Aminoglycosides:
What have we learned about toxicity ?

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Symposium # 153:
Back to the Future: The Aminoglycosides are Here Again

What were the advantages of aminoglycosides as seen in the mid 1980's?

- Microbiology
  - wide spectrum, but especially active against Gram (-) organisms including "difficult" ones (*P. aeruginosa*, *Serratia*, etc…)
  - concentration-dependent bactericidal activity (related to peak) with prolonged post-antibiotic effect ...
  - low propensity to cause resistance (and possibility to rotate among derivatives with distinct resistance patterns)
  - synergy with cell-wall acting agents with no cross-resistance ...
- Pharmacokinetics:
  - no metabolism, few drug interactions, rapid elimination (except kidney) ...
  - linear pharmacokinetics and predictable blood levels
  - several fast methods for monitoring
- Pharmaceutics:
  - excellent shelf stability
  - cheap to make …

adapted from Price, Antimicrob Agents Chemother. 1986 Apr;29(4):543-8.

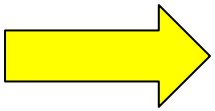
Aminoglycosides in the 80's:
Questions raised ...

- Can they be really be used without fearing resistance ?
- What is the real risk (and liabilities) of toxicity ?
  - ➔ nephrotoxicity (reversible ...)
  - ➔ ototoxicity (irreversible !)
- All seem to have quite similar biophysical, chemical, microbiological and pharmacokinetic properties, but...
  - are they (some and real) differences in toxicities that may suggest the preferential use of one over the others (beyond differences in susceptibility to resistance mechanisms) ?
  - can we further dissociate activity and toxicity ?
  - what is/are the mechanism(s) of these adverse effects ?
  - can we protect patients ?

Clinical signs of (impending) aminoglycoside nephro- and ototoxicity

- Nephrotoxicity
  - polyuria
  - increase in creatinine or BUN (nonoliguric renal failure)

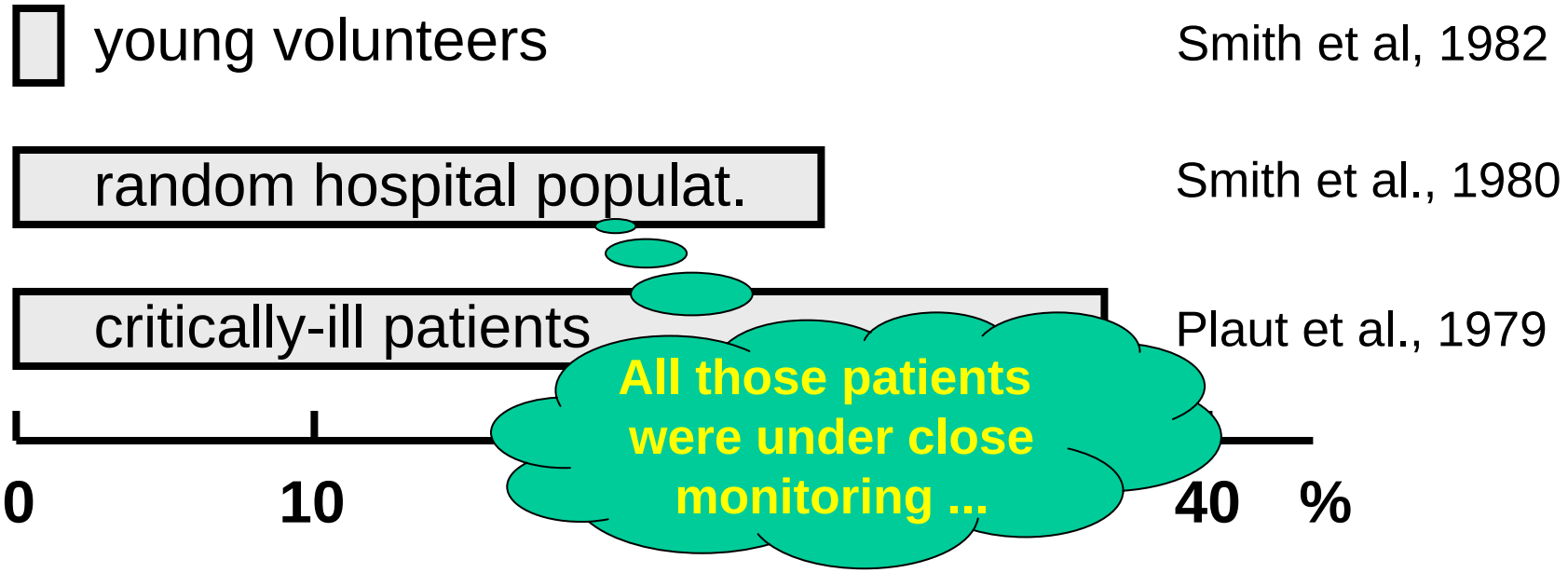
- Ototoxicity
  - cochlear damage (hearing loss beginning with high frequencies; tinnitus or a sensation of “fullness may represent early injury.)
  - vestibular damage: dizziness and loss of equilibrium (with frequent nausea, vomiting)



These symptoms indicate (and are associated with) widespread organ lesions (acute tubular necrosis / haircells destruction)

Aminoglycosides toxicity in the 80's: huge variations among patient populations

Patients with nephrotoxic reaction after treatment with gentamicin



Ototoxicity may vary from 3% and 14% based patient's complain but may be as high as 62% based on audiograms – Rare patients with mitochondrial DNA mutations (A1555G being most common) are high risk.

Why such variation in nephrotoxicity ?

PROVEN, CLINICALLY RELEVANT RISK FACTORS IN AMINOGLYCOSIDE NEPHROTOXICITY*

Patient-related

- Age
- Large initial creatinine clearance
- Impaired renal function (if dose not adjusted)
- Liver disease
- Critically ill state and shock
- High tissue accumulation

Treatment-related

- High peak levels**
- Sustained elevated levels***
- Total dose
- Duration of treatment
- Coadministration of other potentially nephrotoxic drugs (vancomycin, cephaloridine and perhaps cefalothin, but not other beta-lactams, amphotericin, cisplatin)
- Coadministration of loop diuretics and volume-depleting agents

* Based partly on Refs. 9 and 55 and various reports on animal studies.

** For the schedule of administration considered. Thus, patients treated once a day may have much higher peak levels than patients treated three times a day, without signs of toxicity. Determination of standards for peak levels in the once-a-day regimen have, however, not yet been determined.

*** Usually determined 8 h after last administration; sustained levels usually related to inadequate elimination, tissue storage and/or too frequent dosing and are therefore highly indicative of potential toxicity.

Toxicology Letters, 46 (1989) 107–123

High doses in animals showed that gentamicin accumulates in renal cortex and cause renal tubular necrosis and dysfunction (and regeneration) prior to renal failure

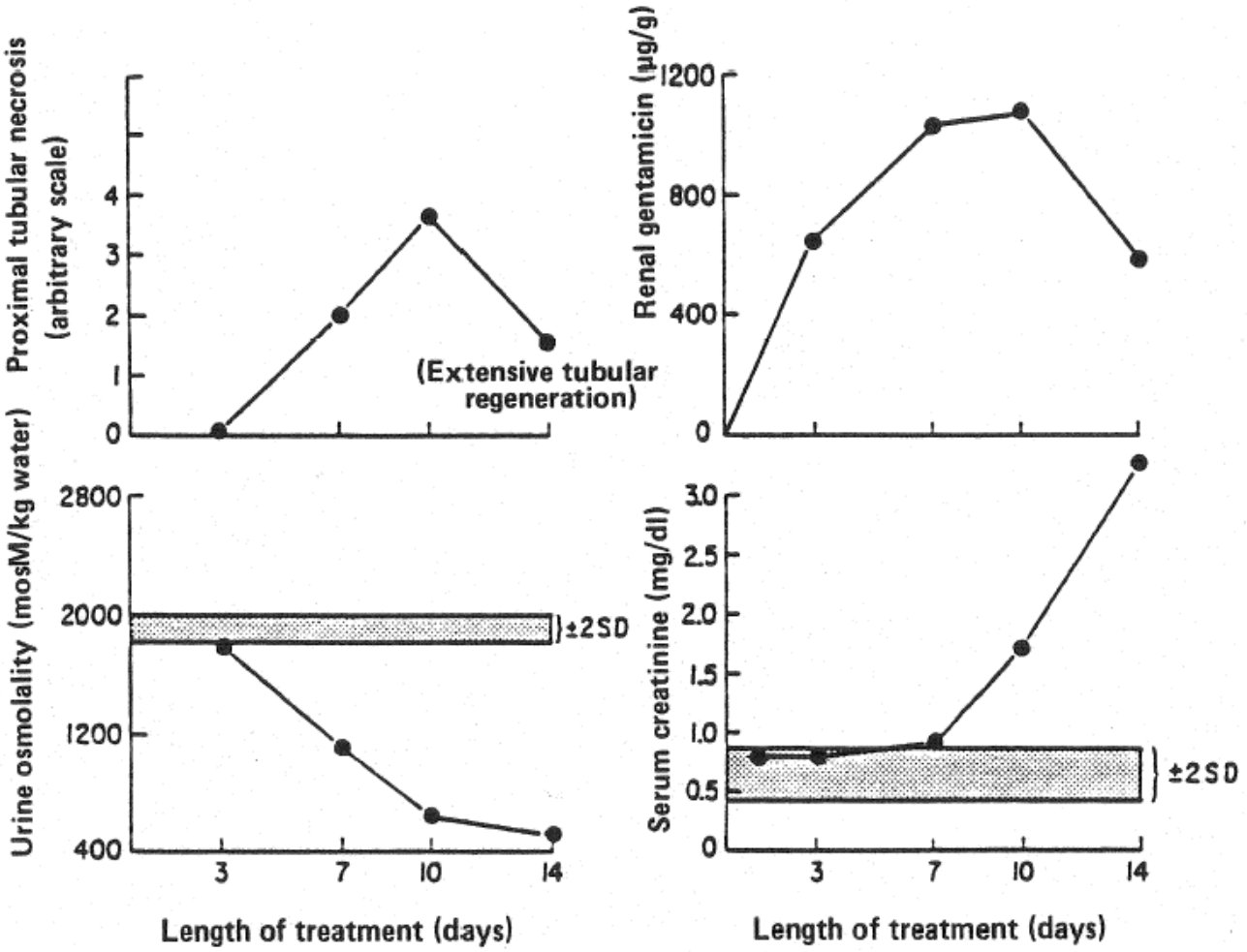


Fig. 1. Renal changes in Fischer 344 rats after gentamicin (40 mg/kg per day in two injections per day). From Ref. 13.

13 Parker, R.A., Bennett, W.H. and Porter, G.A. (1982) Animal models in the study of aminoglycoside nephrotoxicity. In: A. Whelton and H.C. Neu (Eds.), The Aminoglycosides: Microbiology, Clinical Use and Toxicology. Marcel Dekker, New York, pp. 235–267.

And low doses studies allowed to observe a clear succession of events …

PATHOLOGICAL FEATURES OF AMINOGLYCOSIDE INTOXICATION IN ANIMALS AND HUMANS TREATED WITH LOW, THERAPEUTIC DOSES OF AMINOGLYCOSIDES*

Fate of the drug

Glomerular filtration and partial binding to the brush border (low-affinity, high-capacity binding)
Sequestration in lysosomes of proximal tubules (intralysosomal concentrations reach values of 10 g/l and above)

Early alterations (zero to six days)

Accumulation of phospholipids in and enlargement of lysosomes
Inhibition of activities of lysosomal phospholipases and sphingomyelinase
Decreased reabsorption and/or intracellular lysosomal sequestration and digestion of exogenous proteins, mostly cationic (lysozyme, β_2-microglobulin)**
Shedding of brush-border enzymes (e.g., alanylaminopeptidase) and release of lysosomal enzymes (e.g., *N*-acetyl-β-D-glucosaminidase)**

Established alterations (after approximately six days)

Degenerative lesions
- Coarse granulation of epithelial cells***
- Focal necroses+ apoptoses and shedding of cell content into the lumen
- Increased phospholipid excretion in urine (in humans only)++
- Proteinuria, hypo-osmotic polyuria
- Decreased glomerular filtration and increased blood urea nitrogen and creatinine, without immediate signs of glomerular damage

Regenerative lesions
- Tubular cell proliferation and dedifferentiation
- Tubular dilatation
- Interstitial proliferation (fibroblastic cells) and focal infiltration by inflammatory cells

* From Ref. 30 and the references cited in this paper; see also the review of Humes et al. [6].
** Often used for early detection of aminoglycoside insult; however, their measurement appears of limited practical value in diseased patients.
*** These cells show markedly enlarged lysosomes, with decreased buoyant density and prominent myeloid bodies.
\+ Electron microscopy shows widespread alteration of the cell ultrastructure and subcellular organelles, including mitochondria, endoplasmic reticulum and nuclei.
++ Myeloid bodies abundant in lumen and urine.

