Nephrotoxicity of Aminoglycosides and Comparisons with Cis-Platinum

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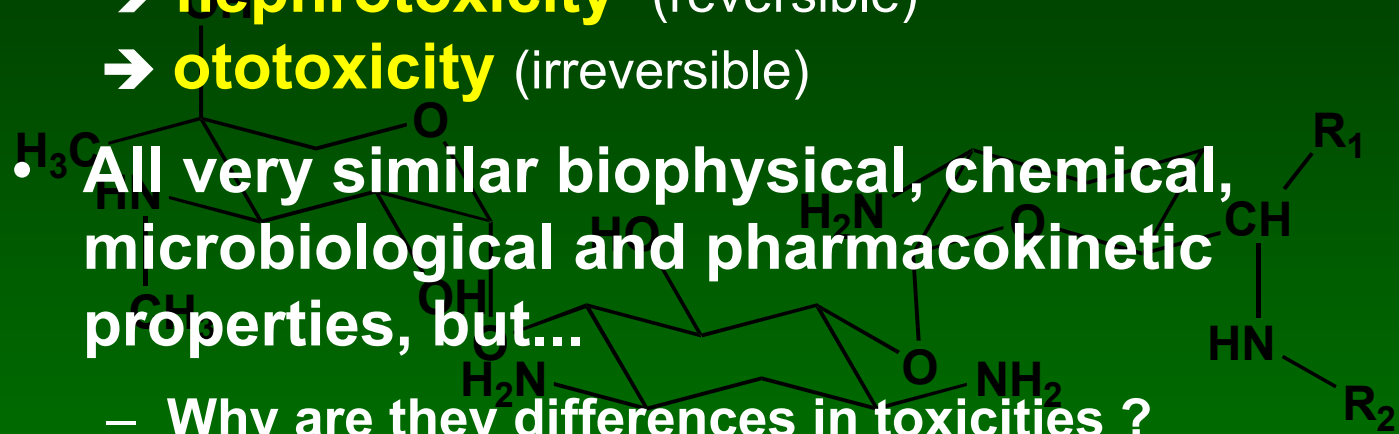
Aminoglycosides in the 70's ...

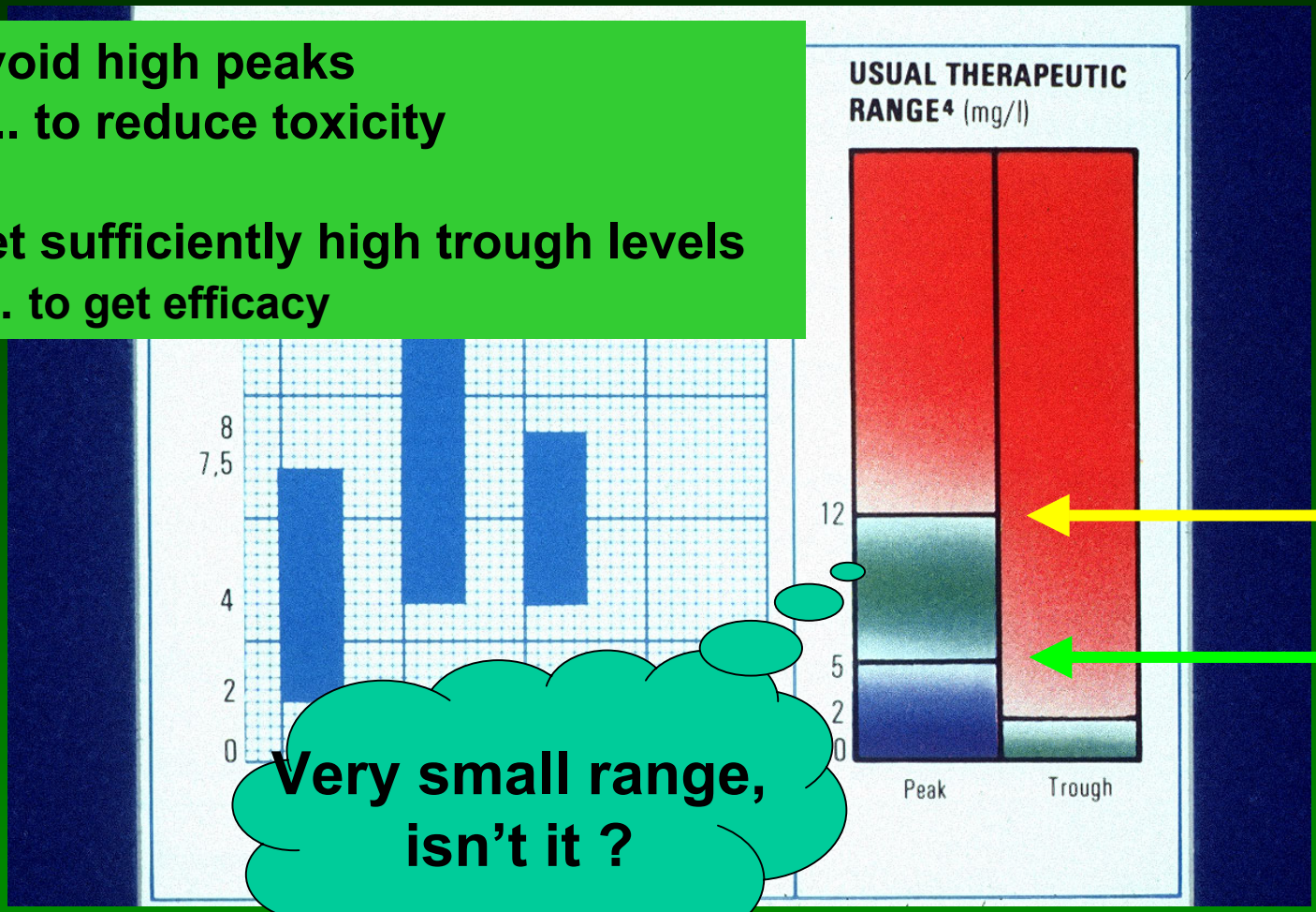
- **Potent antimicrobials but toxic**
  - ➔ **nephrotoxicity** (reversible)
  - ➔ **ototoxicity** (irreversible)
- **All very similar biophysical, chemical, microbiological and pharmacokinetic properties, but...**
  - **Why are they differences in toxicities ?**
  - **Are those differences real ?**
  - **What is/are the mechanism(s) ?**
  - **Can we protect patients ?**



Aminoglycosides monitoring in the 80's ...

avoid high peaks
... to reduce toxicity

get sufficiently high trough levels
… to get efficacy



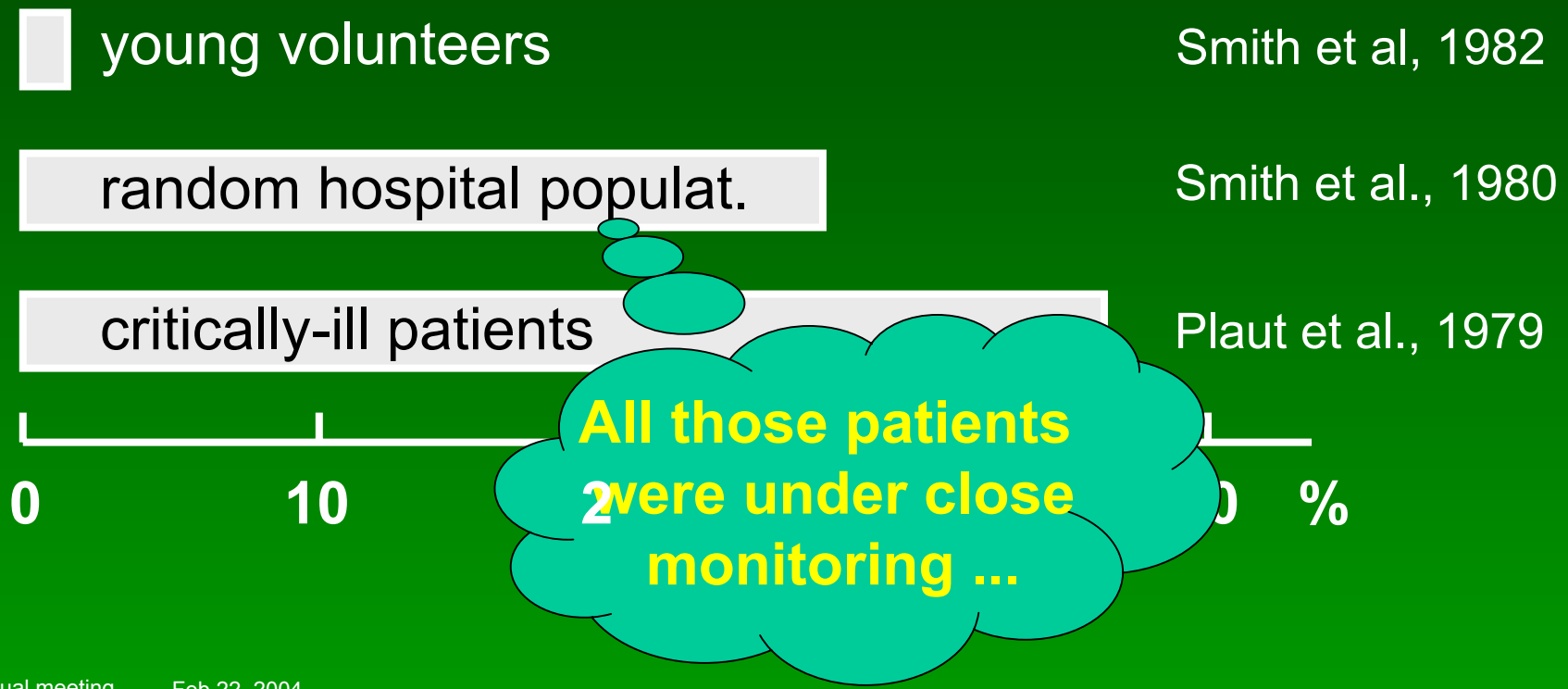
toxicity !!

lack of efficacy

Abott TdX manual, 1986

Aminoglycosides toxicity incidence is highly variable among patient populations

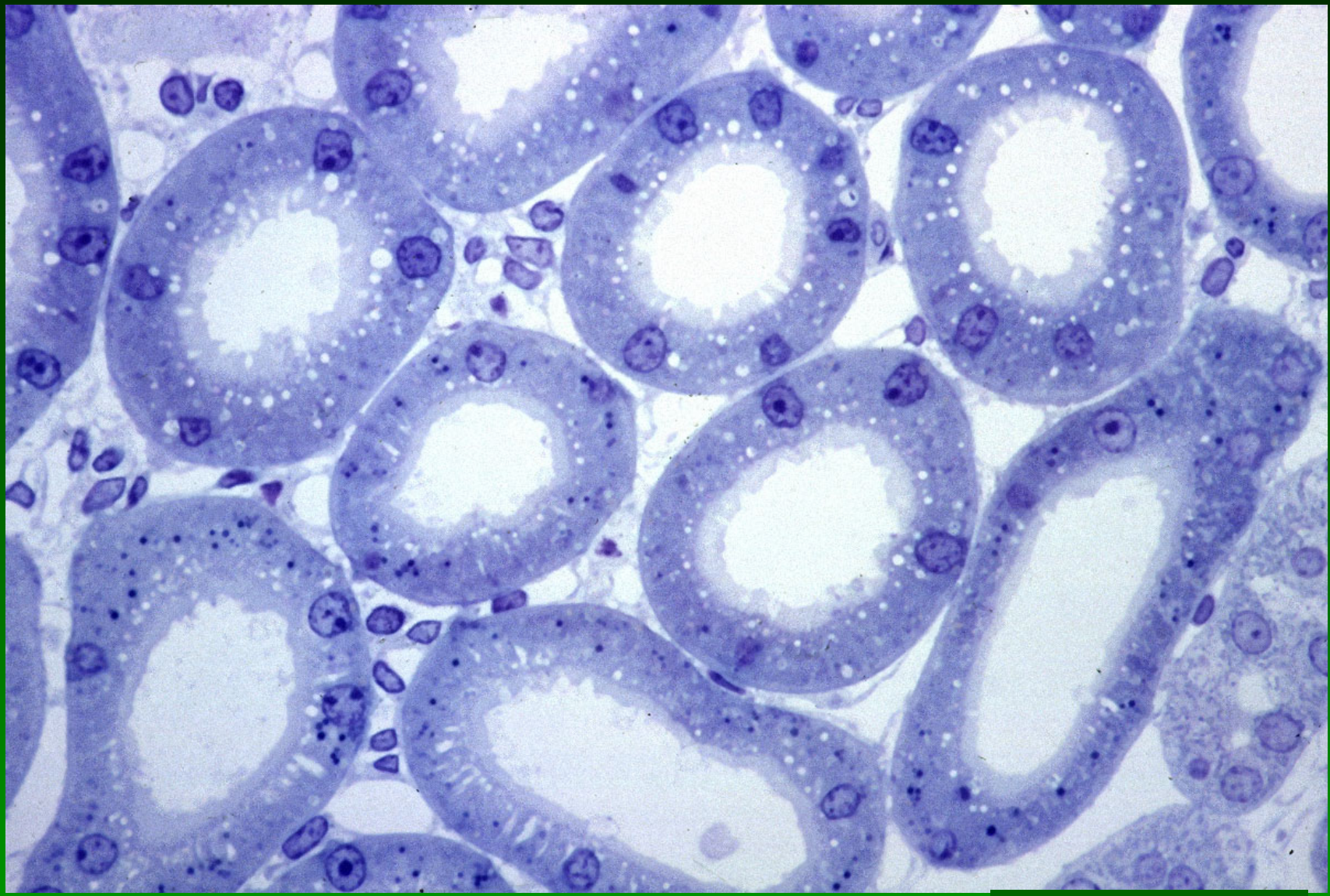
Patients with nephrotoxic reaction after treatment with gentamicin



