Aminoglycosides:
a new look at old but probably faithful antibiotics *

* if you can use them properly …

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What did I do (and where did I do it) **?**

- Teaching of Pharmacology and Pharmacotherapy
- Post-graduate training on Drug Development
- Launching of Clinical Pharmacy in Europe
- Web-based courses on anti-infective Pharmacology
- 20 graduating students, doctoral fellows and post-graduate fellows working on anti-infective therapy (laboratory and clinical applications)

- Toxicity, medicinal chemistry, and improved schedules of aminoglycosides
- novel beta-lactams, and continuous infusion
- fluoroquinolones efflux and PK/PD
- Novel glycopeptides and derivatives thereof and models of intracellular infection

www.facm.ucl.ac.be

A partial view of our University Clinic (900 beds) and the Education and Research buildings (5,000 students), in the outskirts of Brussels, Belgium

- Editorial board of AAC
- Member of the General Committee of EUCAST (for ISC) and of its Steering ommittee (2008-10)
- Member of the Belgian Antibiotic Policy Coordination Committee
- Founder and Past President of the International Society of Antiinfective Pharmacology (ISAP)

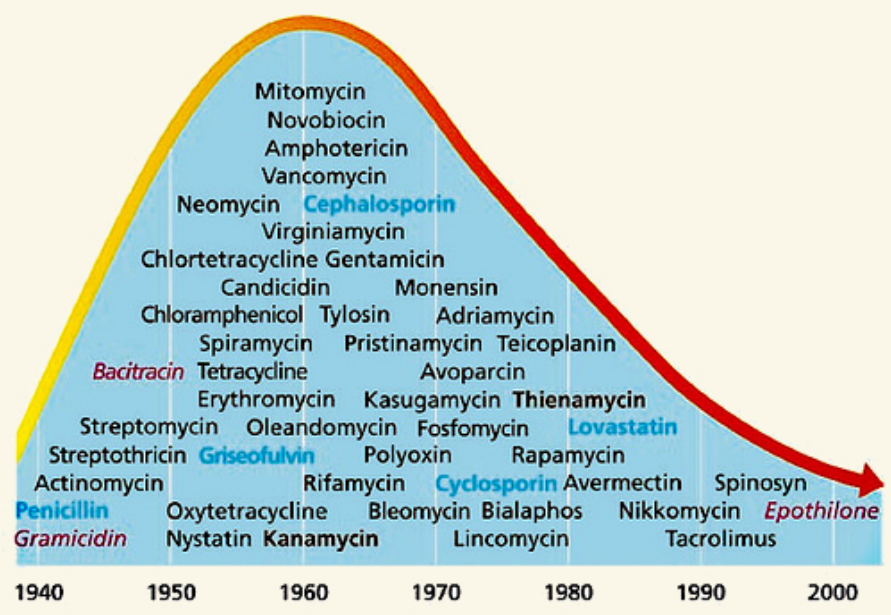
www.isap.org

Program, please !

- A bit of history
- Activity and resistance
- Toxicity
- A real clinical application

I hope you will follow …

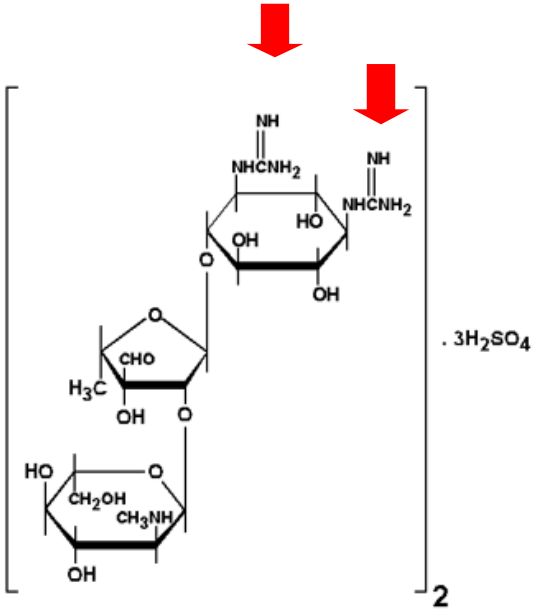
Part 1 : A bit of history



The antibiotic saga !

Aminoglycosides is a cyclic story

Streptomycin: the first aminoglycoside

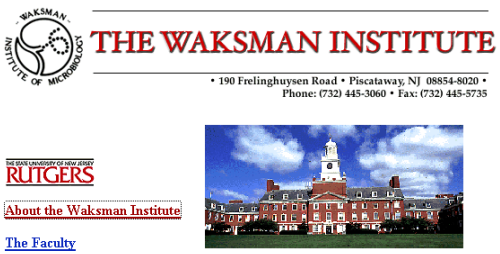


- discovered by par Waksman at Rutgers University in New Jersey in 1943
- broad spectrum including Gram (+) and Gram (-), and *Mycobacterium tuberculosis*
- highly bactericidal
- but gave rapidly rise to resistance (ribosomal alteration [target modification]
- well know for its ototoxic pontential (more for dihydrostreptomycine), but largely due to its use for prolonged treatments
- rarely used nowadays except for tuberculosis (2d or 3d line), tularemia, plague, and, sometimes, endocarditis

Streptomycin was the firts antibiotic to be discovered by systematic screening

streptomyces grisaeus

Waksman and Fleming …

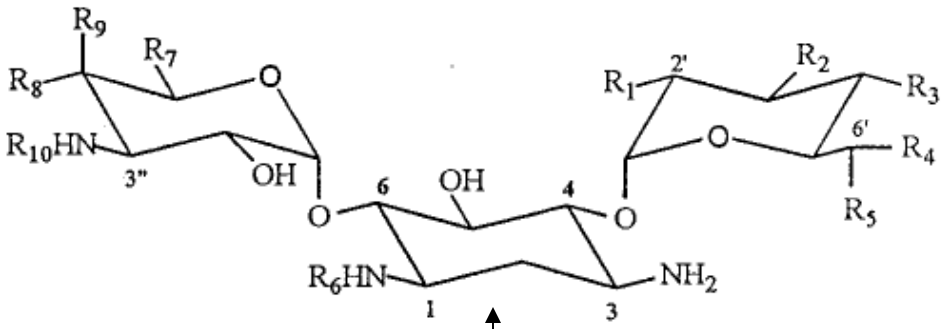


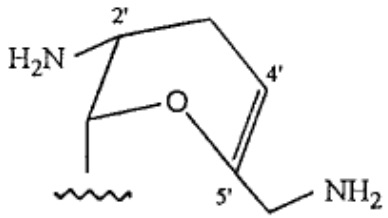
From the point of view of human benefit, never was a Nobel prize so justifiably awarded as was the award to Selman Waksman for the discovery of streptomycin and other antibiotics produced from *Streptomyces spp*. Waksman and his talented team (many of whom went on to make important antibiotic discoveries in their own right) developed the concept of **systematic screening** of microbial culture products for biological activity, a technology which has provided the foundation of the antibiotic industry, and for this alone his name should rank high in any pantheon of microbiology.

J. Davies: In Praise of Antibiotics, ASM News
http://www.asm.org/memonly/asmnews/may99/feature6.html

Main clinically-usable aminoglycosides in the 80's ...

4,6-DISUBSTITUTED DEOXYSTREPTAMINE





(a)

"

'

build up around a 4,6 substituted 2-deoxystreptamine

flanked with 2 aminosugars

Aminoglycoside	R_1	R_2	R_3	R_4	R_5	R_6*	R_7	R_8	R_9	R_{10}
Kanamycin A	OH	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
Kanamycin B	NH_2	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
Kanamycin C	NH_2	OH	OH	H	OH	H	CH_2OH	OH	H	H
Amikacin	OH	OH	OH	H	NH_2	COR'	CH_2OH	OH	H	H
Tobramycin	NH_2	H	OH	H	NH_2	H	CH_2OH	OH	H	H
Dibekacin	NH_2	H	H	H	NH_2	H	CH_2OH	OH	H	H
Arbekacin	NH_2	H	H	H	NH_2	COR'	CH_2OH	OH	H	H
Gentamicin C_1	NH_2	H	H	CH_3	$NHCH_3$	H	H	CH_3	OH	CH_3
Gentamicin C_{1a}	NH_2	H	H	H	NH_2	H	H	CH_3	OH	CH_3
Gentamicin C_2	NH_2	H	H	CH_3	NH_2	H	H	CH_3	OH	CH_3
Gentamicin C_{2b}	NH_2	H	H	H	$NHCH_3$	H	H	CH_3	OH	CH_3
Gentamicin B	OH	OH	OH	H	NH_2	H	H	CH_3	OH	CH_3
Isepamicin	OH	OH	OH	H	NH_2	COR	H	CH_3	OH	CH_3
Sisomicin	---	---	---	---	---	H	H	CH_3	OH	CH_3
Netilmicin	---	---	---	---	---	CR''	H	CH_3	OH	CH_3

* R = $CHOHCH_2NH_2$; R' = $CHOH(CH_2)_2NH_2$; R'' = CH_2CH_3 (a) = primed sugar for sisomicin and netilmicin

What were the advantages of aminoglycosides as seen in the mid 80'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 mechanims) ?
  - can we further dissociate activity and toxicity ?
  - what is/are the mechanism(s) of these adverse effects ?
  - can we protect patients ?

Part 2: Activity and resistance …

Aminoglycosides: mode of action (the classical view)…

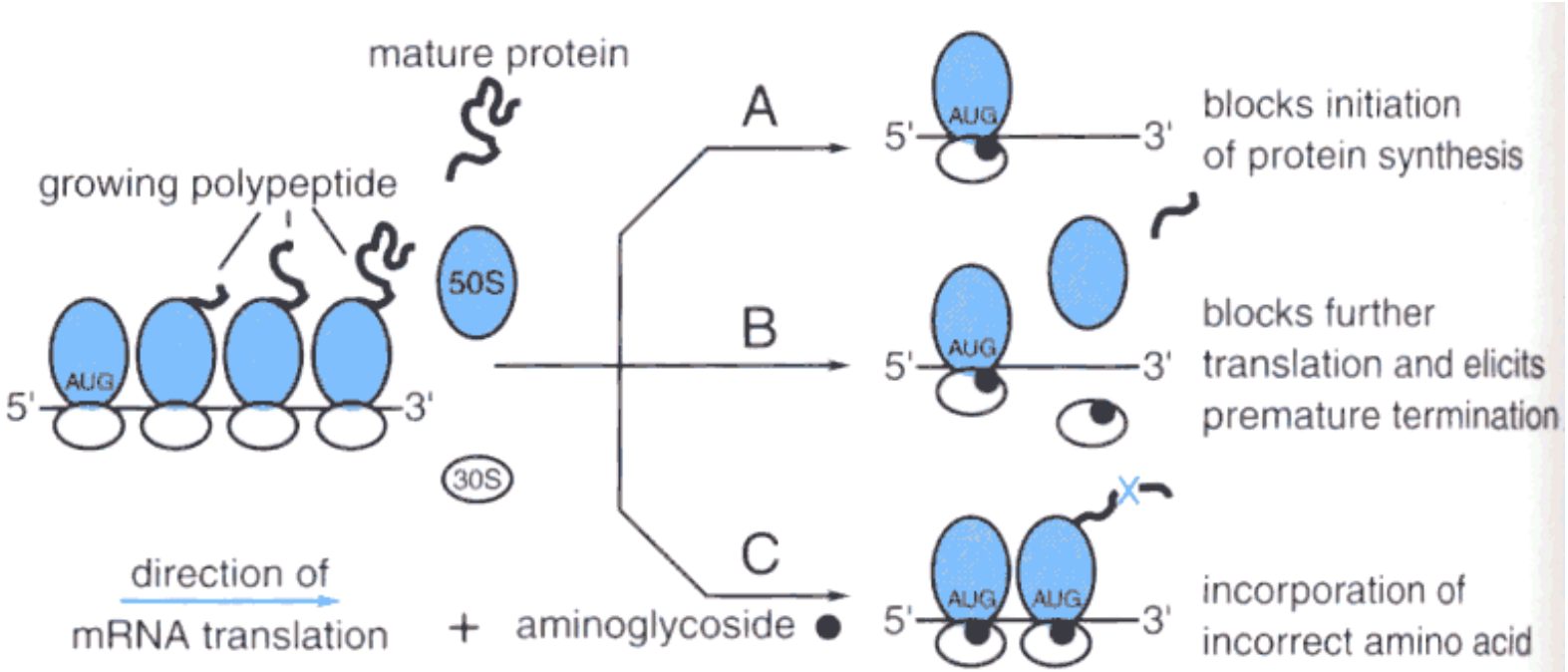


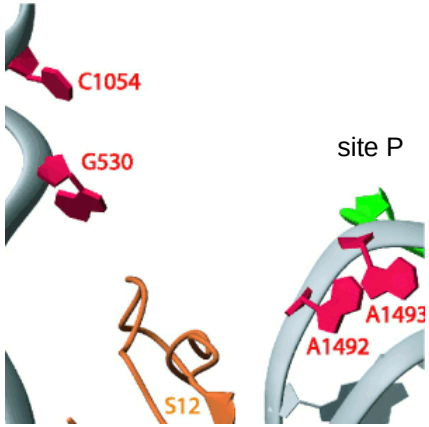
Figure 46–2. Effects of aminoglycosides on protein synthesis.

A. Aminoglycoside (represented by closed circles) binds to the 30 S ribosomal subunit and interferes with initiation of protein synthesis by fixing the 30 S–50 S ribosomal complex at the start codon (AUG) of mRNA. As 30 S–50 S complexes downstream complete translation of mRNA and detach, the abnormal initiation complexes, so-called streptomycin monosomes, accumulate, blocking further translation of message. Aminoglycoside binding to the 30 S subunit also causes misreading of mRNA, leading to ***B.*** premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein, or ***C.*** incorporation of incorrect amino acids (indicated by the "X"), resulting in the production of abnormal or nonfunctional proteins.

Goodman & Gilman's, 10th ed. p 1222

More recent views on the mode of action of aminoglycosides

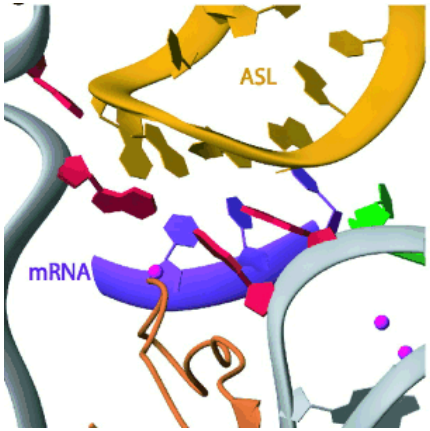
resting stage



energy-dependent deformation (GTP)

protrusion of A1492 and A1493 only if the pairing mRNA – tRNA is correct

tRNA



the code is confirmed !!

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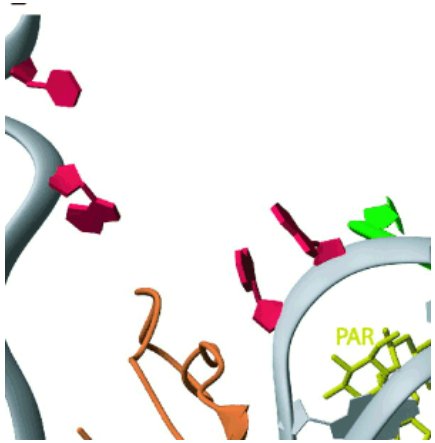
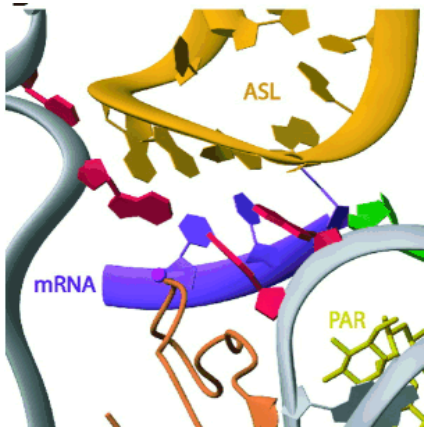
in the presence of an aminoglycoside



the binding of the aminoglycoside causes a protrusion of protrusion of A1492 et A1493 without need for energy

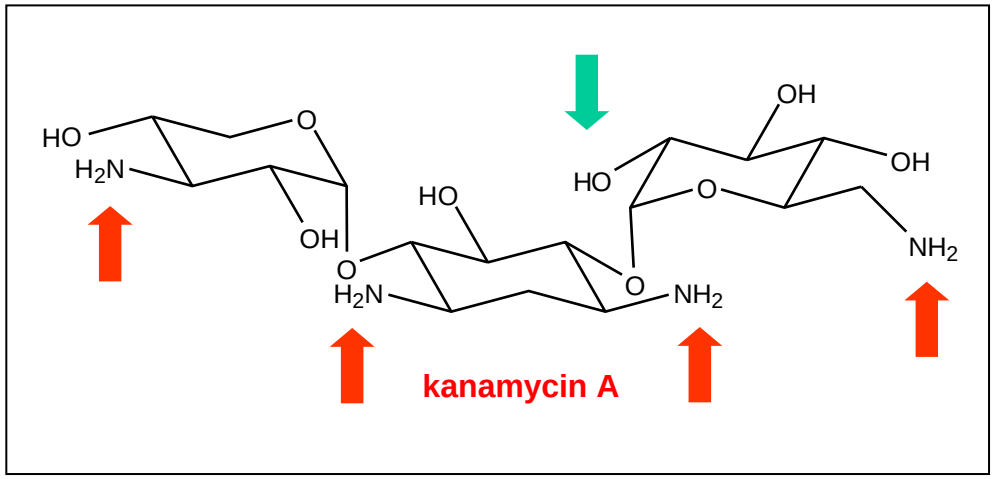
the pairing will always be considered as correct !!



the code is "confirmed" but the actual pairing may be incorrect (wrong tRNA…)

J. M. Ogle et al., Science 292, 897 -902 (2001)

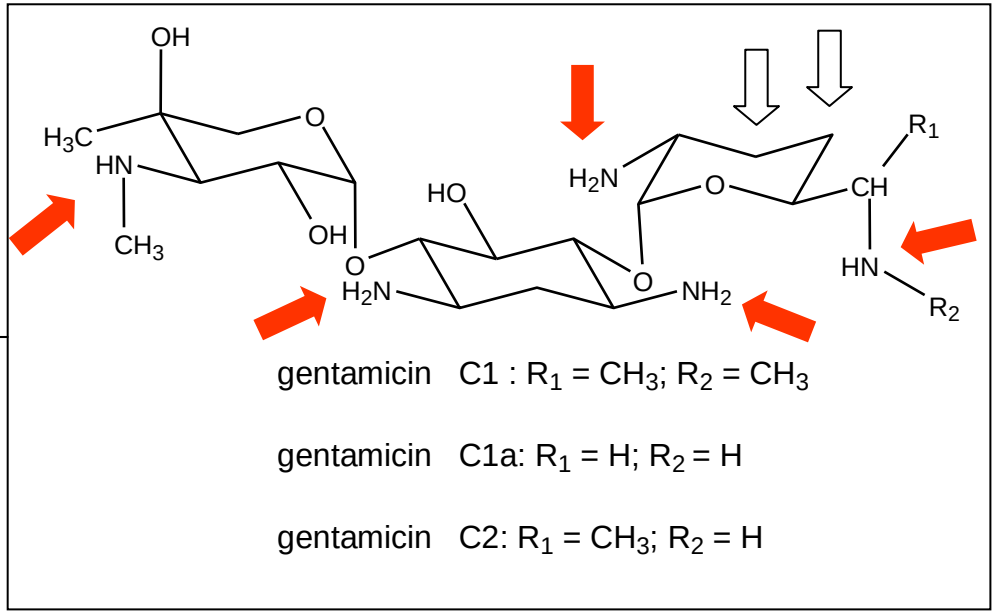
How and why were the main aminoglycosides used in the 90's (and still now) developed ?



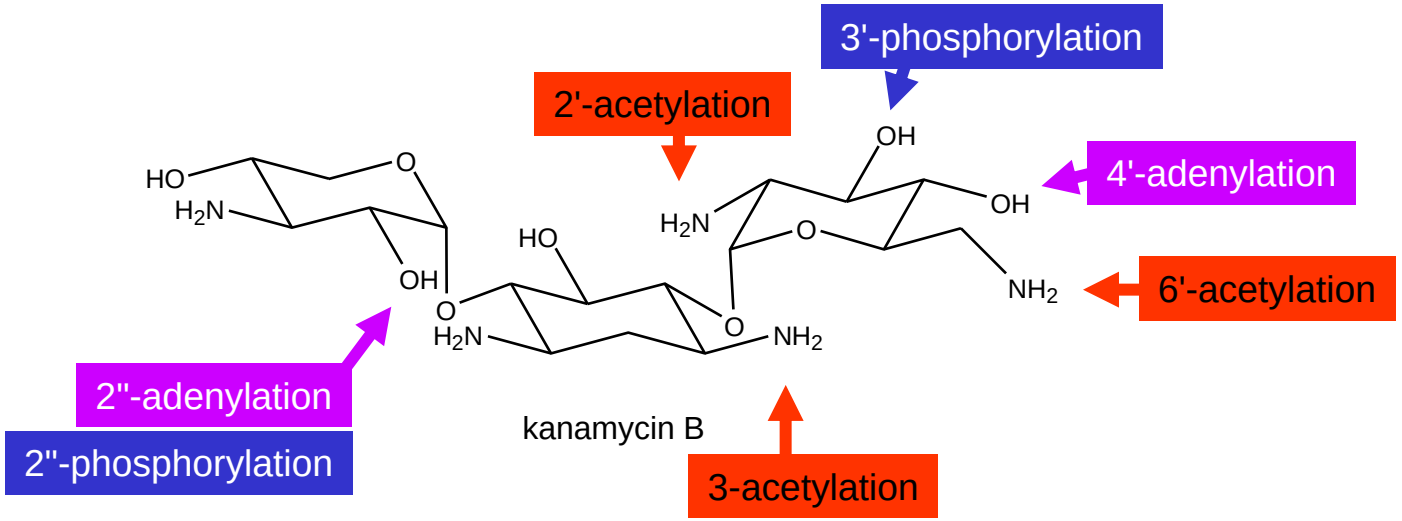
- reasonable activityagainst Gram (-) organisms resistants to SM
- moderate toxicity

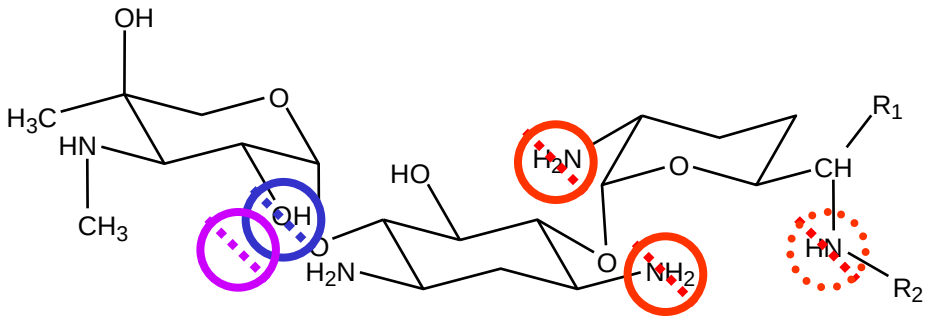
➔large commercial sucess (1960-1980),

largest commercial success since its launch in 1965 !!
The "**gentamicin**" …



Emergence of resistance through enzymatic inactivation (drug modification)

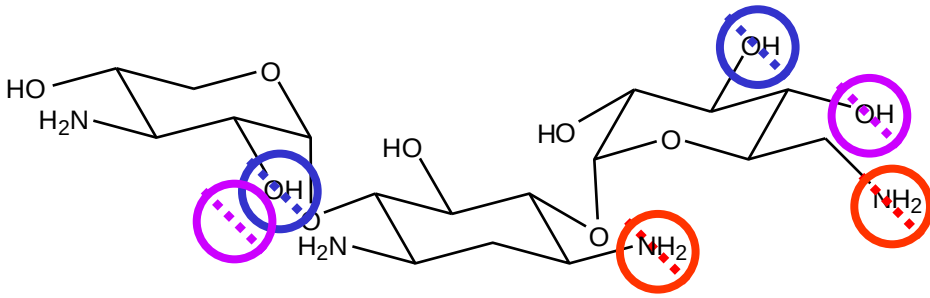




gentamicin C1 : $R_1 = CH_3$; $R_2 = CH_3$

gentamicin C1a: $R_1 = H$; $R_2 = H$

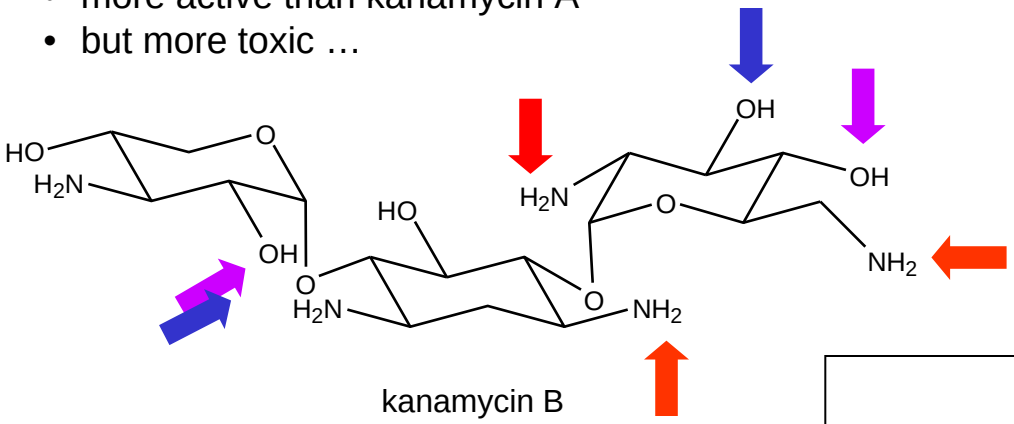
gentamicin C2: $R_1 = CH_3$; $R_2 = H$



kanamycin A

A partial response

- more active than kanamycin A
- but more toxic …

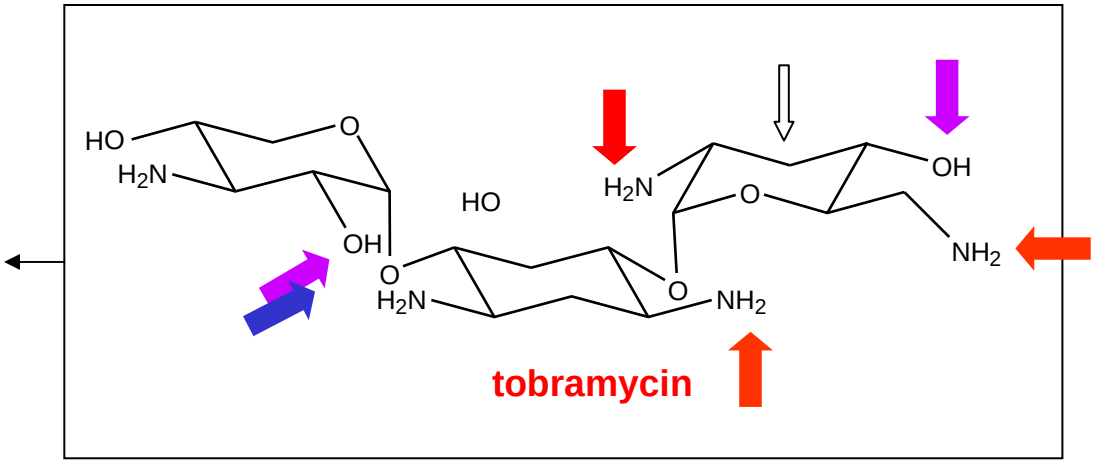


kanamycin B

…

- less toxic
- resistant to 3'phosphotransferases (rares)
- more active against *Pseudomonas*

