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# Inhibition of gentamicin-induced nephrotoxicity by lithium in rat

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**Summary.** Daily intraperitoneal injection of gentamicin in doses of 2, 4 and 10 mg/kg/day for 5 consecutive days produced proximal tubular necrosis in male albino rats as assessed by ultrastructural findings from electron microscopic observations. With respect to nephrotoxicity, aminoglycoside antibiotics (AGs) have been shown to concentrate in the lysosomes of kidney proximal tubular cells to inhibit the activities of phospholipases A and C, including a phospholipidosis, characterized by the formation of myeloid bodies. It has been suggested that the nephrotoxicity of AGs is related to the extent of this phospholipidosis. The concurrent therapy of lithium in doses of 5 and 10 mEq/kg/day, administered subcutaneously, 24 hours prior to gentamicin administration for the same period, proved effective in reducing the gentamicin-induced phospholipidosis in kidney as judged by reduction in lysosomal myeloid bodies to an amount of 26-45 percent. It is well known that lithium interferes with phosphatidylinositol turnover and reduces the cellular availability of myoinositol which is needed for the resynthesis of membrane poliphosphoinositides. Thus, the inhibitory effect of lithium on gentamicin-induced nephrotoxicity may be due to interference of lithium with phosphoinositide cycle.

**Key words:** Aminoglycosides, Gentamicin, Lithium, Nephrotoxicity, Phospholipidosis

## Introduction

Aminoglycoside antibiotics (AGs) remain essential in clinical practice for the treatment of infections caused by gram-negative bacteria, because of their excellent chemotherapeutic properties (Smith et al., 1980; Mingeot-Leclerq et al., 1988). One of the major consequences of AG use is drug-induced nephrotoxicity (Koren et al., 1988; Garrison and Rotschafer, 1989). Morphologically, treatment with gentamicin is associated with an accumulation of myeloid bodies in kidney cortex (Kacew, 1989). Metabolic evidence of gentamicin-induced functional disturbances in the kidney cortex include a decrease in the activities of $Na^+$-$K^+$- ATPase, alkaline phosphatase, phospholipase C accompanied by a significant rise in phospholipid content (Tulkens, 1986; Kacew, 1989). Furthermore, it has been suggested that the nephrotoxic effect of AGs such as gentamicin are related to their ability to bind to phosphatidylinositol 4, 5 bisphosphate ($PIP_2$) (Torner et al., 1988; Gabev et al., 1989). Several risk factors associated with the development of nephrotoxicity have been identified, including age, sepsis, shock, volume depletion, potassium depletion, prior AG administration and concurrent drug therapy (Macdougall, 1988; Mattie et al., 1989).

Lithium, a drug in widespread for the treatment of affective disorders, has attracted considerable attention because its prolonged administration has been associated with the development of renal insufficiency ( Åberg-Wistedt et al., 1988; Jorkasky et al., 1988). A high proportion of patients taking lithium preparations develop a urinary concentrating impairment. Furthermore, it is well-known that lithium interferes with phosphatidylinositol turnover by blocking the activity of the myo-inositol-1-P-phosphatase and reducing the cellular availability of myoinositol which is needed for the resynthesis of membrane polyphosphoinositides (Mantelli and Ledda, 1988). Thus, this study was undertaken to determine whether lithium interferes with gentamicin-induced nephro-toxicity as manifested by changes in ultrastructural findings.

## Materials and methods

Experimental design: Male albino rats weighing 150-200 g were used for this study. The animals were housed in groups of 2 in stainless steel cages, where food and tap water were available *ad libitum*. Gentamicin sulphate was supplied by Schering Co.

All experimental animals received intraperitoneal

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doses of 2, 4 and 10 mg/kg/day of gentamicin for a duration of 5 consecutive days. Controls received an equivalent volume of saline.

In a separate experiment, animals received a subcutaneous injection of lithium chloride in doses of 5 and 10mEq/kg/day, 24 hours prior to gentamicin administration, for the same period. Controls received the same amount of saline. Injections were performed simultaneously. Through ventral incision, under ether anaesthesia animals were then perfused via aorta through heart ventricle, with 0.1M sodium cacodylate-buffered 2.5% glutaraldehyde, pH 7.4, at room temperature.