A look in the microscope …

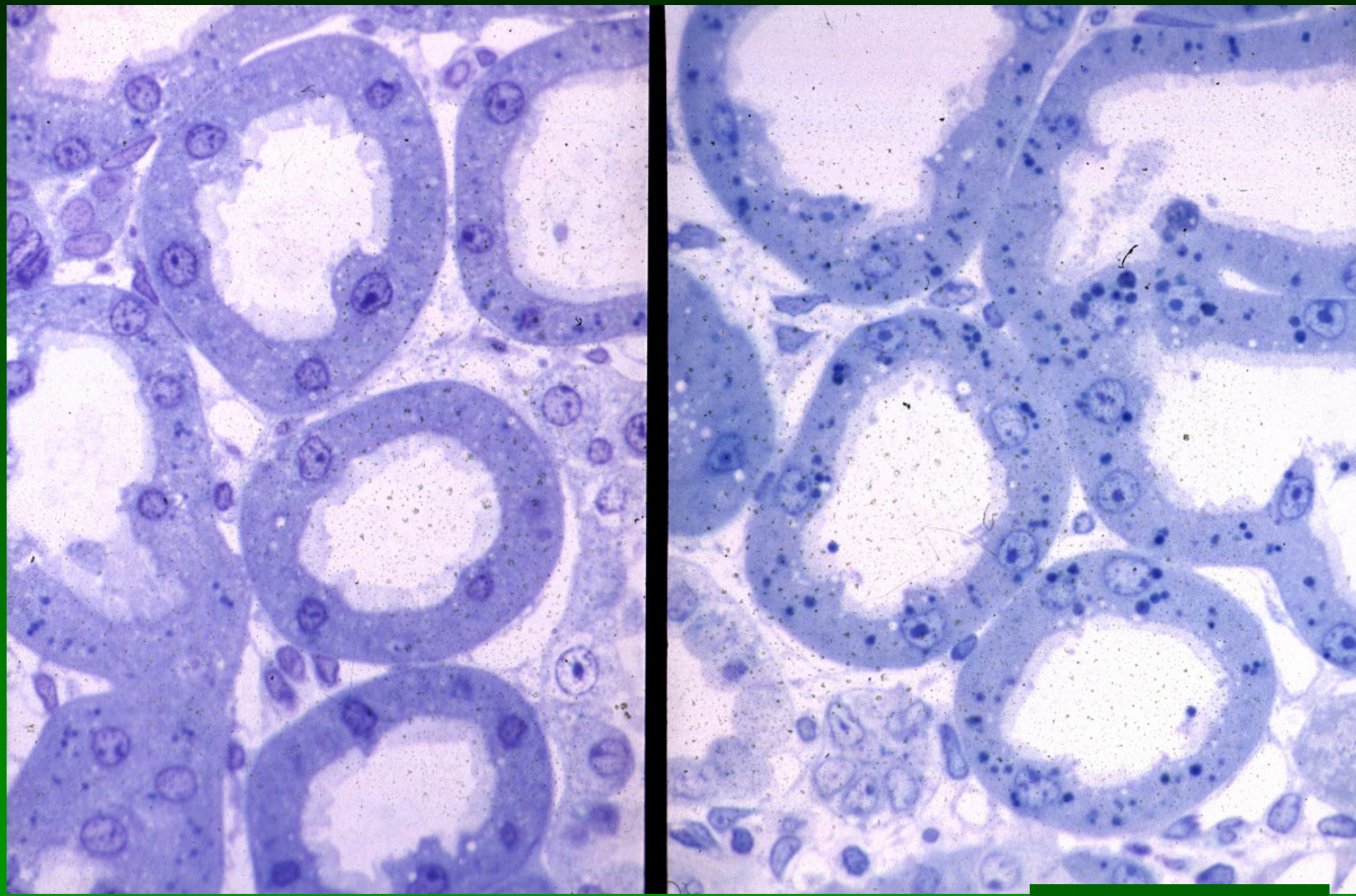
P. Maldague, unpublished

Somewhat closer …



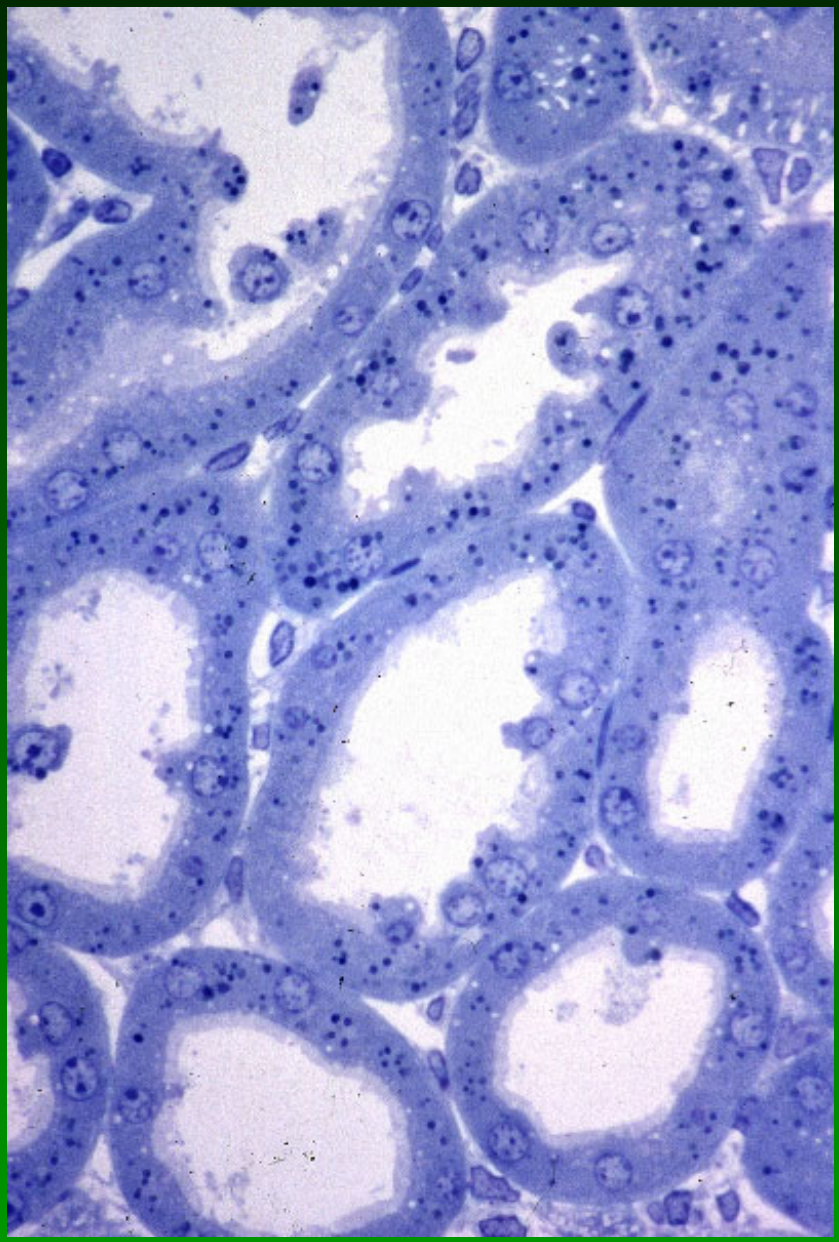
P. Maldague, unpublished

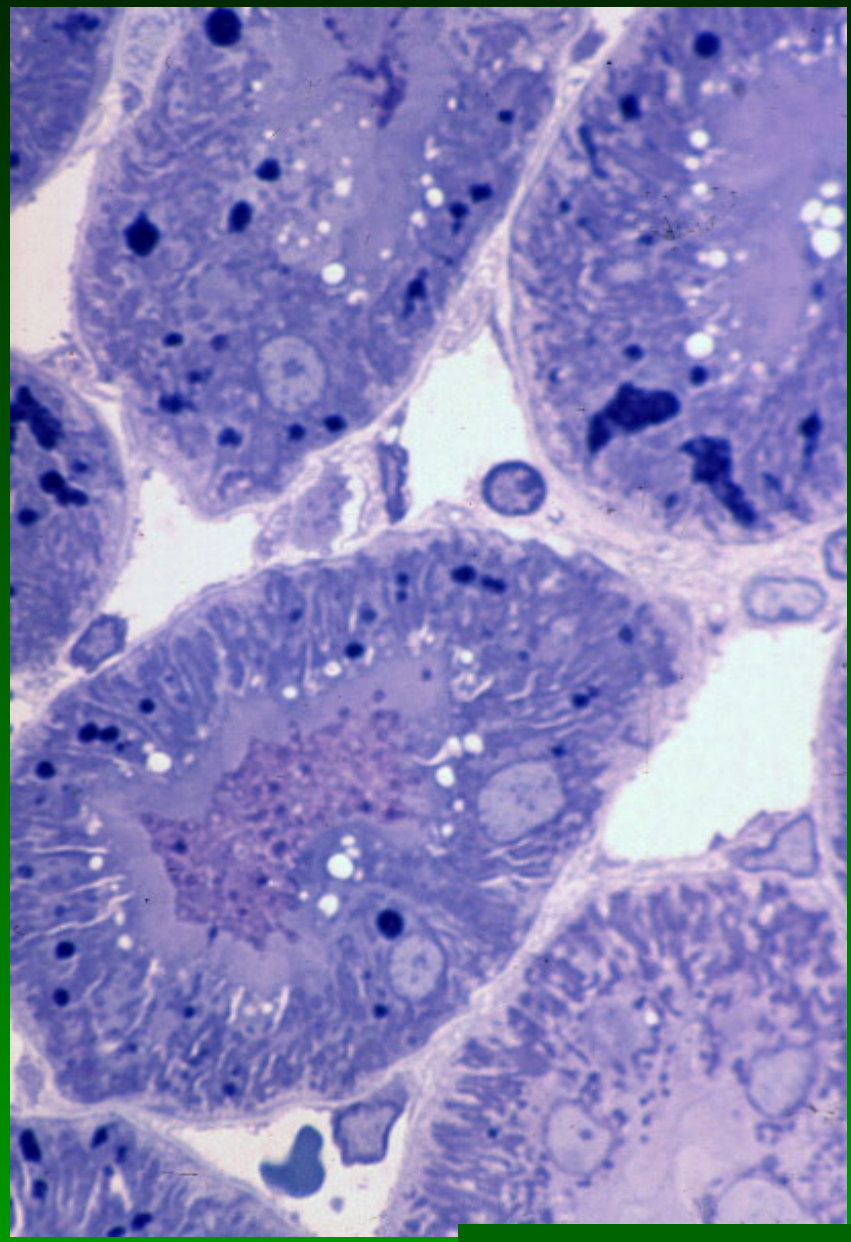
Compare …



P. Maldague, unpublished

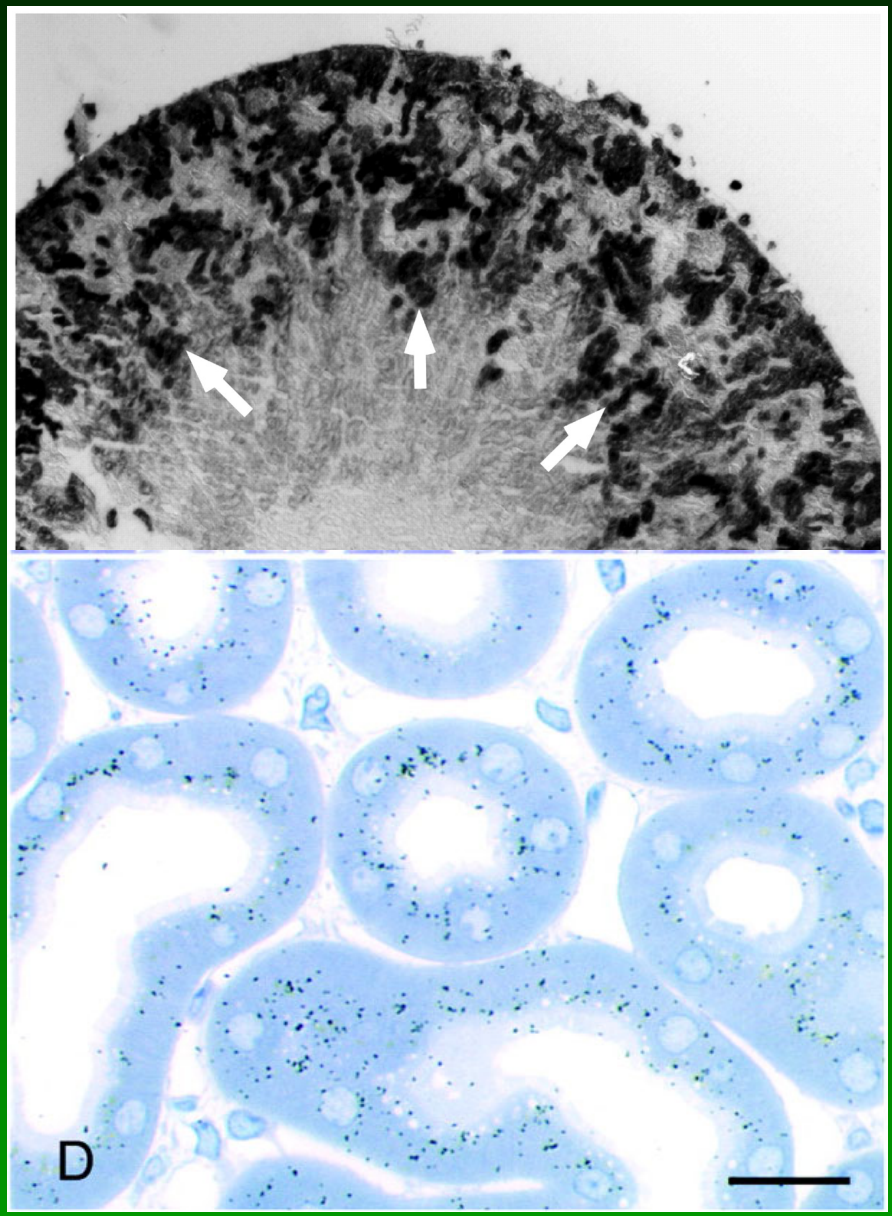
And examine …

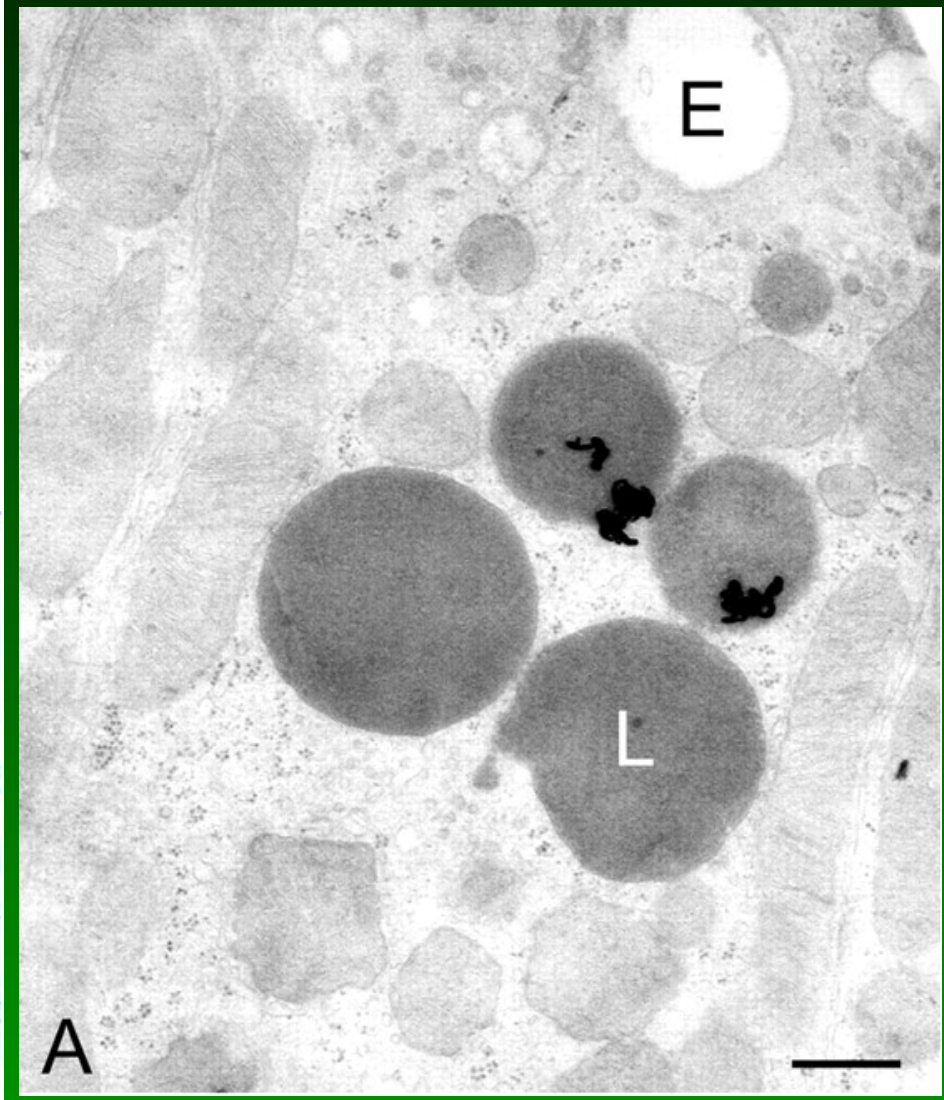




P. Maldague, unpublished

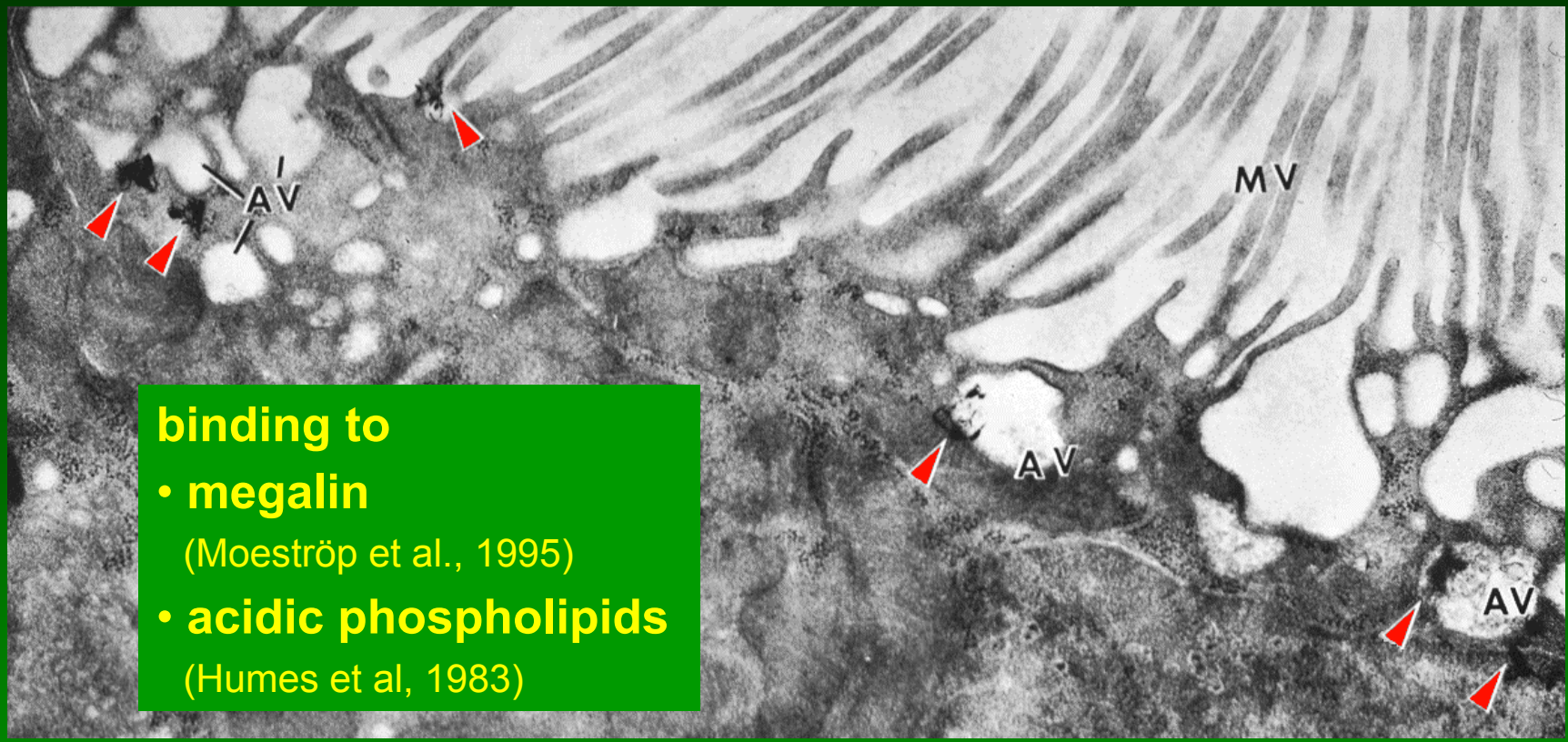