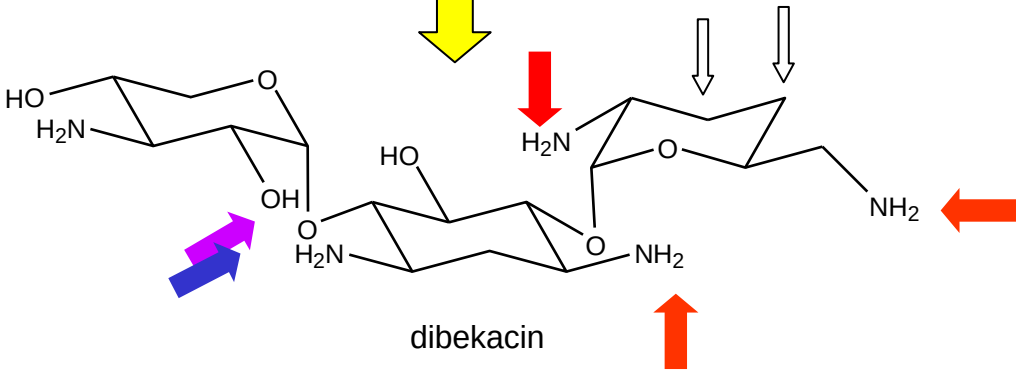
➔ large clinical success (1975-1995)



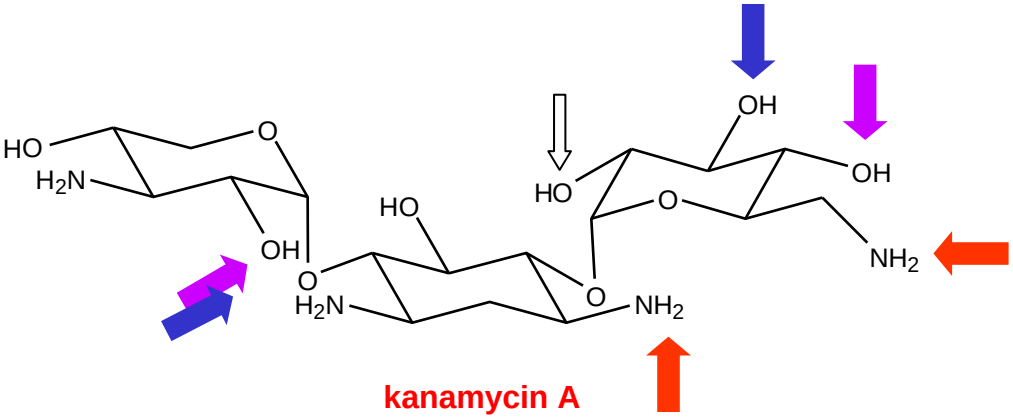
tobramycin

- more toxic
- resistant to 3' phospho- et 4'adenylyl transferases (rares)
- no advantage for *Pseudomonas*
- weak towards *Serratia*

➔ no success outside Japan (1975-1995)

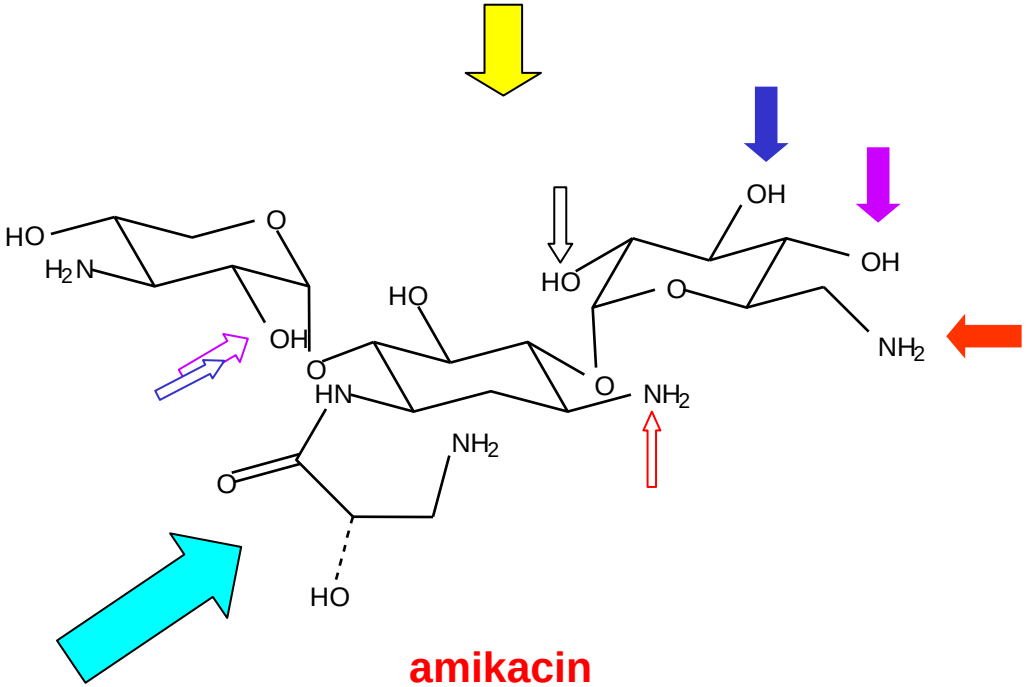


dibekacin



kanamycin A

A more fundamental response ...

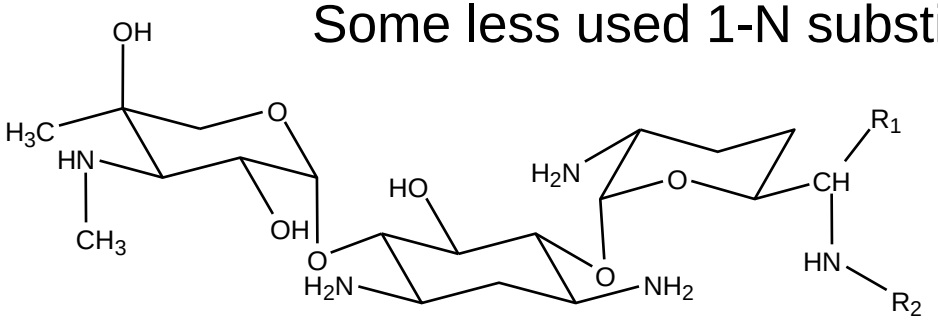


amikacin

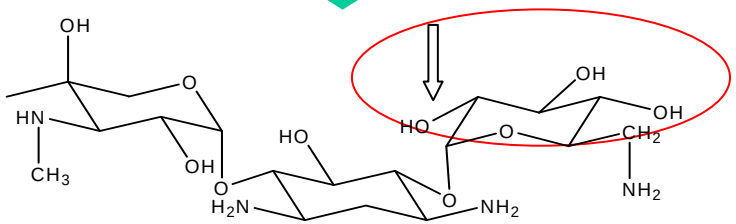
- activity largely maintained
- decreased toxicity (but disputed)
- resistance to enzymes acting on 2" and 3 (frequents) et naturally insensitive to those acting on 2' (frequents)

➔large clinical success from 1985…

Some less used 1-N substituted aminoglycosides ...



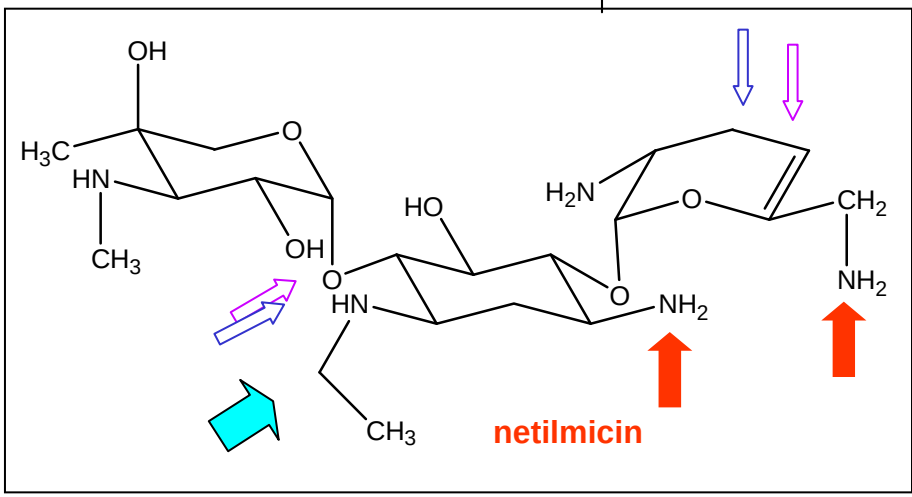
gentamicine C1a: R_1 = H; R_2 = H



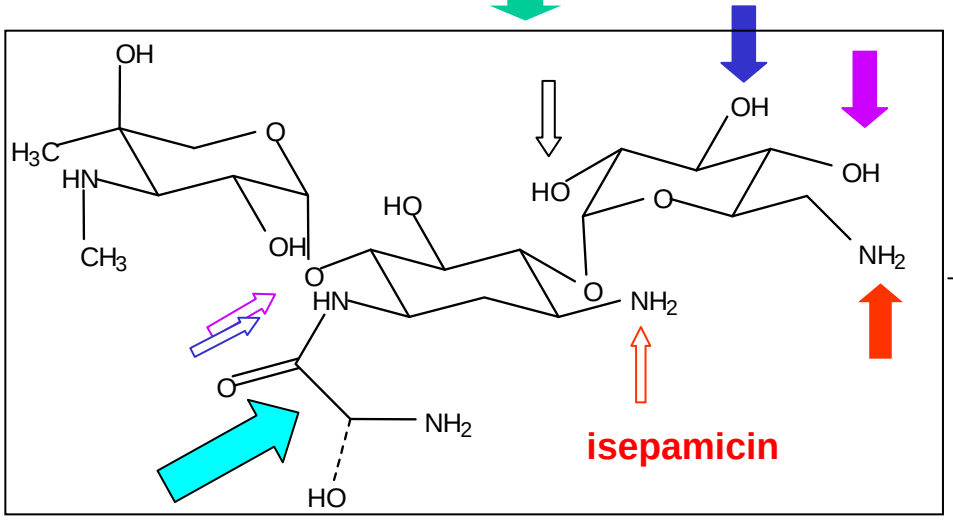
gentamicine B

- reasonable activity
- resistant to some enzymes (less than amikacin)
- toxicity largely controversial

➔variable success (1985…)



netilmicin

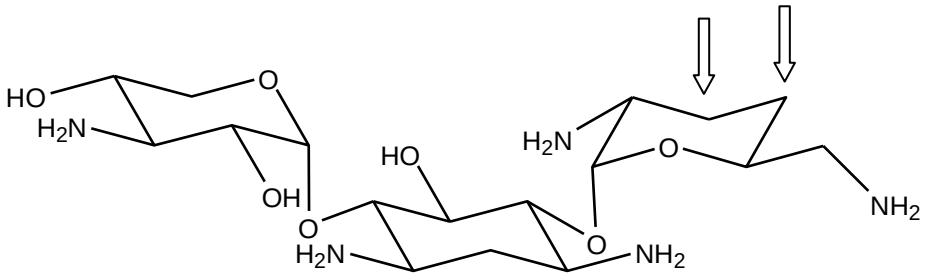


isepamicin

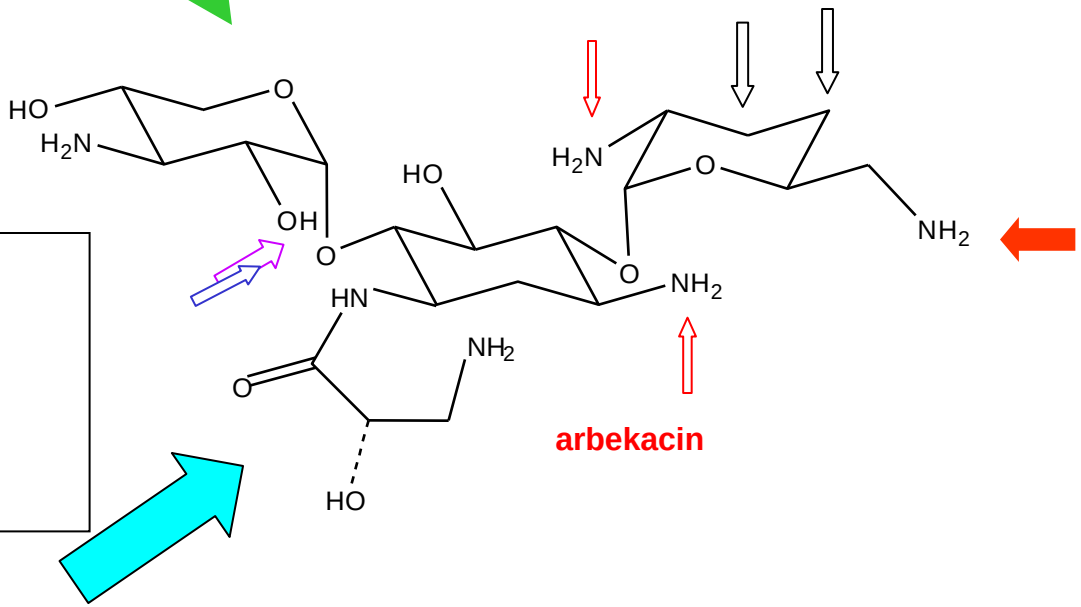
- activity = amikacin
- toxicity ≤ amikacin
- resistant to inactivation ≥ amikacin

➔good clinical success in Japan only

And still another less used 1-N substituted aminoglycoside ...



dibekacin

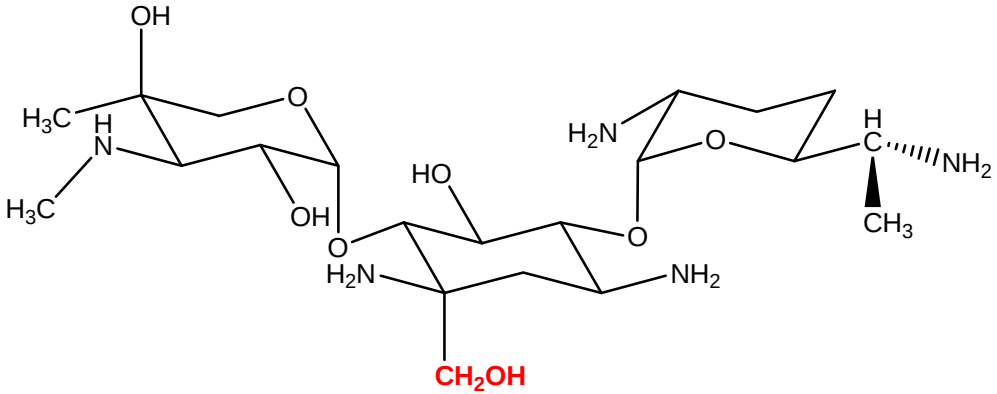


arbekacin

- activity > amikacin
- toxicity ? (controversies)
- resistant to inactivation ≥ amikacin and to the bifunctional enzymes

➔used sparingly in Japan

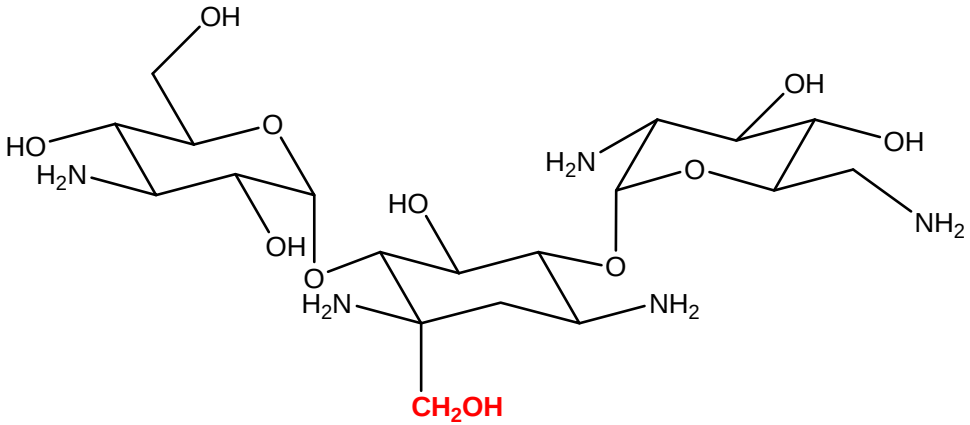
A failed attempt : substitution in C1...



1-C-hydroxymethyl-gentamicin C2 (Sandoz – 1986)

protection against almost all enzymes known at that time

1-C-hydroxymethyl-kanamycin B (1990)



Van Schepdael et al. J. Med. Chem. 1991; 34:1483-1492

No protection in kanamycins !

Enzyme-mediated resistance in the late 90's…

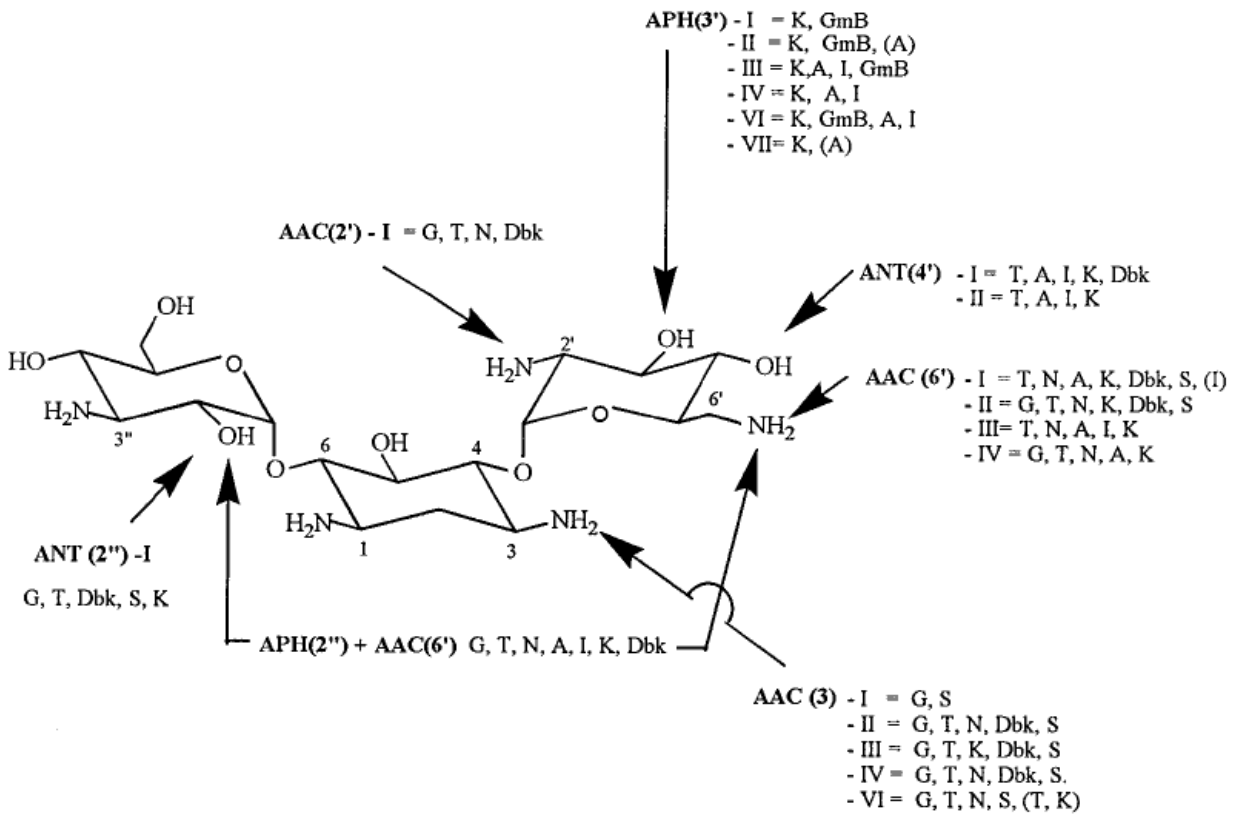


FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (phenotypic classification; each phenotype comprises several distinct gene products [denoted by lowercase letters after the roman numeral in the text]); at least one enzyme is bifunctional and affects both positions 2″ (*O*-phosphorylation) and 6′ (*N*-acetylation)). The main clinically used aminoglycosides on which these enzymes act are as follows: amikacin (A), dibekacin (Dbk), commercial gentamicin (G) (see text), gentamicin B (GmB), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S), and tobramycin (T) (see text for discussion of arbekacin, sagamicin, and dactimicin). The drug abbreviations which appear in parentheses are those for which resistance was detectable in vitro even though clinical resistance was not conferred. Based on the data of Shaw et al. (89).

The situation in the mid-90's

- **gentamicin:** faces the largest rate of resistance but still remain active in a large number of situations
- **tobramycin:** becomes mostly reserved for *P. aeruginosa* infections (because of lower MIC's), although it is said to be less toxic than gentamicin;
- **amikacin:** becomes widely used (active against resistance strains; probably less toxic [although this is hotly debated]; and … good marketing…);
  isepamicin (which is slightly superior to amikacin) remains confined to Japan

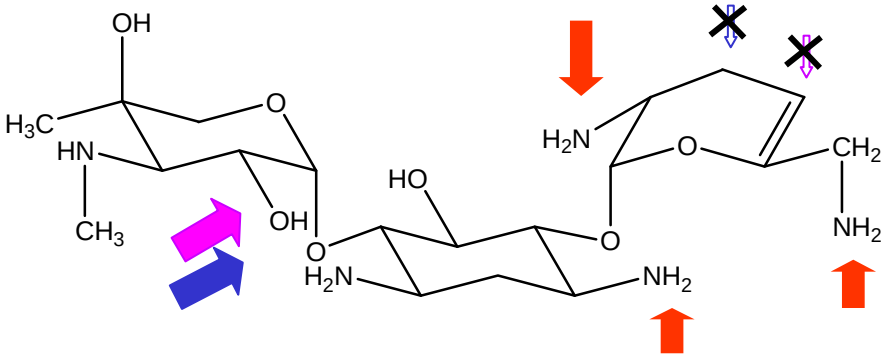
- **arbekacin:** (HABA derivative of dibekacin) acquires a special niche in Japan because of an unanticipated activity againts methicillin-resistant S. aureus (active against the bifunctional enzyme)

- the non "4,6 disubstituted 2-deoxystreptamine" aminoglycosides ("non classical") are almost not used in human medicine but have niches in veterinary medicine and/or are used for resistance diagnostic and research purposes

2005: the renewal of aminoglycosides ?

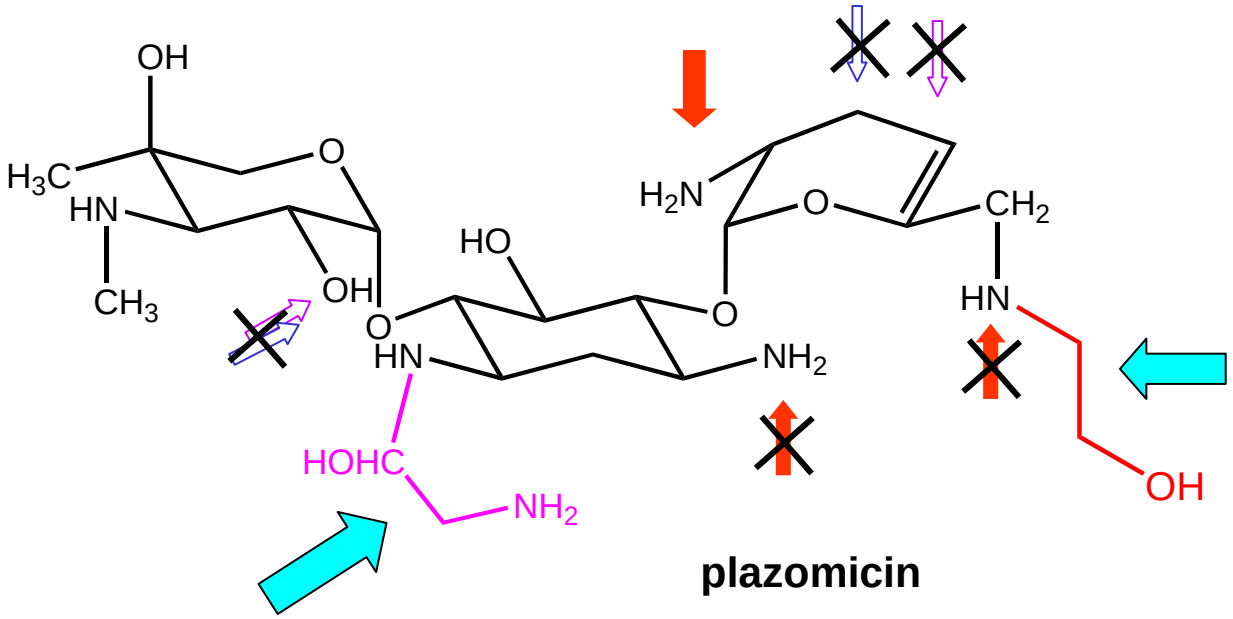
- Achaogen (a small drug discovery company) launches a program aimed at discovering new aminoglycosides, capitalizing on the knownledge of
  - resistance mechanisms
  - toxicity targets and practical means of alleviating them (see later)
- The program is initially supported by the Biomedical Advanced Research and Development Authority (BARDA) for $64.5 million as a potential medical countermeasure against the biothreat pathogens, Yersinia pestis and *Francisella tularensis*.
  - use of an injectable antibiotic to ensure adequate protection
  - obtain a compound active against strains that could be engineered to carry known aminoglycoside resistance mechanisms
- Clinical developments (phase II) were later on partially supported by the Wellcome Trust

Plazomicin (ACHN-490): made from sisomicin



sisomicin

still susceptible
to AAC(2')
(but rare enzyme)



plazomicin

Plazomicin (ACHN-490) : milestones

- first description in 2006
- phase I uneventful (2008)
- phase II completed in 2012

South San Francisco, CA, May 15, 2012 -- Achaogen, Inc. announced today that all objectives were met in the company's multi-national Phase 2 study of plazomicin compared to levofloxacin for the treatment of complicated urinary tract infections (cUTI) and acute pyelonephritis in adults.

The Phase 2 study met its objectives of assessing safety and efficacy of plazomicin in comparison to levofloxacin. Plazomicin was well-tolerated and demonstrated favorable microbiological and clinical outcomes at the Test-of-Cure Visit, 5 to 9 days after the end of therapy, which were the primary and secondary outcome measures in this study, respectively.

Presented at ICAAC 2012 Wednesday, Sep 12, 2012, 9:15 AM -11:15 AM Presentation Title: L2-2118a - Plazomicin Safety and Efficacy in Patients with Complicated Urinary Tract Infection (cUTI) or Acute Pyelonephritis (AP)

Resistance from the mid-90's to now …

- enzyme-mediated resistance remains the main mechanism and gets highly complex by the simultaneous presence of distinct enzymes, and the occurrence of bifunctional ones…

- **efflux** has been described in *P. aeruginosa* and explains the relatively low activities observed against this species
  - **Plazomicin is ineffective against *P. aeruginosa* (because of efflux)**

- two new mechanisms of **ribosomal methylation** (armA and armB) has been described that causes resistance to all 2,4 disubstituted deoxystreptamine-containing aminoglycosides and to fortimicin (but not to paromomycin and similar derivatives). It is plasmid-mediated and seems to spread
  - **Plazomicin is also ineffective against arm+ strains**

Efflux…

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2000, p. 2242–2246 Vol. 44, No. 9
0066-4804/00/$04.00+0

Contribution of the MexX-MexY-OprM Efflux System to Intrinsic Resistance in *Pseudomonas aeruginosa*

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Received 18 October 1999/Returned for modification 20 February 2000/Accepted 26 May 2000

To test the possibility that MexX-MexY, a new set of efflux system components, is associated with OprM and contributes to intrinsic resistance in *Pseudomonas aeruginosa*, we constructed a series of isogenic mutants lacking *mexXY* and/or *mexAB* and/or *oprM* from a laboratory strain PAO1, and examined their susceptibilities to ofloxacin, tetracycline, erythromycin, gentamicin, and streptomycin. Loss of either MexXY or OprM from the MexAB-deficient mutant increased susceptibility to all agents tested, whereas loss of MexXY from the MexAB-OprM-deficient mutant caused no change in susceptibility. Introduction of an OprM expression plasmid decreased the susceptibility of the *mexAB-oprM*-deficient-/*mexXY*-maintaining mutant, yet caused no change in the susceptibility of a *mexAB-oprM*- and *mexXY*-deficient double mutant. Immunoblot analysis using anti-MexX polyclonal rabbit serum generated against synthetic oligopeptides detected expression of MexX in the PAO1 cells grown in medium containing tetracycline, erythromycin, or gentamicin, although expression of MexX was undetectable in the cells incubated in medium without any agent. These results suggest that MexXY induced by these agents is functionally associated with spontaneously expressed OprM and contributes to the intrinsic resistance to these agents.