Once the perfusion was completed, the pieces of 1 $mm^3$ of renal cortex were excised and immersed for an additional 2 hours in the same fixative. After rinsing in buffer, postfixation was done with 1% osmium tetroxide in the same buffer for 2 hours. Then the specimens were directly dehydrated in a graded series of ethanol and embedded in Epon 812. Semithin sections of 1 µm were cut using glass knives on an LKB nova ultramicrotome and examined under light microscope after being stained with toluidine blue.

Ultrathin sections were cut with the same instrument, collected from water surface on uncoated copper grids of 400 mesh, doubly stained with uranyl acetate and lead citrate and examined using a Zeis EM 10 C transmission electron microscope (Carl Zeiss, Oberkochen), operated

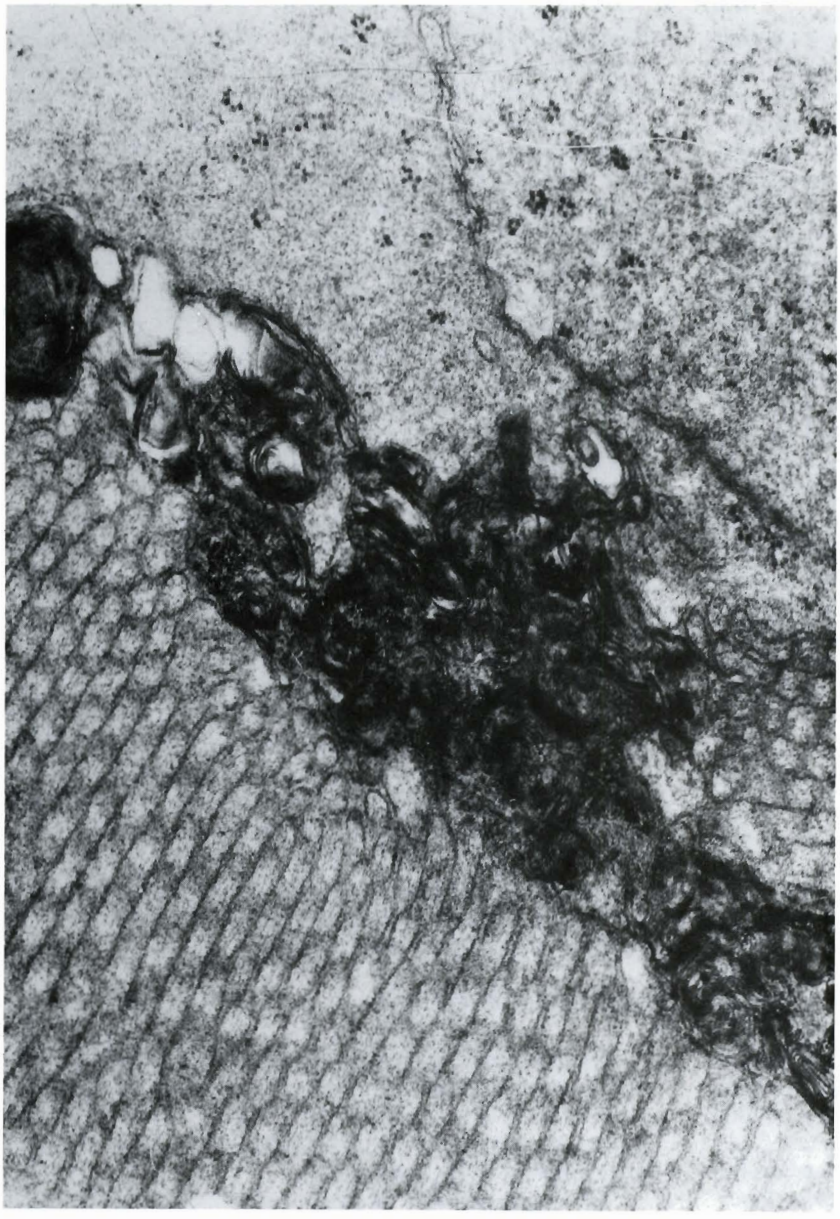

**Fig. 1.** Representative electron micrograph of apical portion of proximal tubular cells treated with gentamicin (4 mg/kg/day for 5 days). Myeloid lamellar structures are clearly seen between the brush border projections. x 32,800

at 60 KV.

For evaluation of the lysosomal alterations, scoring of myeloid bodies was done as follows: For each animal 5 pieces of cortex kidney were sampled and the phospholipidosis was evaluated by two observers who were unaware of treatment given until final pooling of the results. The affected lysosomes are expressed as percent of lysosomes containing myeloid bodies to total lysosomes in each section.