Gentamicin accumulates in lysosomes of proximal tubular cells





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

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

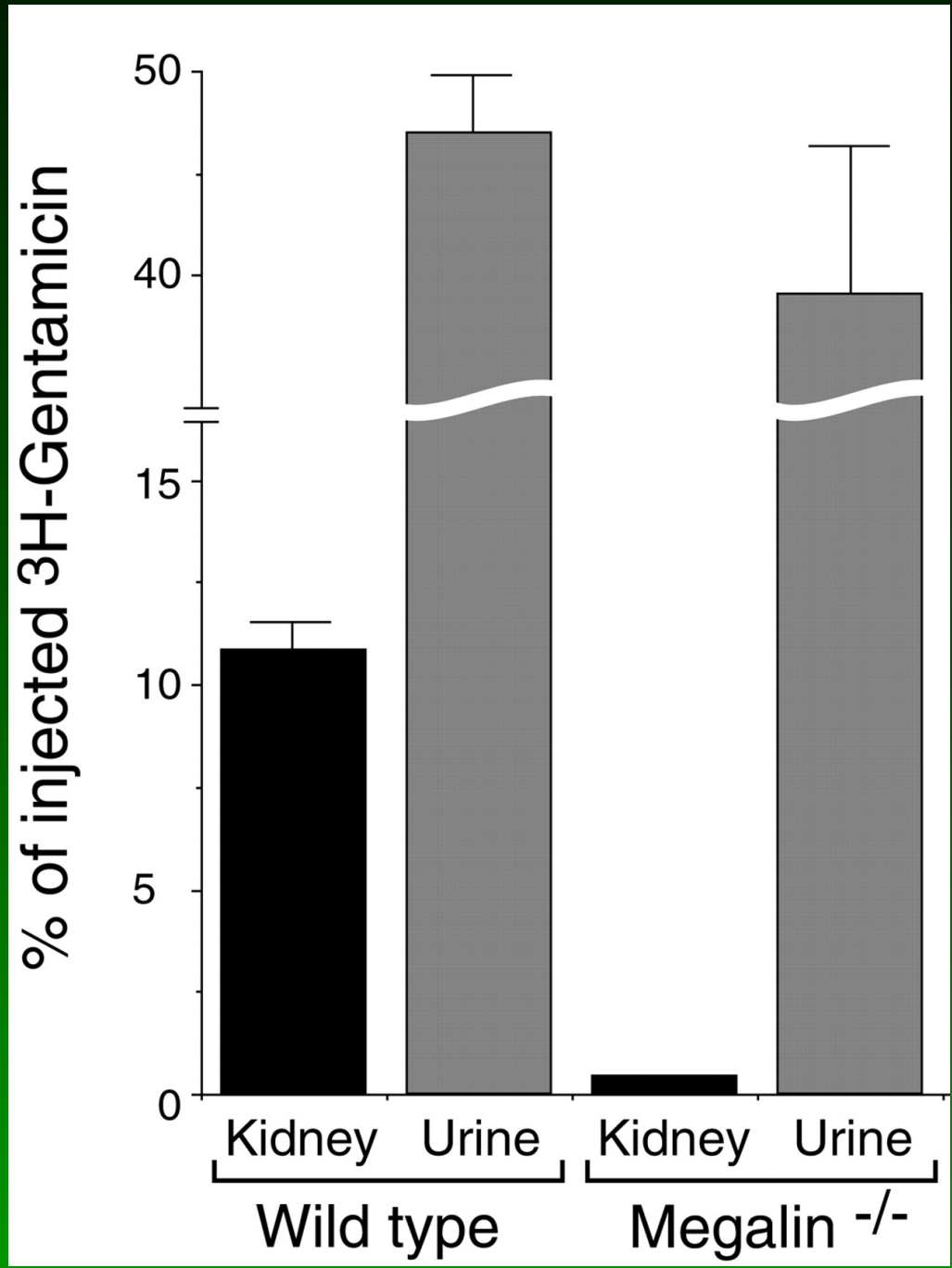


binding to

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

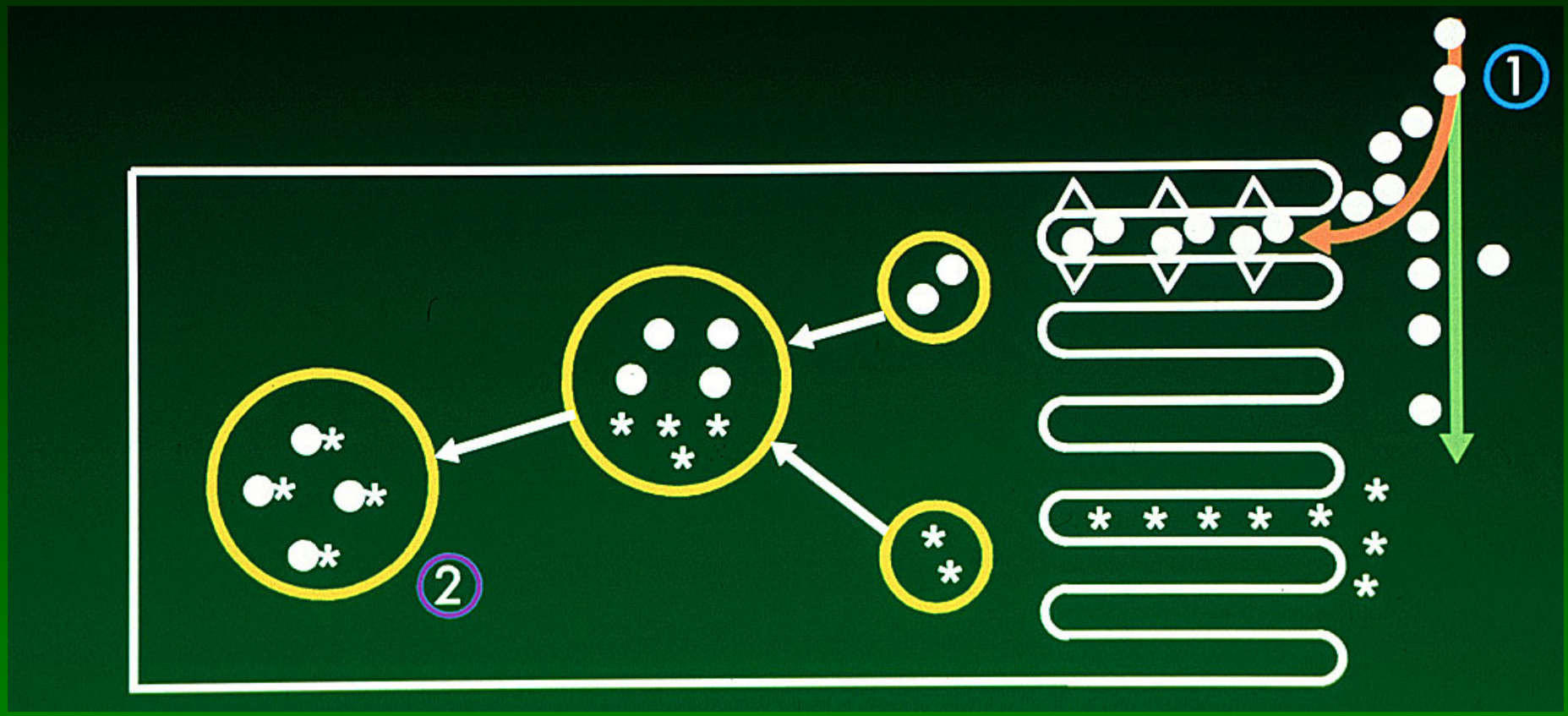
* Just *et al*, Naunym Schmied. Arch. Pharmacol, 1977
Silverblatt & Kuehen, Kidney Intern., 1979