Efflux…

Observed in

- *S. aureus* (MdeA [MFS])
- *E. coli* (MdfA, SetA [MFS]; AcrD [RND])
- *S. maltophilia* (SmeE [RND])
- *P. aeruginosa* (MexXY [RND];constitutively expressed but may be overproduced in resistant strains)

Responsible for

- low intrinsic susceptibility ... (intrinsic resistance)
- adaptative resistance (post-exposure effects)
- cross resistance to most 4,6 disubstituted-2-deoxystreptamine containing aminoglycosides (previously considered as permability mutants)

see review in Van Bambeke et al., J. Antimicrob. Chemother. 51:1055-65, 2003

armA resistance ...

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2003, p. 2565–2571 Vol. 47, No. 8
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.8.2565–2571.2003

Plasmid-Mediated High-Level Resistance to Aminoglycosides in *Enterobacteriaceae* Due to 16S rRNA Methylation

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Received 5 February 2003/Returned for modification 20 April 2003/Accepted 17 May 2003

A self-transferable plasmid of ca. 80 kb, pIP1204, conferred multiple-antibiotic resistance to *Klebsiella pneumoniae* BM4536, which was isolated from a urinary tract infection. Resistance to β-lactams was due to the bla_{TEM1} and bla_{CTX-M} genes, resistance to trimethroprim was due to the *dhfrXII* gene, resistance to sulfonamides was due to the *sul1* gene, resistance to streptomycin-spectinomycin was due to the *ant3″9* gene, and resistance to nearly all remaining aminoglycosides was due to the *aac3-II* gene and a new gene designated *armA* (aminoglycoside resistance methylase). The cloning of *armA* into a plasmid in *Escherichia coli* conferred to the new host high-level resistance to 4,6-disubstituted deoxystreptamines and fortimicin. The deduced sequence of ArmA displayed from 37 to 47% similarity to those of 16S rRNA m^7G methyltransferases from various actinomycetes, which confer resistance to aminoglycoside-producing strains. However, the low guanine-plus-cytosine content of *armA* (30%) does not favor an actinomycete origin for the gene. It therefore appears that posttranscriptional modification of 16S rRNA can confer high-level broad-range resistance to aminoglycosides in gram-negative human pathogens.

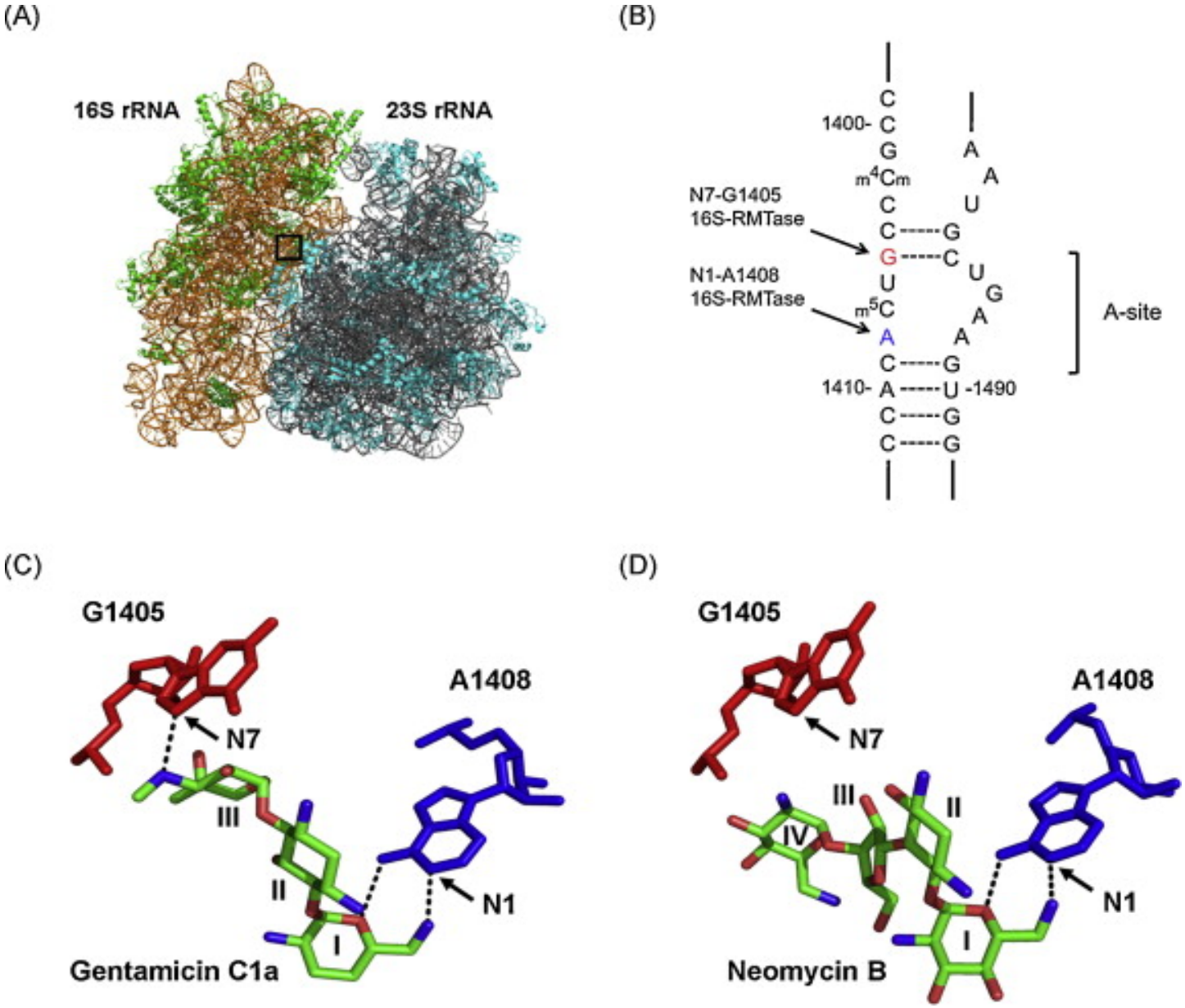
armA resistance ... and other methylases ...

- *armA* originally in Klebsiella pneumoniae together with the bla(TEM1) and bla(CTX-M) genes
  - act by methylation of the 16S RNA (target modification)
  - affects all aminoglycosides except streptomycin
  - difficult to detect specifically in clinical microbiology laboratoriesunless including a fortimicin susceptibilty test (non classical aminoglycsoside)
  - may be more widespread than originally tought and could spread fast because it is carried on a conjugative plasmid flanked by putative transposable elements
- but several other plasmid-mediated 16S rRNA methylases identified in pathogenic *Enterobacteriaceae* (RmtC, RmtB, and RmtA)....

➔ The acceleration of aminoglycoside resistance among Gram (-) bacilli by plasmid-mediated 16S rRNA methylases may become an actual clinical hazard in the near future ...

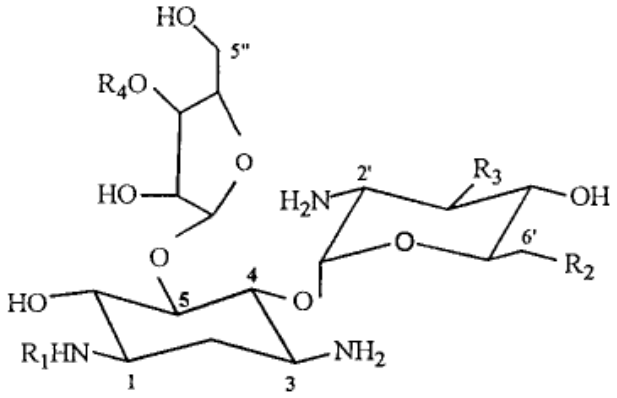
Gallimand et al., Antimicrob. Agents Chemother.2003; 47:2565-2571
Wachino et al., Antimicrob Agents Chemother. 2006; 50:178-84.

The tow main types of methylases



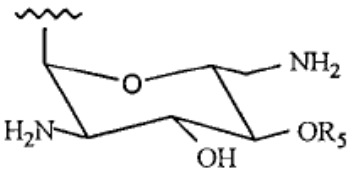
Some non-classical aminoglycosides …

4,5-DISUBSTITUTED DEOXYSTREPTAMINE

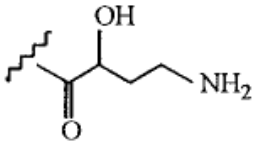


Aminoglycoside	R_1	R_2	R_3	R_4	R_5
Neomycin B	H	NH_2	OH	X	H
Paromomycin I	H	OH	OH	X	H
Lividomycin A	H	OH	H	X	Mannose
Ribostamycin	H	NH_2	OH	H	
Butirosin B	Y	NH_2	OH	H	

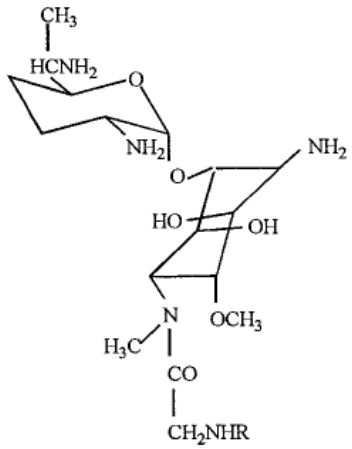
X =



Y =

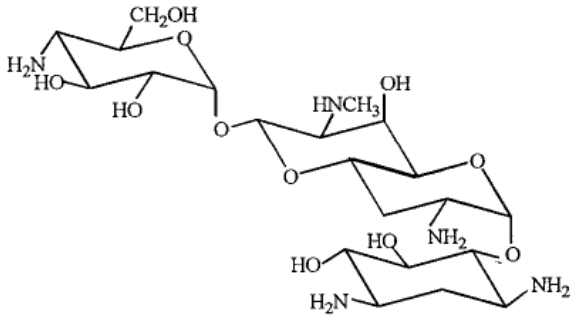


B



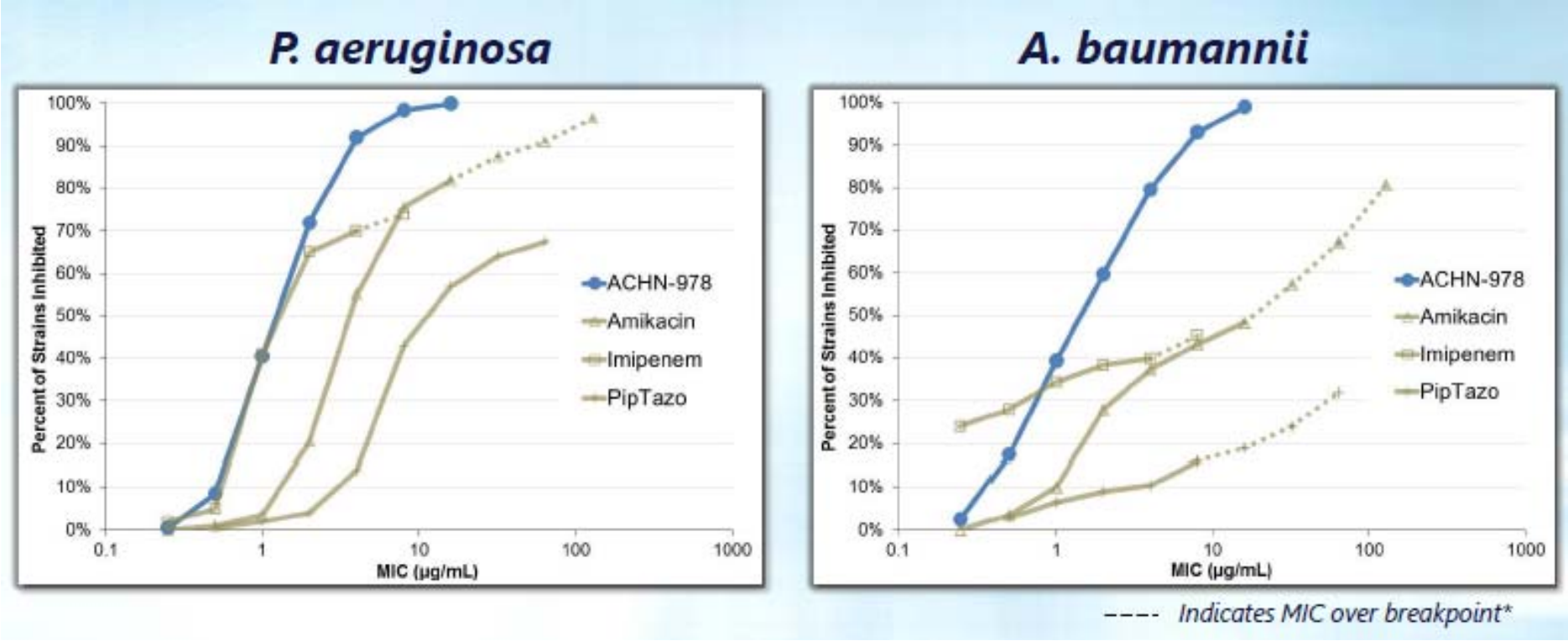
R =	
Fortimicin A	**H**
Dactimicin	**NH-CH=NH**

C



Apramycin

From paromomycin to ACHN-978 …

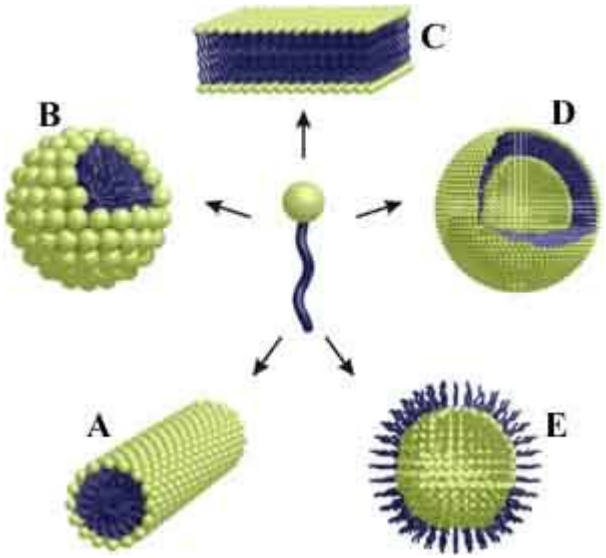


ACHN-978 is active against

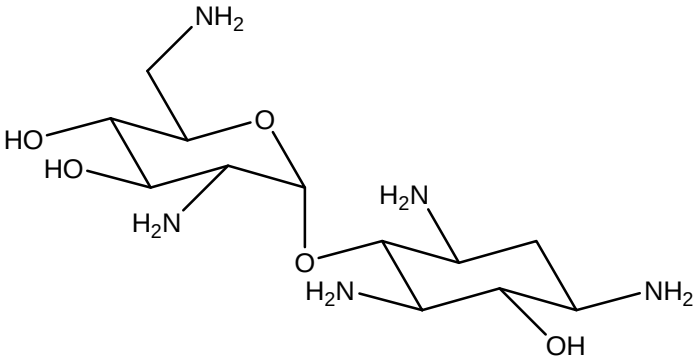
- *Pseudomonas* (wild-type and amikacin-resistant)
- efflux + strains
- arm + strains

but its structure has not been made public so far…

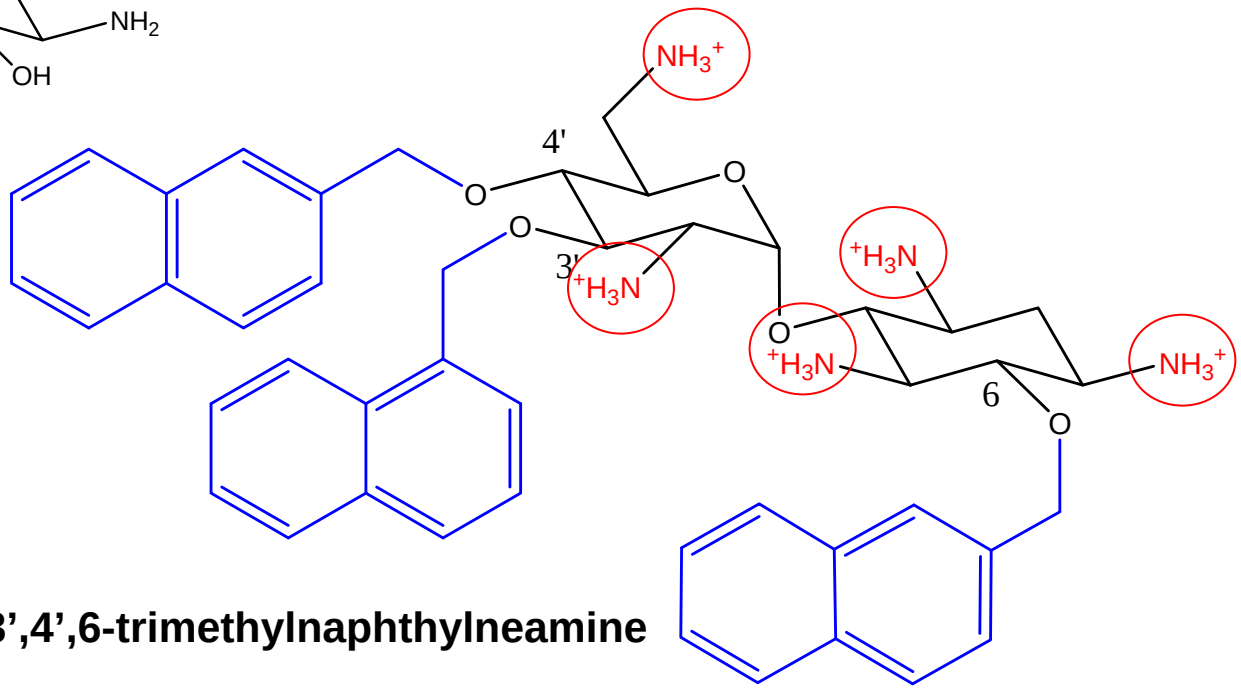
Amphiphilic aminoglycosides ?



Amphiphilic aminoglycosides: derivatives from neamine



neamine



3’,4’,6-trimethylnaphthylneamine

Baussane et al. J. Med. Chem. 2010; 53:119–127

Amphiphilic aminoglycosides

(methylnaphthyl neamine derivatives; Décout and co-workers)

Table 1. Minimum Inhibitory Concentrations against Different *Staphylococcus aureus* Strains for the Neamine Derivatives, Neomycin B, and Neamine

	MIC μg/mL							
aminoglycosides	ATCC 25923	pump NorA	pump MsrA	enzyme APH2″-AAC6′	enzyme APH3′	enzyme ANT4′	ATCC 33592 HA-MRSA	VRSA-VRS-2
neomycin B	2	1	2	1	> 128	32	> 128	128
neamine **1**	32	32	16	16	> 128	> 128	> 128	> 128
3′-mono2NM **2**	> 128	> 128	> 128	> 128	128	> 128	ND	ND
4′-mono2NM **3**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
5-mono2NM **4**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
6-mono2NM **5**	> 128	> 128	> 128	> 128	> 128	> 128	> 128	ND
3′,4′-di2NM **6**	4	8	8	8	4	8	8	4
3′,6-di2NM **7a**	8	8	8	8	4	8	16	16
4′,5-di2NM **8**	64	128	128	128	32	128	64	64
4′,6-di2NM **9**	32	32	32	32	16	16	64	32
3′,4′,6-tri2NM **10a**	4	4	4	4	2	4	2	4
3′,4′,5,6-tetra2NM **11**	32	64	64	64	32	64	32	64
3′,6-diBn **7b**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
3′,6-di2PM **7c**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
3′,6-di2QM **7d**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
3′,4′,6-triBn **10b**	> 128	> 128	> 128	> 128	> 128	> 128	> 128	64
3′,4′,6-triPM **10c**	> 128	> 128	> 128	> 128	> 128	> 128	ND	ND
3′,4′,6-tri2QM **10d**	128	> 128	> 128	128	64	> 128	64	64

Baussane et al. J. Med. Chem. 2010; 53:119–127

Amphiphilic aminoglycosides

(methylnaphthylderivatives; Décout, Mingeot-Leclercq and co-workers)

- no significant inhibition of bacterial protein synthesis at 10 x the MIC
- decreased cell thickness decreased by 50% (Atomic Force microscopy) suggestive of intra-bacterial content leakage
- depolarization of bacterial membrane ($DiSC_3(5)$ probe)
- binding to LPS (displacement of BODIPY-TRcadaverine)
- permabilization of liposomes mimicking *P. aeruginosa* membranes (POPE:POPG:CL; 60:21:11) (calcein release)

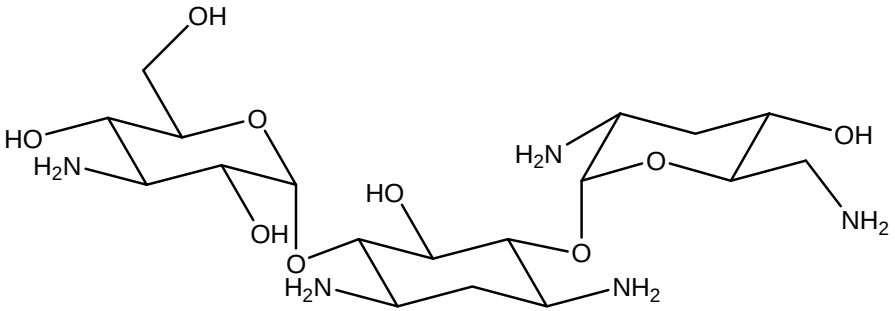
BUT …

- cytotoxicity to eucaryotic cells at 2 to 10 x the MIC !

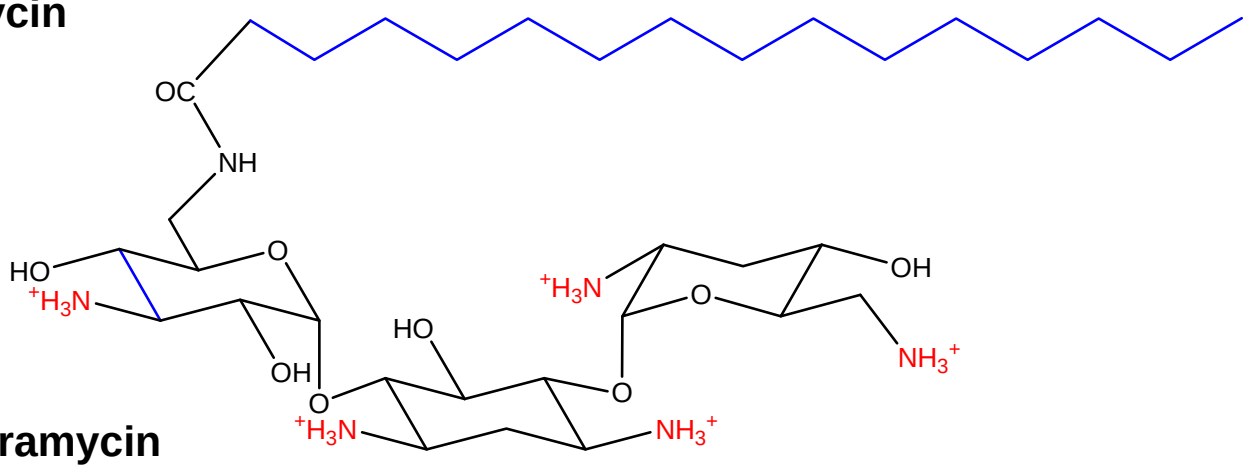
Ouberai et al. Biochimica et Biophysica Acta 1808 (2011) 1716–1727 and unpublished data

Amphiphilic aminoglycosides

(lipid derivatives of tobramycin – Bera and co-workers)



tobramycin



6'NHCO-$C_{15}H_{31}$-tobramycin

Dhondikubeer et al. Journal of Antibiotics (Tokyo) (2012) – E-pub - in press

Amphiphilic aminoglycosides

(lipid derivatives of tobramycin – Bera and co-workers)

- MICs are between 4 (*Staphylococci*, *Enterococci*…) and 256 (*Acinetobacter*)
  → preferential anti-Gram + spectrum
- amphiphilicity is critical for antibacterial activity
- the pentacationic tobramycin-based headgroup appears to be optimal (vs. kanamycin, e.g.)
- MICs are increased (4 to 8 x) by addition of 4% albumin (binding)

BUT

- concentration-dependent hemolytic activity (37% at 100 mg/L)
  [can be reduced by replacement of the lipid tail by a fluorinated lipid tail ($C_2H_4C_8F_{17}$) but is still 27 % at 500 mg/L]

Dhondikubeer et al. Journal of Antibiotics (Tokyo) (2012) – E-pub - in press

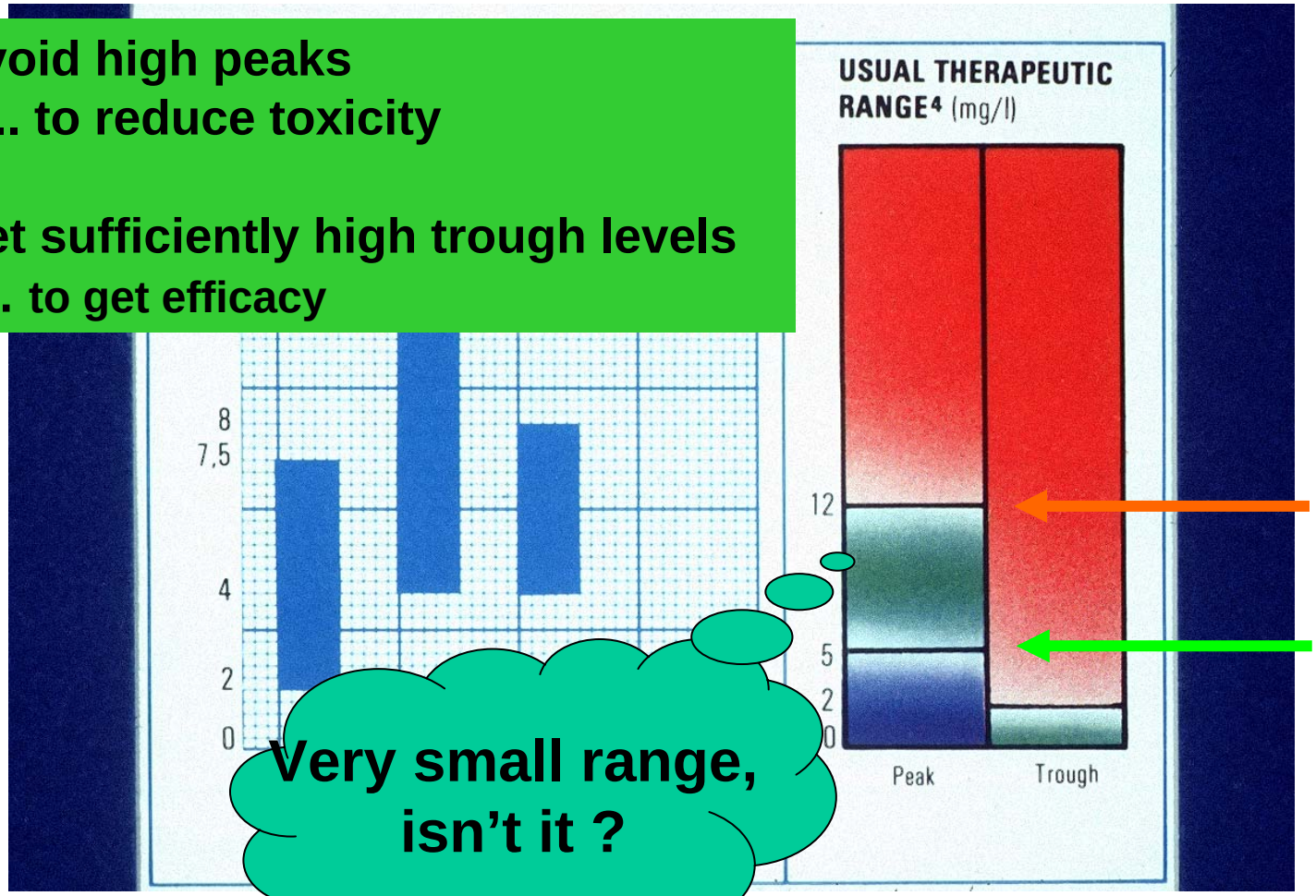
Part 3: Toxicity …

This is where disputes come into the picture…

Aminoglycosides monitoring in the 80's ...

avoid high peaks
... to reduce toxicity

get sufficiently high trough levels
… to get efficacy



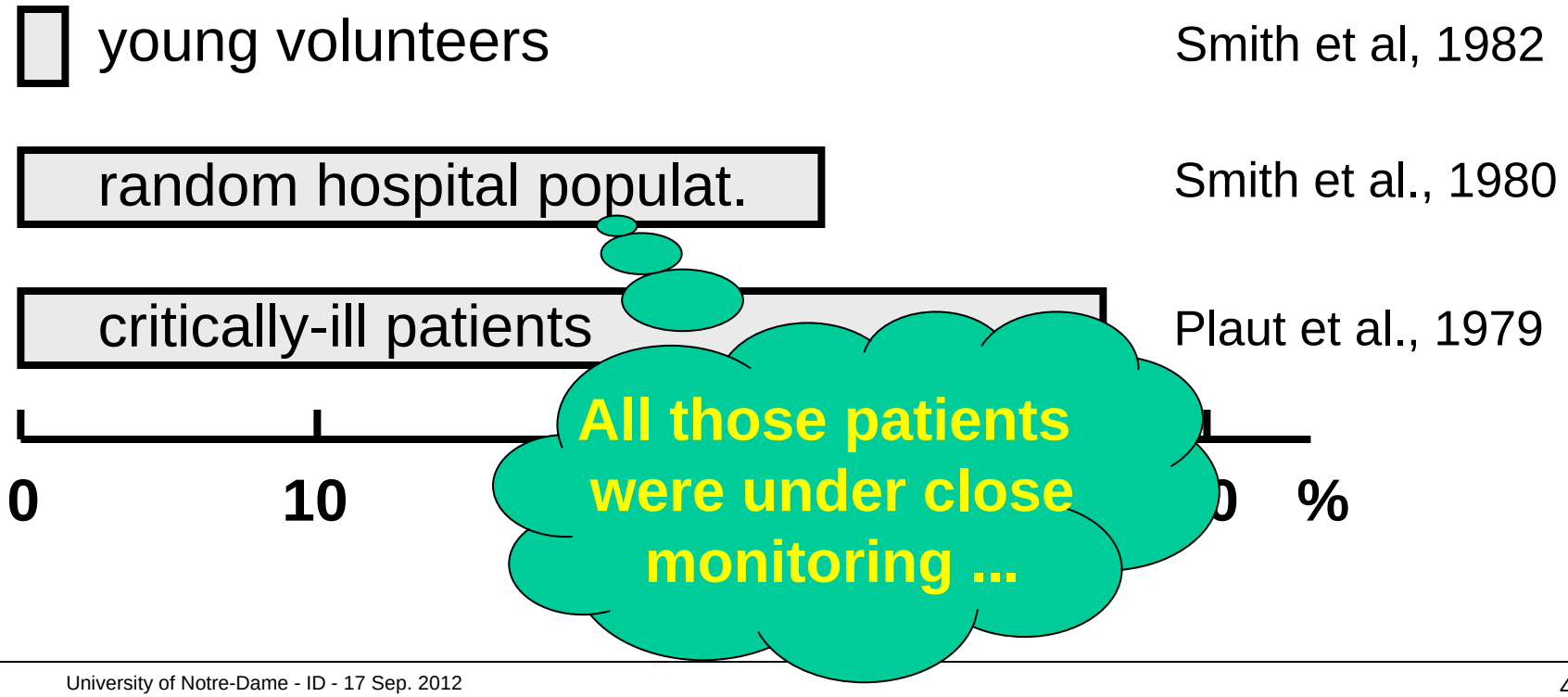
toxicity !!

lack of efficacy

Abott TdX manual, 1986

Aminoglycosides toxicity incidence is highly variable among patient populations

Patients with nephrotoxic reaction after treatment with gentamicin



Why do we see such a variation ?