## Results

As assessed by electron microscopic observation, the lysosomal myeloid bodies were accumulated almost exclusively in renal proximal tubular cells, even though the initial site of drug interaction with the kidney was the plasma membrane of the cells (Fig. 1). Appearance of lamellar material inside the brush border indicated primary binding sites, at which they gained access to the cell.

Necrosis occurred in succession and was induced by gentamicin in a dose-dependent manner.

Doses of 2 mg/kg/day resulted in the formation of 66% of lysosomal cytosegresomes, containing prominent myeloid bodies in proximal tubules (Table 1). At the same dosage, distal tubules manifested fewer myeloid figures.

In doses of 4 mg/kg/day, proximal tubular necrosis

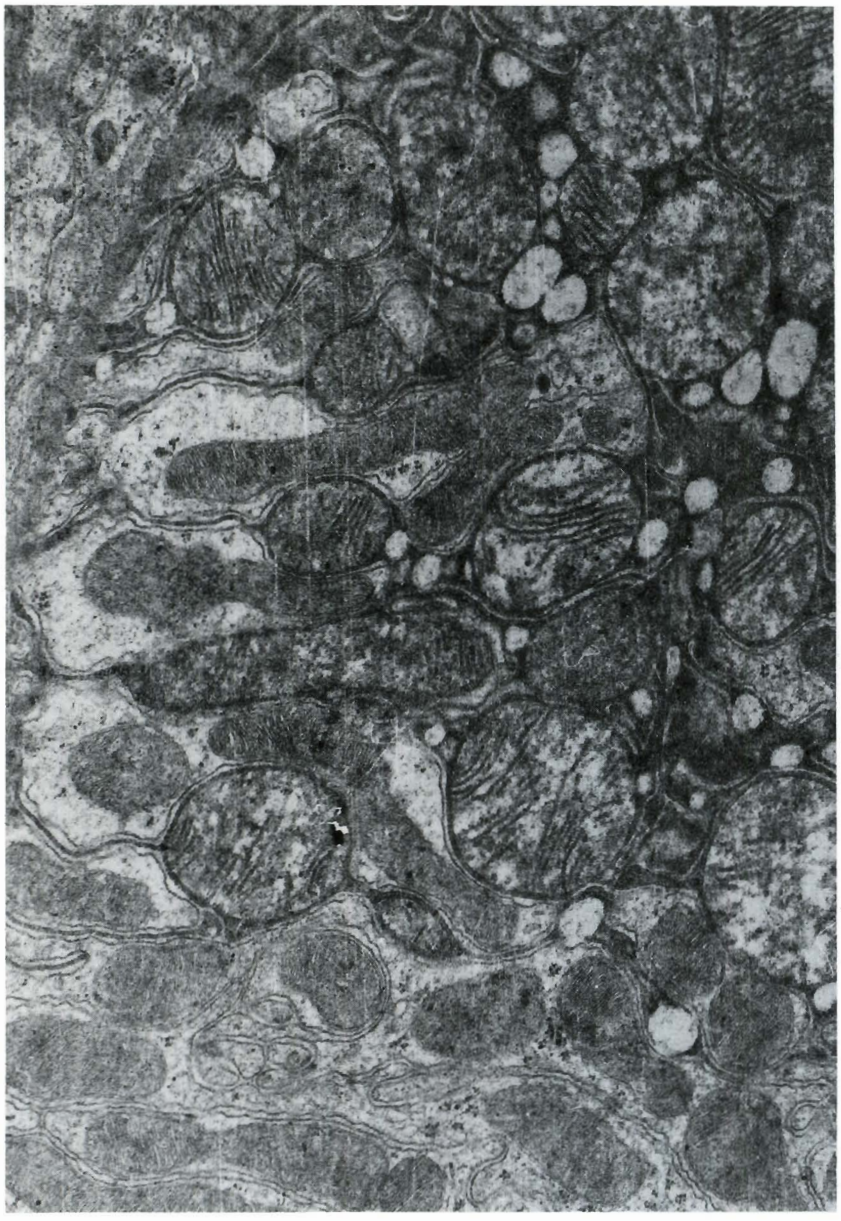

**Fig. 2.** Representative electron micrograph of basal part of proximal tubular cells treated with gentamicin (4 mg/kg/day for 5 days), containing irregular, swollen mitochondria. x 16,000