Toxicology Letters, 46 (1989) 107–123

The American Journal of Medicine
Volume 80 (suppl 6B) June 30, 1986 105

Two first things we learned…

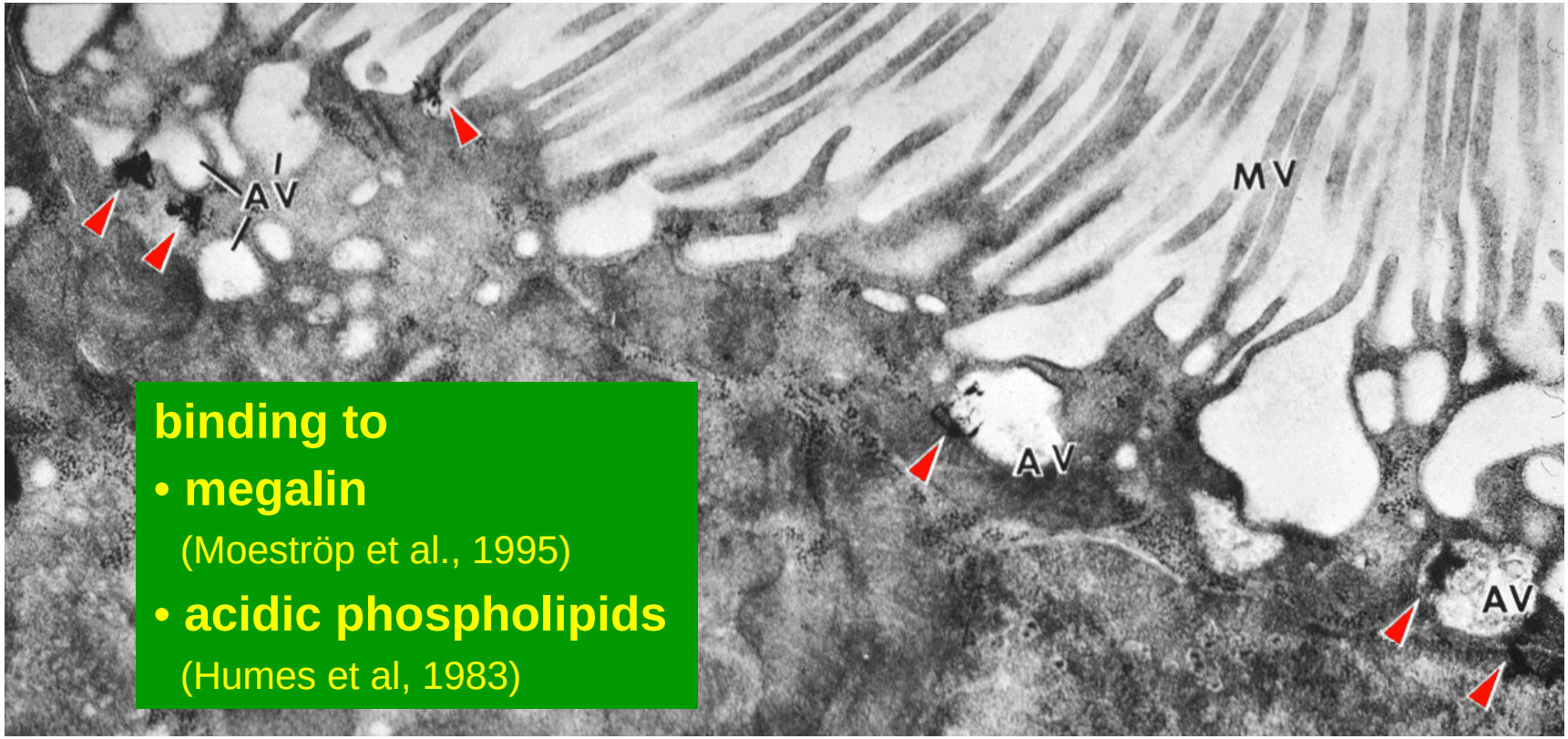
- clinical toxicity was badly controlled by drug monitoring alone if insisting on keeping peak levels low and maintaining trough levels high …

  ➔ was the 3 times daily schedule correct ?

- subclinical changes occur at therapeutic doses in animals … but translate in functional alterations at large doses only … with signs of extensive regeneration

  ➔ could what we see in humans (increase in creatinine, polyuria, glomerular and tubular dysfunction) be late events due to insufficient regeneration capabilities ?

Aminoglycoside entry in proximal tubular cells is via brush border binding *...

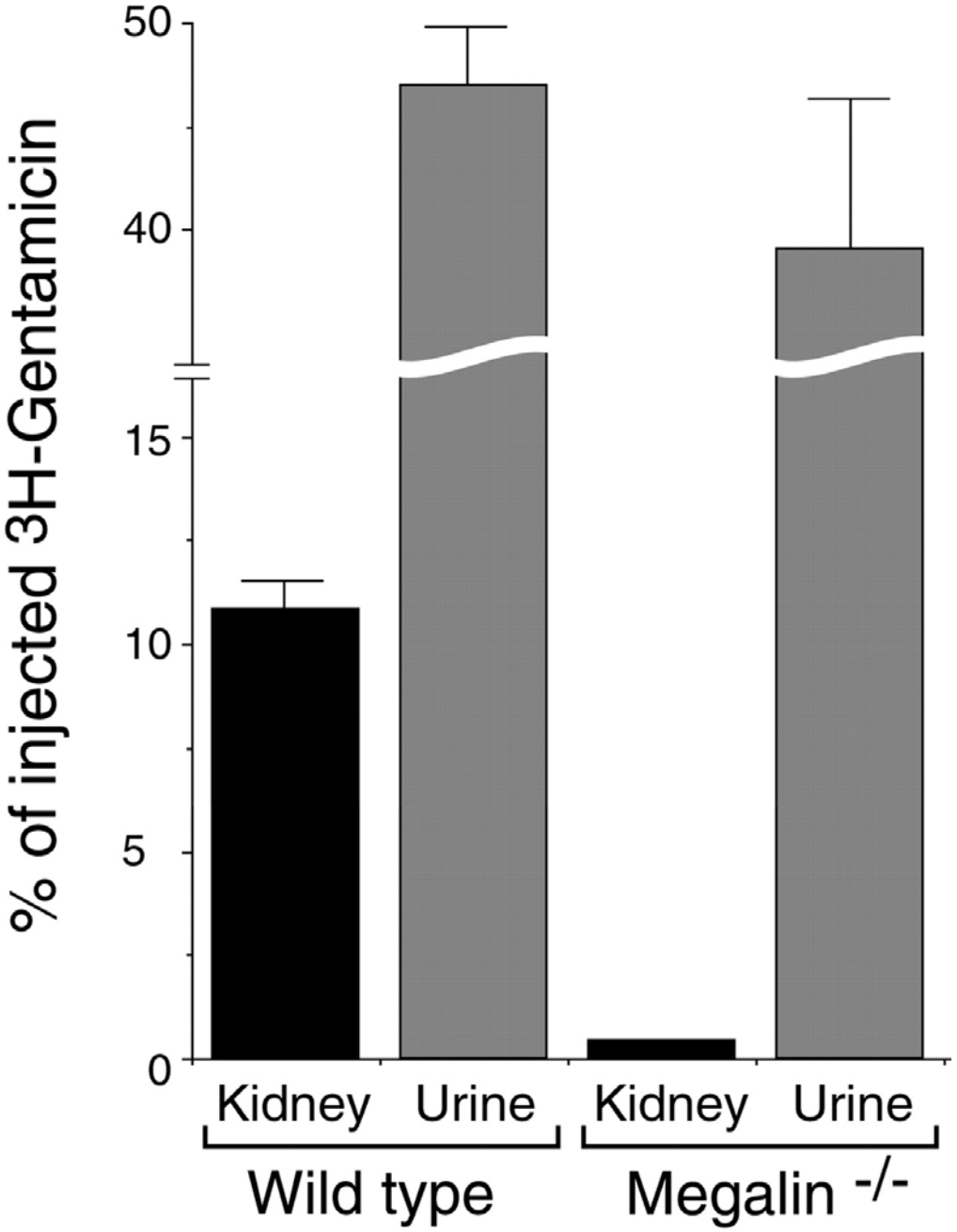


binding to

- **megalin**
  (Moeström et al., 1995)
- **acidic phospholipids**
  (Humes et al, 1983)

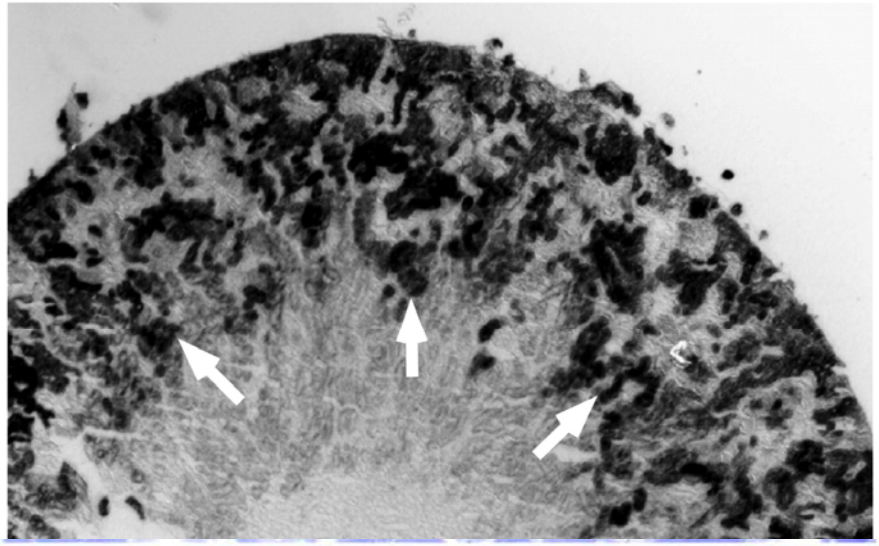
* Just *et al*, Naunym Schmied. Arch. Pharmacol, 1977
Silverblatt & Kuehen, Kidney Intern., 1979

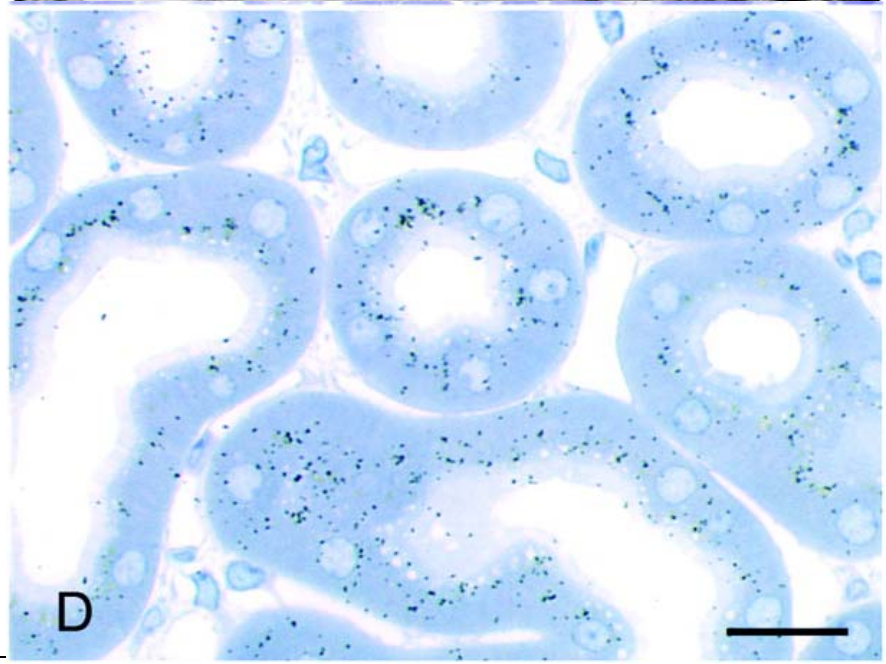
Mice deficient in megalin do not accumulate gentamicin in kidney