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 cause precipitous renal nevrosis, tubular dysfunction, and renal failure associated with regeneration

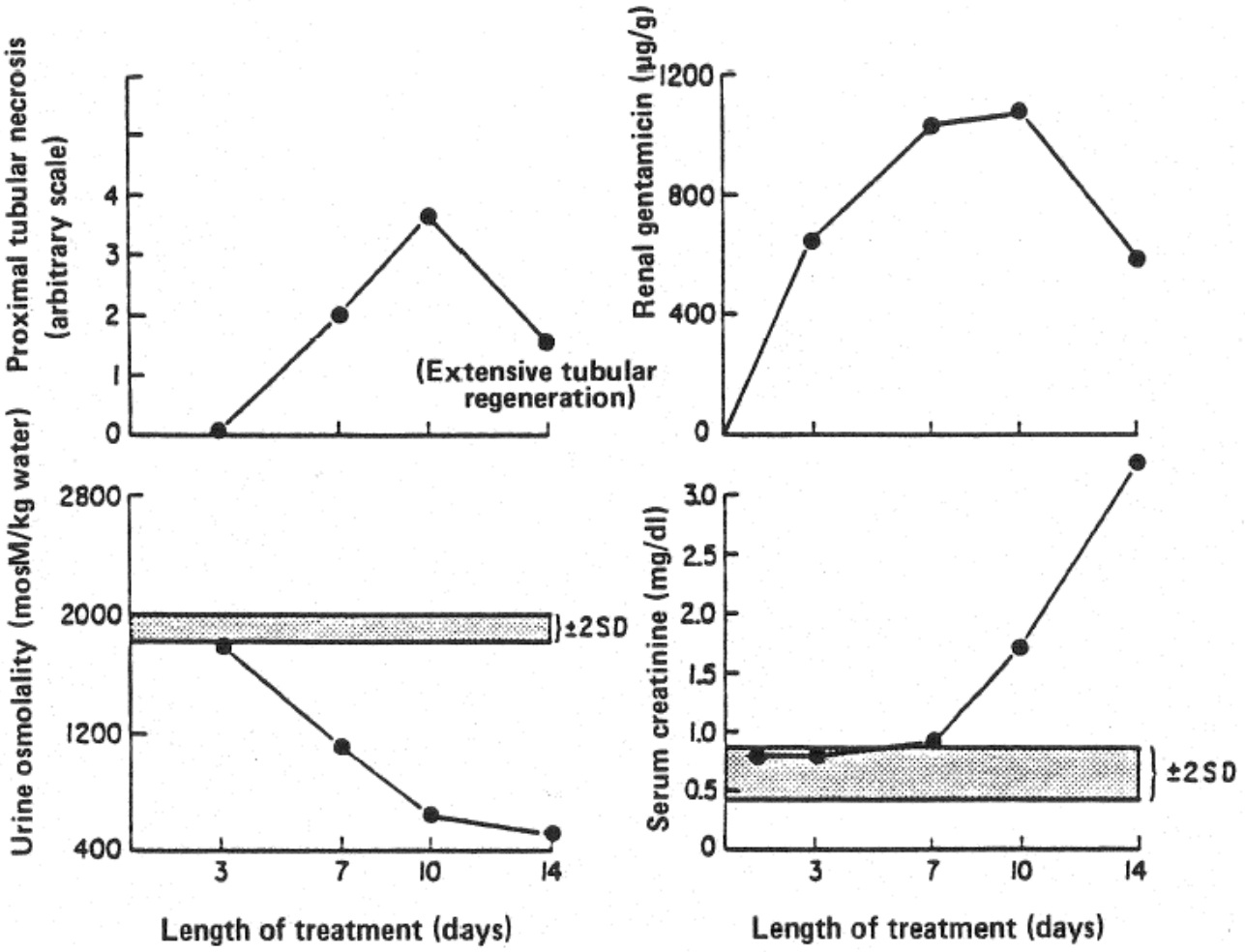


Fig. 1. Renal changes in Fischer 344 rats after gentamicin (40 mg/kg per day in two injections per day). From Ref. 13.

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.

But low doses allow 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

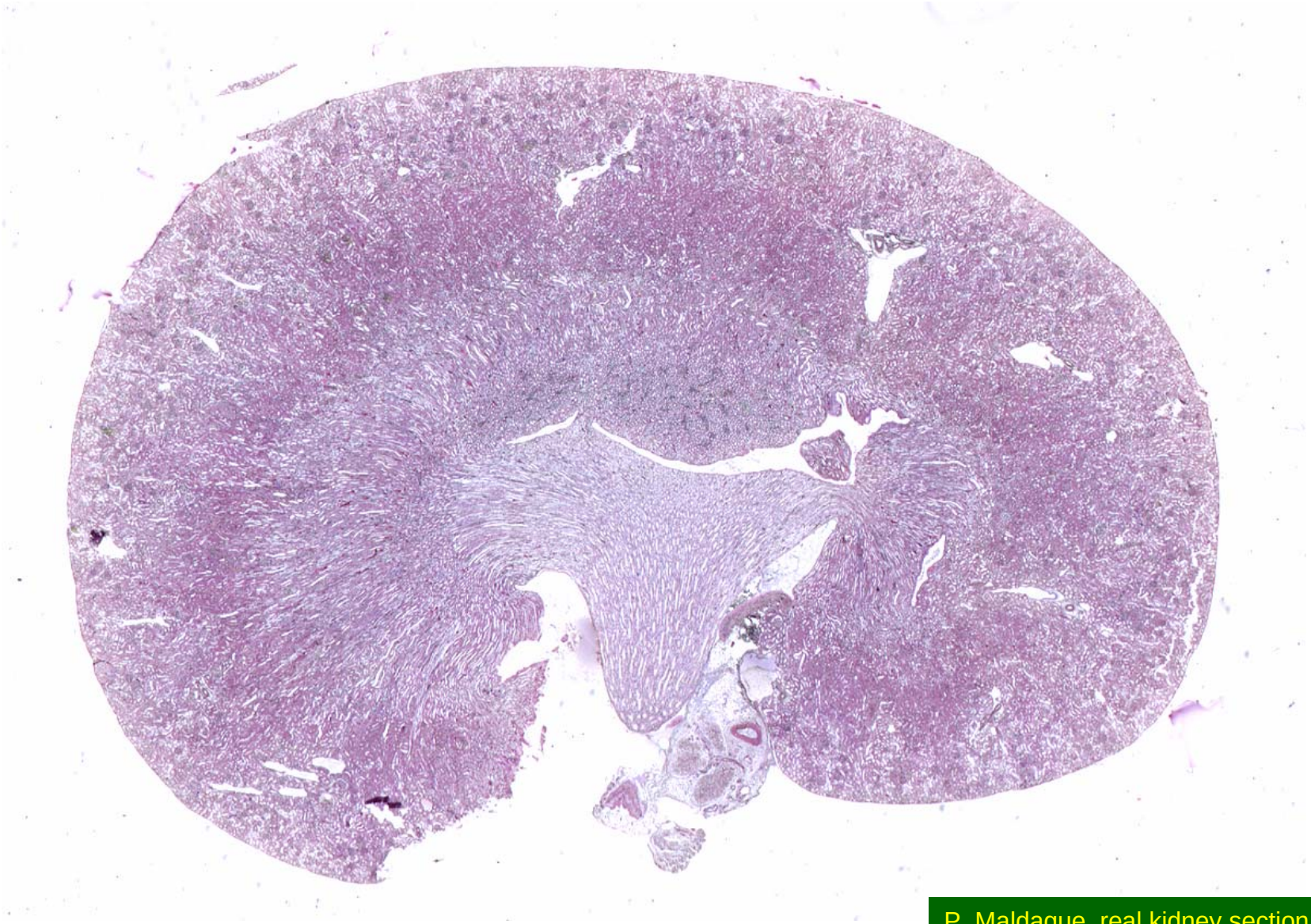
Regenerative lesions
- Tubular cell proliferation and dedifferentiation
- Tubular dilatation
- Interstitial proliferation (fibroblastic cells) and focal infiltration by inflammatory cells

* 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

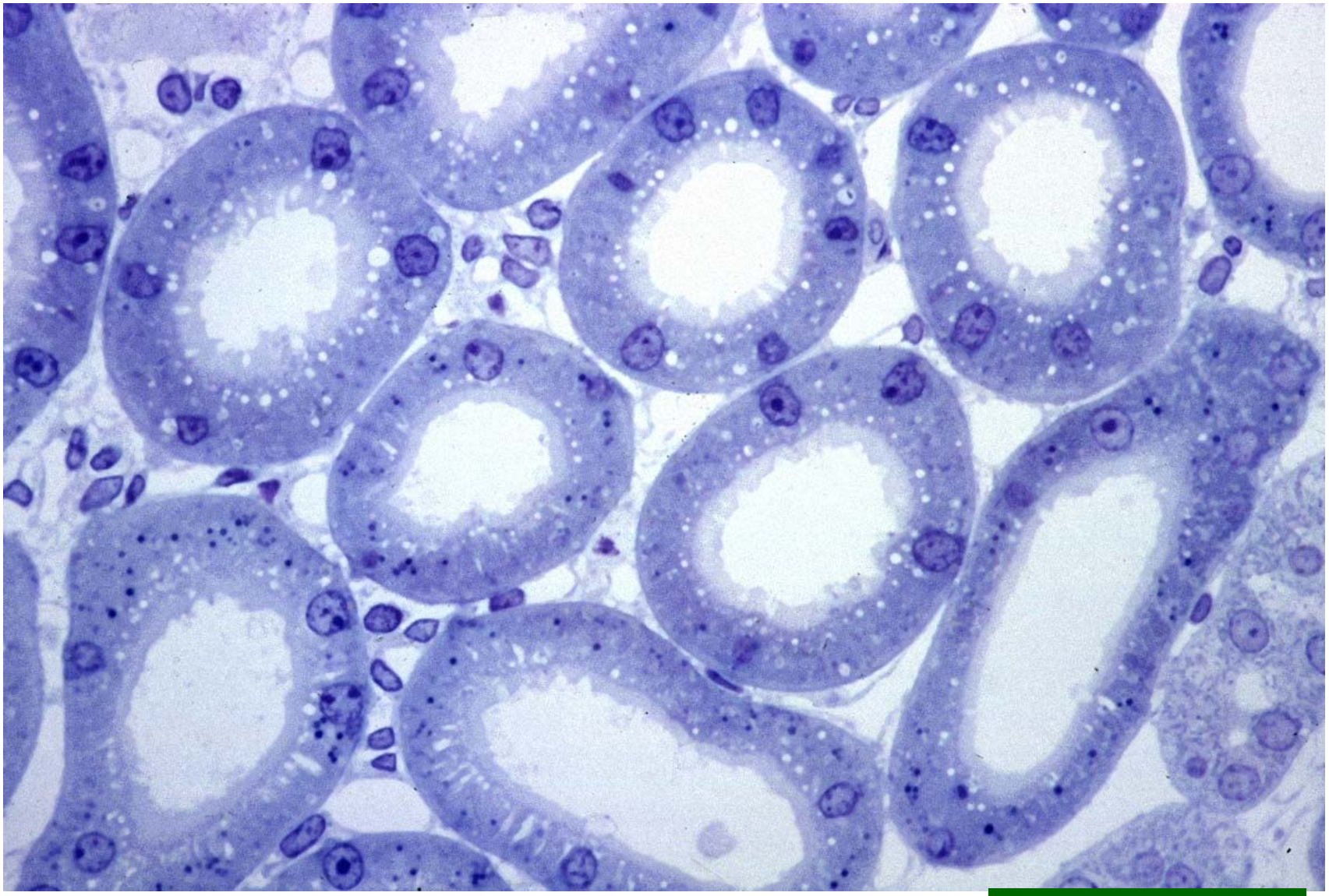
The American Journal of Medicine
Volume 80 (suppl 6B) June 30, 1986 105

A look in the microscope in a rat treated with low doses … (10mg/kg)



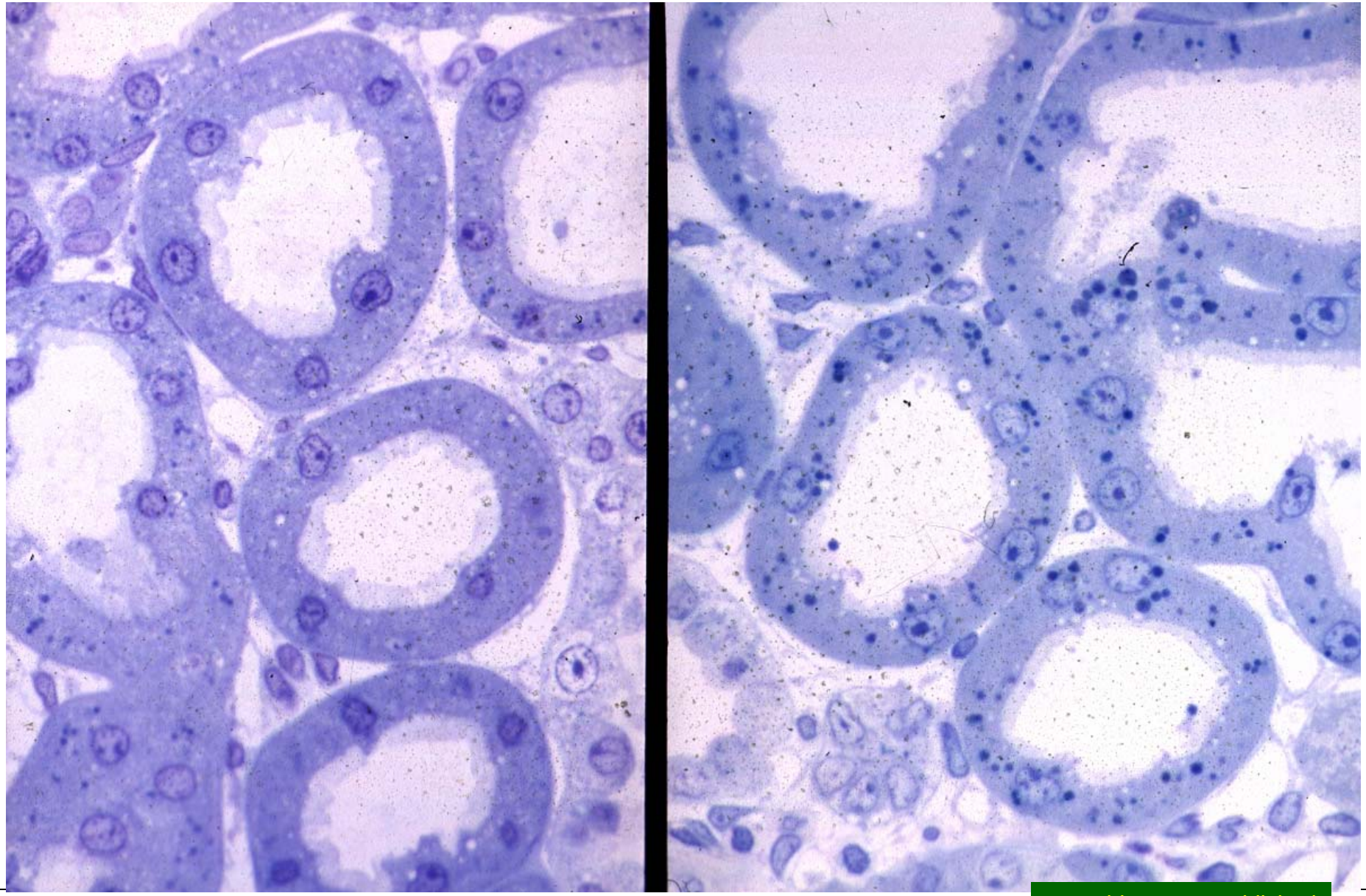
P. Maldague, real kidney section…

Somewhat closer in the control …



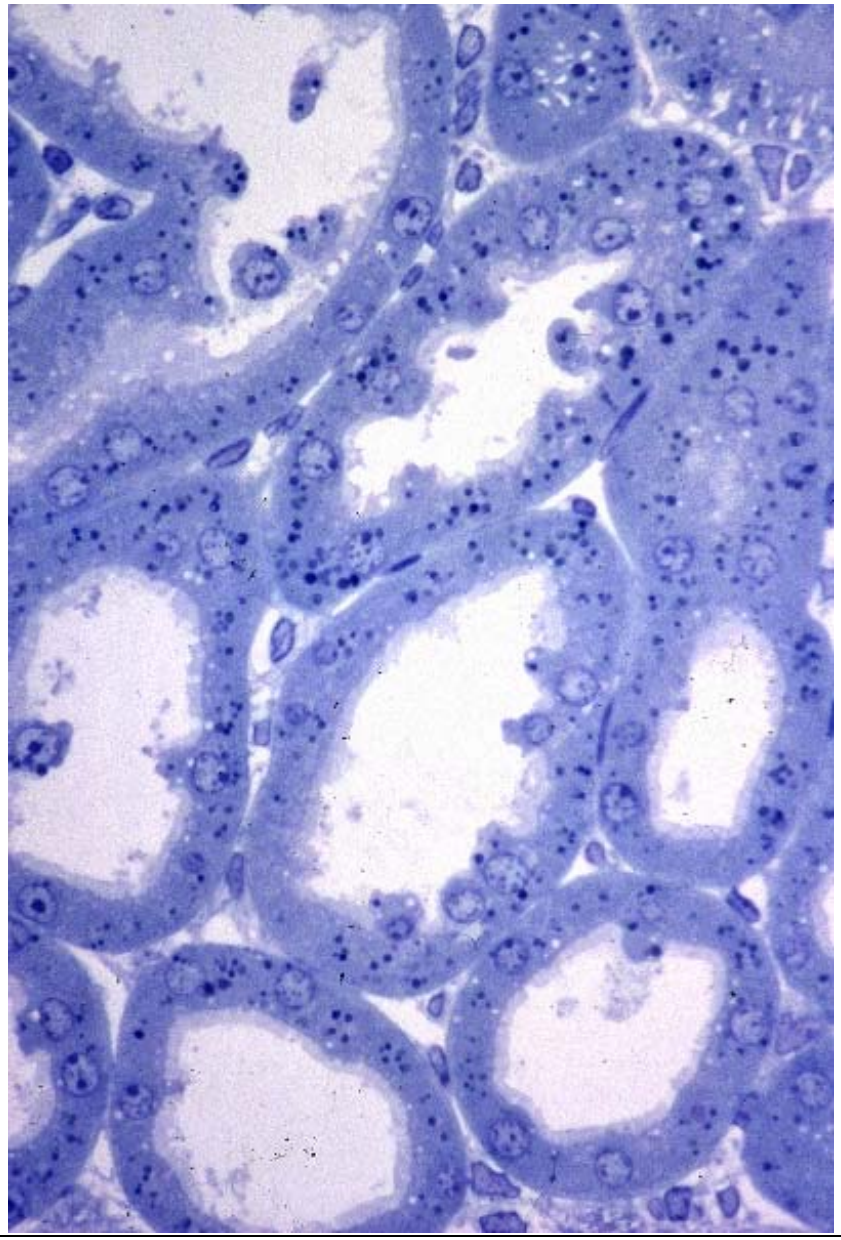
P. Maldague, unpublished

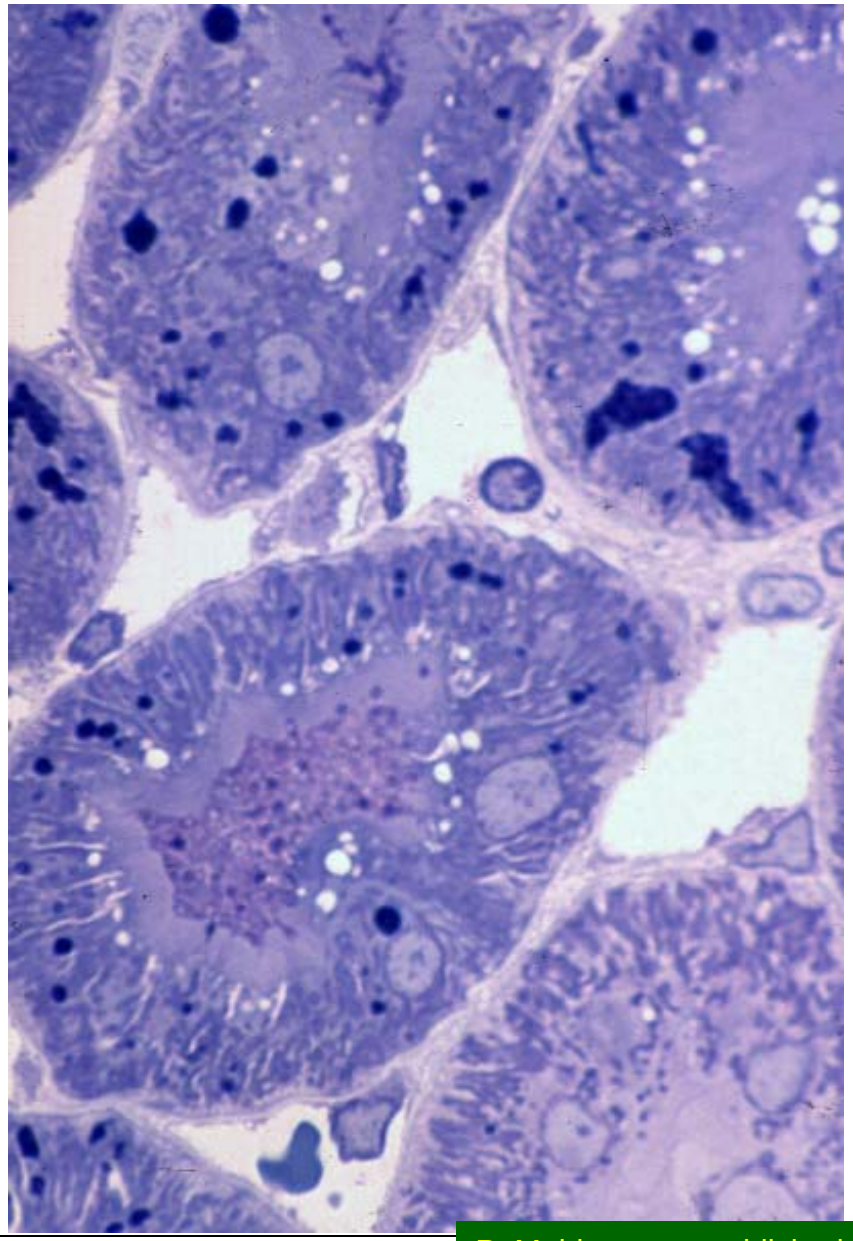
Compare ...