**Table 1.** Effect of gentamicin alone or in combination with lithium on myeloid body formation. The results obtained from at least 5 experiments. All values shown are mean±SEM

| GROUP | DRUFS/KG/DAY | %OF LYSOSOME CONTAINING MYELOID BODIES |
|---|---|---|
| 1 | Saline/Gentamicin 2 mg | 66.66±1.45 |
| 2 | Lithium 5 mEq/Gentamicin 2 mg | 39.66±5.17* |
| 3 | Lithium 10 mEq/Gentamicin 2 mg | 24±3.05** |
| 1 | Saline/Gentamicin 4 mg | 79.66±2.73 |
| 2 | Lithium 5 mEq/Gentamicin 4 mg | 53.33±0.91* |
| 3 | Lithium 10 mEq/Gentamicin 4 mg | 31±1.53** |
| 1 | Saline/Gentamicin 10 mg | 90.16±2.73 |
| 2 | Lithium 5 mEq/Gentamicin 10 mg | 90±0.58 NS |
| 3 | Lithium 10 mEq/Gentamicin 10 mg | 83±1*** |

*: p less than 0.01 compared to gentamicin alone; **: p less than 0.001 compared to gentamicin alone; ***: p less than 0.05 compard to gentamicin; NS: non significant

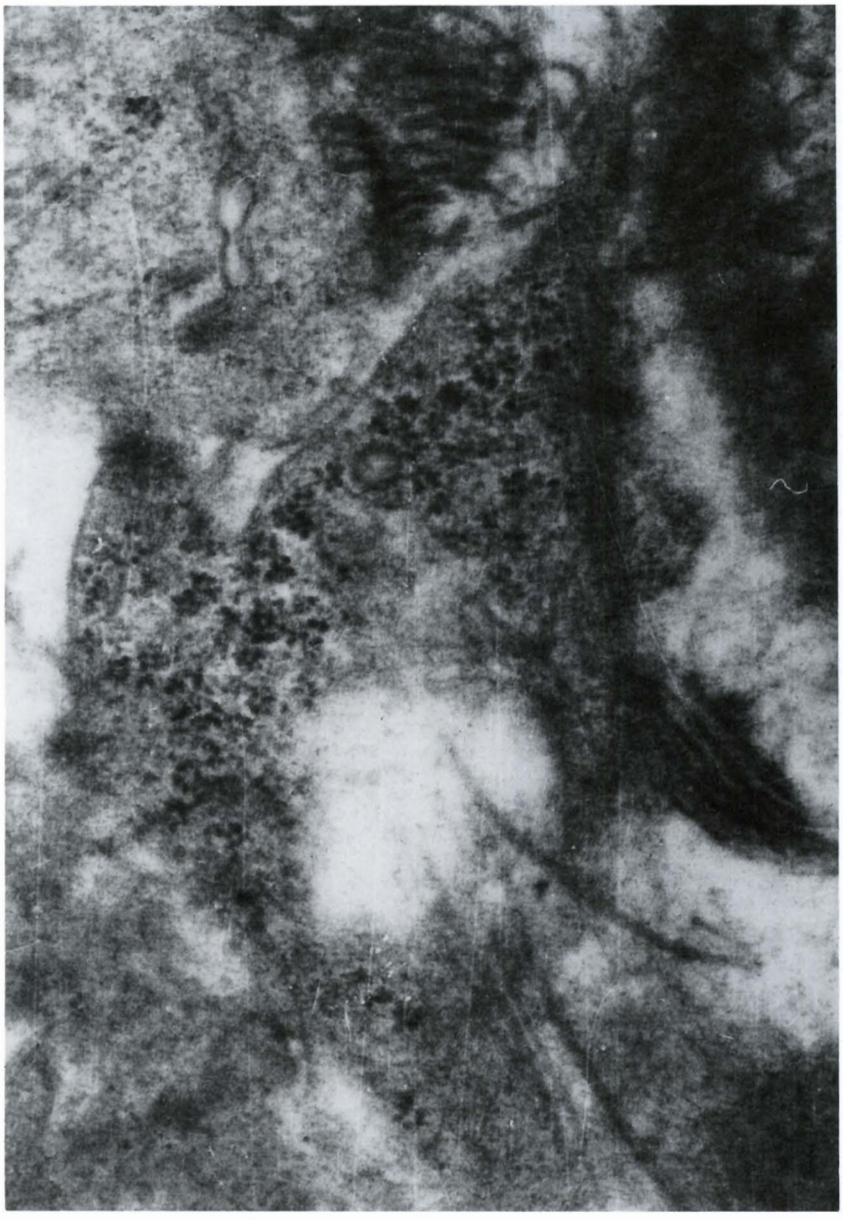

**Fig. 3.** Representative electron micrograph of interstitial cells containing collagen fibres and ribosomes increased after treatment with gentamicin (2 mg/kg/day for 5 days). Magnification. x 100,000