Mice deficient in megalin do not accumulate gentamicin in kidney



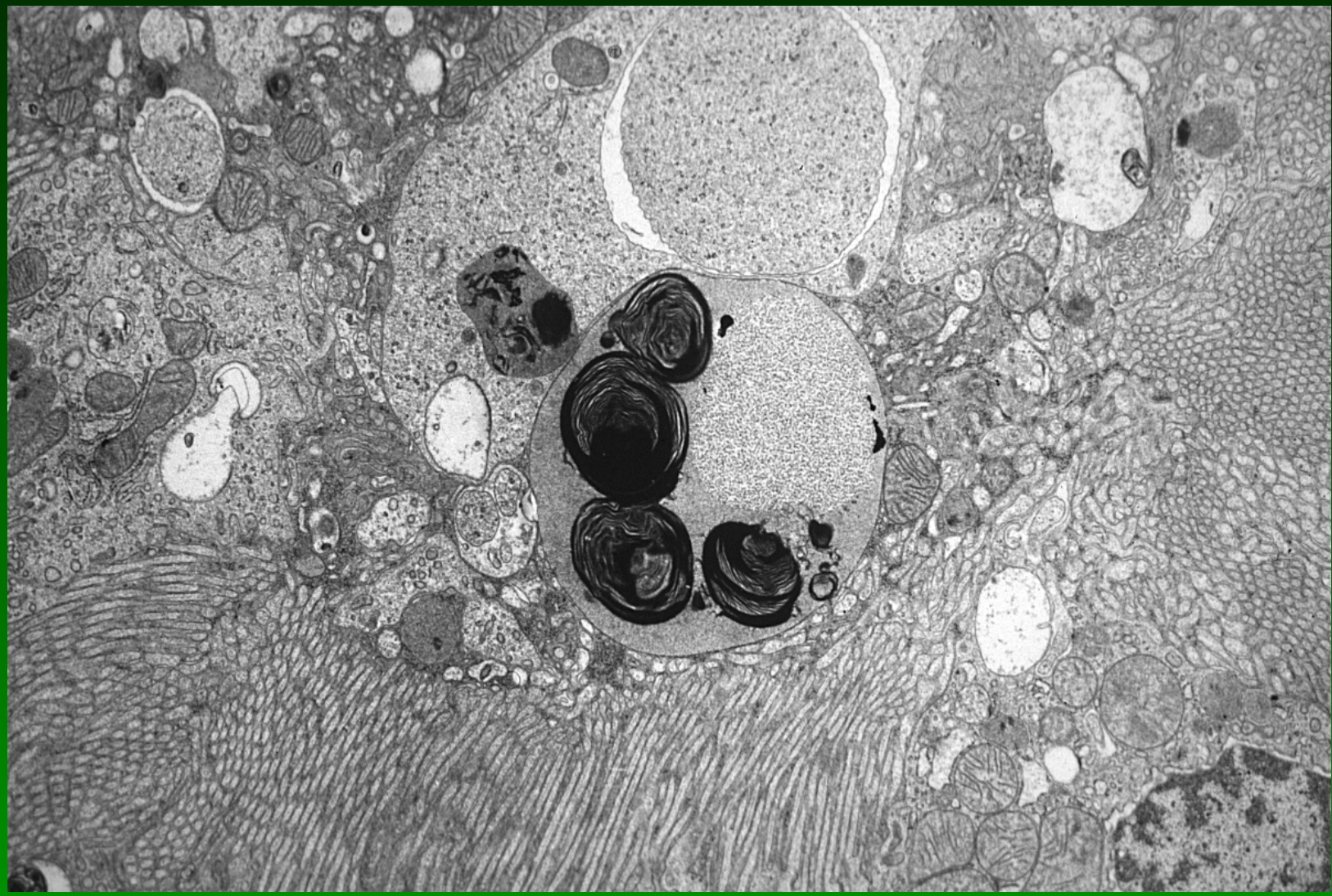
Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Towards a mechanism …



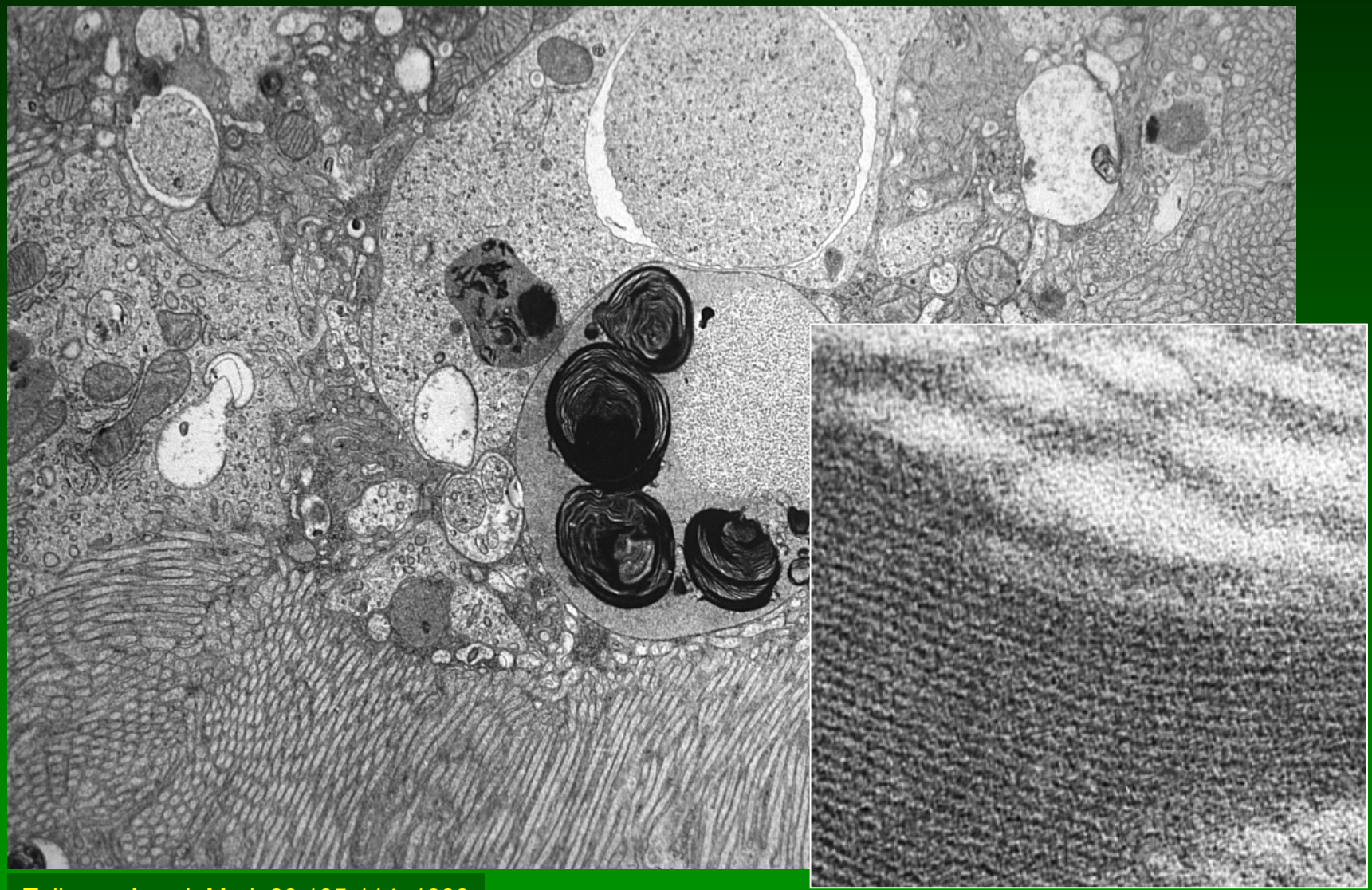
1. **binding to brush border**
2. **accumulation in lysosomes**