P. Maldague, unpublished

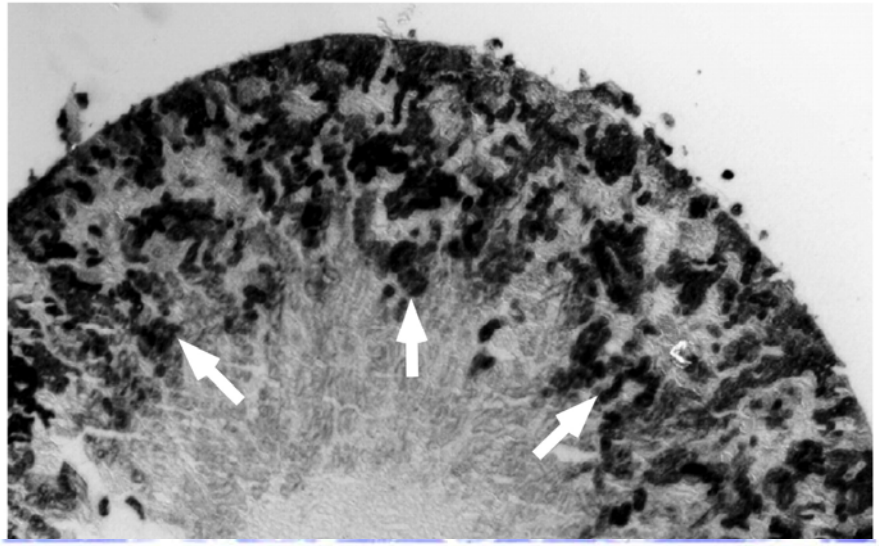
And examine …

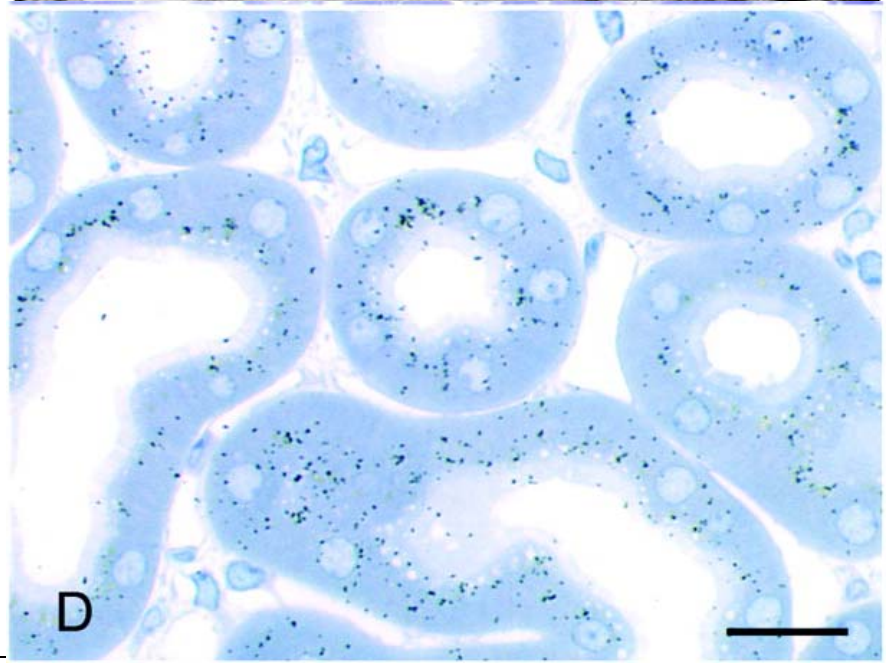


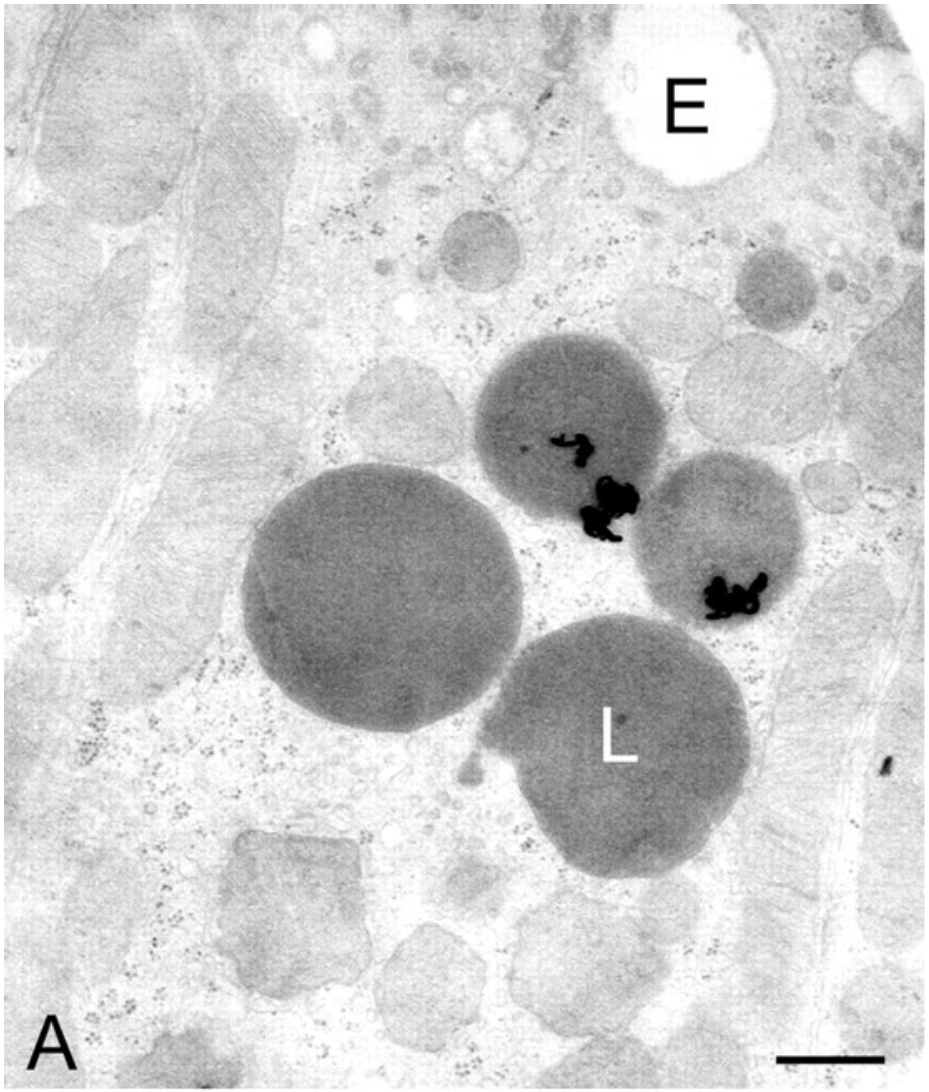


P. Maldague, unpublished

Gentamicin accumulates in lysosomes of proximal tubular cells

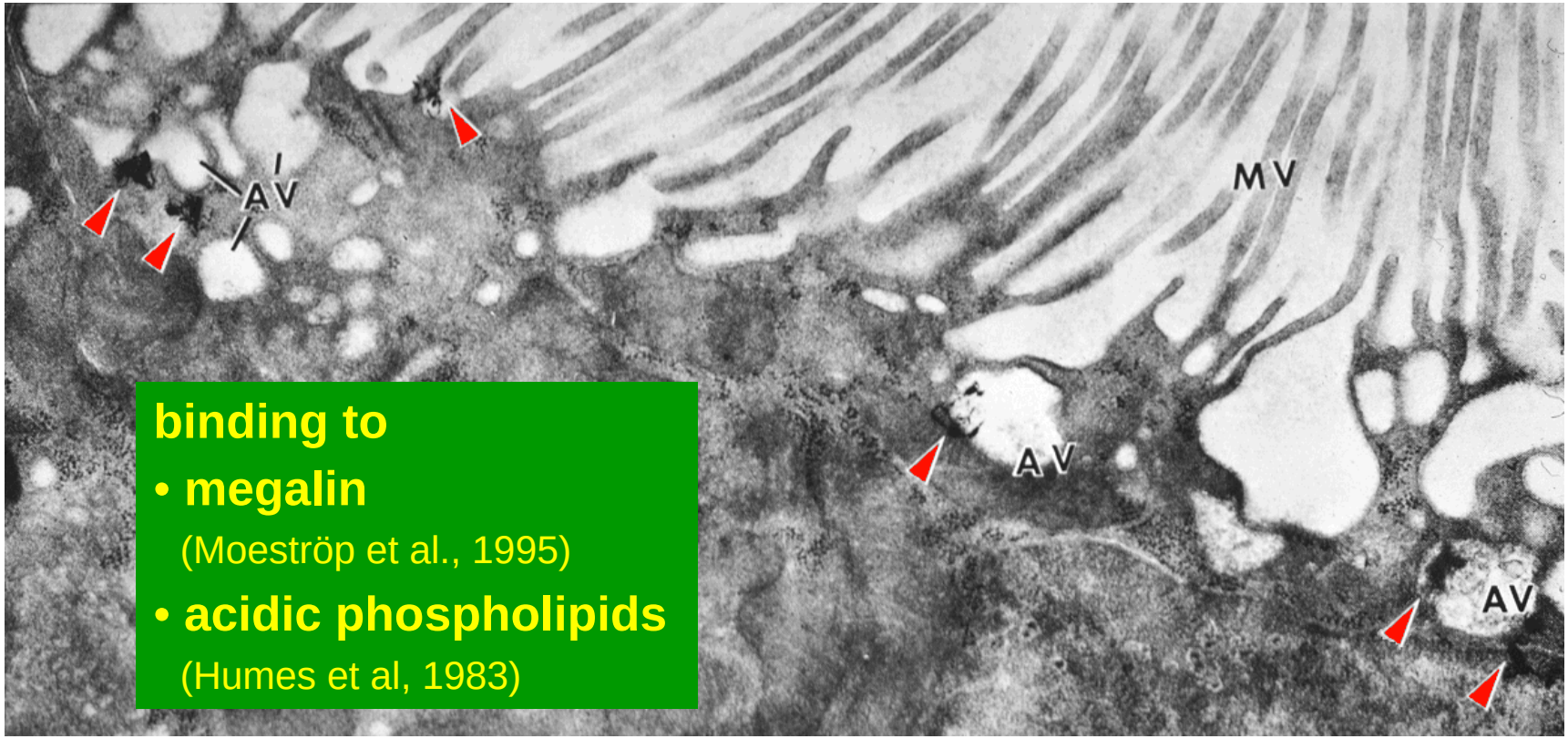






Schmitz et al., J. Biol. Chem. 277:618-622, 2002

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

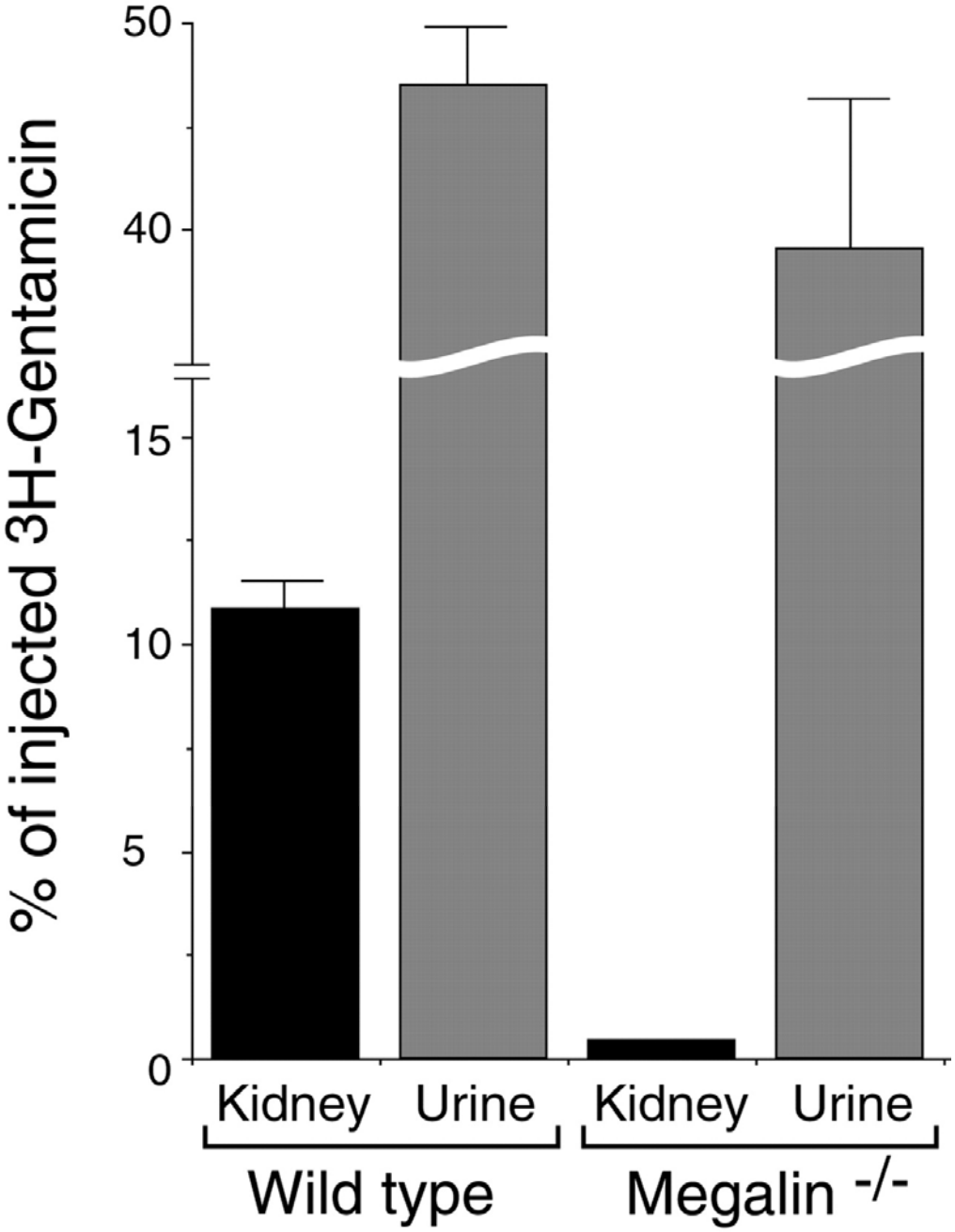


binding to

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

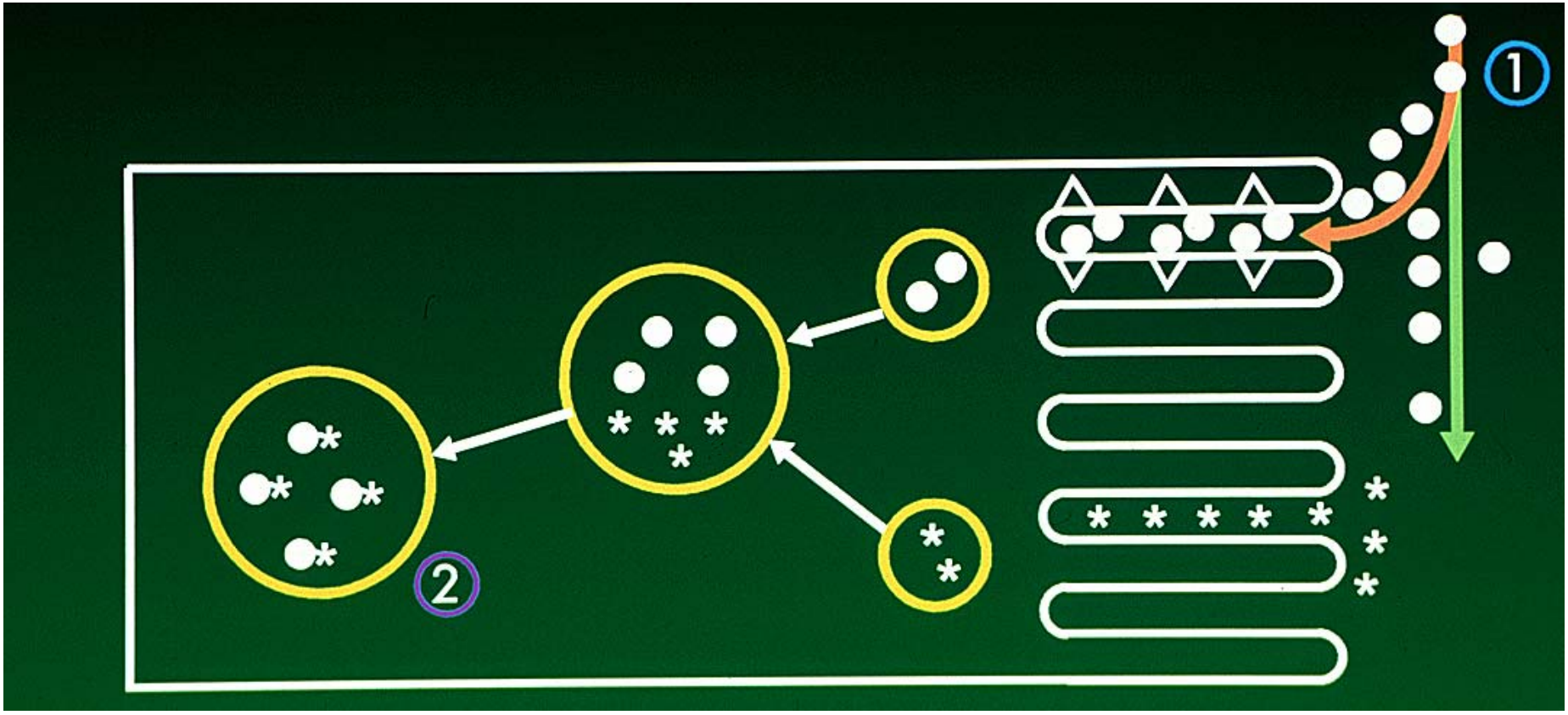
* Just *et al*, Naunym Schmied. Arch. Pharmacol, 1977
Silverblatt & Kuehen, Kidney Intern., 1979

Mice deficient in megalin do not accumulate gentamicin in kidney



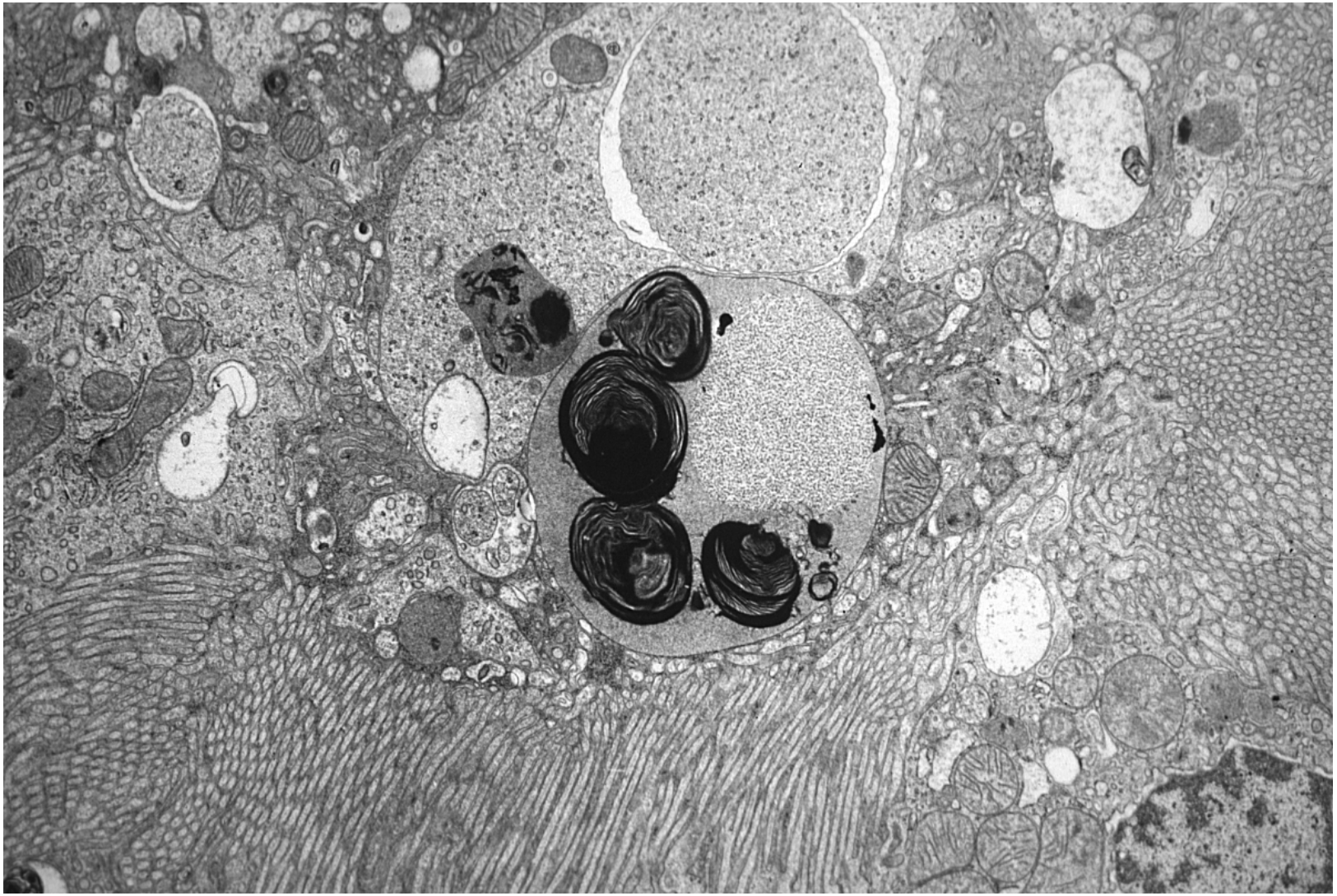
Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Towards a mechanism …



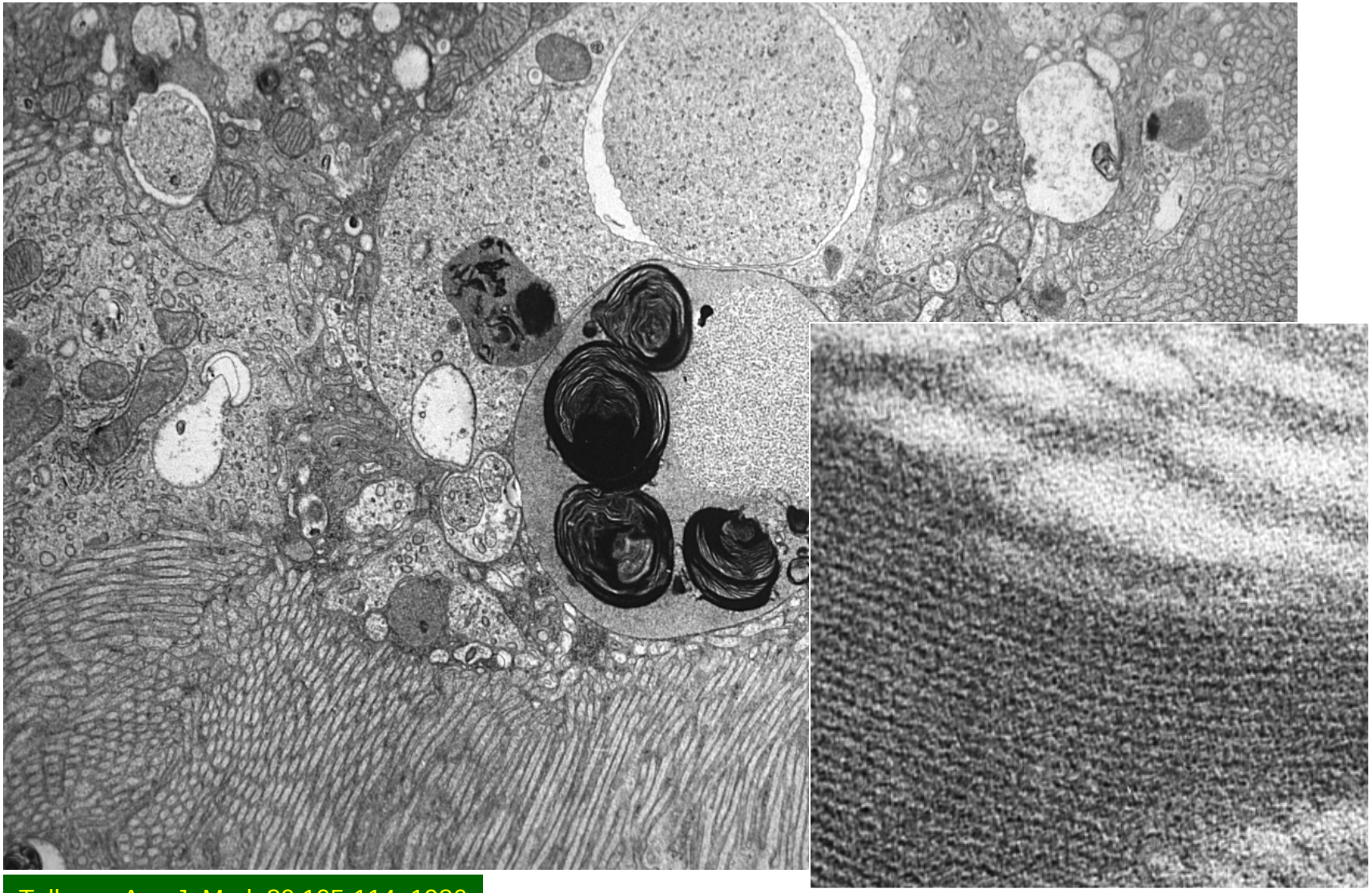
1. **binding to brush border**
2. **accumulation in lysosomes**

Intralysosomal gentamicin causes phospholipidosis



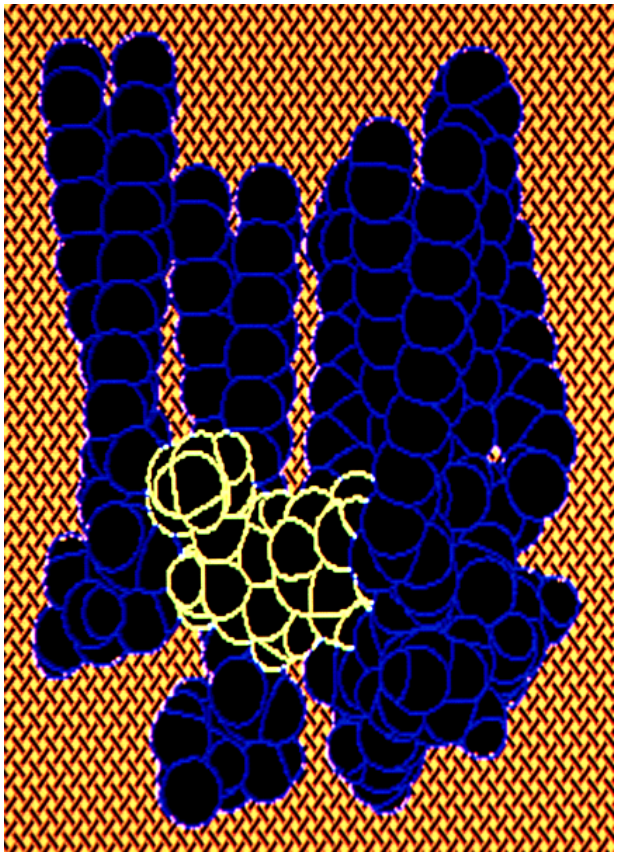
Tulkens, Am. J. Med. 80:105-114, 1986

Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis

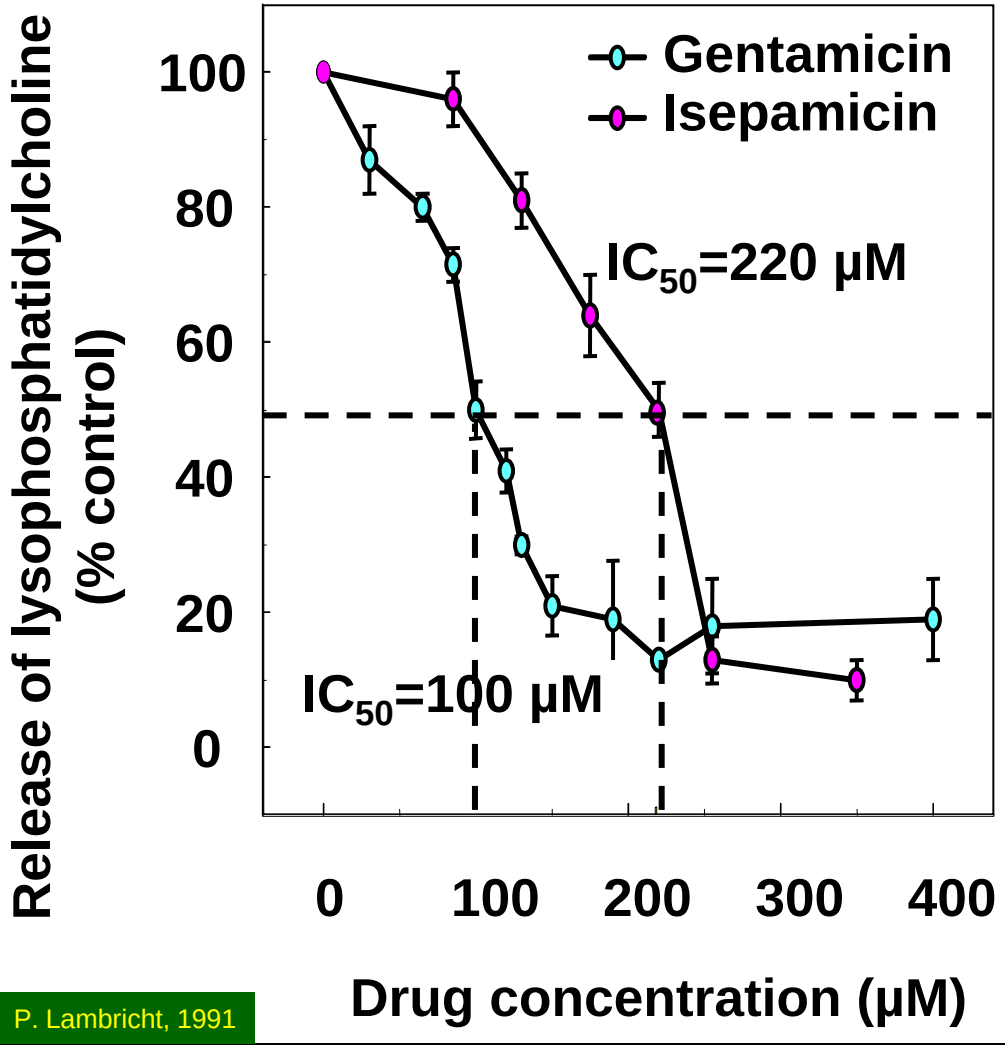


Tulkens, Am. J. Med. 80:105-114, 1986

Phospholipidosis is related to the binding of gentamicin to acidic phospholipids and subsequent inhibition of lysosomal phospholipases