increased and the number of lysosomal myeloid bodies reached 79% (Table 1). The mitochondria were irregular in shape and sometimes enlarged as a group of swollen organelles (Fig. 2). Necrotic cellular debris was found within the lumen of some tubules. In most proximal tubular cells, ribosomes increased in number, whereas lysosomal phospholipidosis increased in both proximal and distal tubular cells even in doses of 2 mg/kg/day (Fig. 3).

Doses of 10 mg/kg/day resulted in the number of myeloid bodies reaching 90% and caused extensive changes in both proximal and distal tubular cells, (Figs. 4, 5, and Table 1).

The accumulation of phospholipidosis in the enlarged lysosomes caused the widespread alteration of the cell ultrastructure and subcellular organelles. These organelles included endoplasmic reticulum, nuclei and mitochondria. Myeloid figures were also present in the foot processes and dense material was observed in the basal region of the lateral processes (Fig. 6).

Other features of the effect of gentamicin were the proliferation of interstitial cells, collagen fibres and ribosomes (Fig. 3).

The concurrent therapy of lithium (in doses of 5 and 10 mEq/kg/day) and gentamicin, reduced lysosomal myeloid bodies to an amount of 26-45% (Table 1).

In lithium-treated cells, mitochondrial damage was present. However, the enlarged lysosomes did not contain myeloid bodies (Fig. 7). Also, dense material disappeared in the basal region of proximal tubular cells.