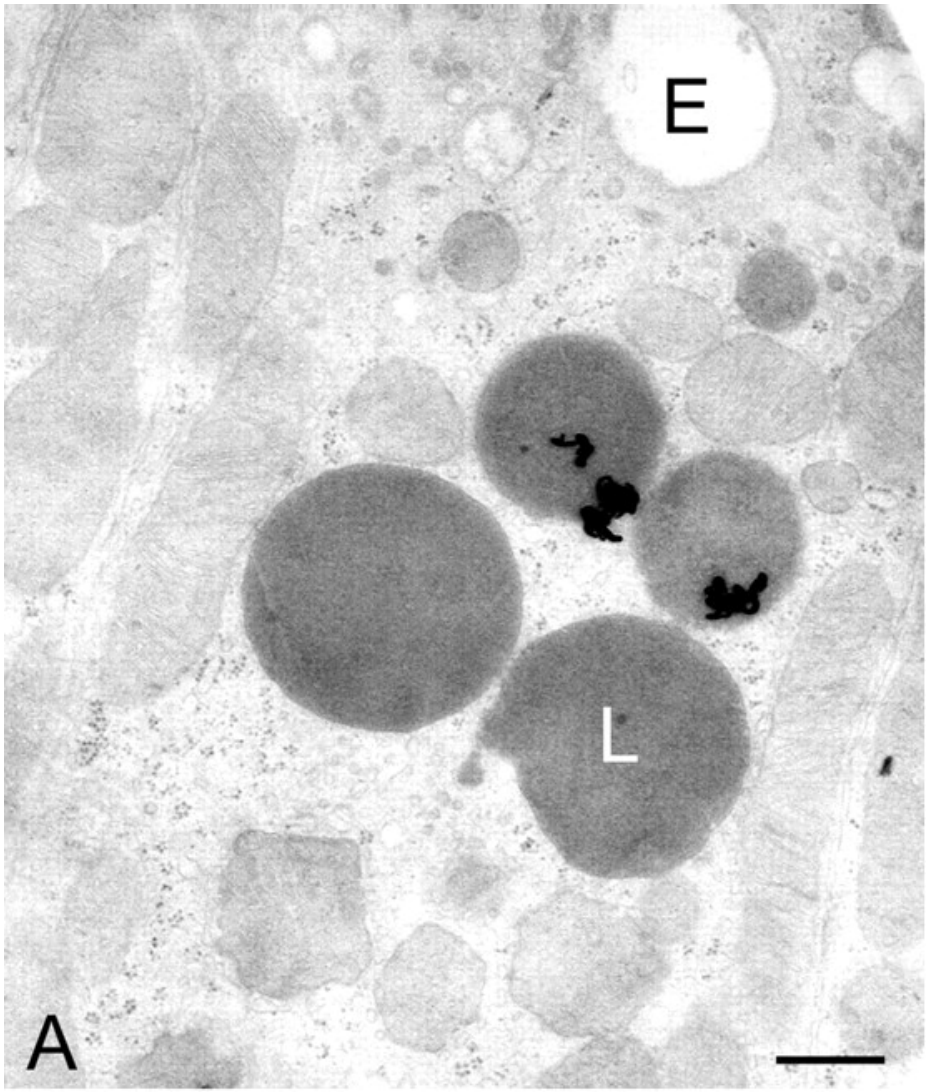


Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Gentamicin accumulates in lysosomes of proximal tubular cells

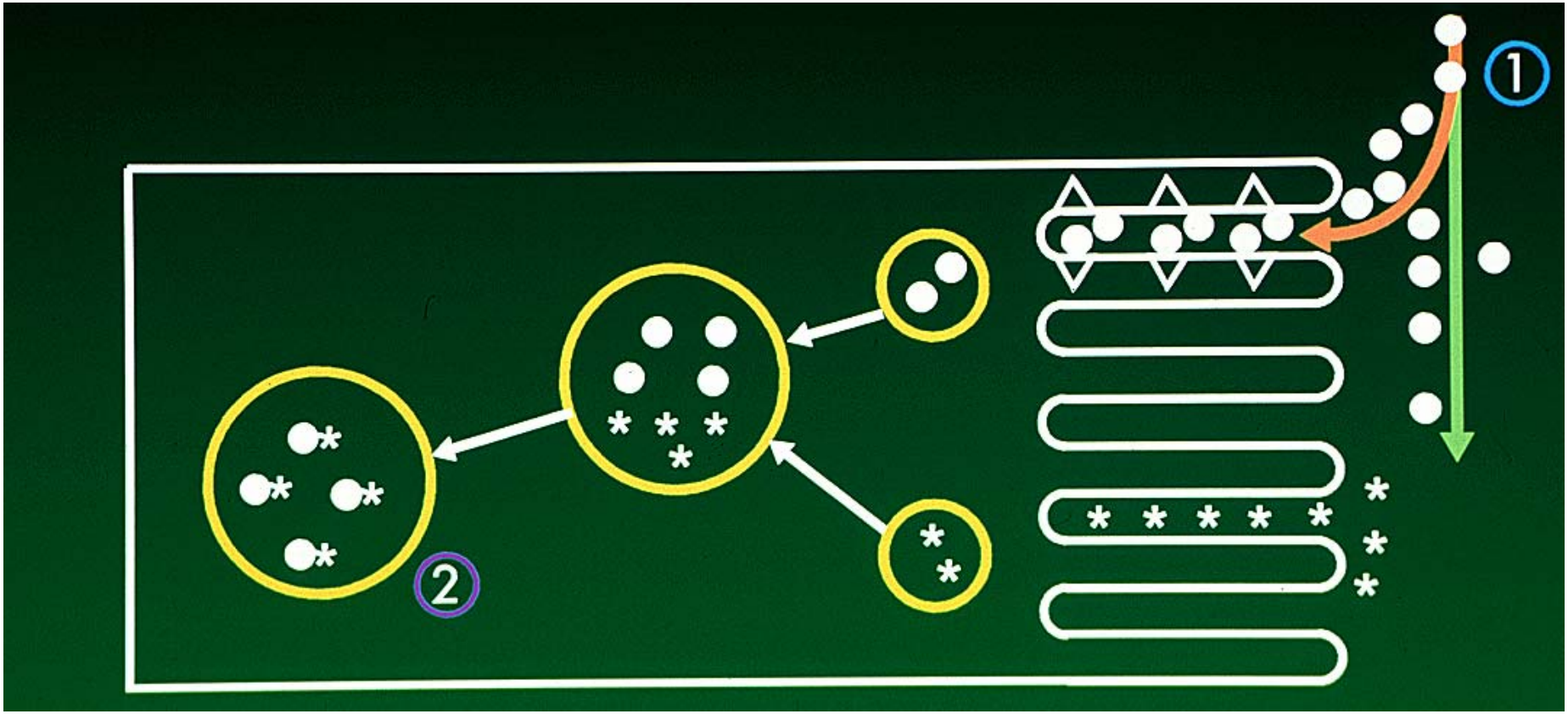






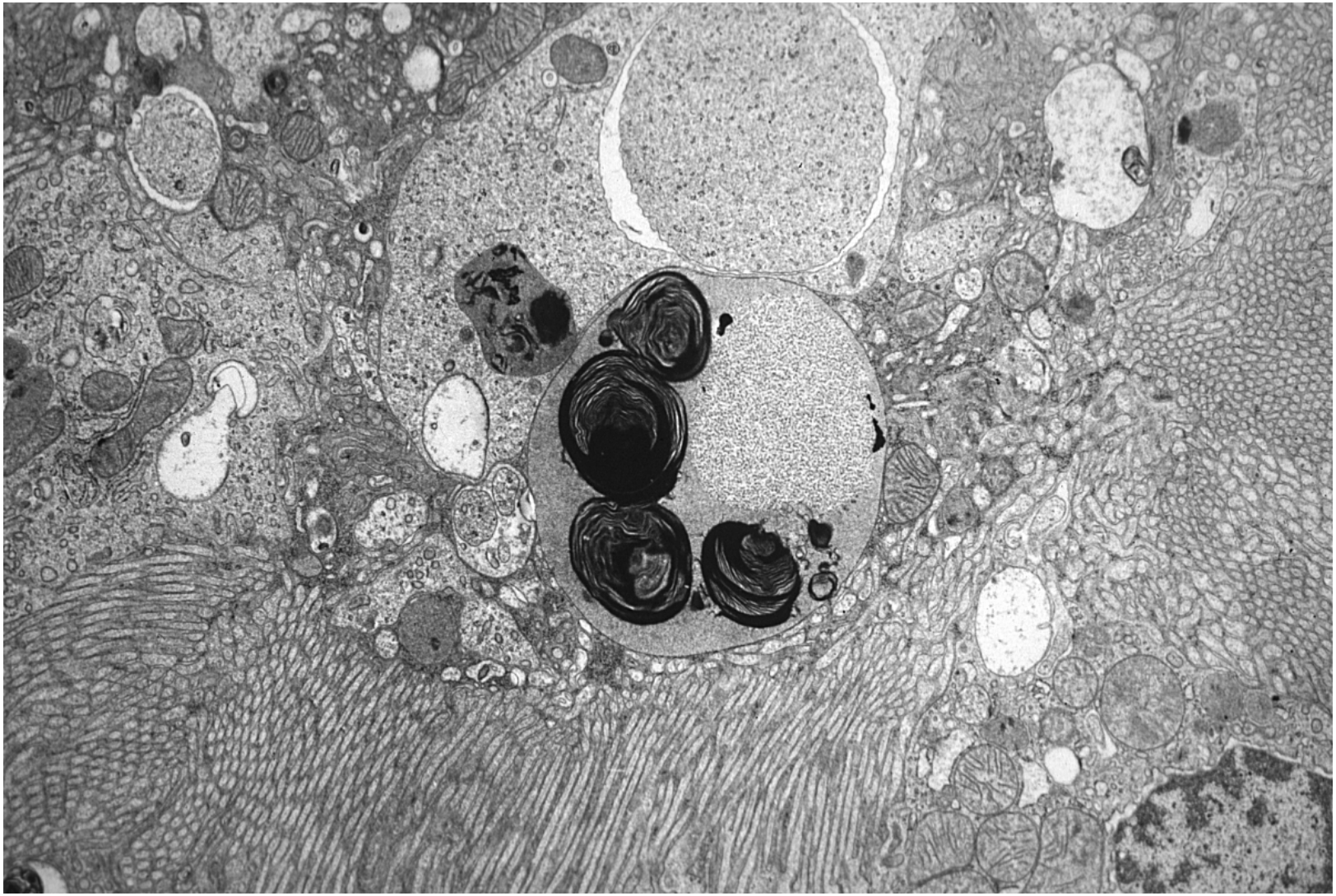
Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Towards a mechanism of entry and early alterations…



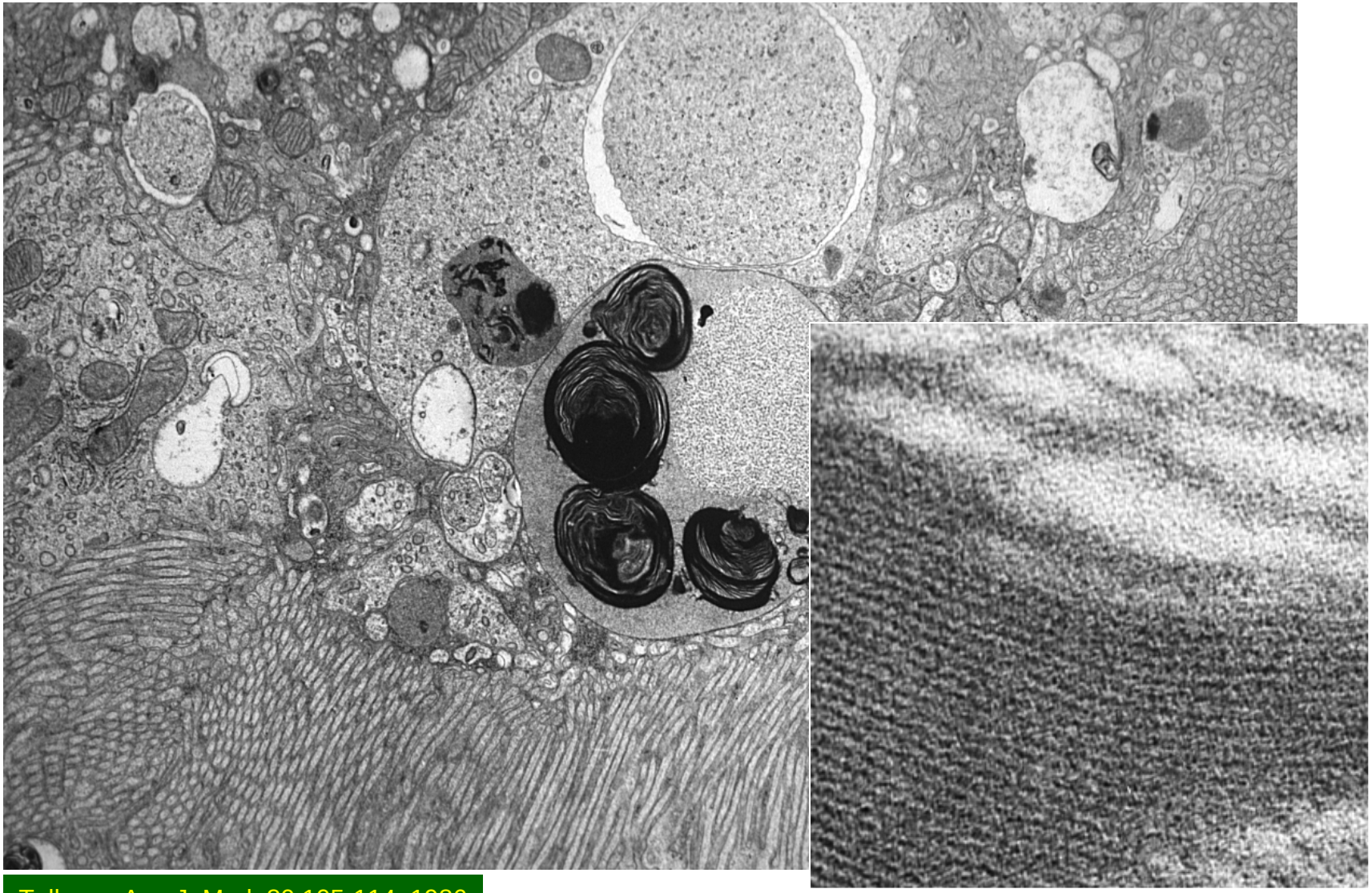
1. **binding to brush border**
2. **accumulation in lysosomes**