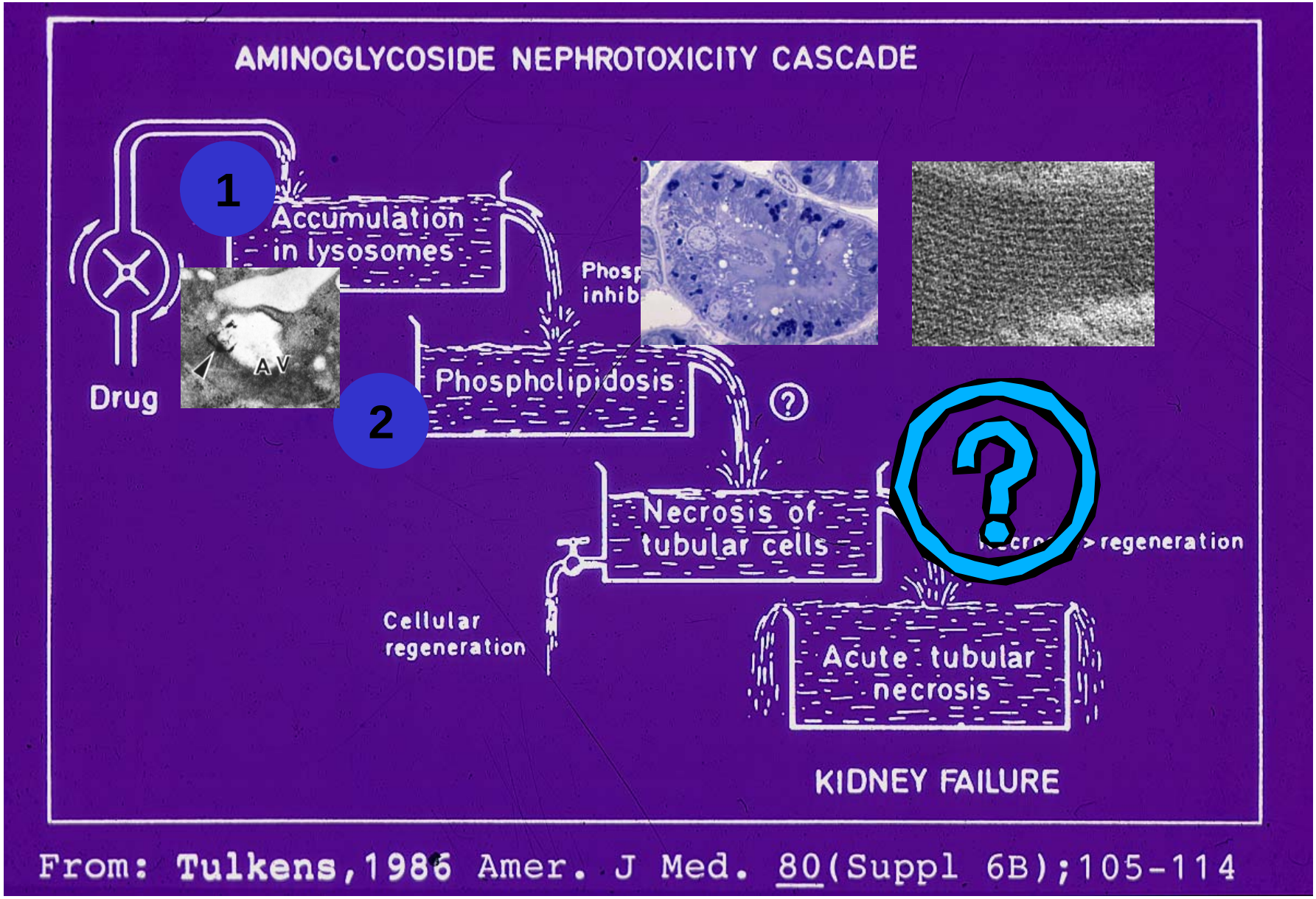


Adapted from Brasseur et al., 1989



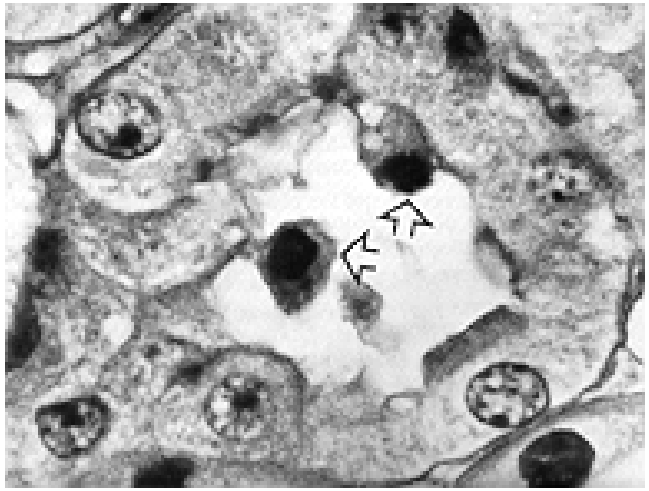
P. Lambricht, 1991

A first global hypothesis ?...



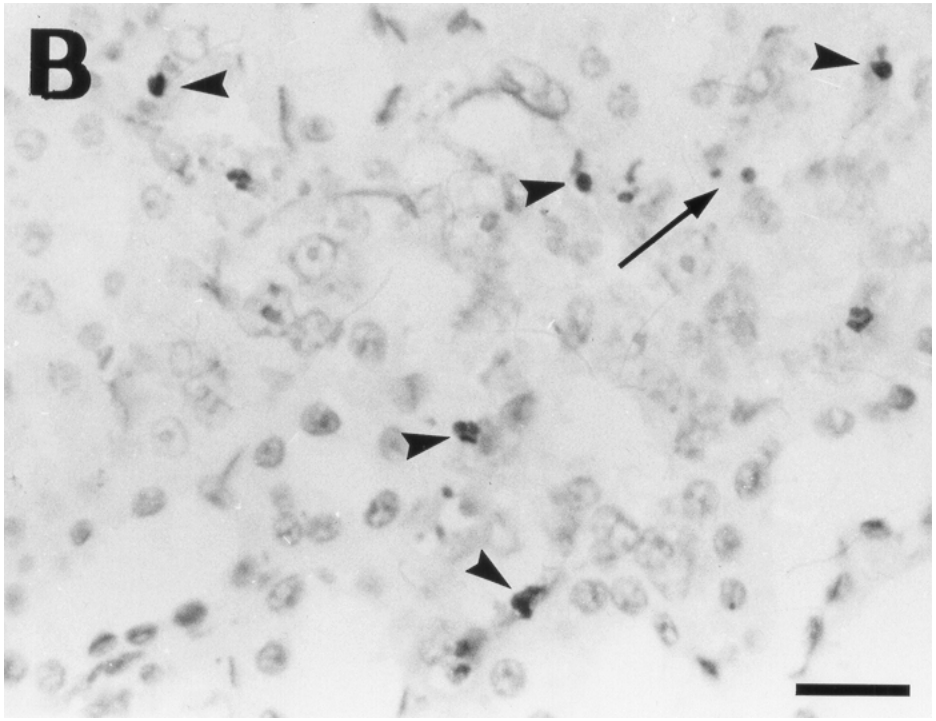
Gentamicin causes apoptosis at low, therapeutically-relevant dosages

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 ?

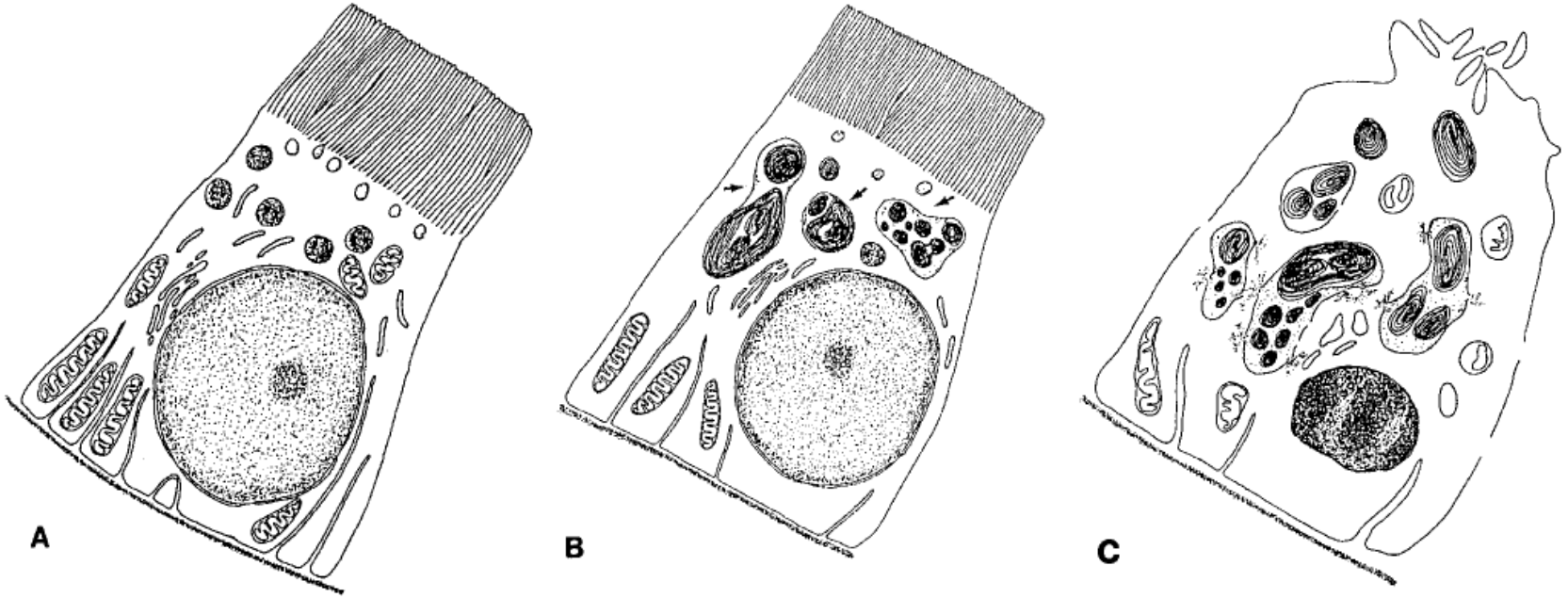
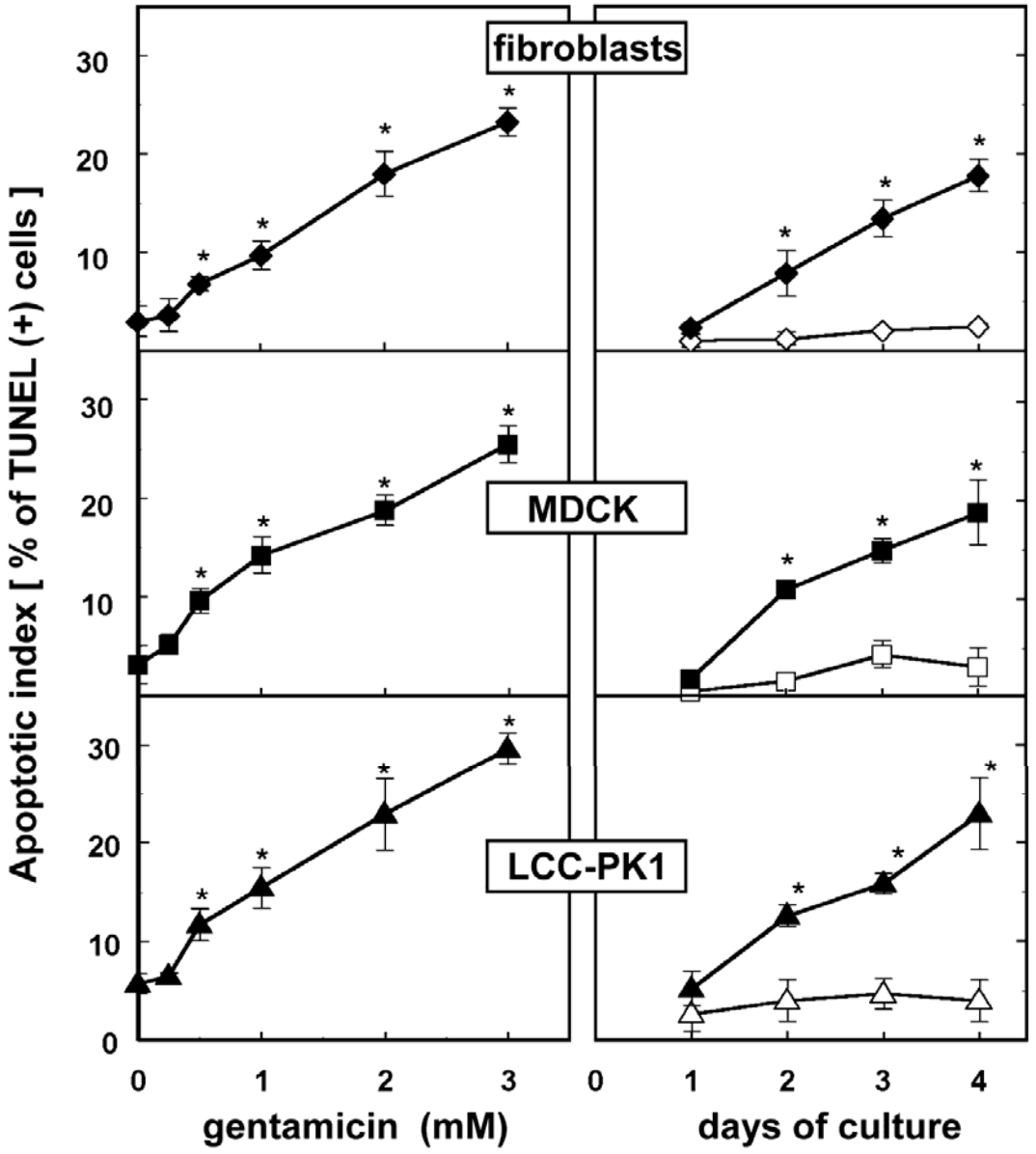


FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive 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.

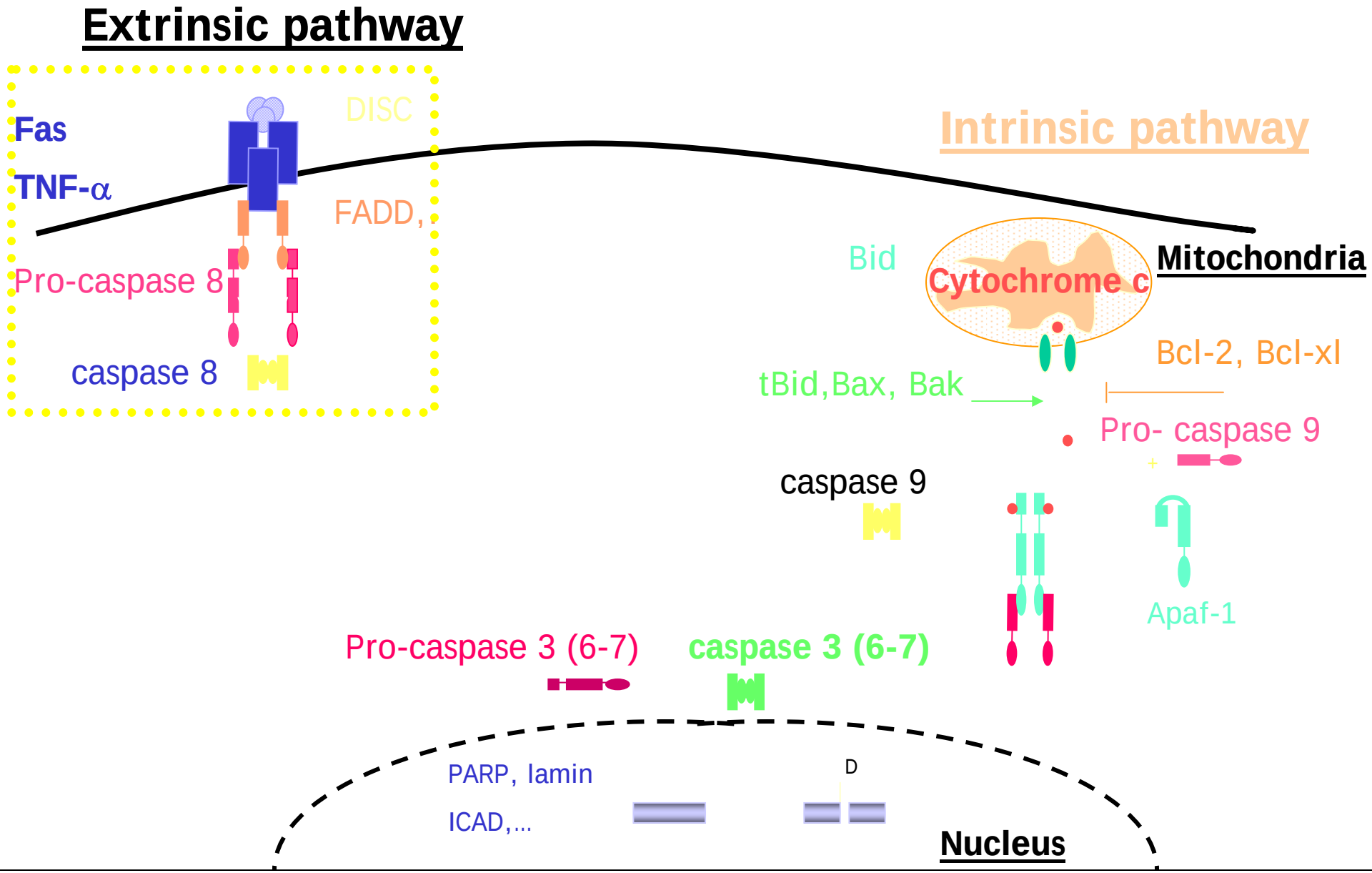
Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. (1999) 43:1003-1012

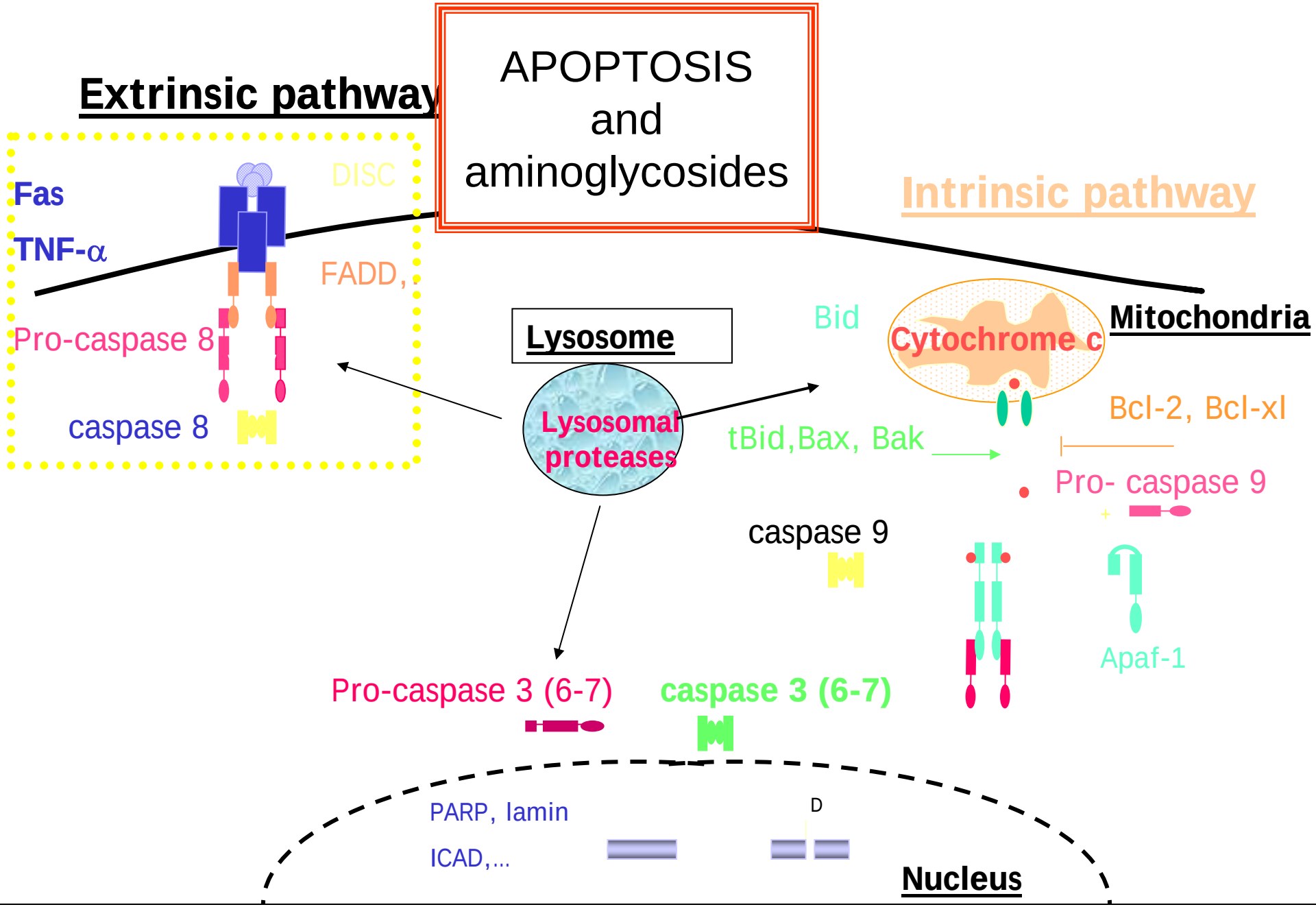
Gentamicin-induced apoptosis can be reproduced with cultured kidney and non-kidney cells …



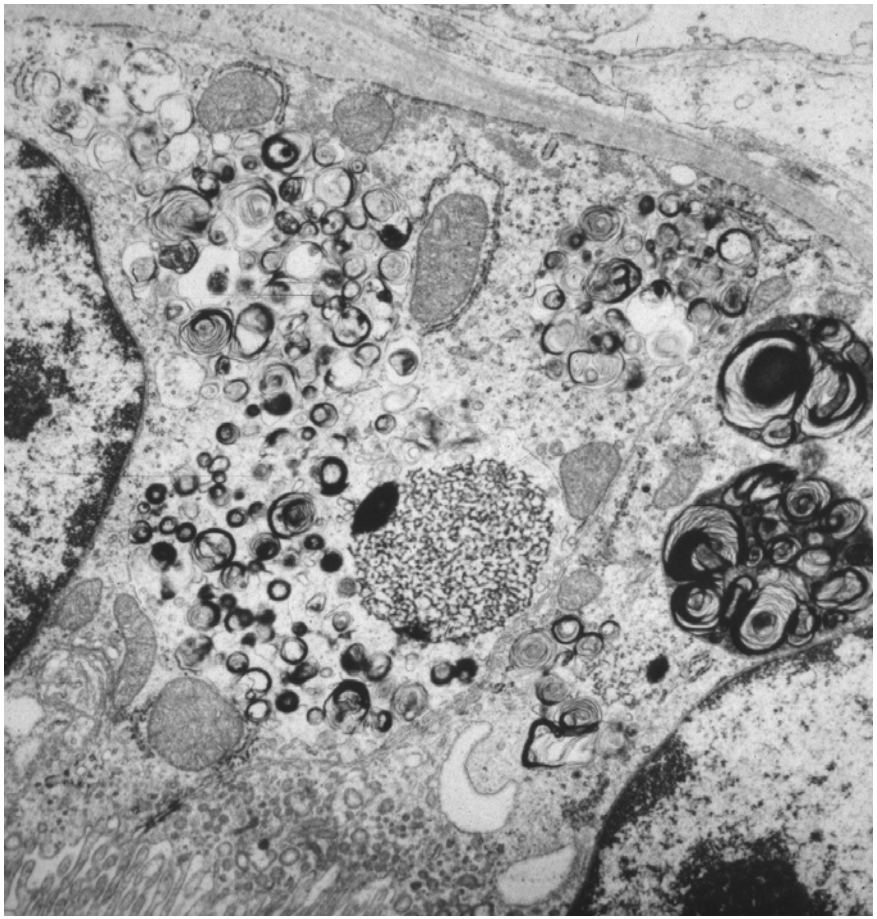
El Mouedden et al.,
Toxicol. Sci., 56:229-239, 2000

APOPTOSIS: main signaling pathways ...

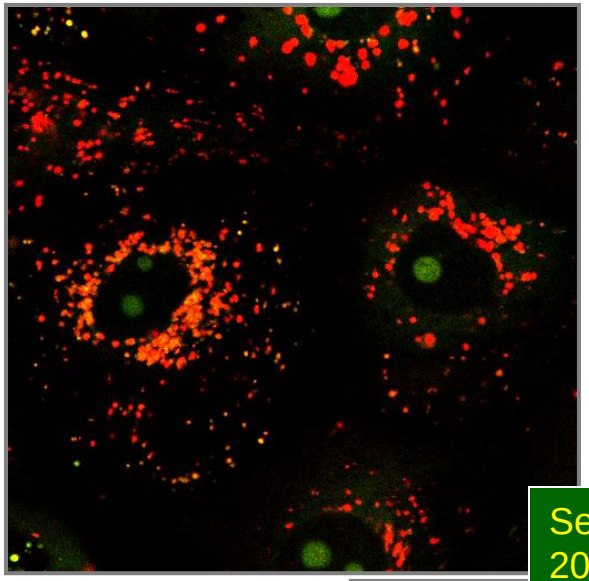



APOPTOSIS
and
aminoglycosides
Extrinsic pathway
Intrinsic pathway
Fas
TNF-α
DISC
FADD,
Pro-caspase 8
caspase 8
Lysosome
Lysosomal proteases
Bid
Cytochrome c
Mitochondria
Bcl-2, Bcl-xl
tBid,Bax, Bak
Pro- caspase 9
caspase 9
Apaf-1
Pro-caspase 3 (6-7)
caspase 3 (6-7)
PARP, lamin
ICAD,...
D
Nucleus

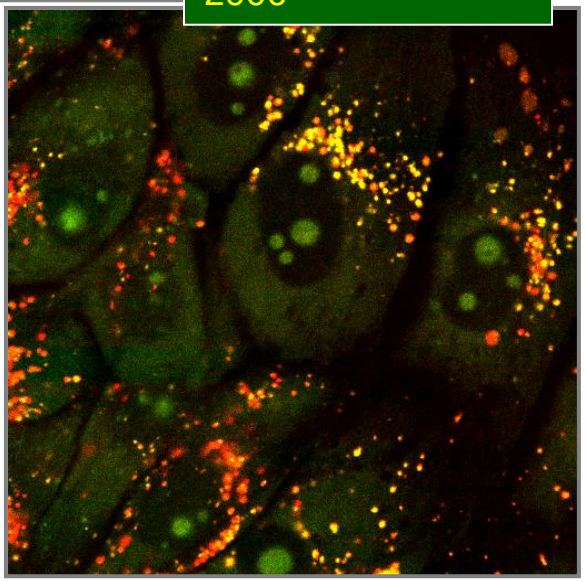
Could lysosomal rupture cause apoptosis and necrosis ?