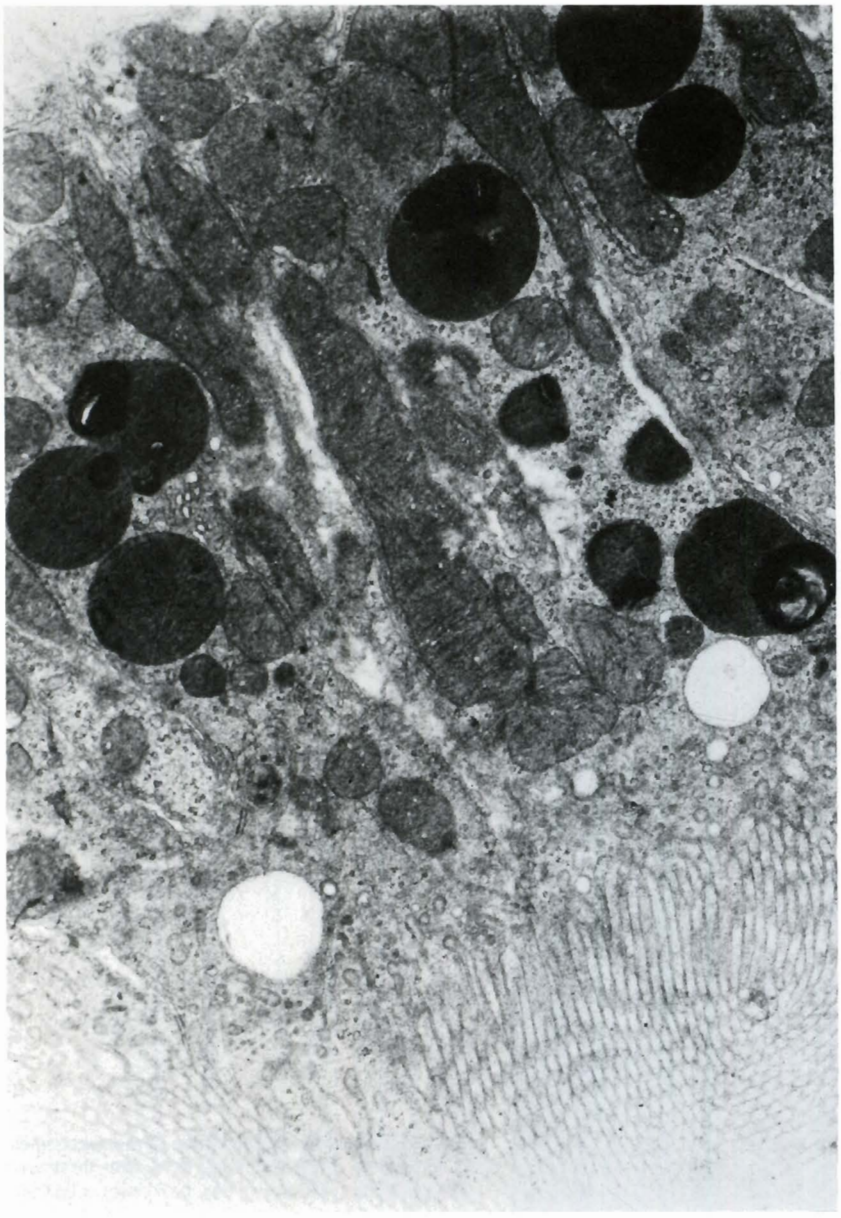

**Fig. 4.** Ultrastructural appearance of myeloid bodies accumulating in lysosomes of basal cytoplasm after treatment with gentamicin (10 mg/kg/day for 5 days). x 16,000

## Discussion

In spite of the introduction of new classes of highly potent, wide-spectrum antibiotics, AGs are still an essential part of our armamentarium against severe, life-threatening infections (Tulkens, 1989). Unfortunately, the clinical usefulness of these drugs is limited due to the development of nephrotoxicity (Kacew, 1989; Commandeur and Vermeulen, 1990). Estimates of nephrotoxic frequency vary widely but generally range between 10% to 25% of patients given these antibiotics (Macdougall, 1988). Given in sufficiently high daily doses, the AGs cause reversible acute proximal tubular necrosis and acute renal failure in experimental animals and in humans over 1-2 weeks (Houghton et al., 1988). One of the most important prerequisities for the development of AG nephrotoxicity is the accumulation of these drugs in the renal cortex (Chahoud et al., 1988; Aramaki and Tsuchiya, 1989). It has been proposed that the first step of accumulation of AGs into renal tubular cells is the binding of AGs to biomembranes such as brush-border membranes (BBMs) (Just et al., 1977). The extent of tubular necrosis is undoubtedly an essential determinant in the drug-induced impairment of renal function. Such impairment is generally the only important toxic expression of concern to clinicians; however, it is clear that both necrosis and impaired renal function are only consequences of earlier biochemical and ultrastructural alterations (Tulkens, 1986; Commandeur and Vermeulen, 1990). With respect to nephrotoxicity, AGs such as gentamicin are polycationic molecules capable of binding to phospholipids,