Intralysosomal gentamicin causes phospholipidosis



Tulkens, Am. J. Med. 80:105-114, 1986

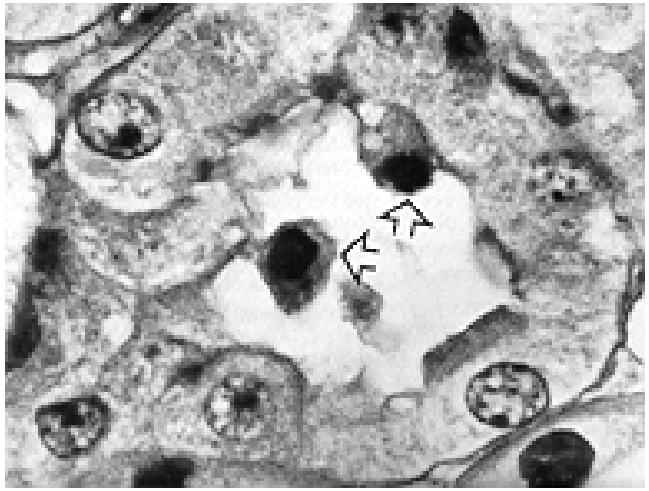
Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis



Tulkens, Am. J. Med. 80:105-114, 1986

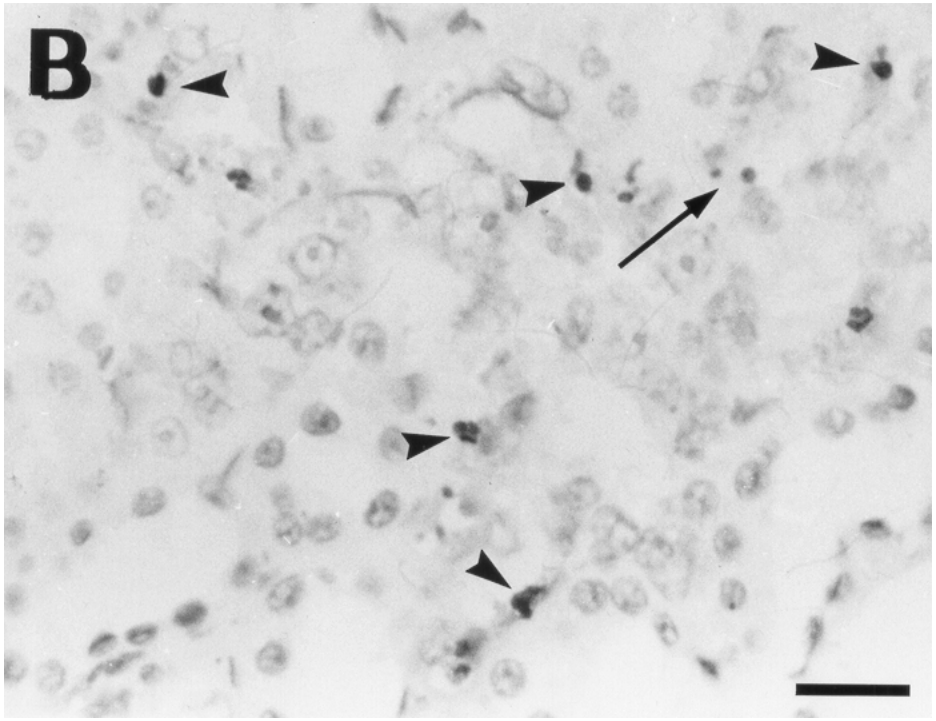
Gentamicin causes apoptosis at low, therapeutically-relevant dosages

Hematoxylin/eosin



Laurent et al.,
Antimicrob. Agents Chemother.,
24:586-593, 1983

Tunel



El Mouedden et al.,
Antimicrob. Agents Chemother.,
44:665-675, 2000

What is the mechanism of gentamicin–induced apoptosis and its relation to necrosis in kidney cortex ?

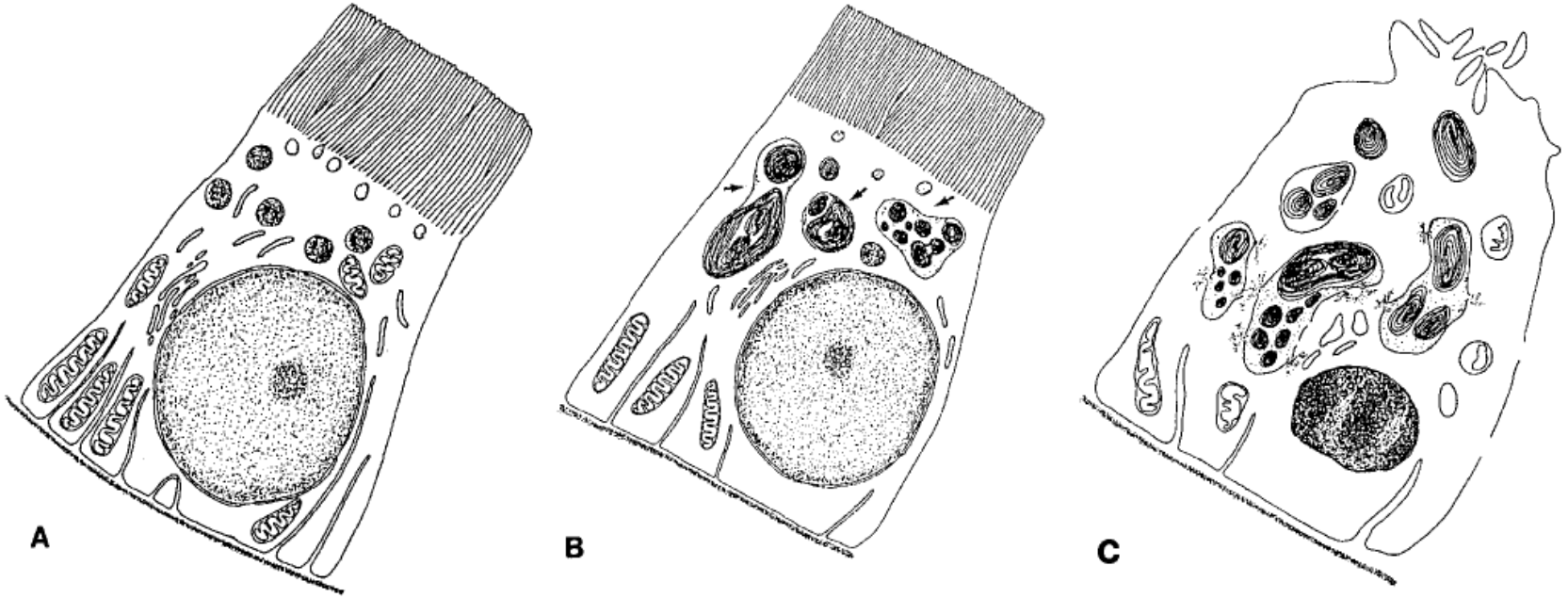
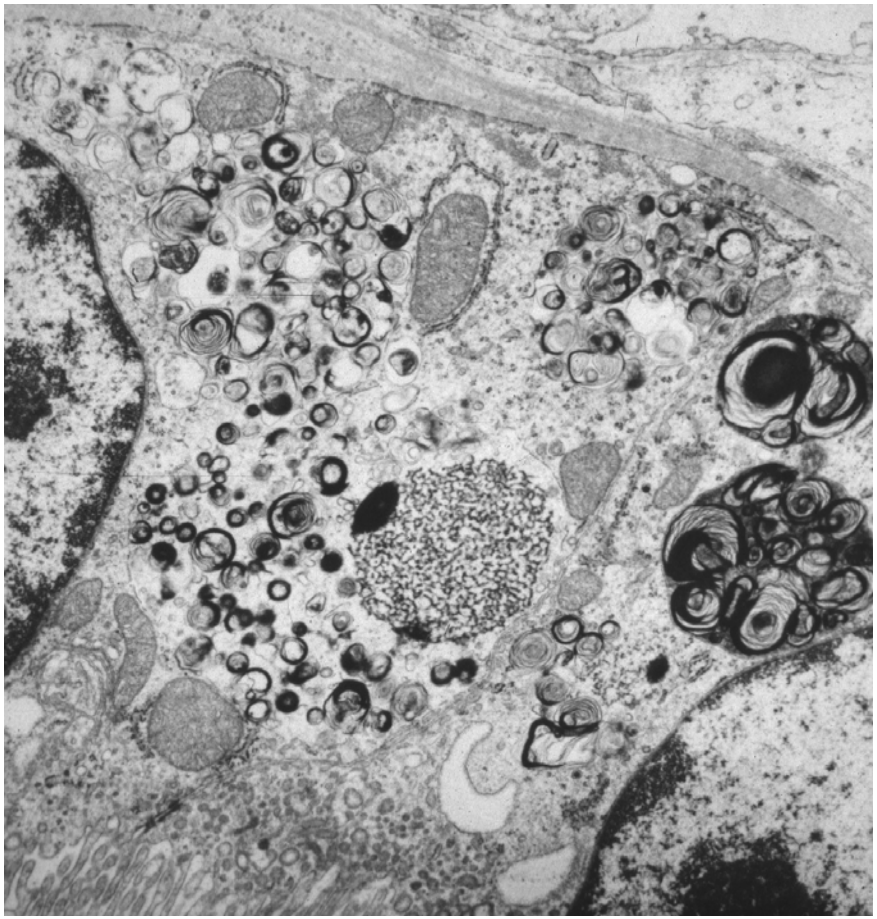


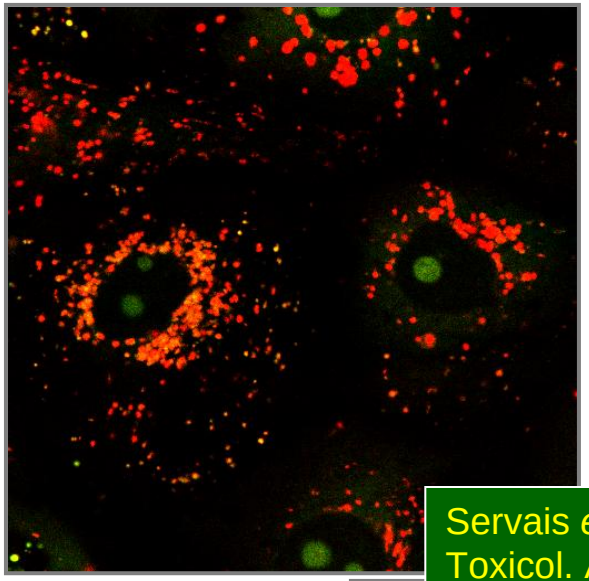
FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-like structures, most commonly referred to as *myeloid bodies*); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses (C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. The figure is adapted from reference 76 and is based on the typical descriptions given in references 38, 40, 71, 76, 77, 127, and 138.

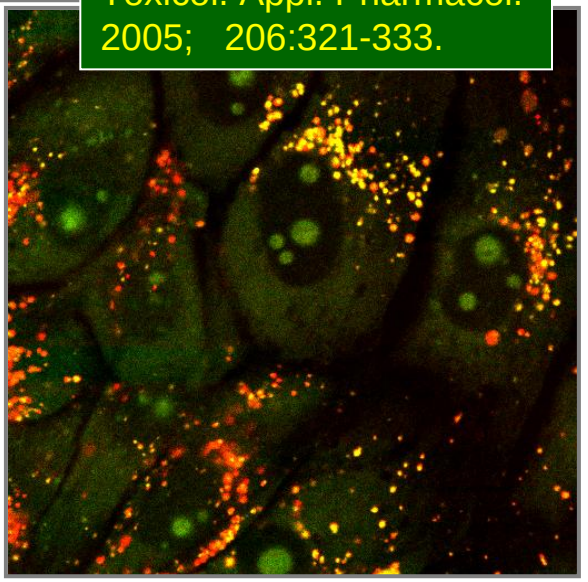
Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. (1999) 43:1003-1012

Could lysosomal rupture cause apoptosis and necrosis ?