Intralysosomal gentamicin causes phospholipidosis



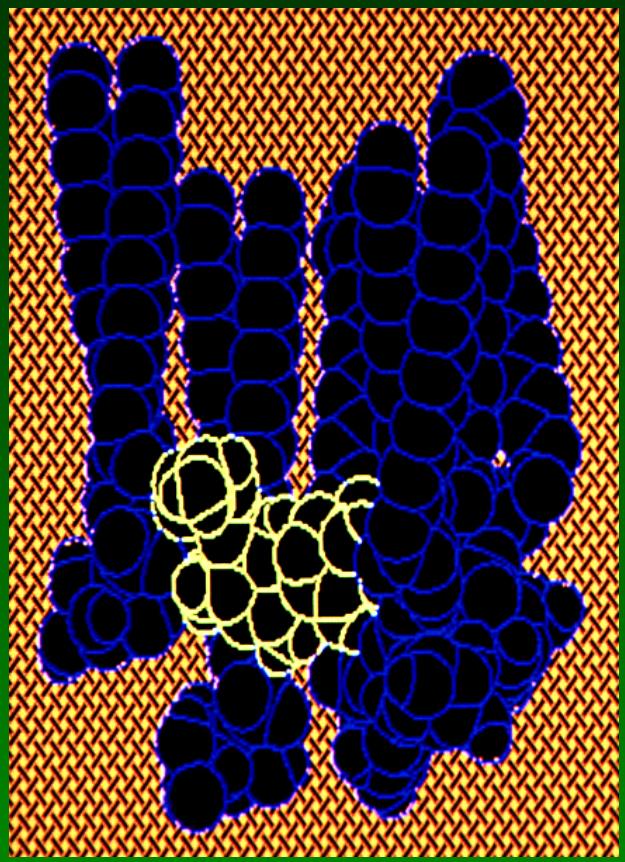
Tulkens, Am. J. Med. 80:105-114, 1986

Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis

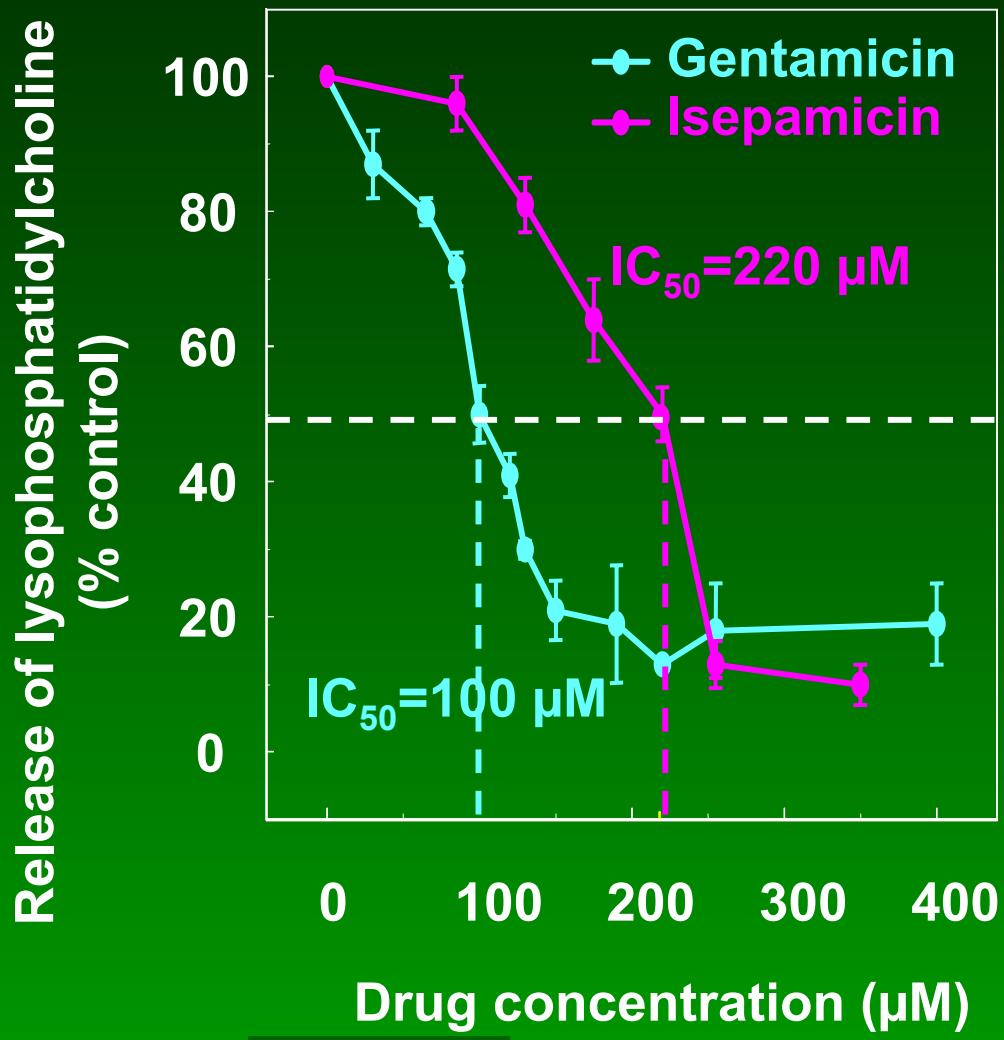


Tulkens, Am. J. Med. 80:105-114, 1986

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

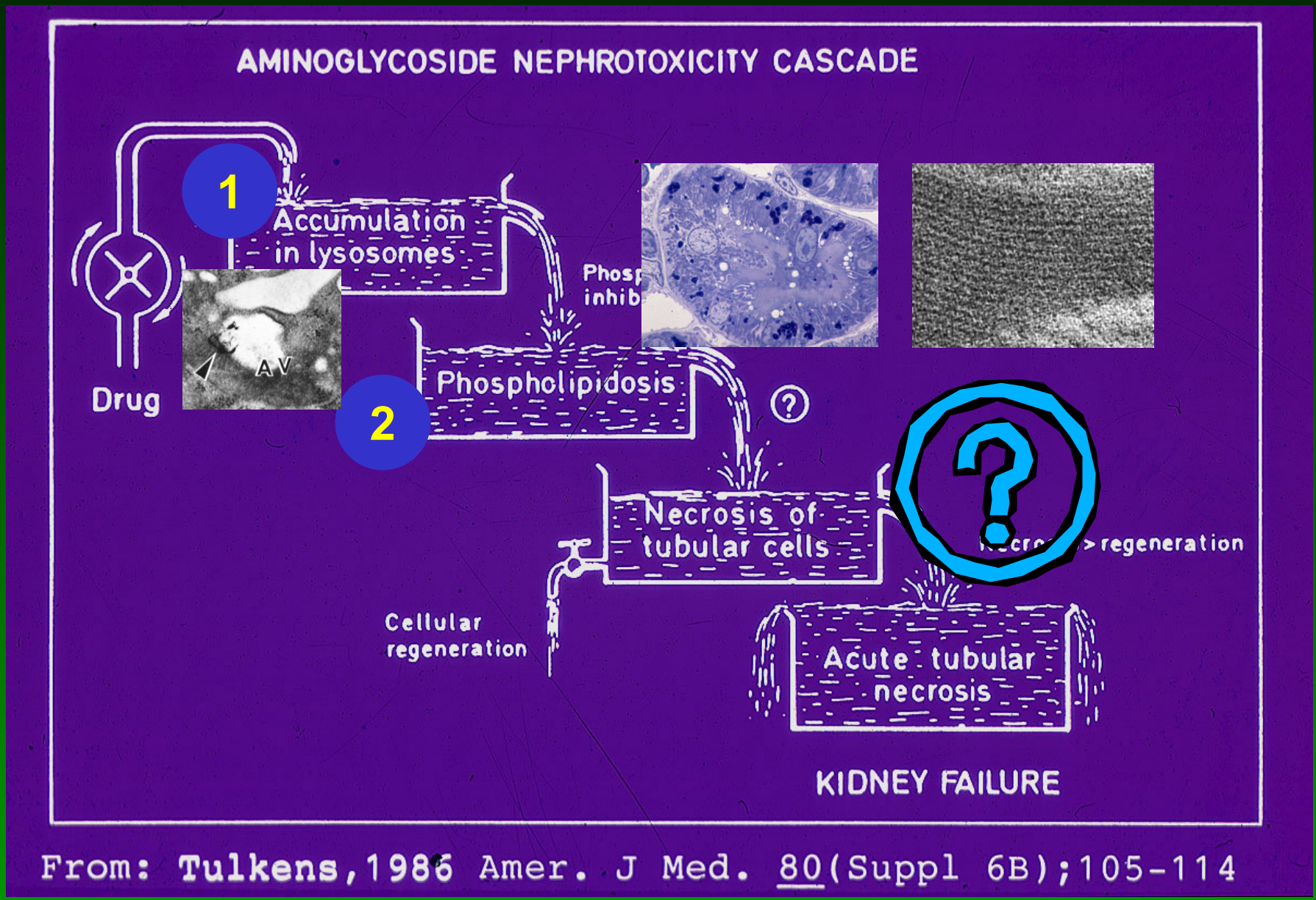


Adapted from Brasseur et al., 1989



P. Lambricht, 1991

A first global hypothesis ?...

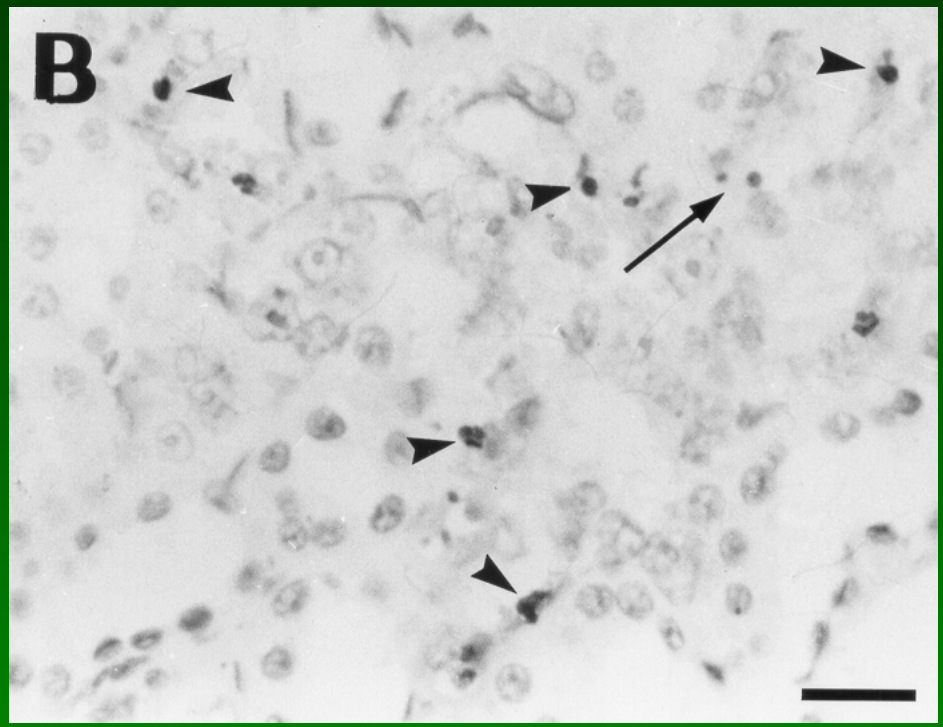


Gentamicin causes apoptosis at low, therapeutically-relevant dosages

Hematoxylin/eosin

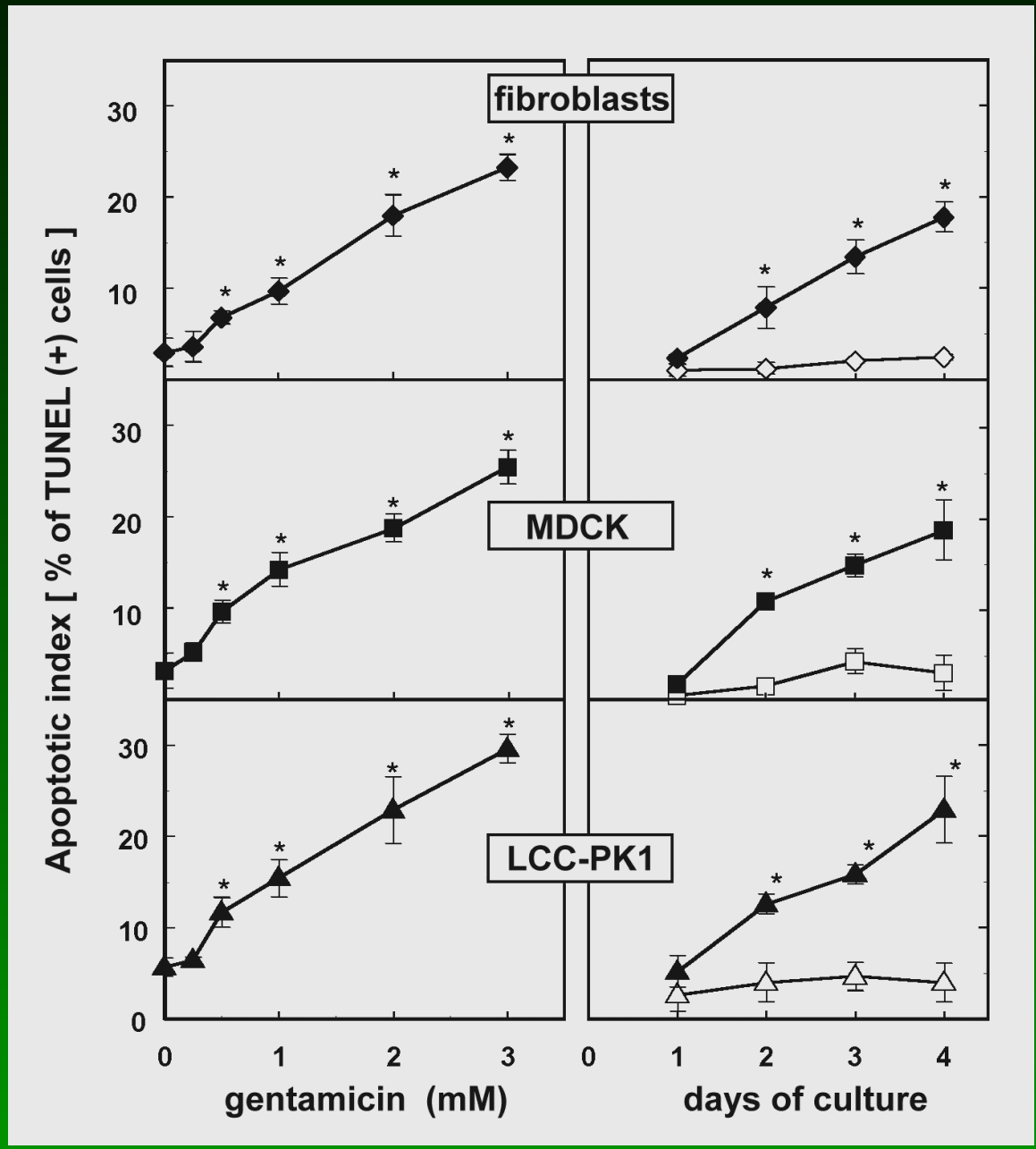
Laurent et al.,
Antimicrob. Agents Chemother.,
24:586-593, 1983

Tunel



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

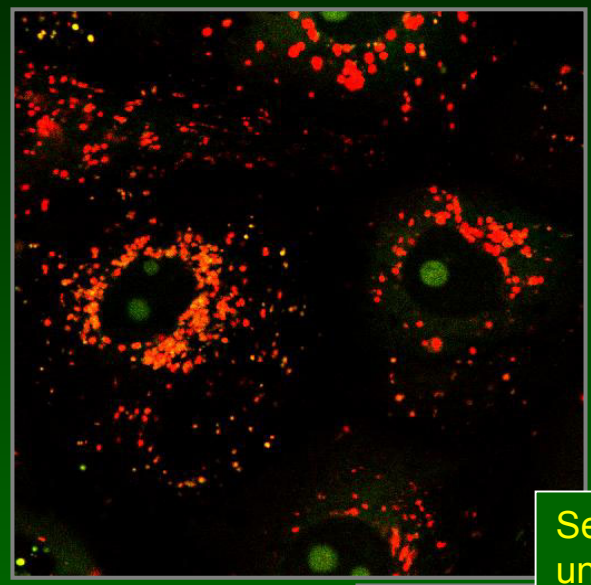
Gentamicin-induced apoptosis can be reproduced with cultured kidney and non-kidney cells …

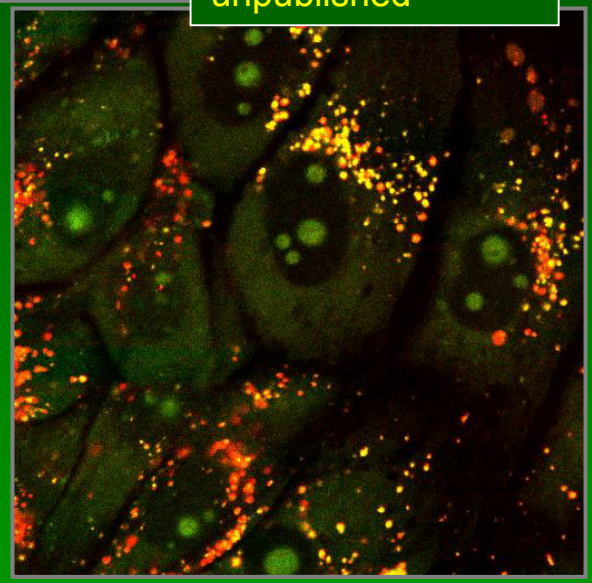


El Mouedden et al.,
Toxicol. Sci., 56:229-239, 2000

Is lysosomal rupture causing apoptosis and necrosis ?