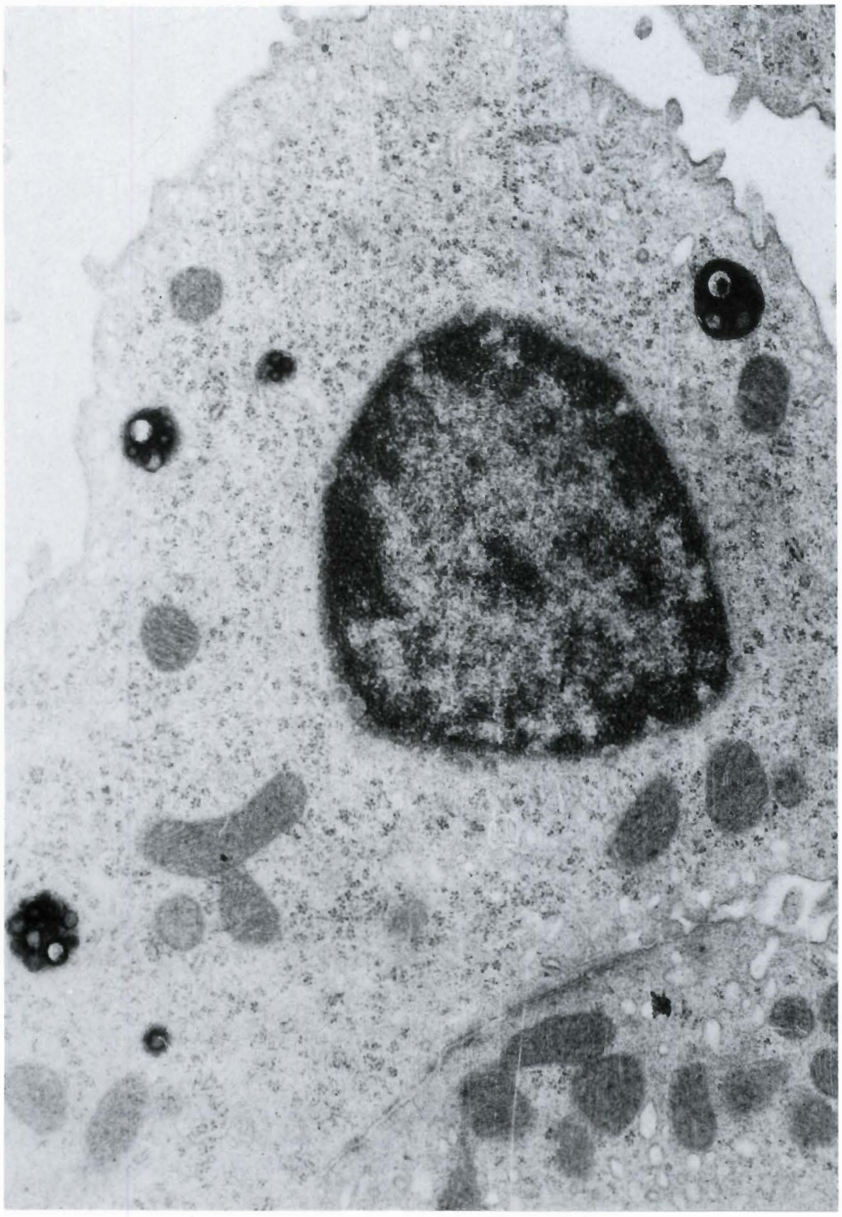

**Fig. 5.** Ultrastructural appearance of myeloid bodies in lysosomes of distal tubular cells after treatment with gentamicin (10 mg/kg/day for 5 days). x 25,000

especially $PIP_2$, thus inhibiting the activities of phospholipases A and C, and sphingomyelinase and inducing a phospholipidosis, characterized by the formation of myeloid bodies (Mingeot-Leclercq et al., 1988; Tysnes et al., 1988). The latter consist of a complex mixture of phospholipids naturally present in the cell, even though they show a significant enrichment in phosphatidylinositol. Certain evidence strongly suggests that the nephrotoxicity of AGs is related to the extent of this phospholipidosis (Tulkens, 1986). Our results are consistent with findings of these investigators and show gentamicin-induced myeloid body formation in a dose-dependent manner (Table 1).

Several important risk factors associated with the development of AG nephrotoxicity have been identified, including age, sepsis, shock, volume depletion, potassium depletion, prior aminoglycoside administration and concurrent drug therapy (Macdougall, 1988; Mattie et al., 1989). In view of the clinical importance of AGs, a concerted effort to identify a therapeutically effective inhibition of nephrotoxicity has been a scientific goal (Hottendorf and Williams, 1986). Although the clinical feasibility is questionable, dietary calcium supplementation or thyroxine administration was found to protect against gentamicin-induced nephrotoxicity (Kacew, 1989). Lee and Michael (1985) noted that the calcium channel blocker, nitrendipine, was effective in the blockage of

**Fig. 6.** Electron micrograph of the ascending part of proximal tubular cells after treatment with gentamicin (10 mg/kg/day for 5 days) showing accumulation of dense material at lateral processes. x 80,000

gentamicin-induced renal damage as judged by less histopathological damage and an increase in inulin clearance compared to antiboiotic alone. Williams and Hottendorf (1985) reported that the polyamino acids polylysine, polyasparagine, and polyaspartic acid (PAA) inhibited binding of ($^3$H) gentamicin to rat renal BBMs. Moreover, it has been reported that latamoxef (LMOX), an oxacephem antibiotic, protects rats from tobramycin-induced nephrotoxicity which is due to inhibition of tobramycin binding to BBMs (Kojima et al., 1990).

In this study we have found that lithium has the ability to interfere with gentamicin-induced nephrotoxicity as manifested by changes in ultrastructural findings.