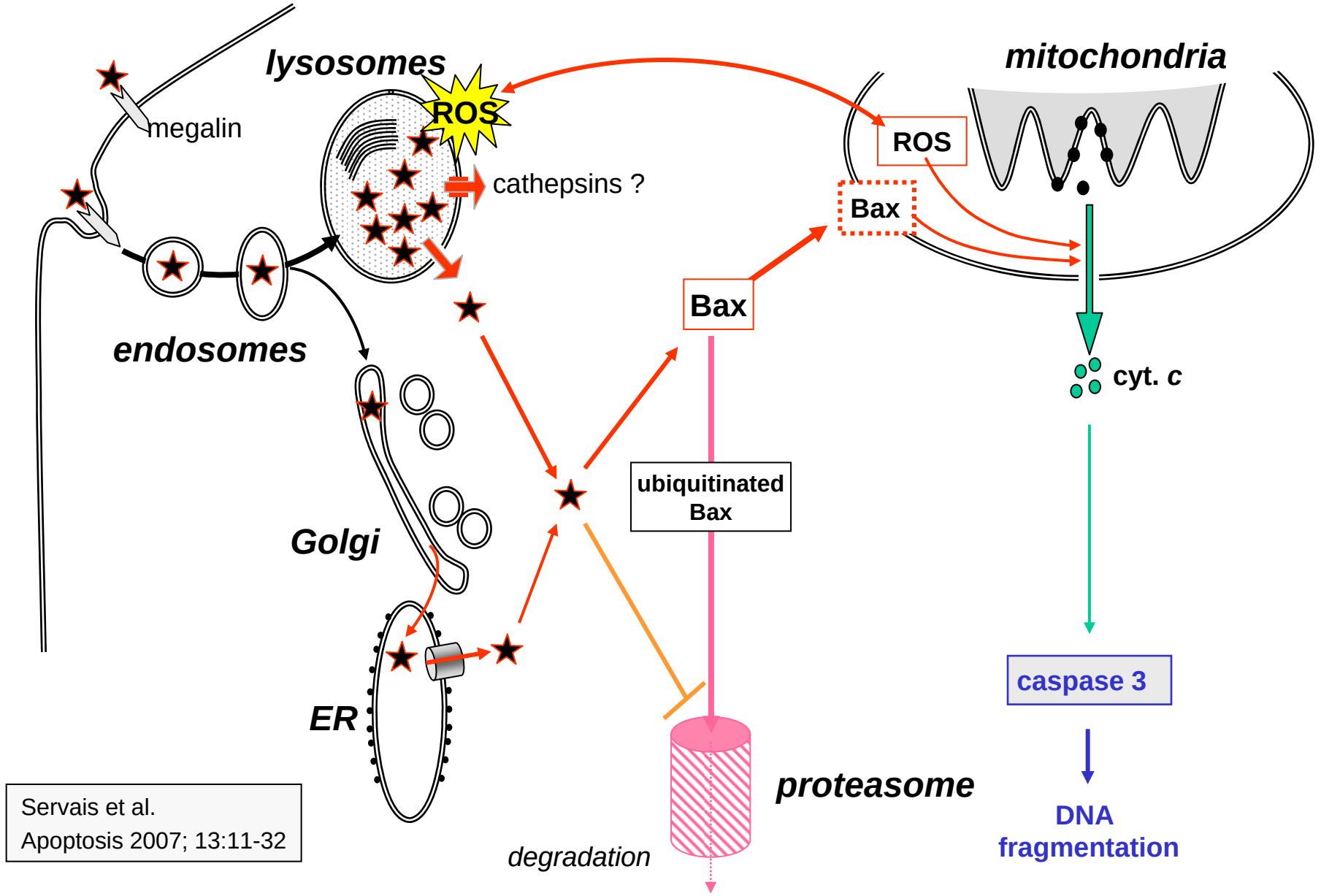
Maldague et al. unpublished





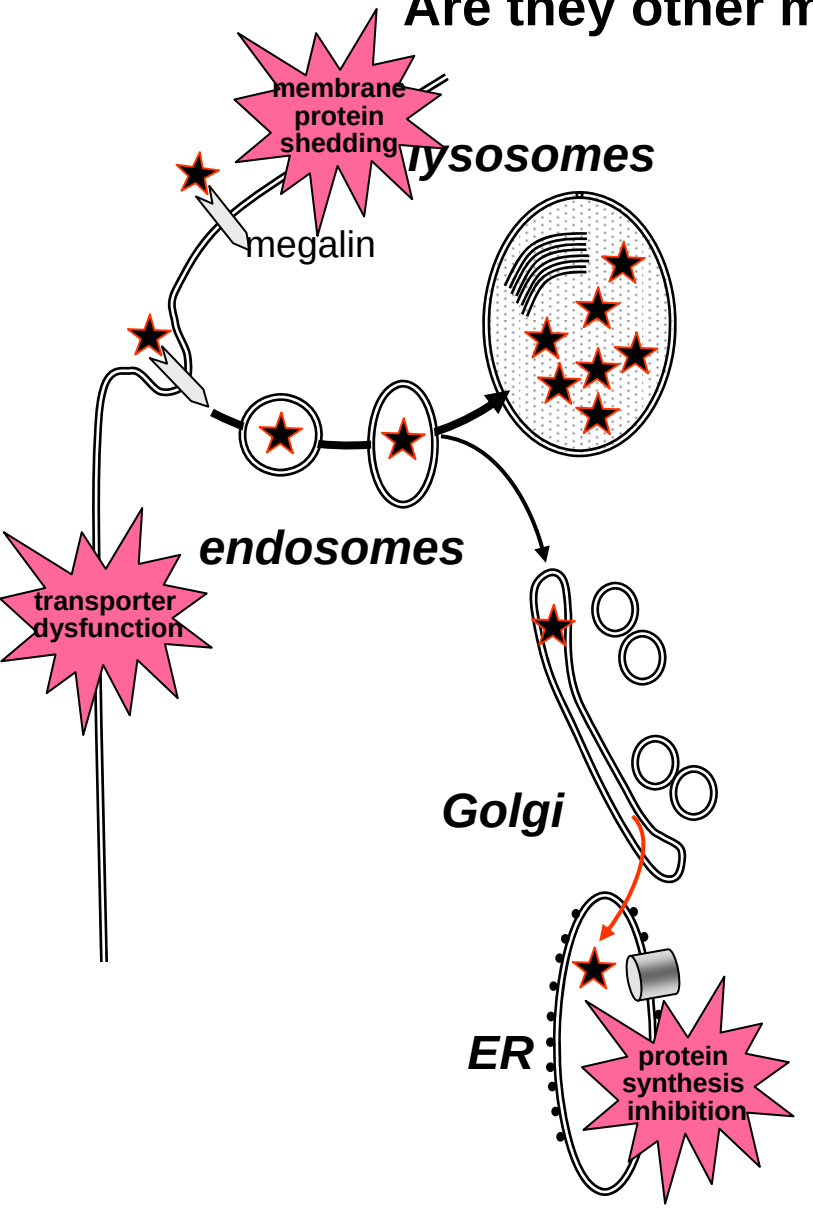
Servais *et al.*,
Toxicol. Appl. Pharmacol.
2005; 206:321-333.

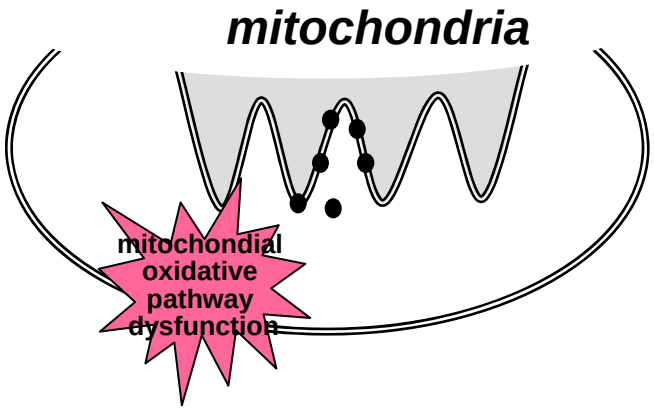
Gentamicin and apoptosis: an overview



Servais et al.
Apoptosis 2007; 13:11-32

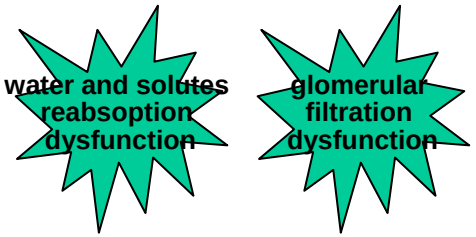
Are they other mechanisms of toxicity proposed ?

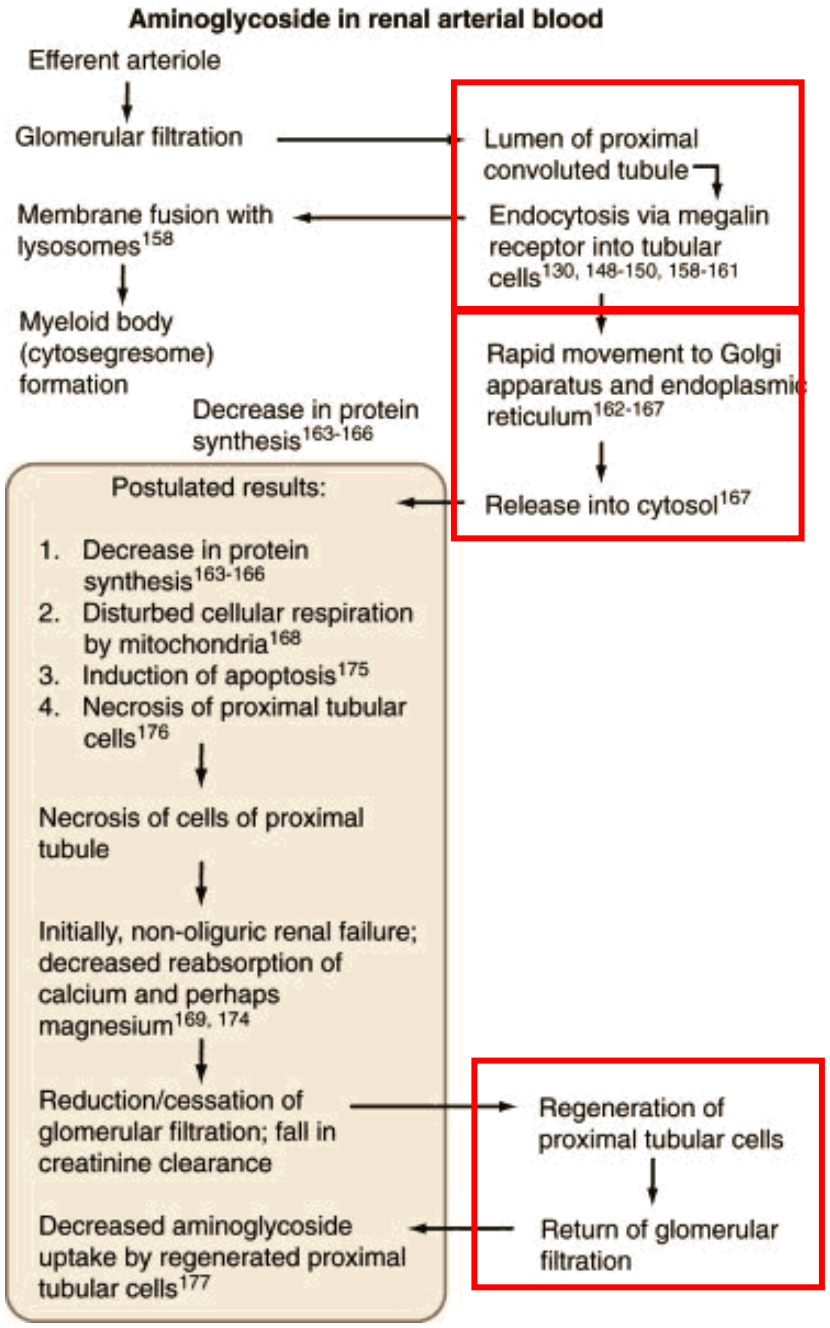




Yes, many others, but the questions are whether alterations described

- are primary (causative) or secondary
- are seen at therapeutically-meaningful doses and concentrations (PK/PD)