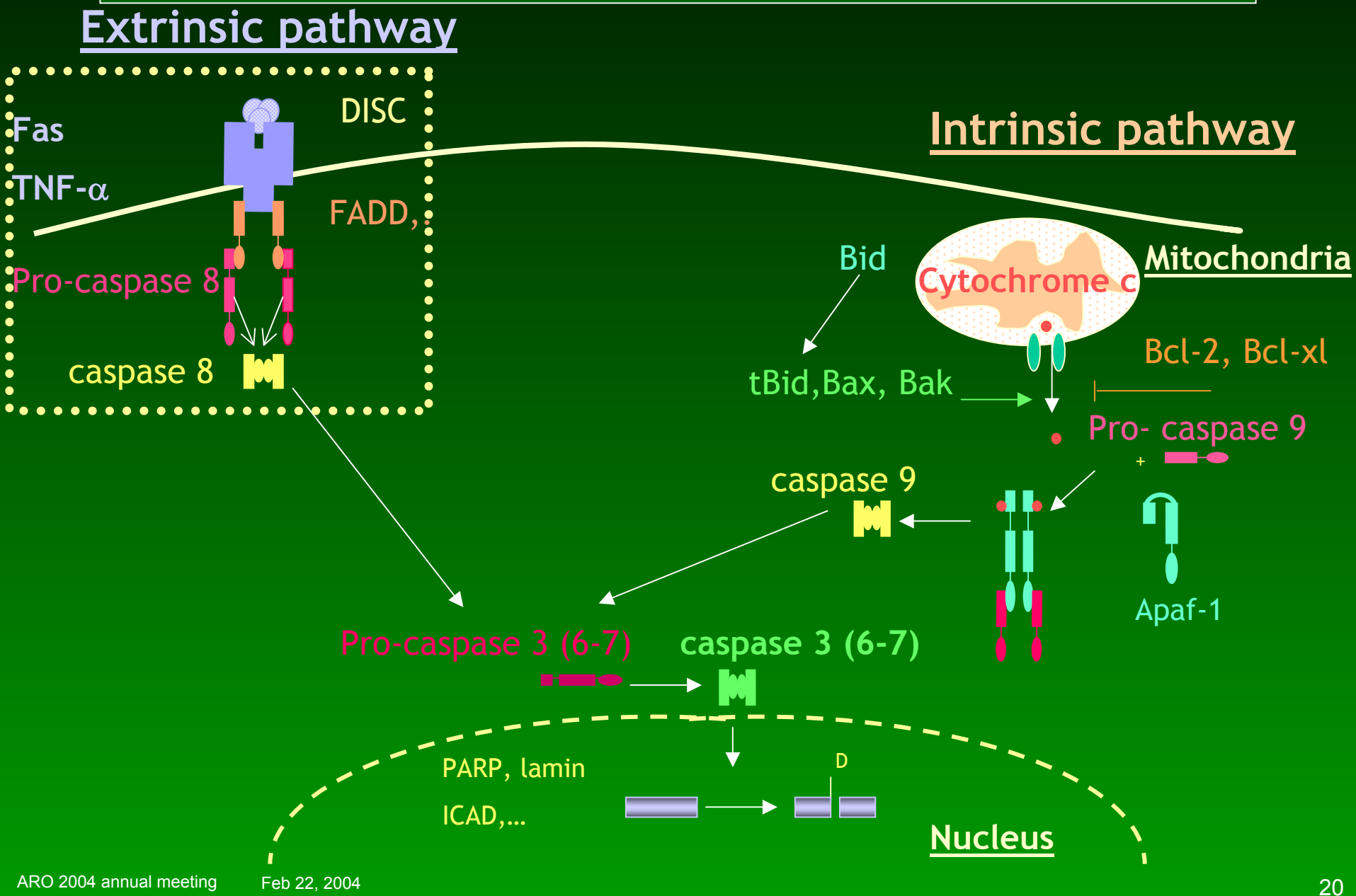
Maldague et al., 1983

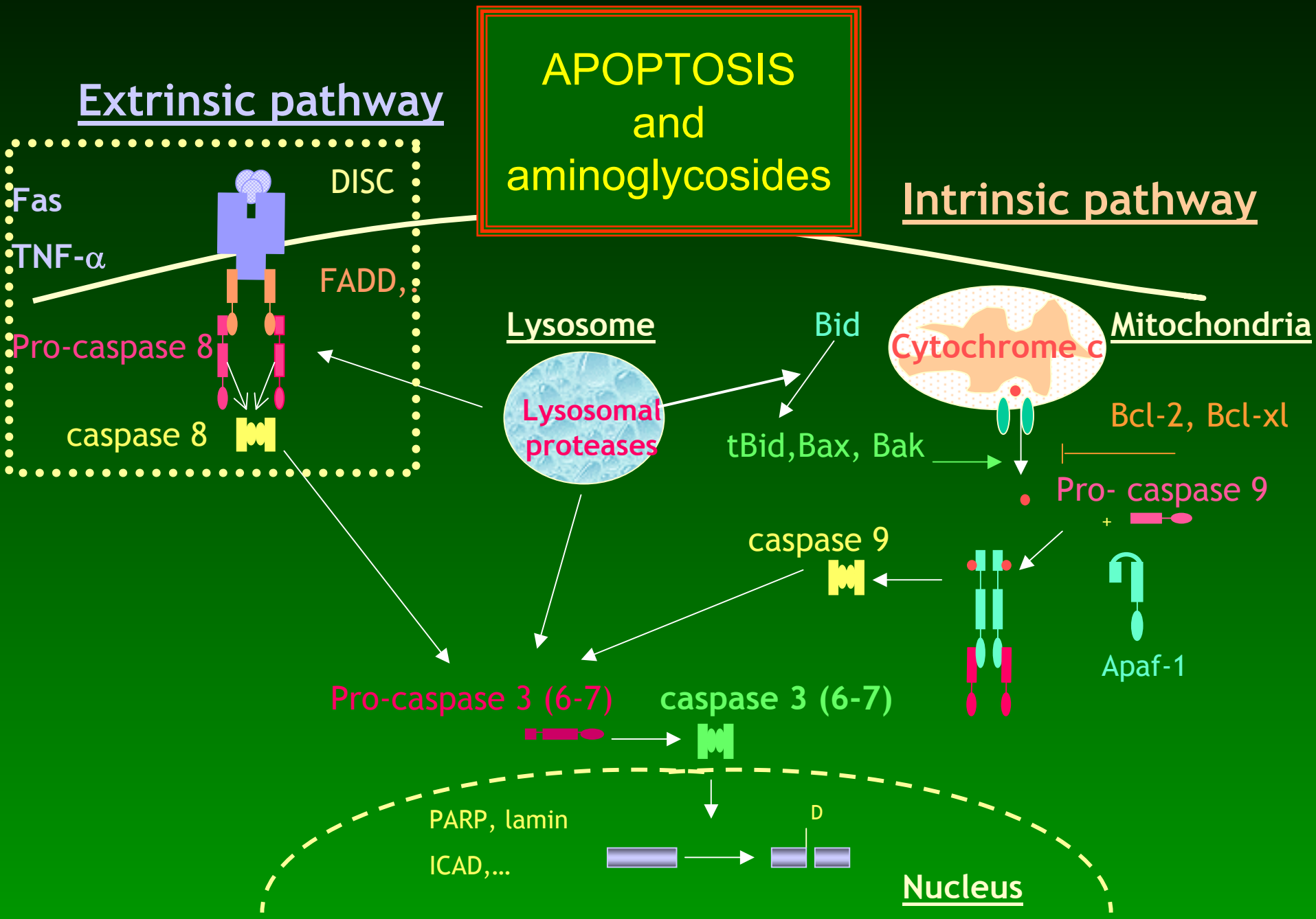




Servais et al., unpublished

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
tBid,Bax, Bak
Cytochrome c
Mitochondria
Bcl-2, Bcl-xl
Pro- caspase 9
caspase 9
Apaf-1
Pro-caspase 3 (6-7)
caspase 3 (6-7)
PARP, lamin
ICAD,...
D
Nucleus

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…

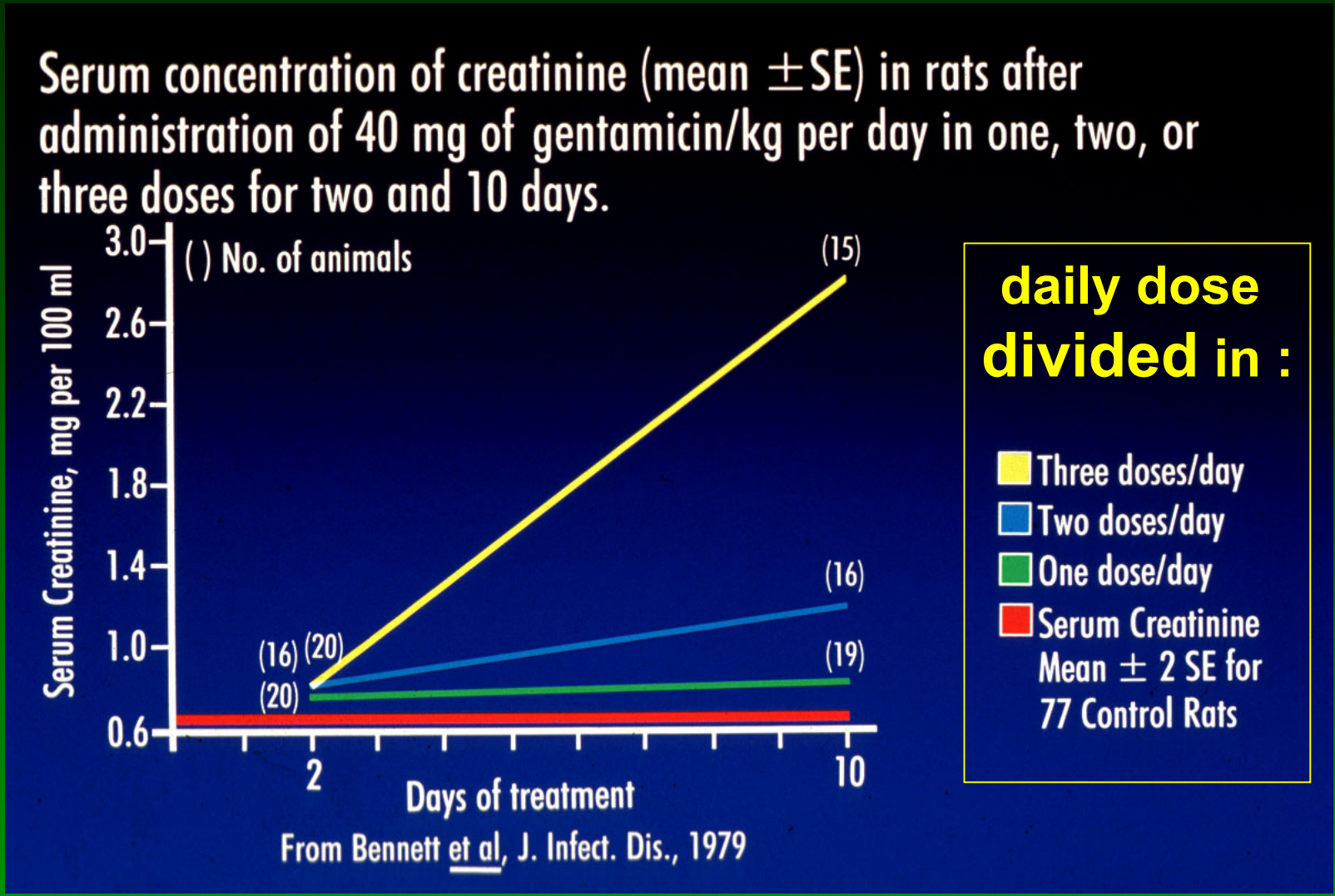
Mingeot & Tulkens, Antimicrob. Agents Chemother. 43:1003-1012, 1999

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

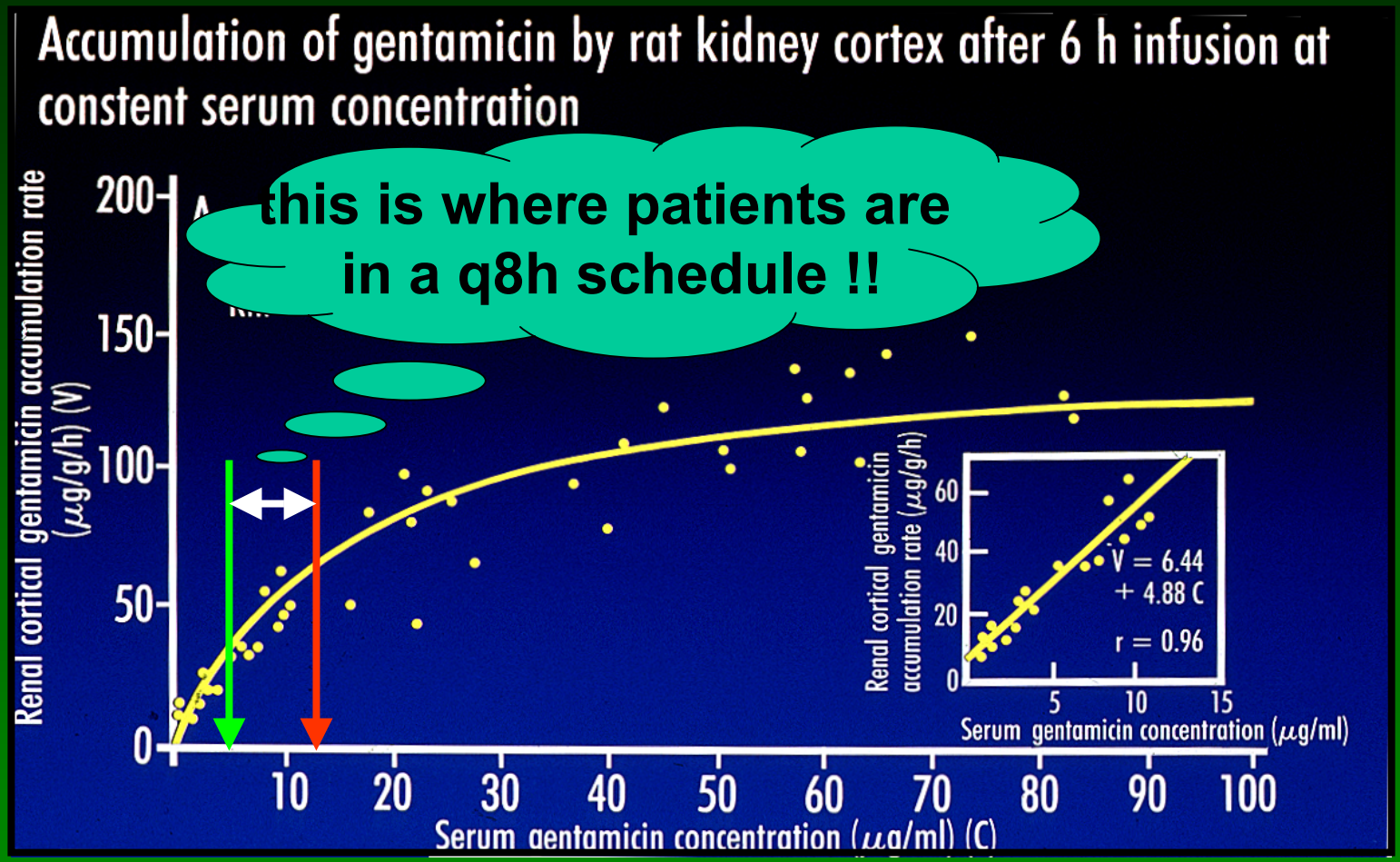
Mechanism	Compound
I. Decrease or prevention of drug accumulation by kidneys	
Intracellular complexation of aminoglycosides	
Polyanionic compounds	Dextran sulfate (59) Inositol hexasulfate (67)
Acidic drugs	Piperacillin (44) Latamoxef-moxalactam (68) Fosfomycin (33, 54) Pyridoxal-5′-phosphate (114)
Competition 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	Flerotaxacin (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) Ducrimicin (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.

