.W.C., Dahlhein, H., Gruner, A., Mason, J. and Grange, P. 1972.
Activation of renin in the single juxtaglomerular apparatus by sodium chloride in the tubular fluid at the macular densa. Circ. Res. 30 (Suppl.) : 182.

Viau, C., Bernard, A., Ouled, A., Lauwerys, S.R. and Tulkens, P. 1984.
Polycationic gentamicin inhibits the tubular reabsorption of plasma proteins according to their electrical charge. In : (Eds) Bach, P.H. and Lock, E.A., Renal heterogeneity and target cell toxicity. Proceedings of the second international symposium on nephrotoxicity. Publ. J. Wiley, Chichester : pp 353-356.

Walenkamp, G., Vree, T., Guelen, P., and Jongman-Nix, B. 1983.
Interaction between the renal excretion rates of alpha$_2$-microglobulin and gentamicin in man. Clin. Chim. Acta. 127 : 229.

Watson, A., Coffey, L., Keogh, B., and McCann, S. R. 1983.
Severe hypomagnesaemia and hypocalcaemia following gentamicin therapy. Ir. Med. J. 76 : 381-383.

Watson, A. J. S., Watson, M. M. R., and Keogh, J. A. B. 1984
Metabolic abnormalities associated with tobramycin therapy. Ir. J. Med. Sci. 153 : 96-99.

Whelton, A. 1979.
Aminoglycoside renal cortical kinetics : a clue to mechanisms of toxicity. Prog. Clin. Biol. Res. 35 :33-41.

Whelton, A., Stout, R. L., Carter, G. G., Craig, T. J., Bryant, H. H., Herbst, D. V. and Walker, W. G. 1981.
Modulation of renal cortical aminoglycoside uptake by concurrent aminoacid administration. in Nephrotoxicity Ototoxicity of drugs. In : (Ed) Fillastre, J.P., Vol 102, Editions Inserm, Rouen.

Whitworth, J. A. 1982.
Interpretation of renal function tests. Australian Family Physician. 11 : 595-601.

Wide, L. and Thoren, L. 1972.
Increased urinary clearance for albumin, Beta$_2$-microglobulin, insulin and luteinising hormone following surgical or accidental trauma. Scand. J. Clin. Lab. Invest. 30 : 275-279.

Wilkinson, R. , Lucas, G. L. , Heath, D. A. , Franklin, I. M. and Broughton, B. J. 1986.
Hypomagnesaemic tetany associated with prolonged treatment with aminoglycosides. Br. Med. J. 292 : 818-819.

Zaloga, G.P., Chernow, B., Pock, A., Wood., Zaritsky, A. and Zucker, A. 1984
Hypomagnesaemia is a common complication of aminoglycoside therapy. Surg. Gyn. Obst. 158 : 561-565.