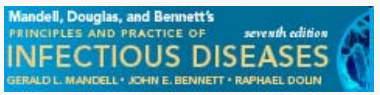


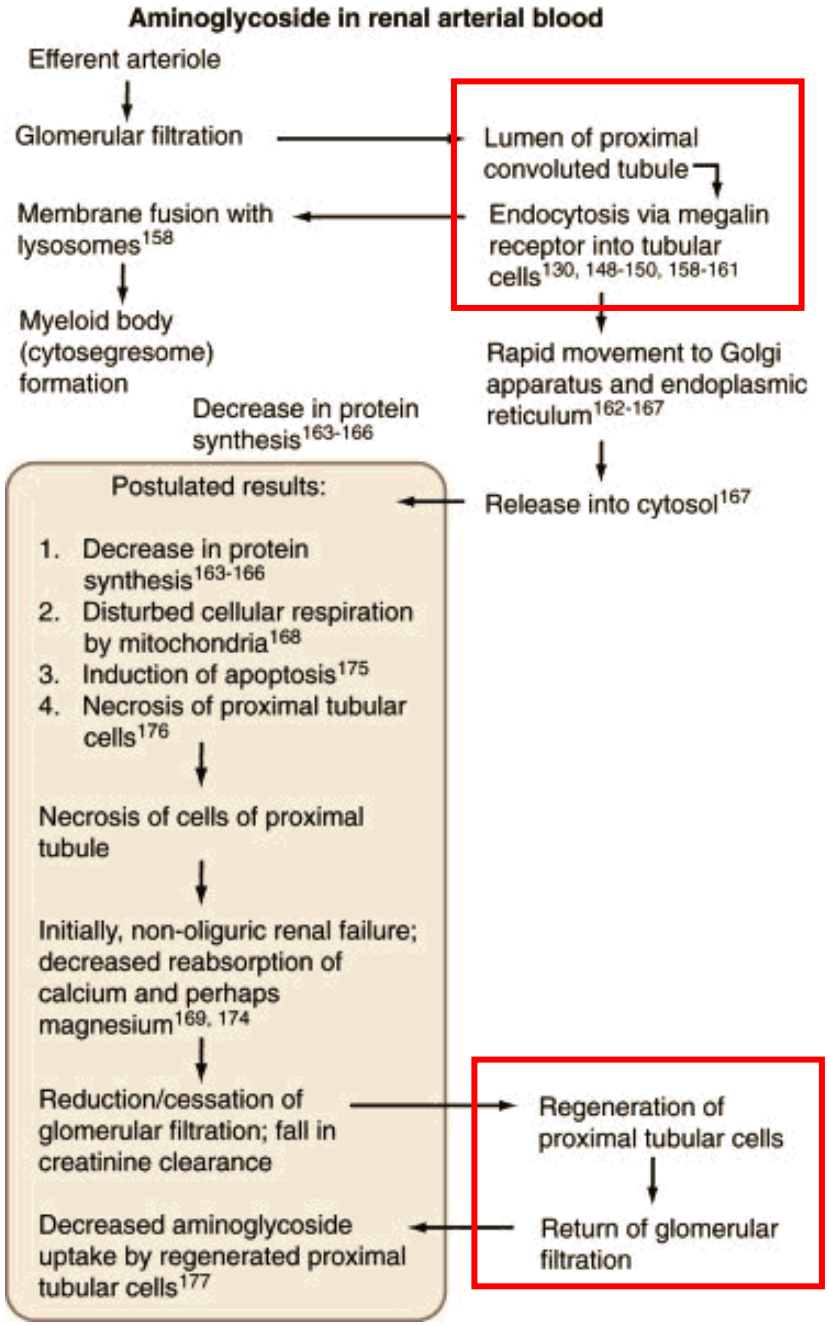


Towards a unifying hypothesis for pathogenesis

- gentamicin enters proximal tubular cells receptor-mediated pinocytosis and ends up in lysosomes…
- a minor part, however, escapes lysosomes (membrane destabilization, ROS production, retrograde transport ?) and reaches the cytosol … where it induces apoptosis and other cell toxicities…
- tubular/glomerular dysfunction is a late consequence of cellular alterations, which
  - take some time
  - can be compensated to a large extent by regeneration
  - varies in intensity and rate among patients based upon their individual risk factors

Gilbert & Leggett 2009





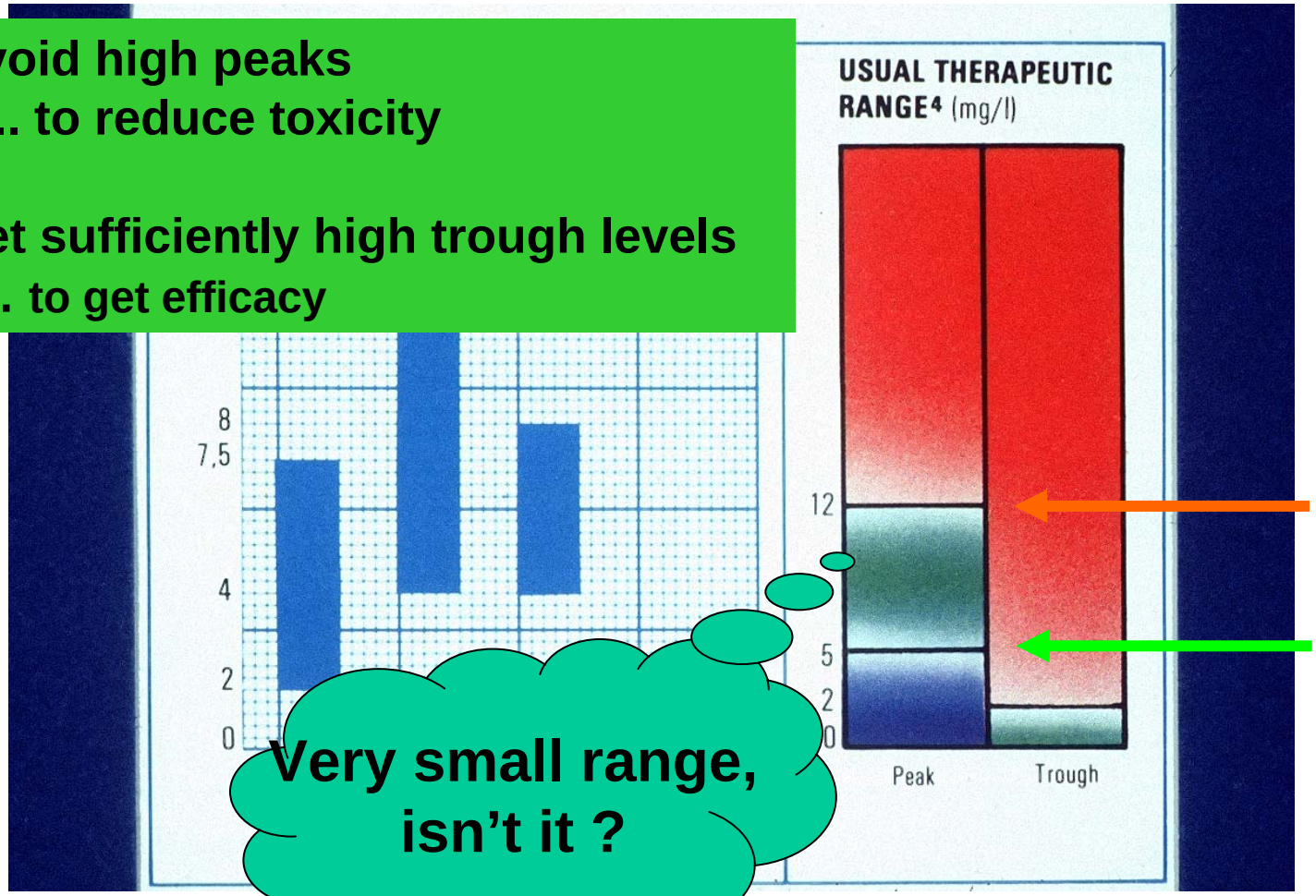
What could the **clinician** do ?…

- block or reduce aminoglycoside accumulation
- avoid conditions that favor the progression from subclinical to clinical/functional alterations

Aminoglycosides monitoring in the 80's ...

avoid high peaks
... to reduce toxicity

get sufficiently high trough levels
… to get efficacy

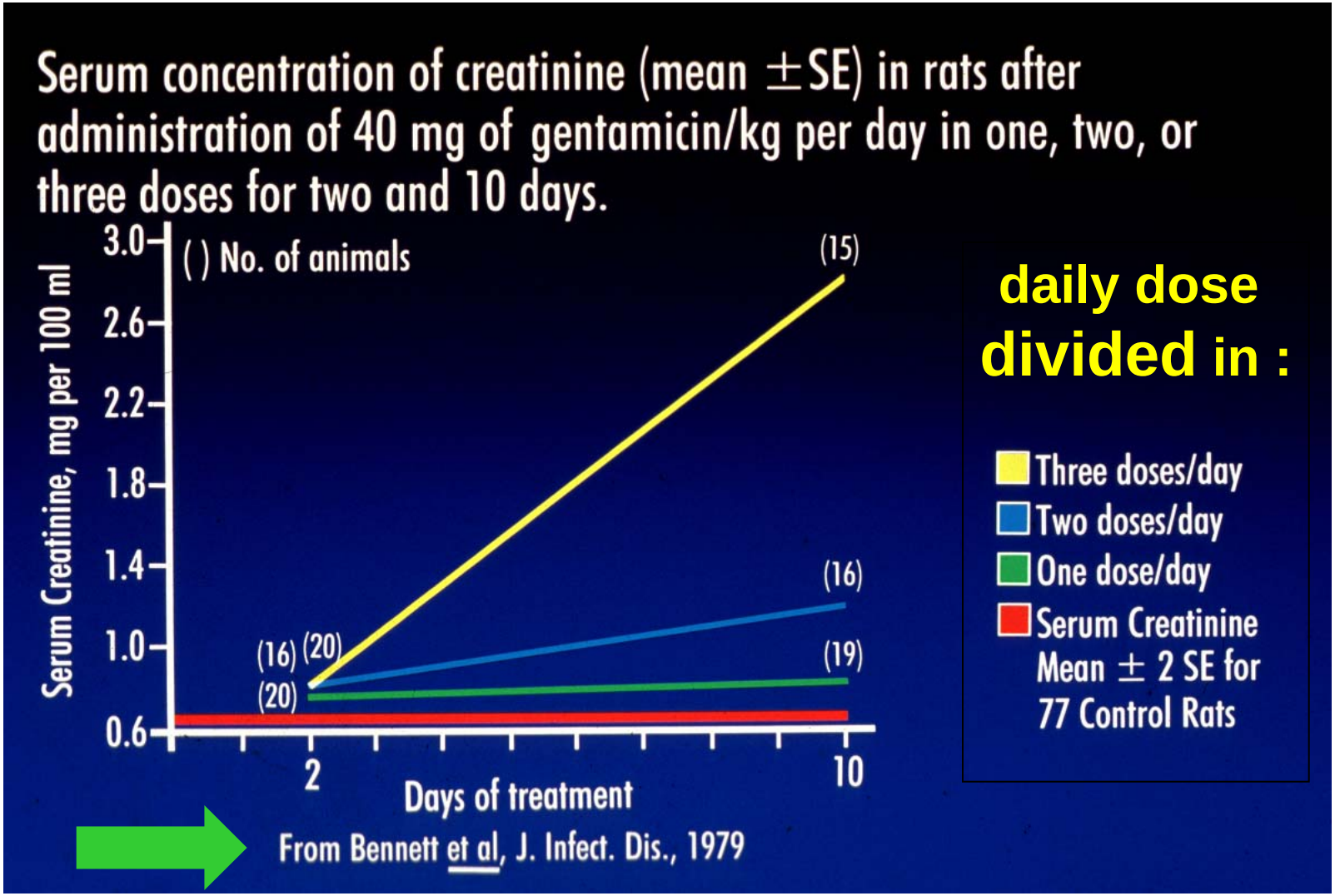


toxicity !!