Aminoglycoside toxicity is not linked to peak ...

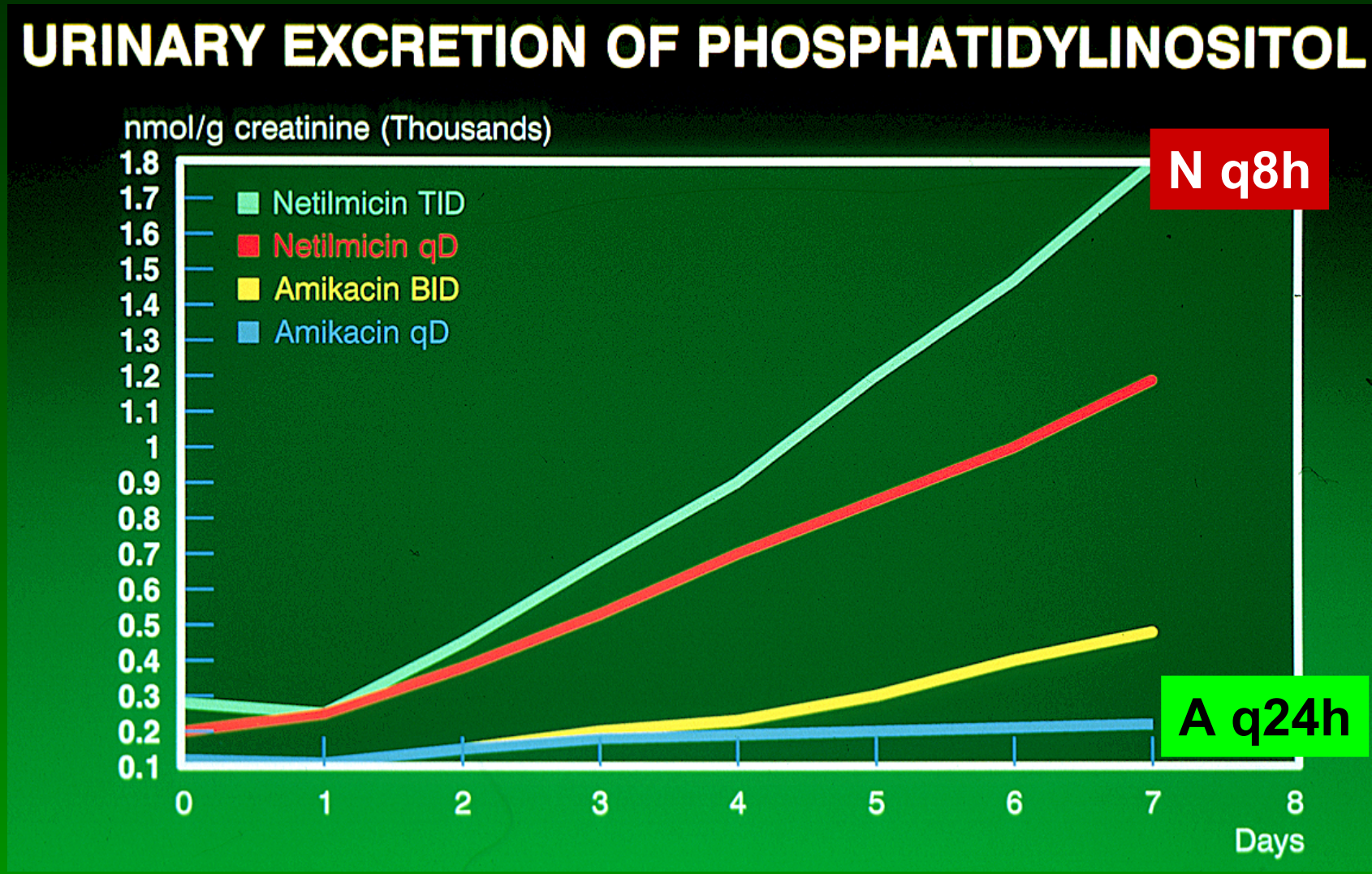


Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...



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

Phospholipiduria …

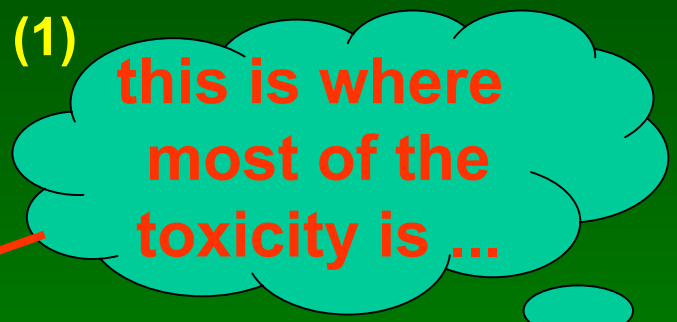


Tulkens et al., 1989

And auditory alterations …

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

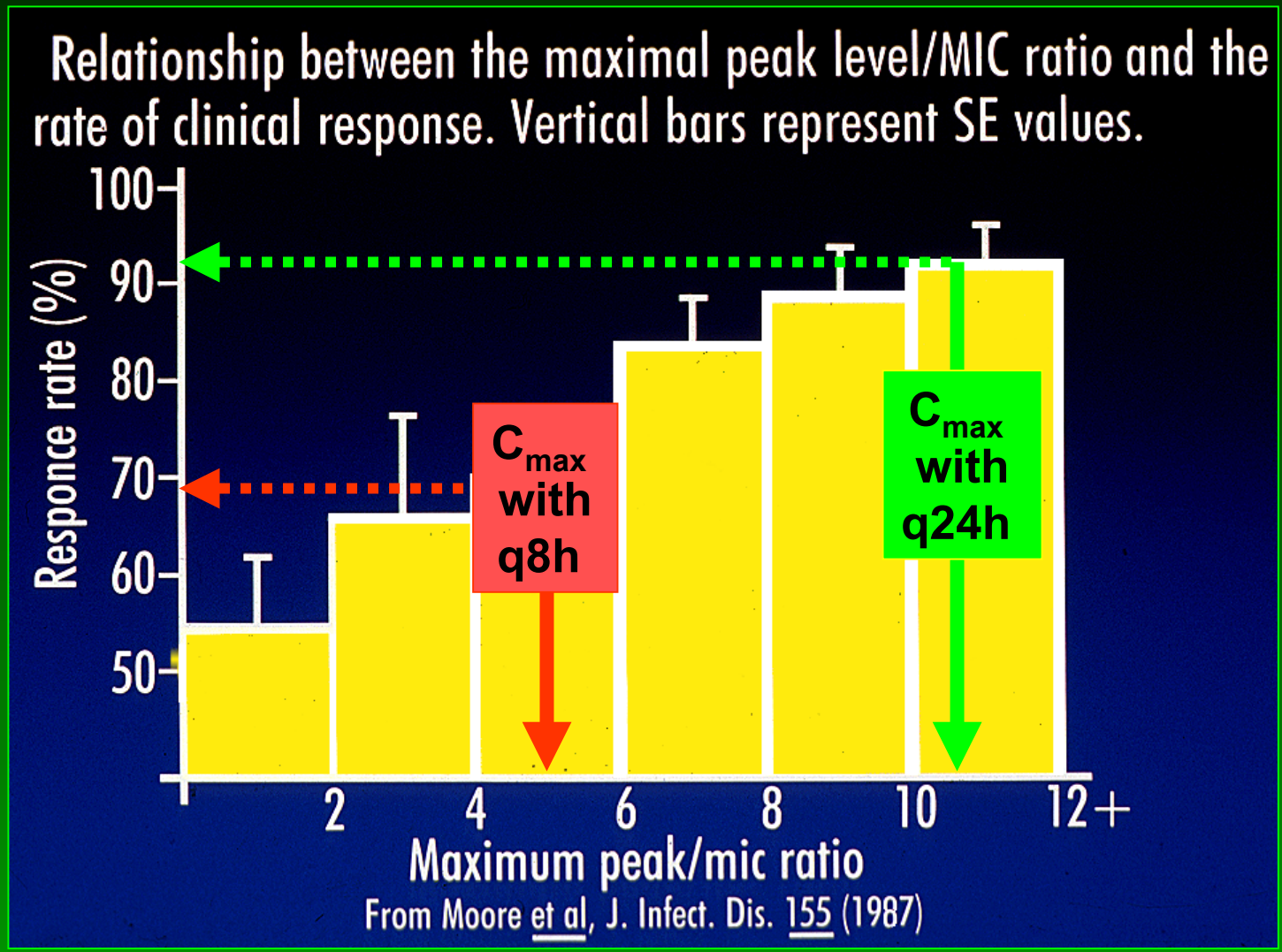
	low tone (0.25-8 kHz)	high tone (10-18 kHz)
amikacin		
• q24h	**1 (1)**	**3 (4)**
• q12h	**0**	**6 (6)**
netilmicin		
• q24h	**0**	**3 (7)**
• q8h	**2 (3)**	**8 (9)**



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

Tulkens et al., 1989

Aminoglycosidepeak /MIC ratio is predictive of clinical efficacy



Is the once-a-day schedule used ?

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

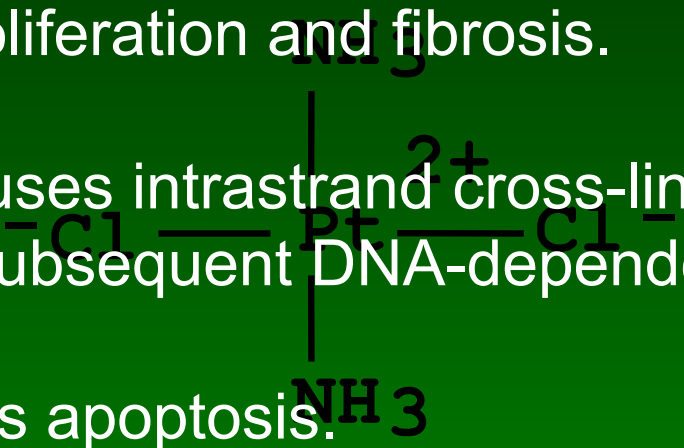
National survey of extended-interval aminoglycoside dosing (EIAD).

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)**

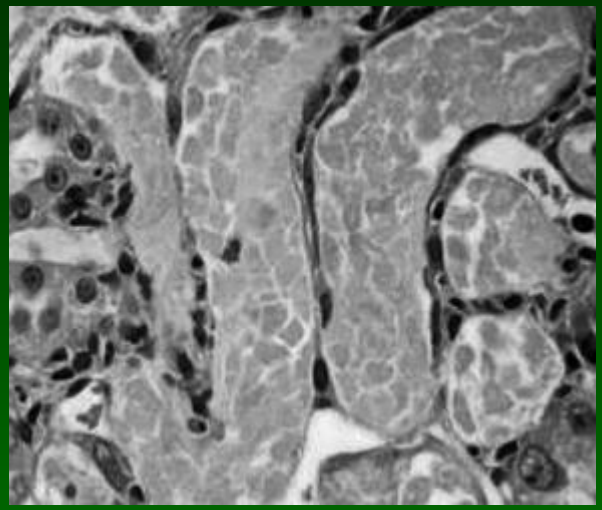
And what bout cis-platin now …

- induces a more chronic type of renal failure, mostly in S3 segment
- with large focal necrosis, cyst formation and marked interstitial proliferation and fibrosis.



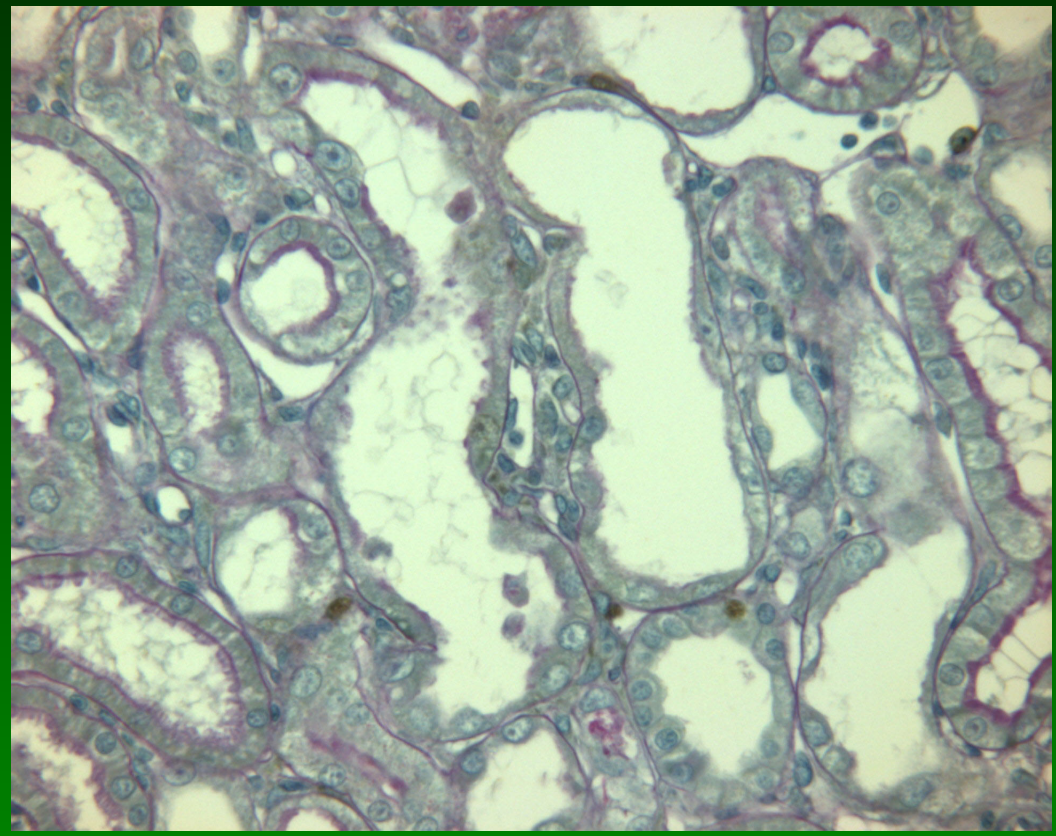
- Cis-platin causes intrastrand cross-linking in DNA, blocking all subsequent DNA-dependent activities.
- It also triggers apoptosis.
- Compared to AG, post-necrosis and post-apoptotic regeneration seem largely impaired in cis-platin renal toxicity.