Maldague et al., 1983



Servais *et al.*, 2006



Are lysosomes disrupted by gentamicin ?

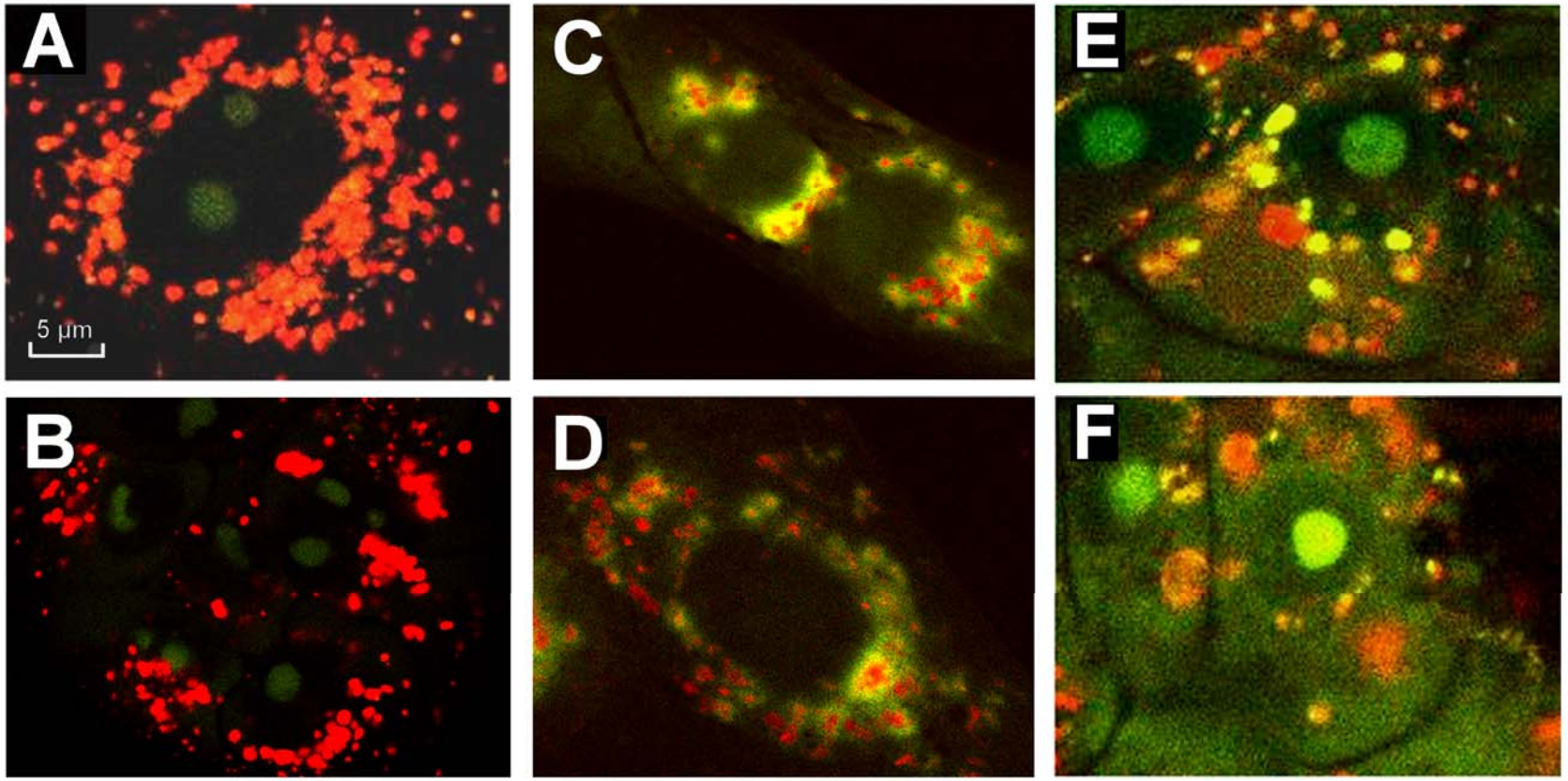
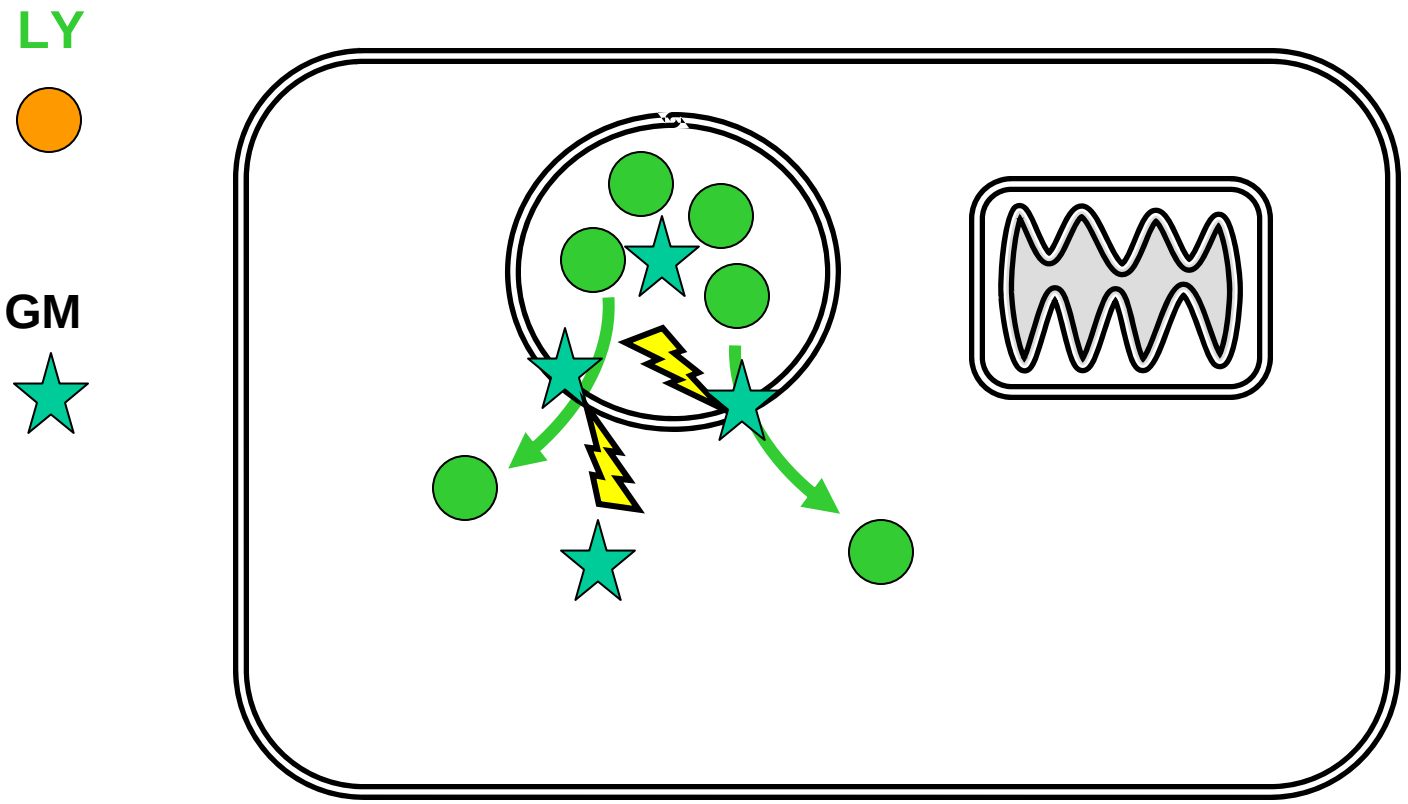


Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 µg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 µM, 3 h).

H. Servais et al. / Toxicology and Applied Pharmacology 206 (2005) 321–333

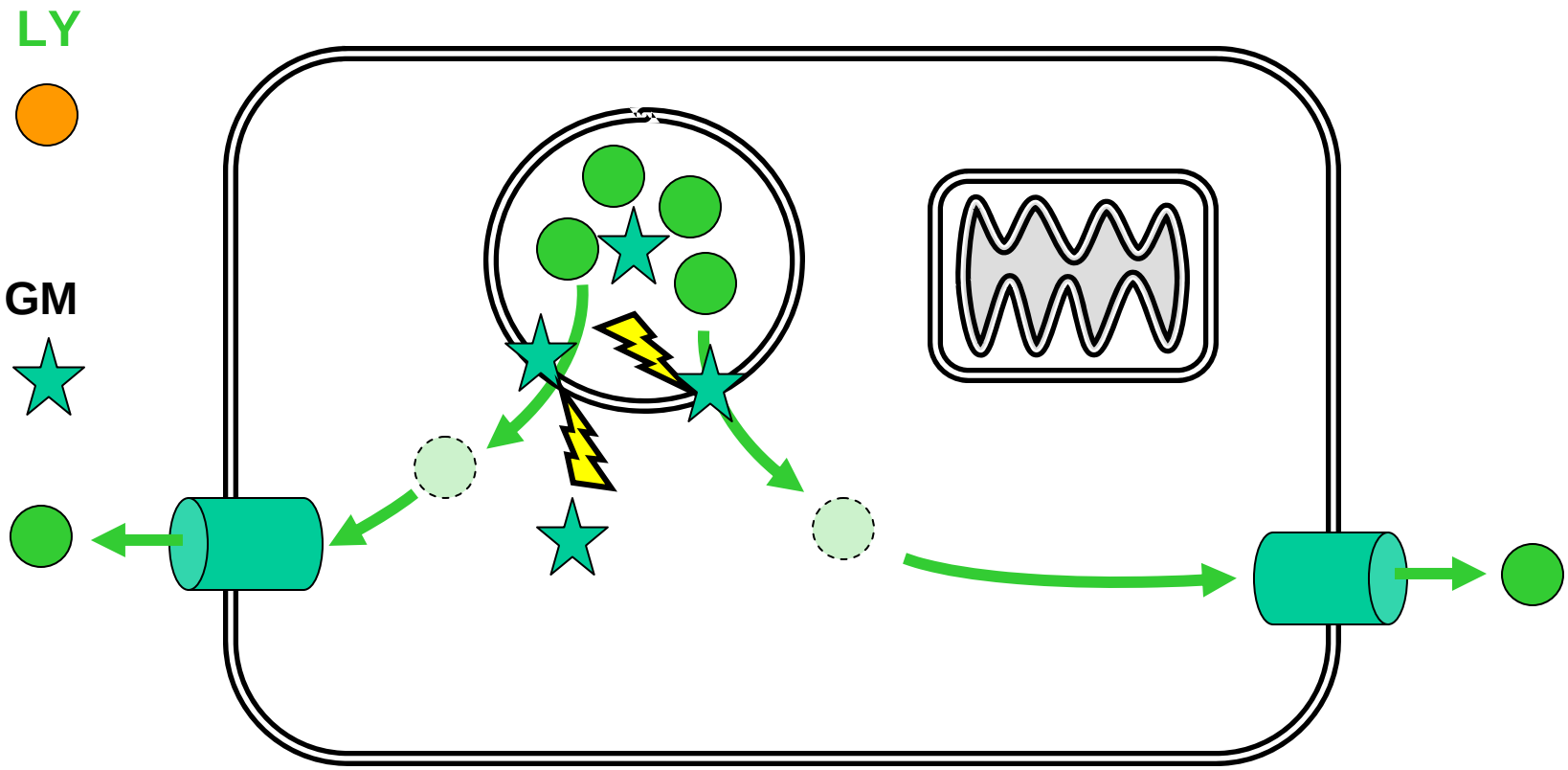
Using Lucifer Yellow to detect lysosome rupture (1)



Loading cells with gentamicin and LY to detect the release of LY

Denamur et al. Free Radic Biol Med. 2011 Jul 23.

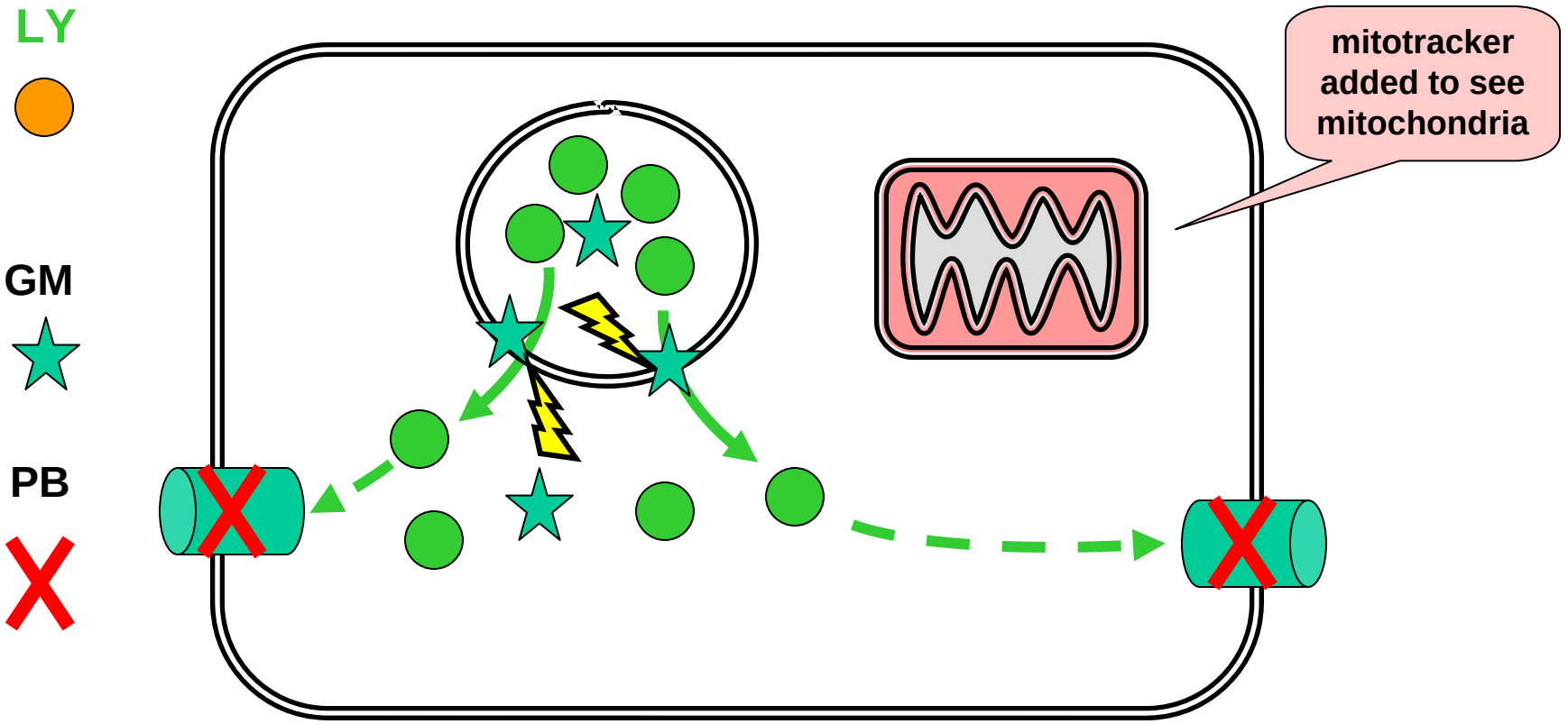
Use of Lucifer Yellow to detect lysosome rupture (2)



But LY is quickly effluxed through an export transporter, so that it never stays long in the cytosol … and cannot be seen

Denamur et al. Free Radic Biol Med. 2011 Jul 23.

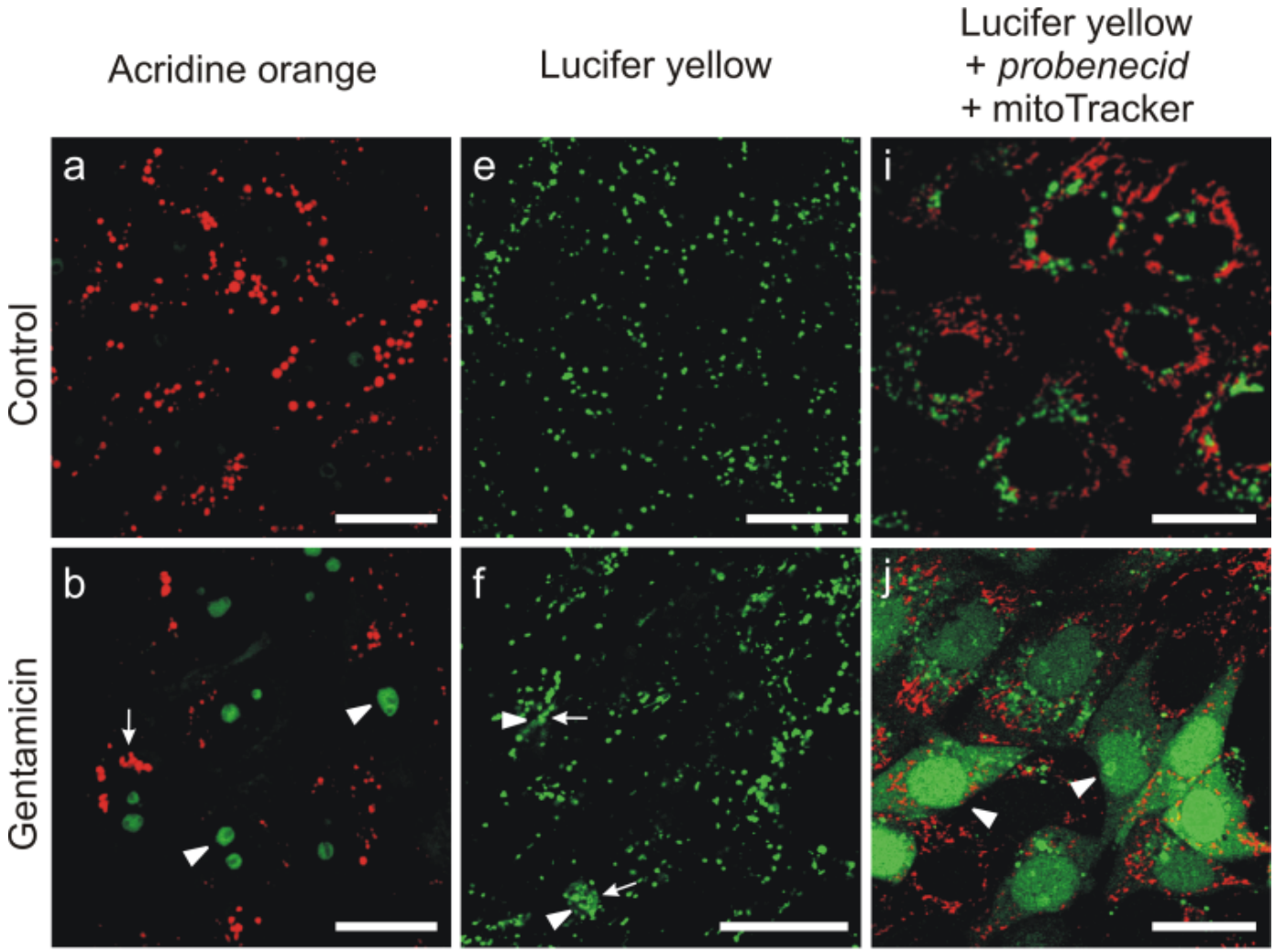
Use of Lucifer Yellow to detect lysosome rupture



Adding probenecid (PB) allows to block LY efflux

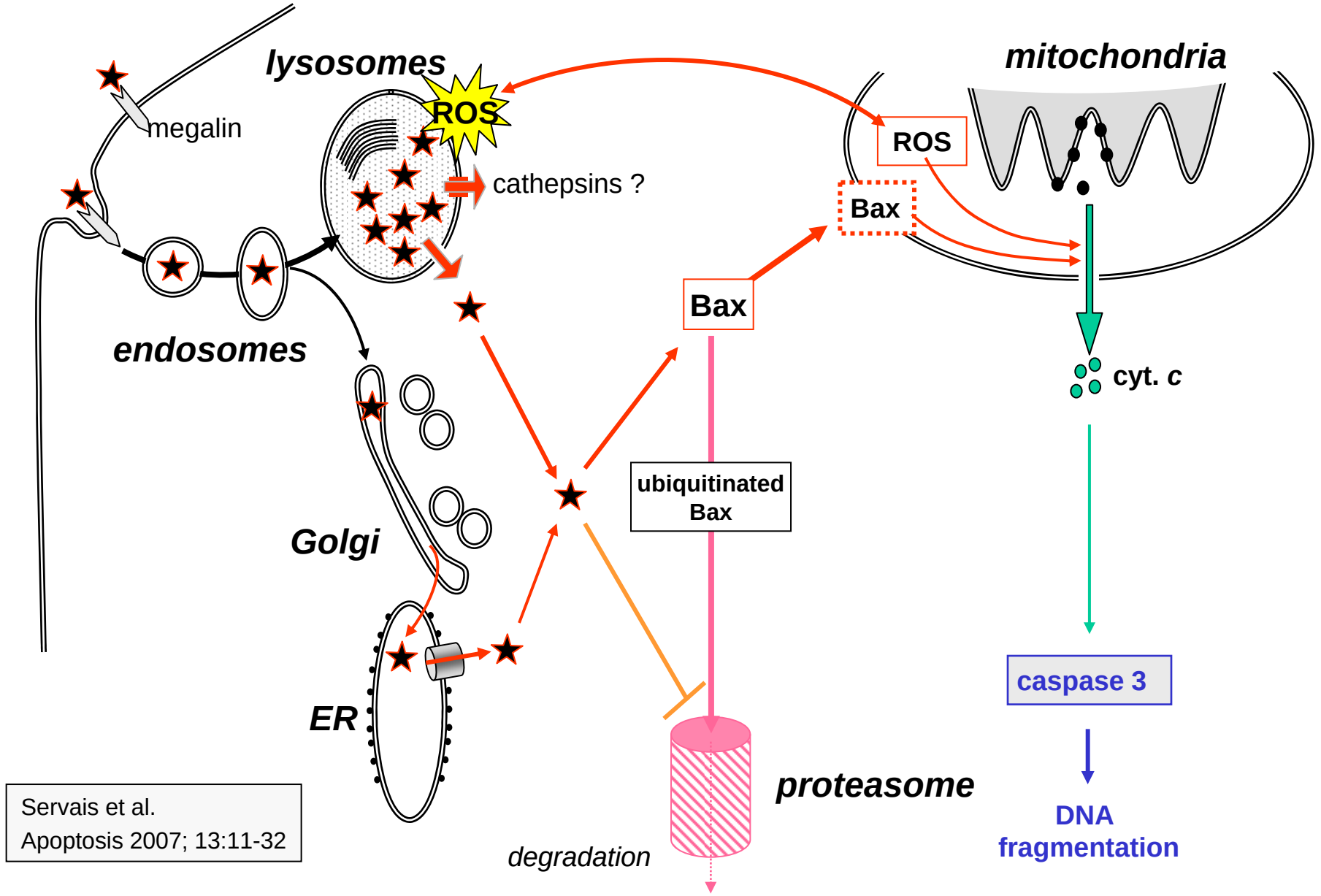
Denamur et al. Free Radic Biol Med. 2011 Jul 23.

A recent demonstration of lysosomal rupture induced by gentamicin



Denamur et al. Free Radic Biol Med. 2011 Jul 23.

Gentamicin and apoptosis: an overview



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

What if you by-pass lysosomes ?

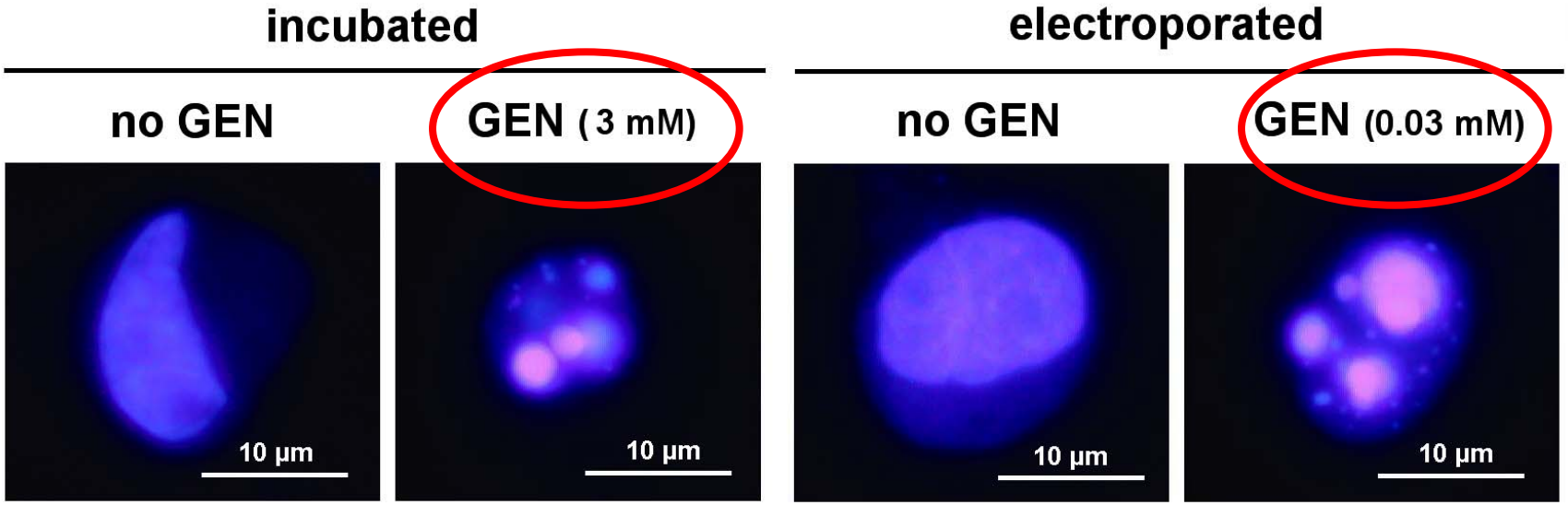
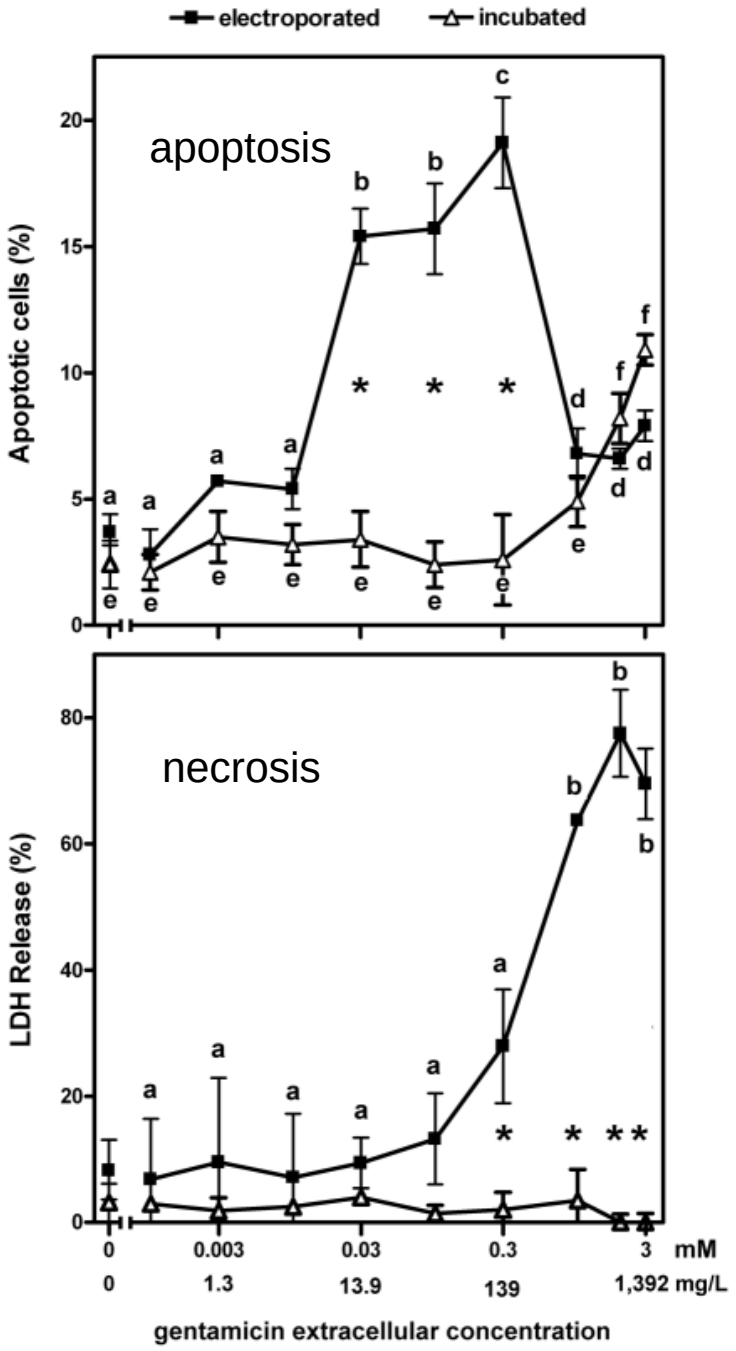


Figure 1: Staining of nuclei of LLC-PK_1 cells by 4',6'-diamidine-2'-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the absence (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (0.03 mM; 13.9 mg/L), and examined 24 h later. In the absence of gentamicin, both electroporated and incubated cells show a diffuse finely reticulated staining characteristic of euchromatin of diploid interphase animal cells. In contrast, cells electroporated or incubated in the presence of gentamicin show typical changes associated with apoptosis, consisting in the condensation and fragmentation of the nuclear material.