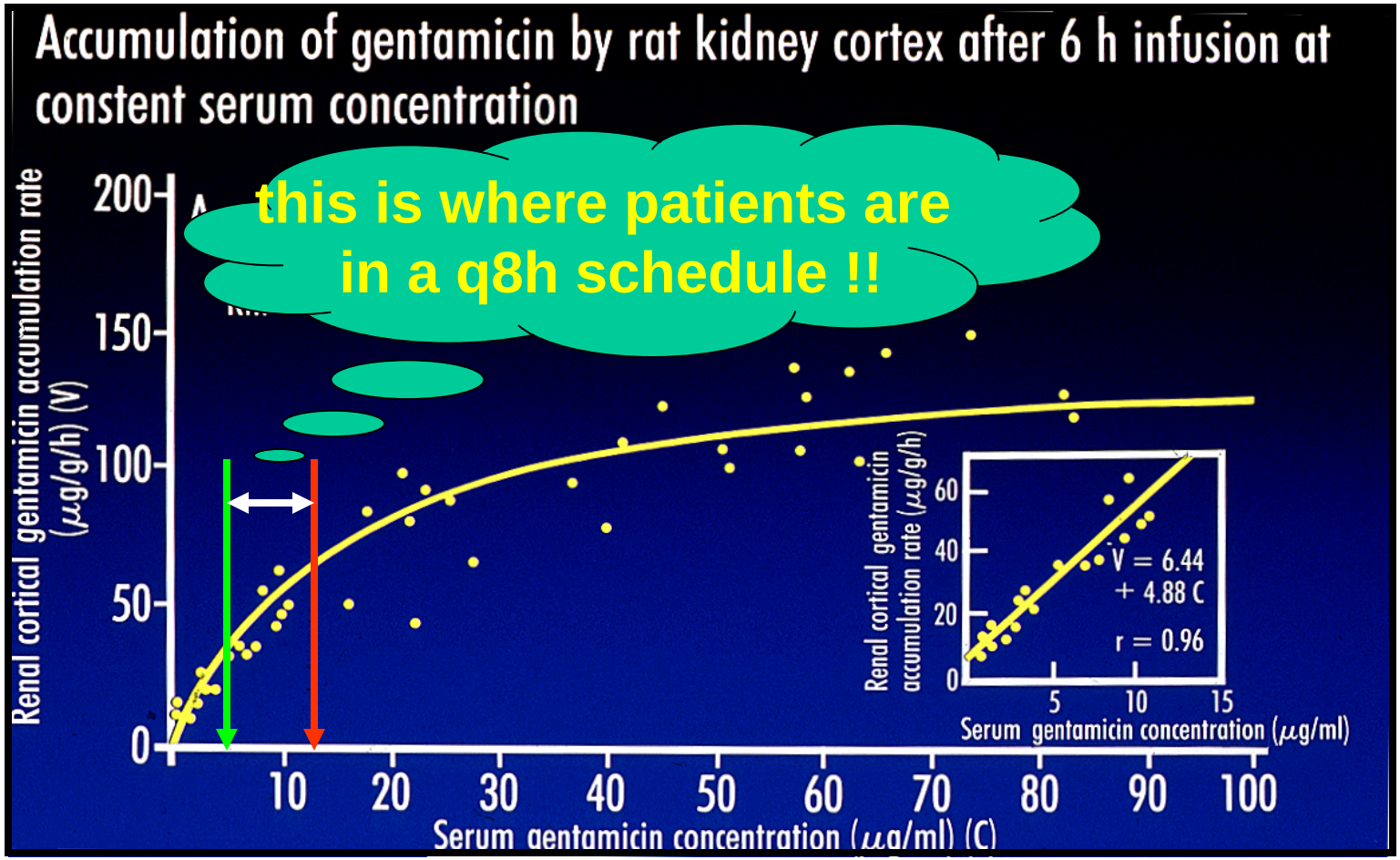
lack of efficacy

Abott TdX manual, 1986

Aminoglycoside toxicity is not linked to peak ...

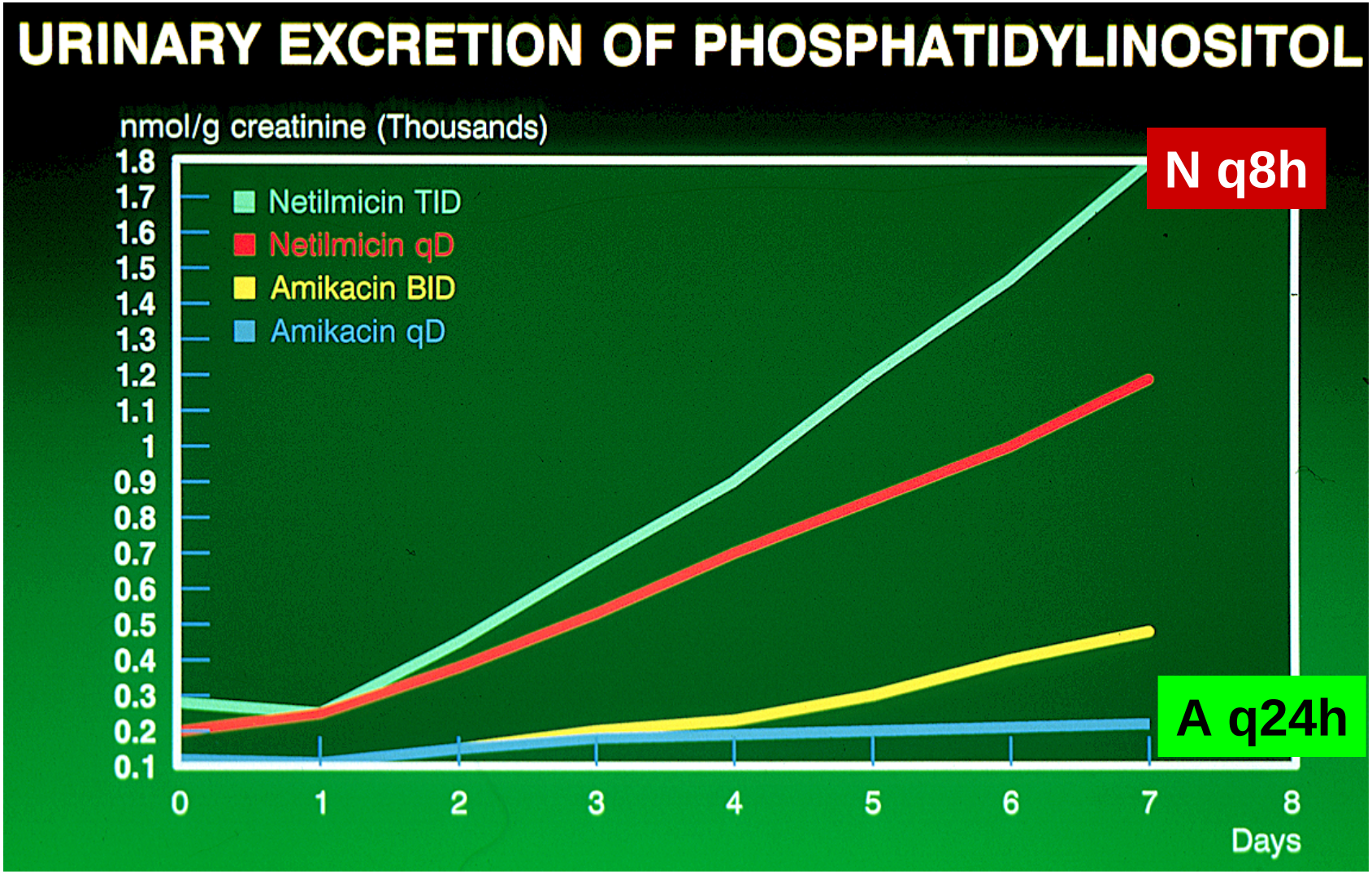


Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...



* Giuliano *et al.*, J. Pharm. Exp. Ther., 1986

Phospholipiduria to monitor early alterations and compare TID/BID to qD…



Tulkens, J Antimicrob Chemother. 1991; 27 Suppl C:49-61.

What about ototoxicity ?

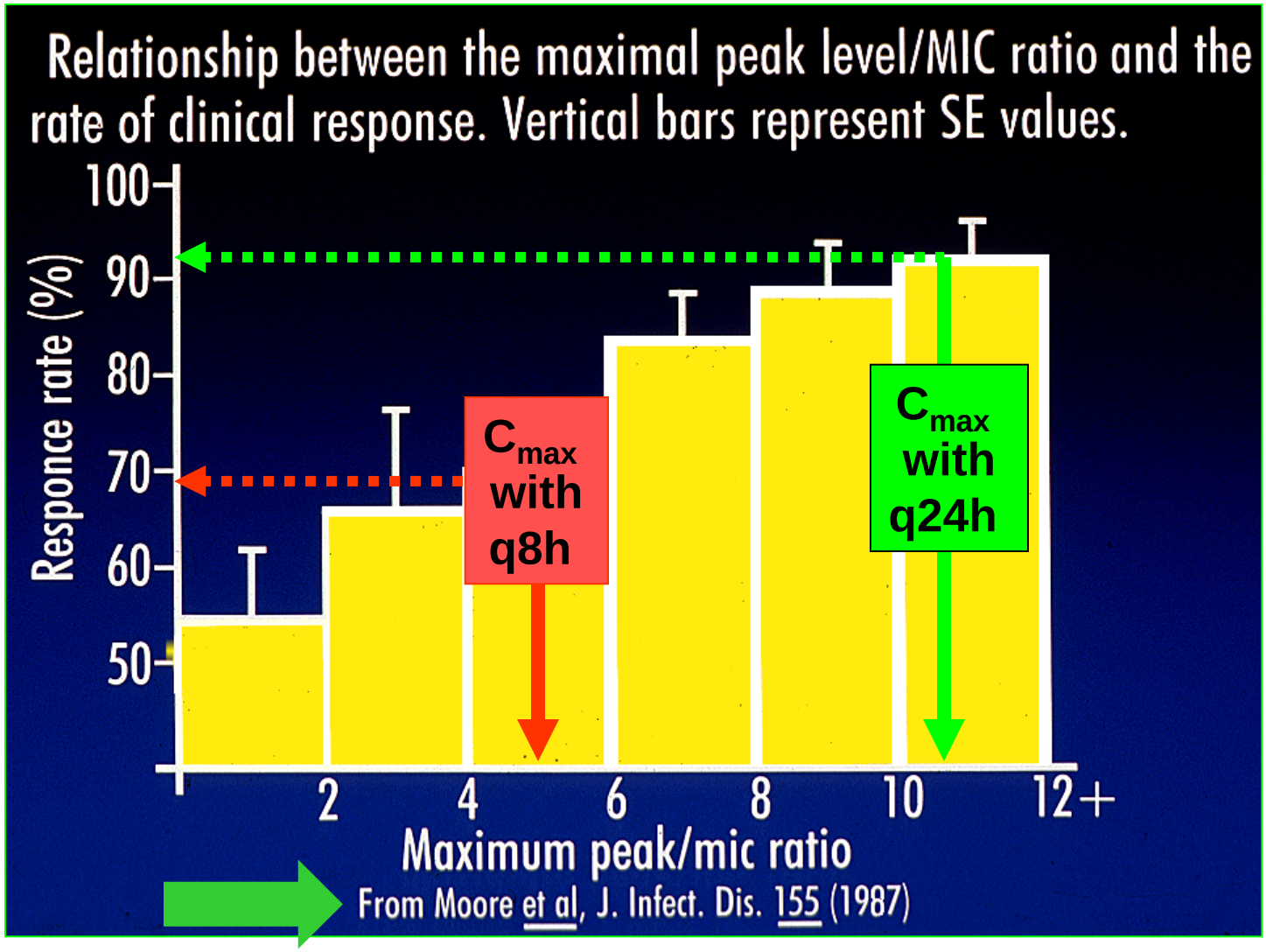
	no. of patients [over 20 in each group] with lesions* and total no. of frequencies affected	
	low tone (0.25-8 kHz)	high tone (10-18 kHz)
amikacin		
• q24h	**1 (1)**	**3 (4)**
• q12h	**0**	**6 (6)**
netilmicin		
• q24h	**0**	**3 (7)**
• q8h	**2 (3)**	**8 (9)**

this is where most of the toxicity was ...

* loss of 15dB or more over baseline (max. loss recorded: 30 dB)

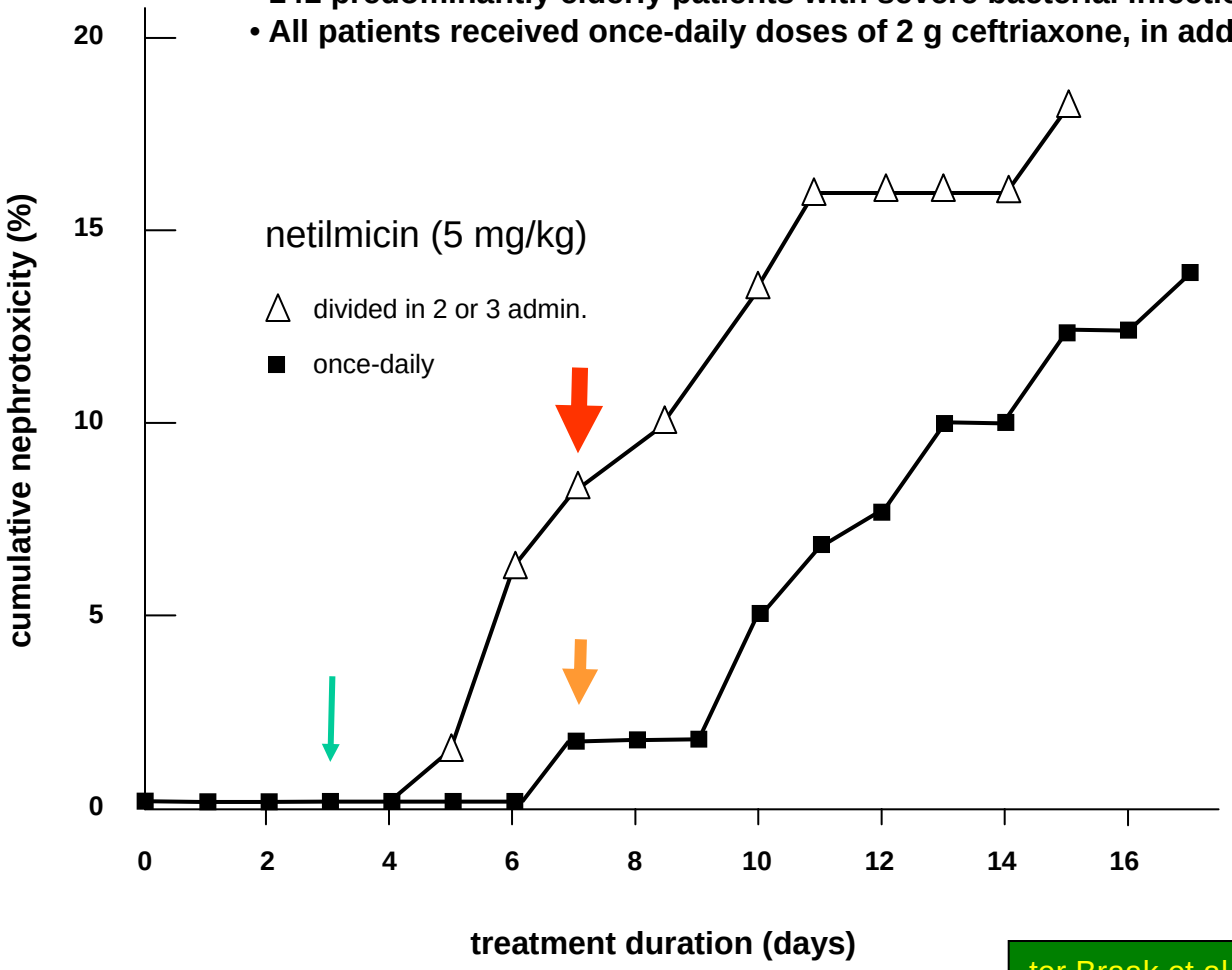
Tulkens, J Antimicrob Chemother. 1991; 27 Suppl C:49-61.