Lithium is a widely used and invaluable drug in the treatment of manic-depressive illness. However, this medication has a low therapeutic index and therefore many attendant side effects (Drummond, 1987; Simard and Gumbine, 1989). A high proportion of patients taking lithium salts develop a urinary concentrating impairment (Jorkasky et al., 1988; Simard and Gumbine, 1989). When administered to intact organisms, it induces subtle alterations in neural activity and early development (teratogenesis) (Berridge and Irvine, 1989). Furthermore, lithium is one of the few agents which interferes with the inositol lipid cycle in a selective manner. Upon long-term treatment, lithium can decrease in inositol and increase in total inositol monophosphates.

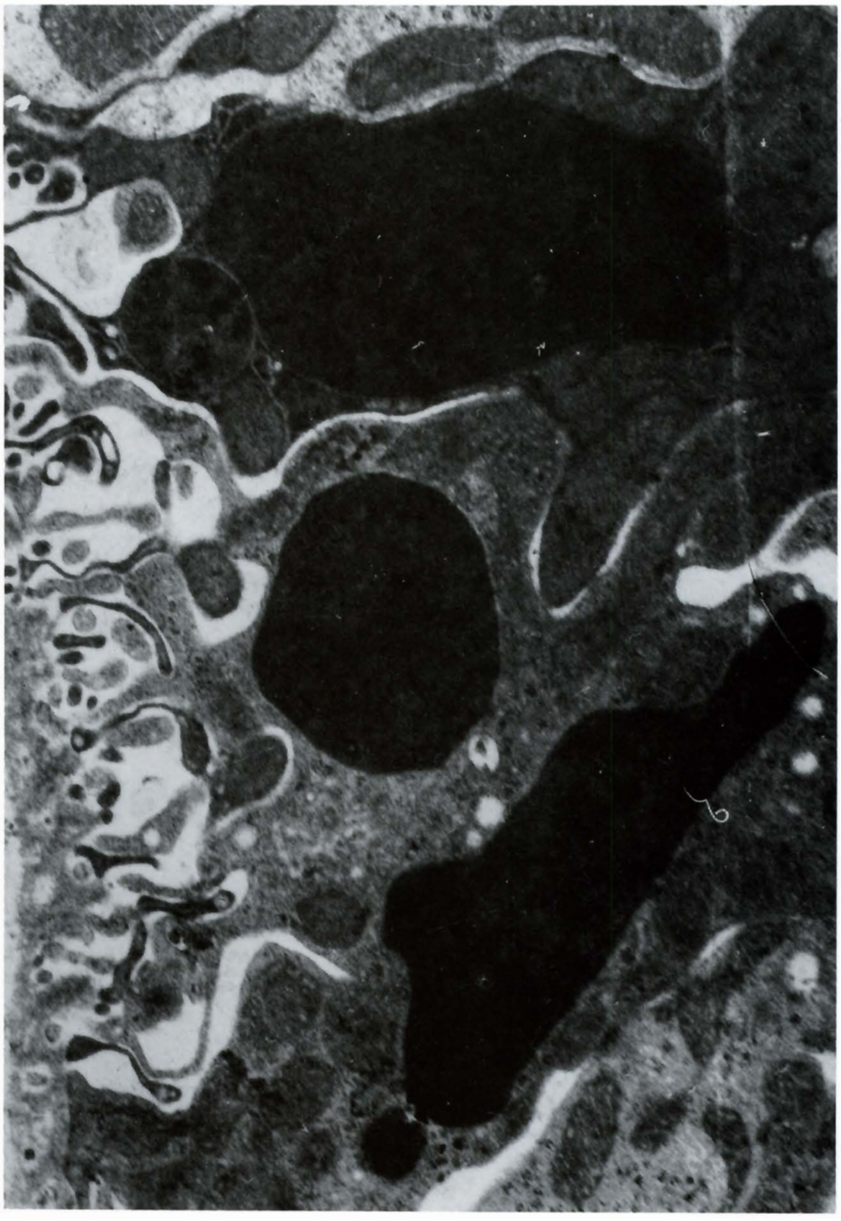

**Fig. 7.** Electron micrograph of basal cytoplasm containing large lysosomes after treatment with lithium (10 mEq/kg/day for 5 days) show no accumulation of myeloid bodies. x 15,750

This is due to the blockage of the metabolic cascade at the monophosphatase level a feature which is evident in intact cells (Drummond, 1987; Berridge and Irvine, 1989). Our findings suggest that myeloid body formation in kidney lysosomes, induced by gentamicin can be reduced significantly by lithium. This effect may be due to the ability of lithium to interfere with phosphoinositide cycle reducing phospholipidosis.

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