Servais et al., Antimicrob. Agents Chemother. 50(4):1213-21, 2006

Bypassing lysosomes in cultured cells make the cells exquisitely sensitive to gentamicin-iduced apoptosis…



Servais et al., Antimicrob. Agents Chemother. 50(4):1213-21, 2006

Part 4: towards the real clinics

The basis of the once-a-day schedule

- gentamicin enters proximal tubular cells by megalin- and acid phospholipids mediated pinocytosis and ends up in lysosomes
- a minor part escapes lysosomes either by membrane destabilization (our hypothesis) or by retrograde transport (Molitoris' hypothesis) to reach the cytososol and the mitochondria … where it induces apoptosis and other toxic disturbances…
- you could prevent toxicity either
  - by impairing the pinocytic uptake of aminoglycosides, or making an aminoglycoside that does not bind to megalin…
    - ➔ block or avoid step one …
  - developing an that does not destabilize lysosomes and/or does not cause apoptosis …
    - ➔ block step 2 and/or its consequences…

Making use of this knowledge to protect patients …

TABLE 2. Main approaches toward reduction of aminoglycoside nephrotoxicity[a]

Mechanism	Compound

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

A long list…

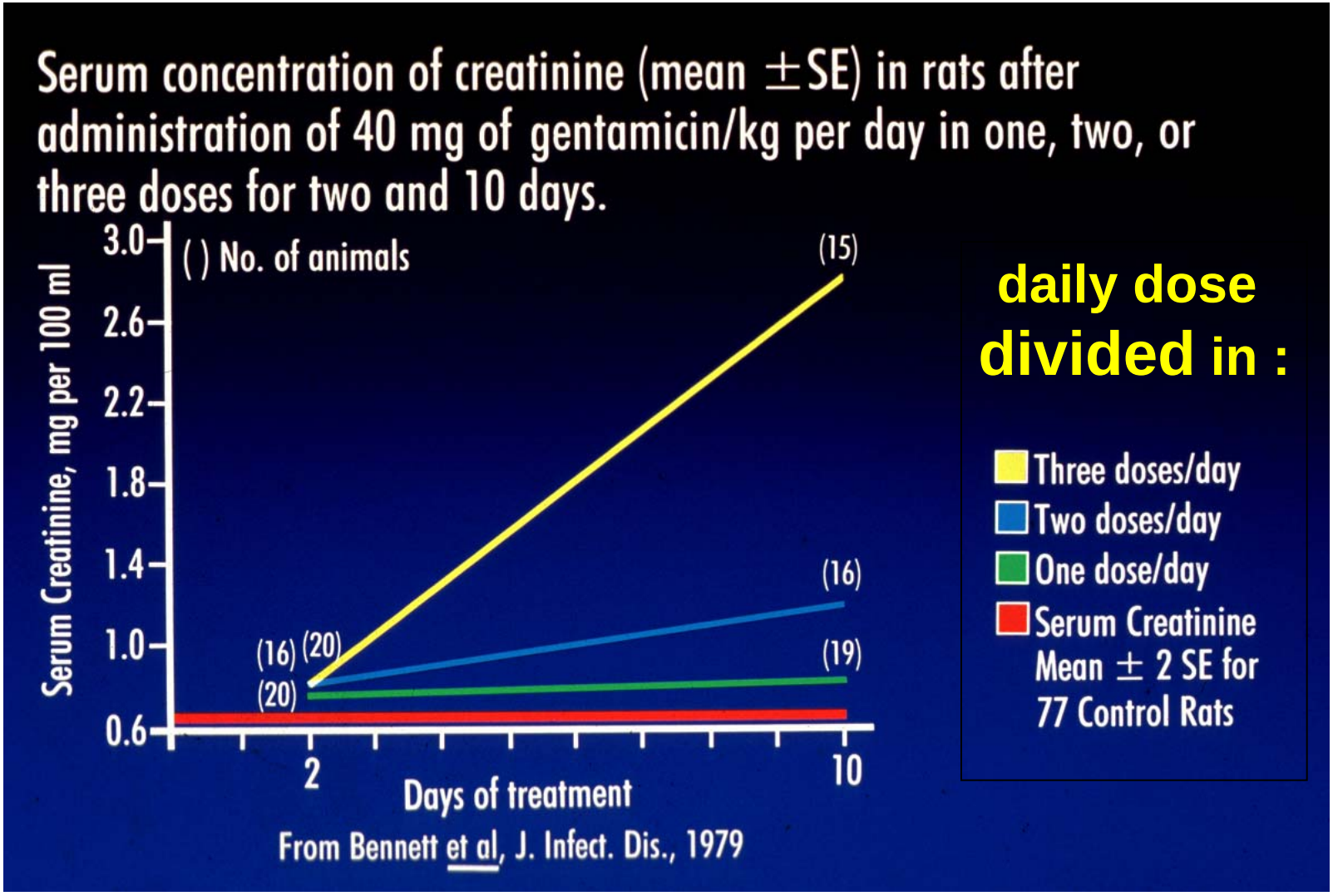
TABLE 2. Main approaches toward reduction of aminoglycoside nephrotoxicity[a]

Mechanism	Compound
I. Decrease or prevention of drug accumulation by kidneys	
Intracellular complexation of aminoglycosides	
Polyanionic compounds	Dextran sulfate (59) Inositol hexasulfate (67)
Acidic drugs	Piperacillin (44) Latamoxef-moxalactam (68) Fosfomycin (33, 54) Pyridoxal-5′-phosphate (114)
Competition with or decrease in aminoglycoside binding to brush border membrane	
Raising the urine pH	Bicarbonate (19, 29)
Competitors	Ca^{2+} (diet supplementation [51] or vitamin D-induced hypercalcemia [21]) Lysine (81) Aminoglycosides (as their own competitors) (39)
Increase in exocytosis	Flerocaxin (9)
II. Prevention or decrease of lysosomal phospholipase inhibition	
Derivatives with lesser intrinsic binding[b]	
N substitution	Amikacin (75), isepamicin (133), arbekacin,[c] 1-*N*- and 6′-*N*-peptidic and aminoacid derivative of kanamycin A and netilmicin (72)
Other substitution	6″-substituted kanamycin B (88)
Fluorinated derivatives[c]	5, 3″ or 3′ fluoro derivatives of tobramycin, dibekacin, arbekacin, or kanamycin[c]
Disaccharidic aminoglycosides	Astromicin (fortimicin) (73) Dactimicin (2-*N*″-formidoyl-astromicin) (53, 73)
Coadministration of agent preventing intralysosomal phospholipidosis	
Intralysosomal sequestration of aminoglycosides	Polyaspartic acid (55, 62)
Increase of membrane negative charge	Daptomycin (41)
Other	Torbafylline (32)
III. Protection against necrosis and other gross cellular alterations	
Antioxidants	Deferroxamine (11) Methimazole (24) Sairei-to (94) Vitamin E + selenium, vitamin C (1, 57) Lower copper feeding (58)
Antioxidant and multifactorial factors	Lipoic acid (107)
IV. Protection against vascular and glomerular effects	
Suppression of renin-angiotensin activation	Deoxycortisone and saline drinking (45)
Protection against Ca^{2+} influx	Ca^{2+} channel blockers (80)
Undefined mechanism	Platelet activation antagonists (104)
V. Increase in kidney regeneration capabilities	
Unspecific mitogenic effect	Ulinastatin (92)
Growth factors	Fibroblast growth factor 2 (78) Heparin-binding epidermal growth factor (106)

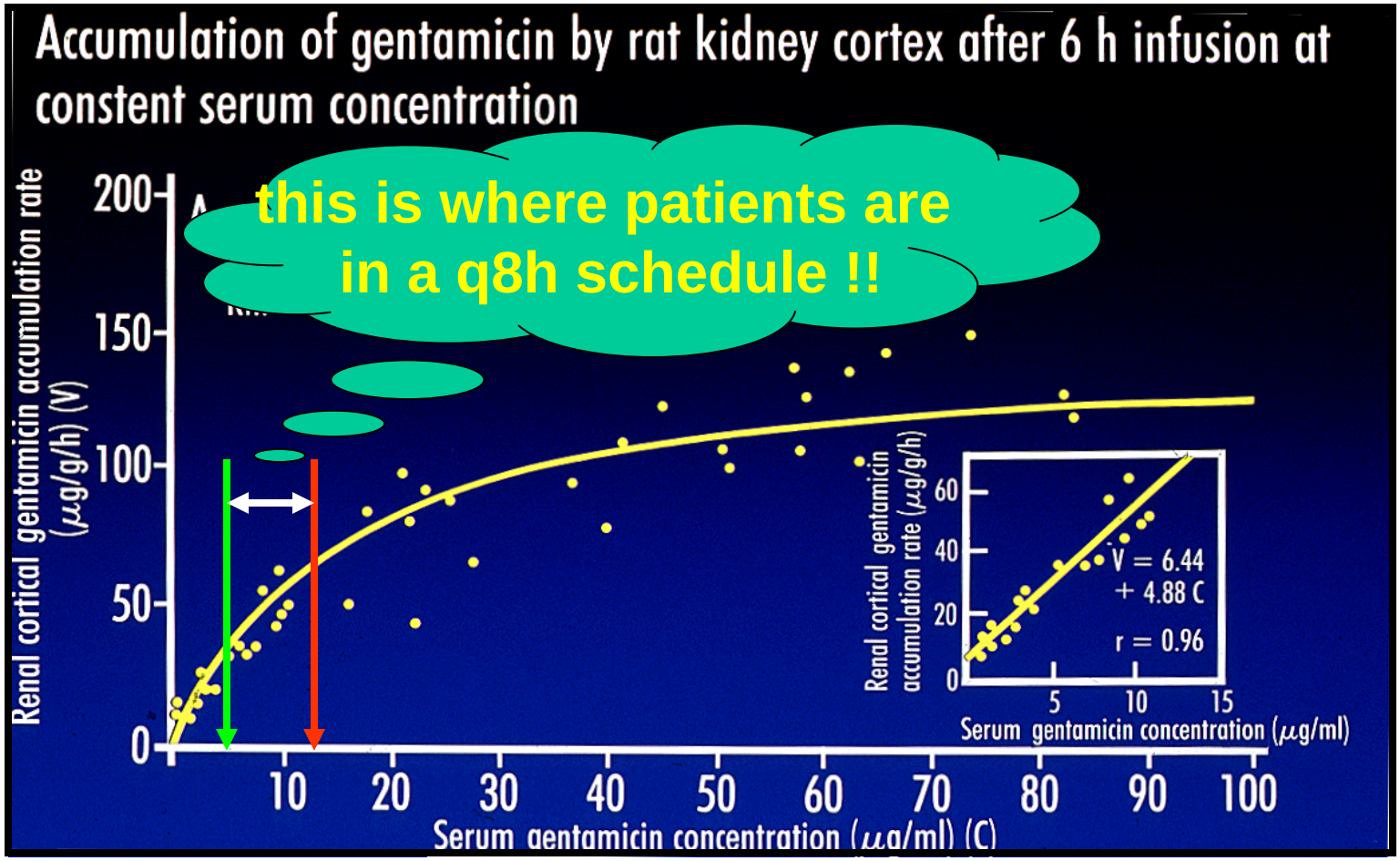
[a] References refer to publications dealing with the proposed mechanism; see text for further details on the extent and characterization of the protection.
[b] See reference 83 for structures.
[c] Mechanism is assumed on the basis of the substitution made (see reference 83 for a discussion and references to original papers), but it has not actually examined.

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

Aminoglycoside toxicity is not linked to peak ...

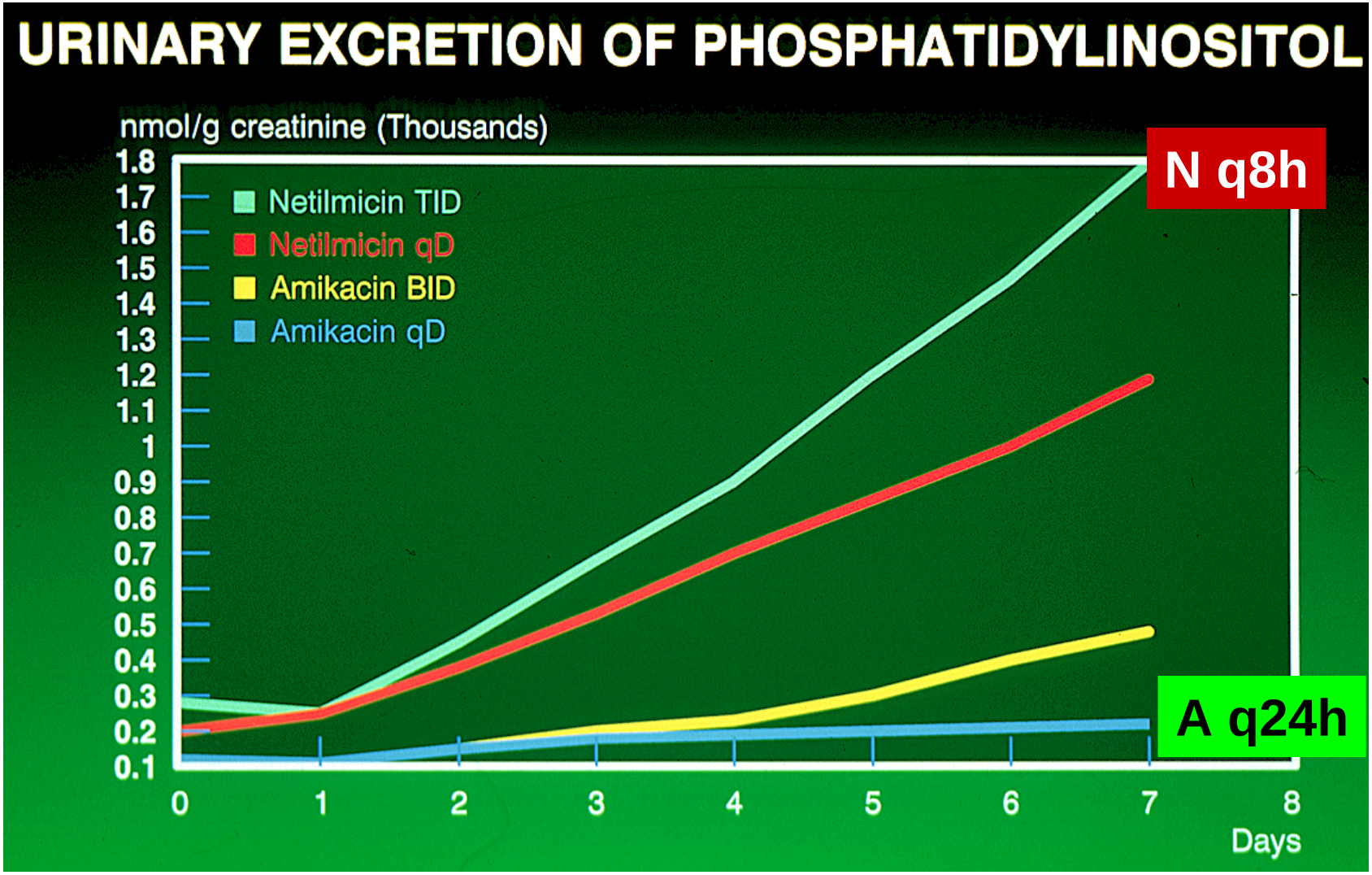


Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...



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

Phospholipiduria …

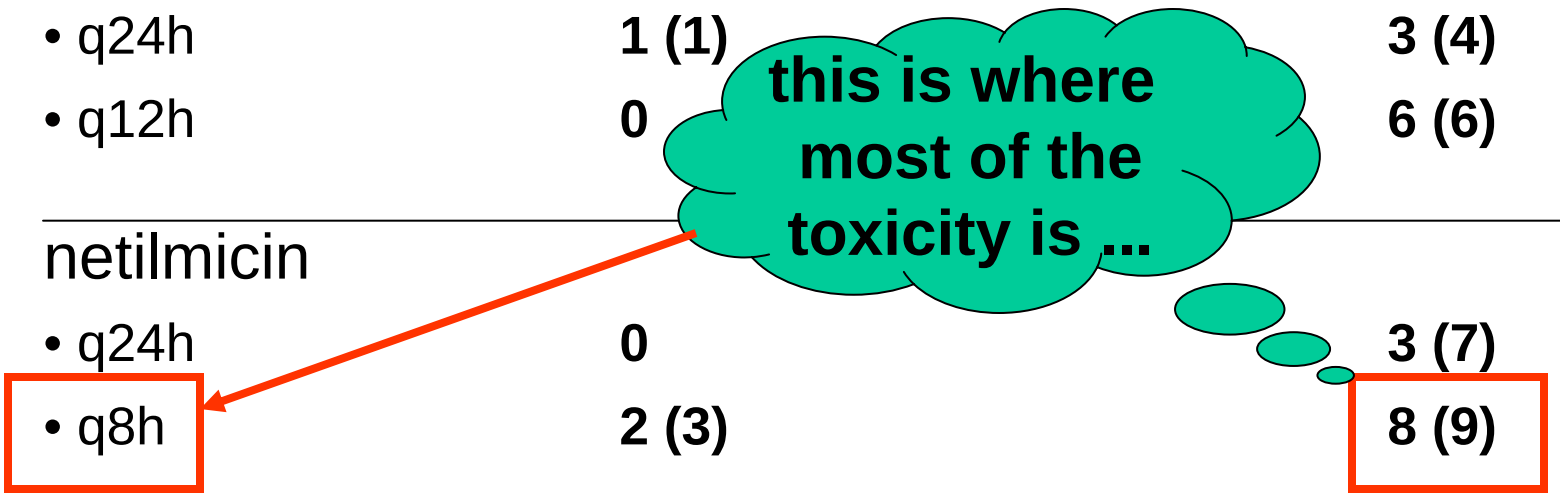


Tulkens *et al.*, 1989

And auditory alterations …

no. of patients [over 20 in each group] with lesions* and total no. of frequencies affected

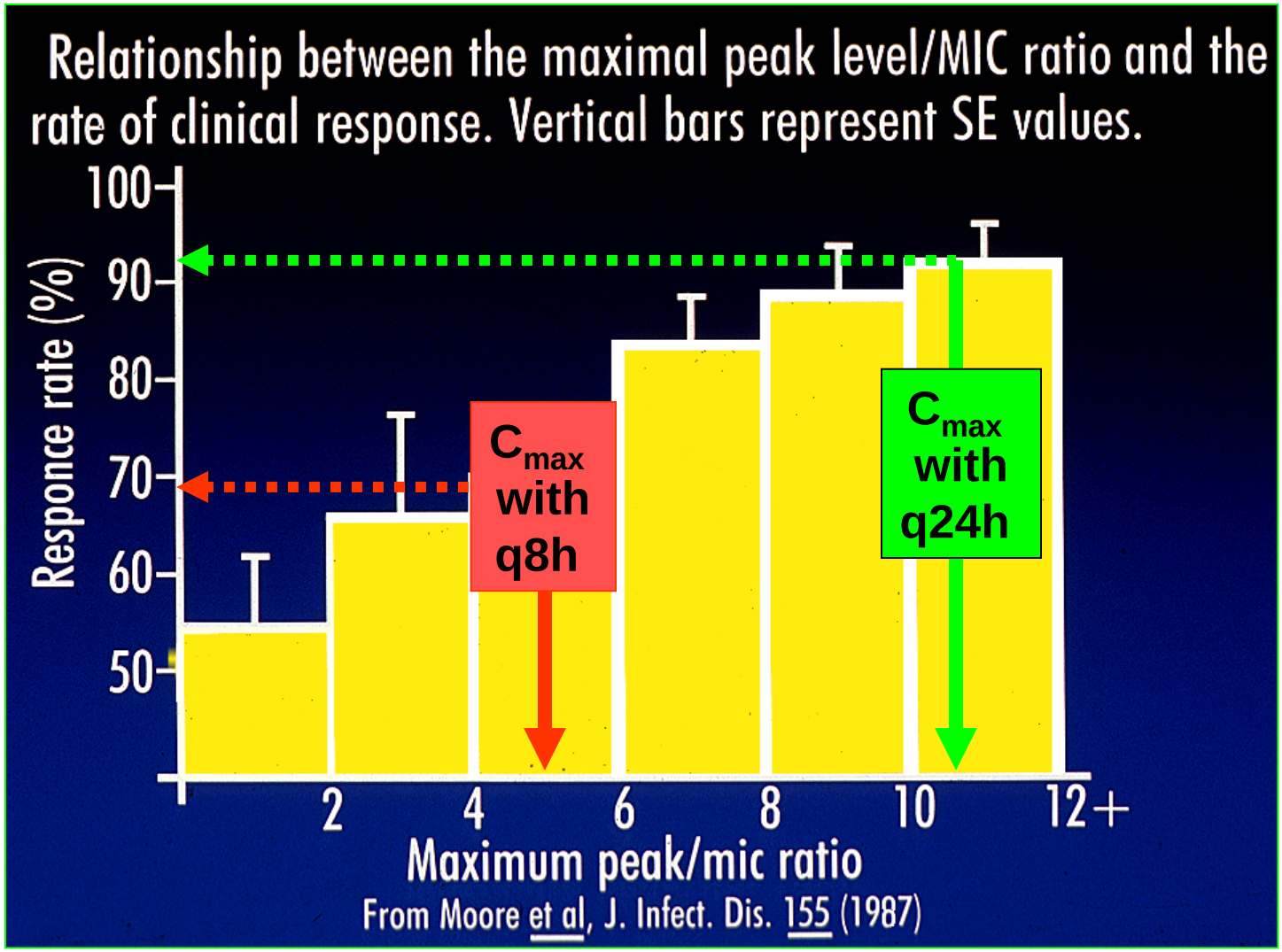
	low tone (0.25-8 kHz)	high tone (10-18 kHz)
amikacin		
• q24h	**1 (1)**	**3 (4)**
• q12h	**0**	**6 (6)**
netilmicin		
• q24h	**0**	**3 (7)**
• q8h	**2 (3)**	**8 (9)**



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

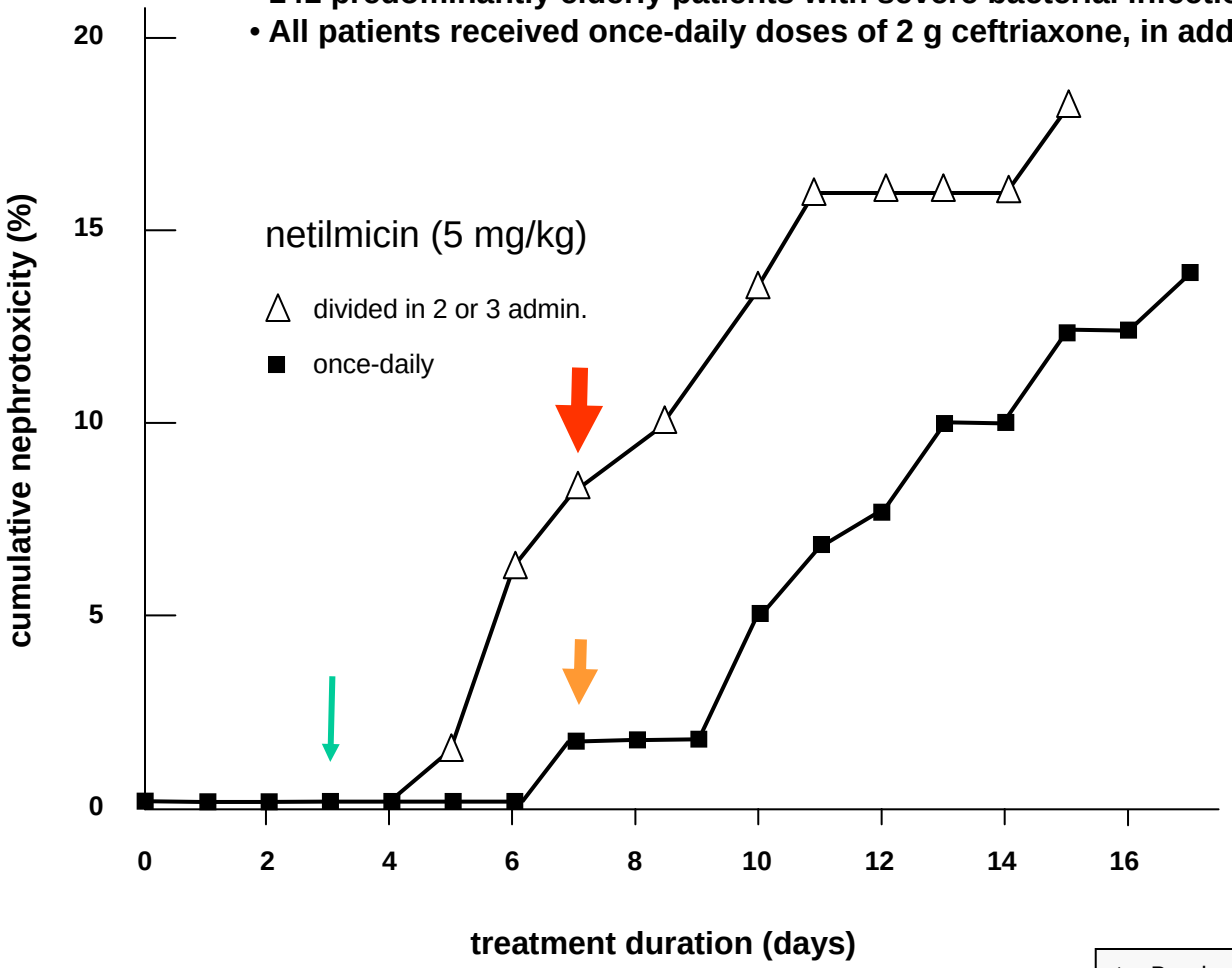
Tulkens *et al.*, 1989

Aminoglycoside peak / MIC ratio is predictive of clinical efficacy



Néphrotoxicity 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.

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)**

Conclusions

- aminoglycosides remain, even in 2012, potent and useful drugs against Gram (-) organisms if
  - appropriate resistance surveillance is in place
  - accepting that they need to be administered by intravenous route
  - toxicity is minimized by using a once-daily (extended interval) schedule and taking the known risk factors in due consideration...
- it may be possible to design/screen for new aminoglycosides with reduced toxicity based on our present knowledge of its mechanisms
- medicinal chemistry is needed to find new ways to avoid resistance (drug inactivation and target mutation...); additional screening may be needed to avoid efflux ...
- new aminoglycosides made along these lines could be important drugs in the future because of the demise of many other classes towards Gram (-) organisms (β-lactams, fluoroquinolones, ...)

Why not ?

It all started only a few years ago …