Aminoglycoside peak /MIC ratio is predictive of clinical efficacy



Nephrotoxicity and schedule of administration … the first large scale clinical trial

- **141 predominantly elderly patients with severe bacterial infections.**
- **All patients received once-daily doses of 2 g ceftriaxone, in addition to netilmicin.**



"Netilmicin-induced toxicity may be reduced by using once-daily dosing regimens and limiting the duration of treatment."

ter Braak et al., Am J Med. 1990 Jul;89(1):58-66.

Once-daily dosing: the first review

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1991, p. 399–405 Vol. 35, No. 3
0066-4804/91/030399-07$02.00/0

MINIREVIEW

Once-Daily Aminoglycoside Therapy

DAVID N. GILBERT

Medical Education and Chiles Research Institute, Providence Medical Center, and Oregon Health Sciences University, 4805 NE Glisan, Portland, Oregon 97213

The once-daily administration of aminoglycosides is an attractive concept. In animal experiments and clinical trials, there is either a reduction in or no influence on the risk of toxicity. Less frequent dosing reduces the contact time between host tissue binding sites and drug. Thanks to the PAE and perhaps other as-yet-unrecognized factors, the fall in the level in serum below the MIC does not appear to impair antibacterial efficacy; in fact, the higher peak level in serum may enhance drug efficacy early in a dosage interval.

Because it will be some time before data from clinical trials in the United States are available, because the results from the international trials are encouraging, and because there is potential benefit to patients, it seems reasonable for infectious diseases consultants to cautiously initiate the educational process necessary to implement once-daily aminoglycoside therapy in their institutions.

Is the once-a-day schedule used ?

Clin Infect Dis 2000 Mar;30(3):433-9

National survey of extended-interval aminoglycoside dosing (EIAD).

Chuck SK, Raber SR, Rodvold KA, Areff D.

- **500 acute care hospitals in the United States**
- **EIAD adopted in 3 of every 4 acute care hospitals**
  - **4-fold increase since 1993**
  - **written guidelines for EIAD in 64% of all hospitals**
- **rationale**
  - **87.1% : equal or less toxicity**
  - **76.9% : equal efficacy**
  - **65.6% :cost-savings**
- **dose: > 5 mg/Kg**
- **47% used extended interval in case of decline in renal function (38% with Hartford nomogram)**

Is the once-daily administration safe in 2009 ?

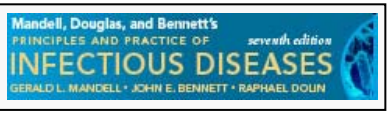
More than 55 published clinical trials (31 prospective randomized) with treatment of all the commonly encountered infections and virtually all populations of patients indicates that once-daily aminoglycoside administration

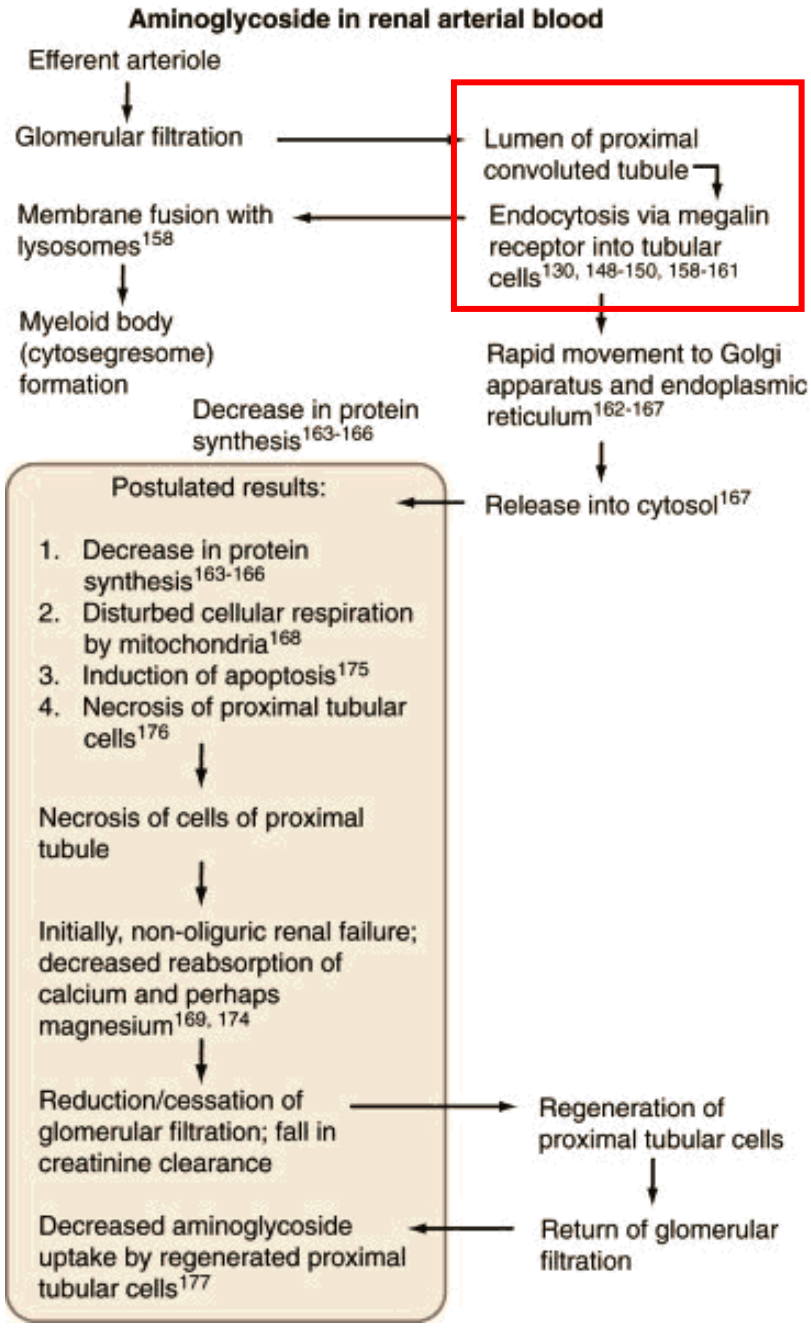
- is as efficacious as the traditional multiple-dose method
- may lower but not eliminate the risk of drug-induced nephrotoxicity and ototoxicity.
- is simpler, less time-consuming, and more cost-effective than multiple-dose regimens
- does not worsen neuromuscular function (if 15-30 min infusion)

Caveats may include

- patients with enterococcal endocarditis.
- pregnant women, CF patients, meningitis caused by aerobic gram-negative bacilli, osteomyelitis

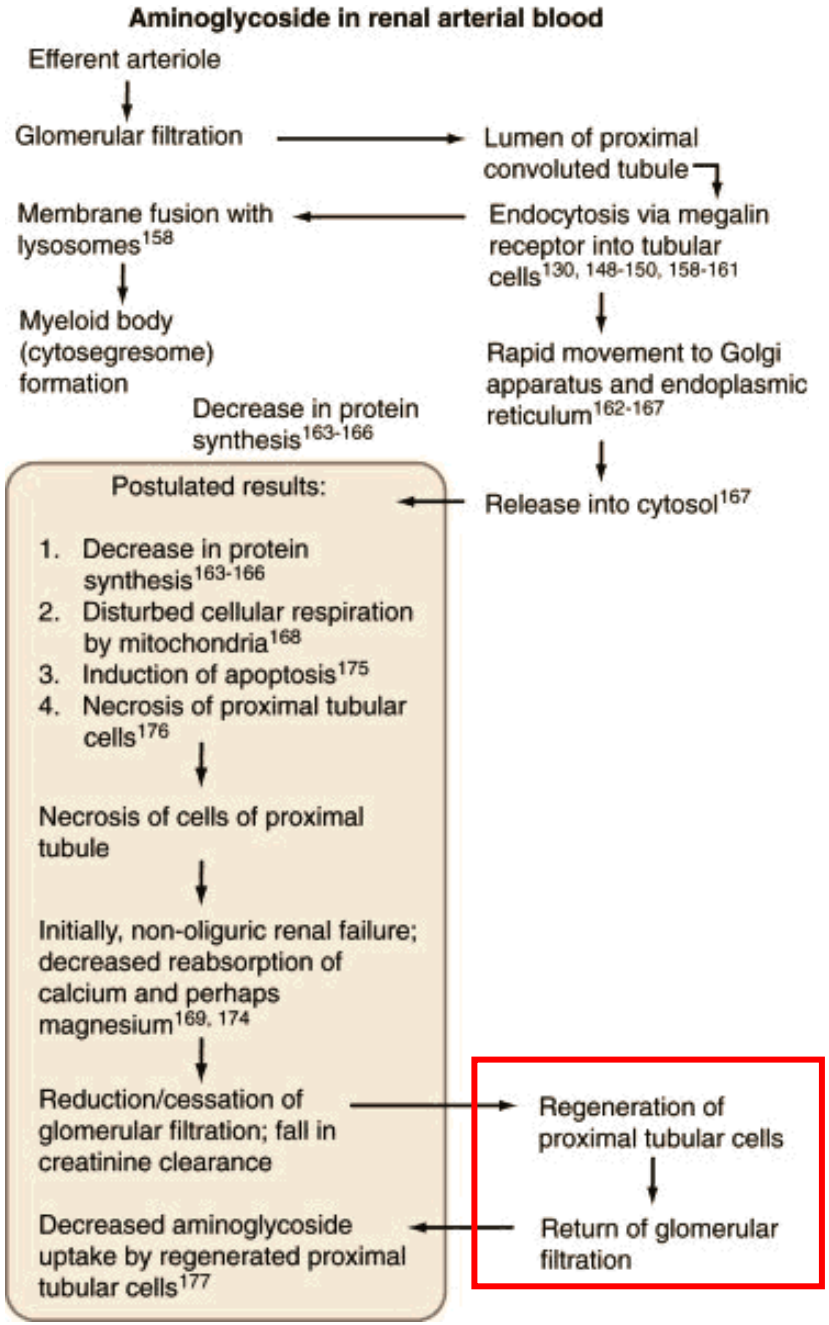
adapted from
Gilbert & Leggett 2009





What has been done ?…

- reduce aminoglycoside accumulation



But can we do this ?

- avoid conditions that favor the progression from subclinical to clinical/functional alterations

you can do this today by reducing treatment duration !

What have we learned ?

- aminoglycoside toxicity can be reduced and brought very low levels (< 5 %) if
  - using a once-daily (extended interval) schedule (with increased interval for patients with decreased renal function)
  - shortening the treatment duration (< 7 days)
  - taking the known risk factors in due consideration...
- to be successful, new aminoglycosides should be
  - designed so as to remain unaffected by the rising resistance mechanisms (enzymes, methylation, efflux)
  - be administered in a the way we know to maximize their efficacy while minimizing their toxicity *

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* - there are many experimental approaches to reduce toxicity, but, apart from the once-daily dosing, none has reached clinical use and approval;
- molecules with lower intrinsic toxicity are being investigated based on present knowledge of the mechanisms of toxicity

Why not ?

Who said you had to turn back your clock today ? *

* this presentation was given on the day of change from daylight saving to standard time

It only started a few years ago …

Stockholm, Sweden, 1989 …

But MANY others participated to this research …
since the early 70's … and I could not cite them all …

And research is still going on in our lab …

biophysical studies

M.P. Mingeot

clinical pharmacy

S. Quing Su

E. Ampe

F. Van Bambeke

cell culture models

phospholipases

H. Aly

megalin inhibitors

M. Ouberaï

lipophilic derivatives

S. Denamur

apoptosis and ROS

M. Riou

microbiology of new molecules

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