Cisplatin induces widespread tubular necroses and destruction



5 days after a single 5 mg/kg body weight injection

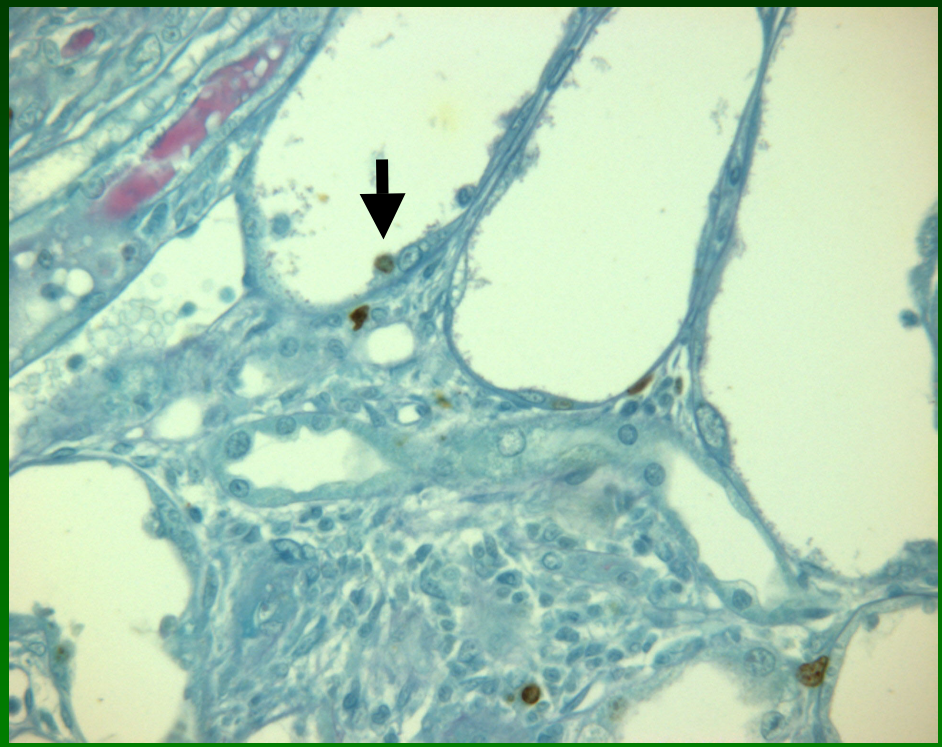
From: Sheikh-Hamad et al., Arch Toxicol. 2003

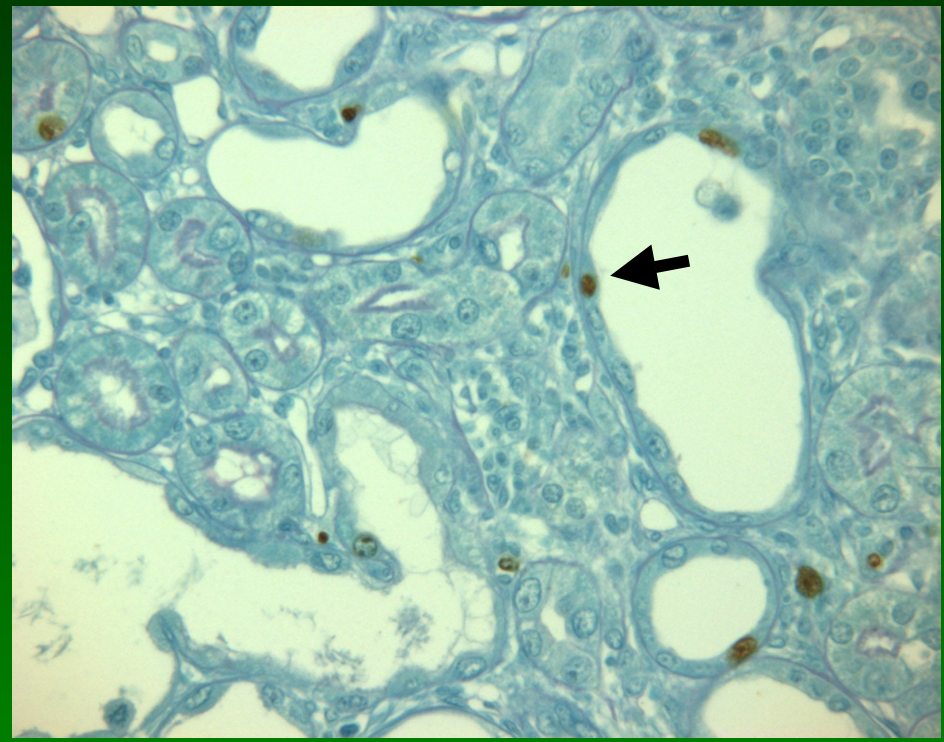


7 days after a single 8 mg/kg body weight injection

Coutesy of G. Laurent

Cyst formation and failing regeneration...

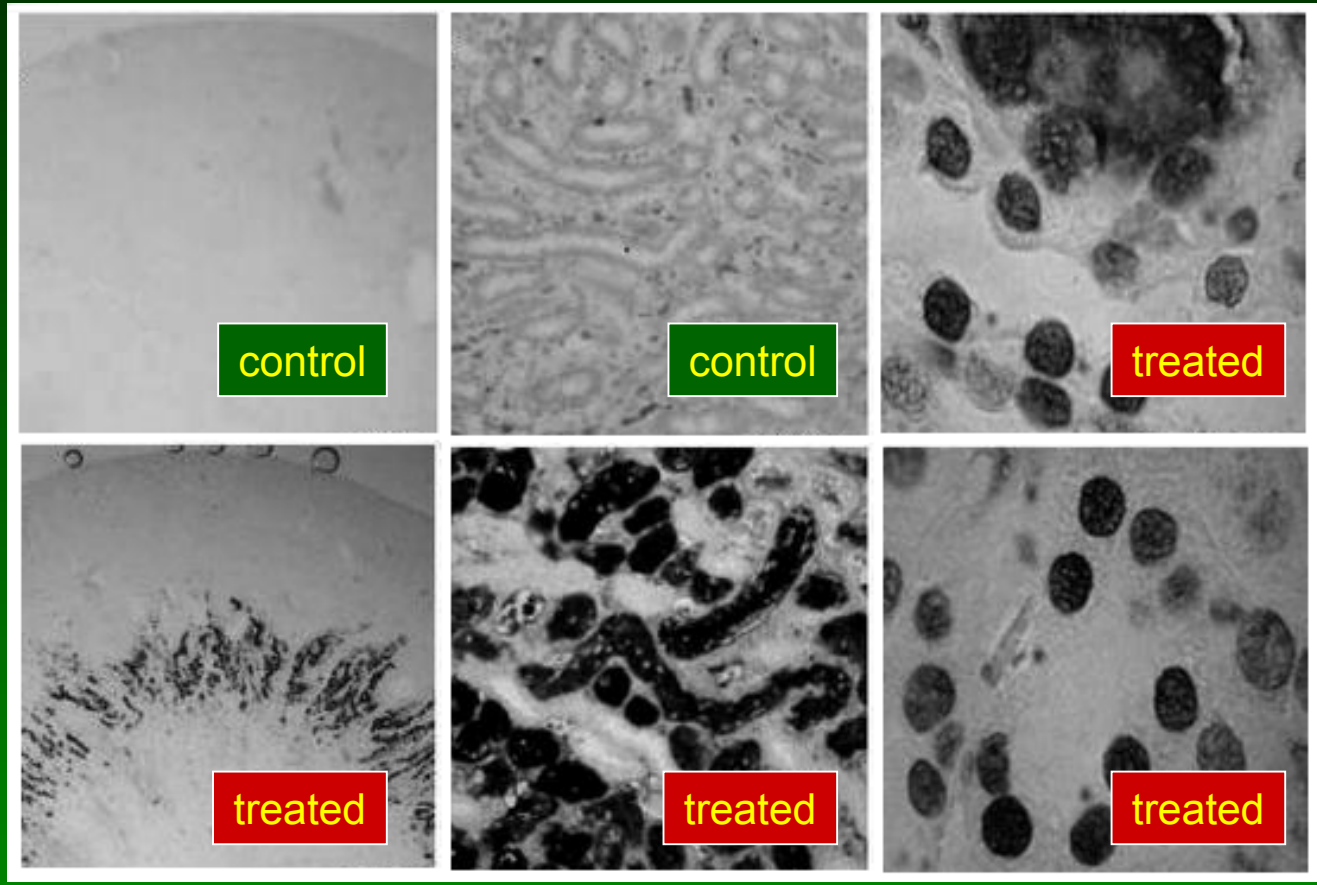




21 days after a single 8 mg/kg body weight injection
(BdU-PAP staining)
Coutesy of G. Laurent, 2004

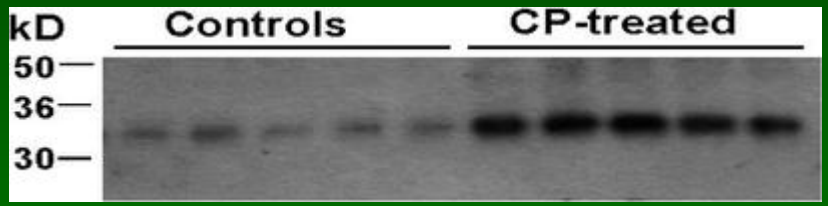
But also extended apoptosis …

Tunel staining

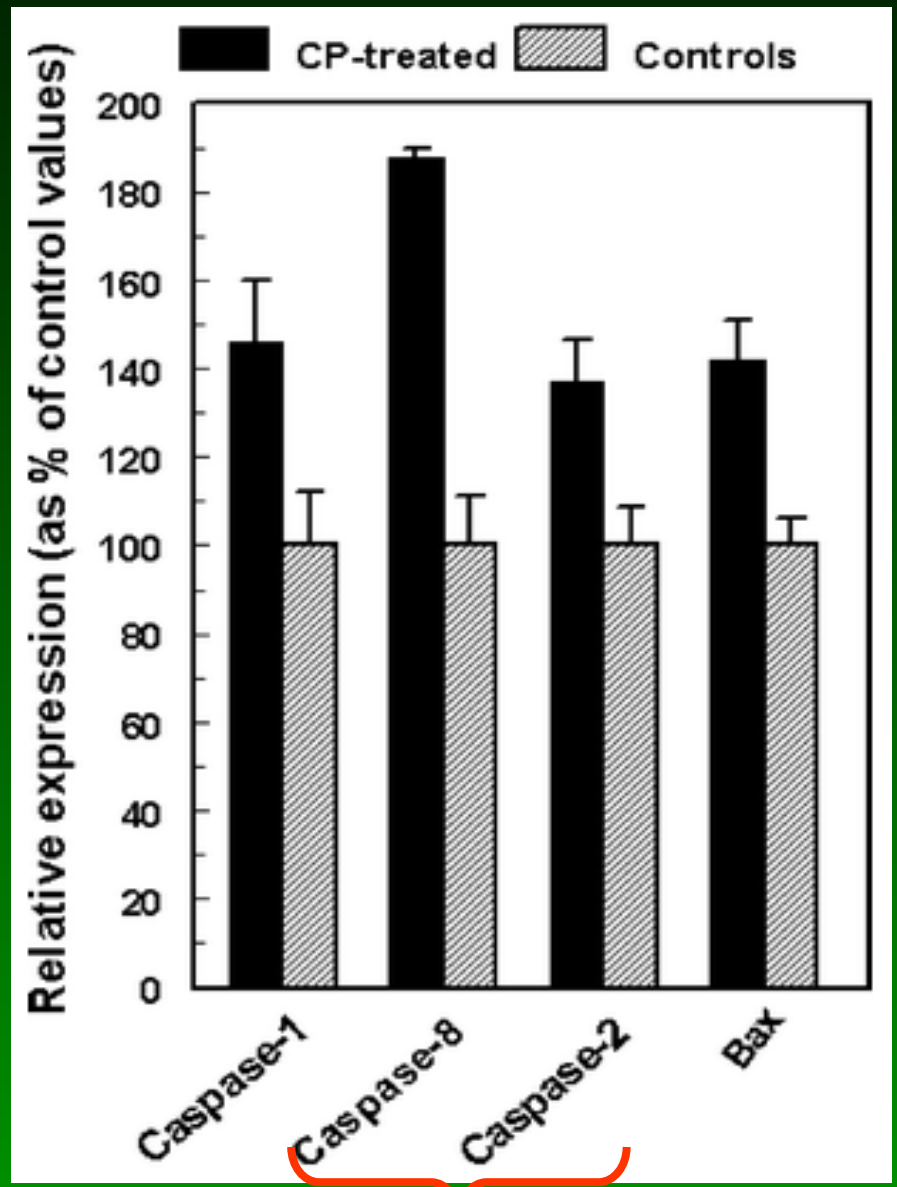


Sheikh-Hamad et al., Arch Toxicol. 2003 Oct 10 [Epub ahead of print]

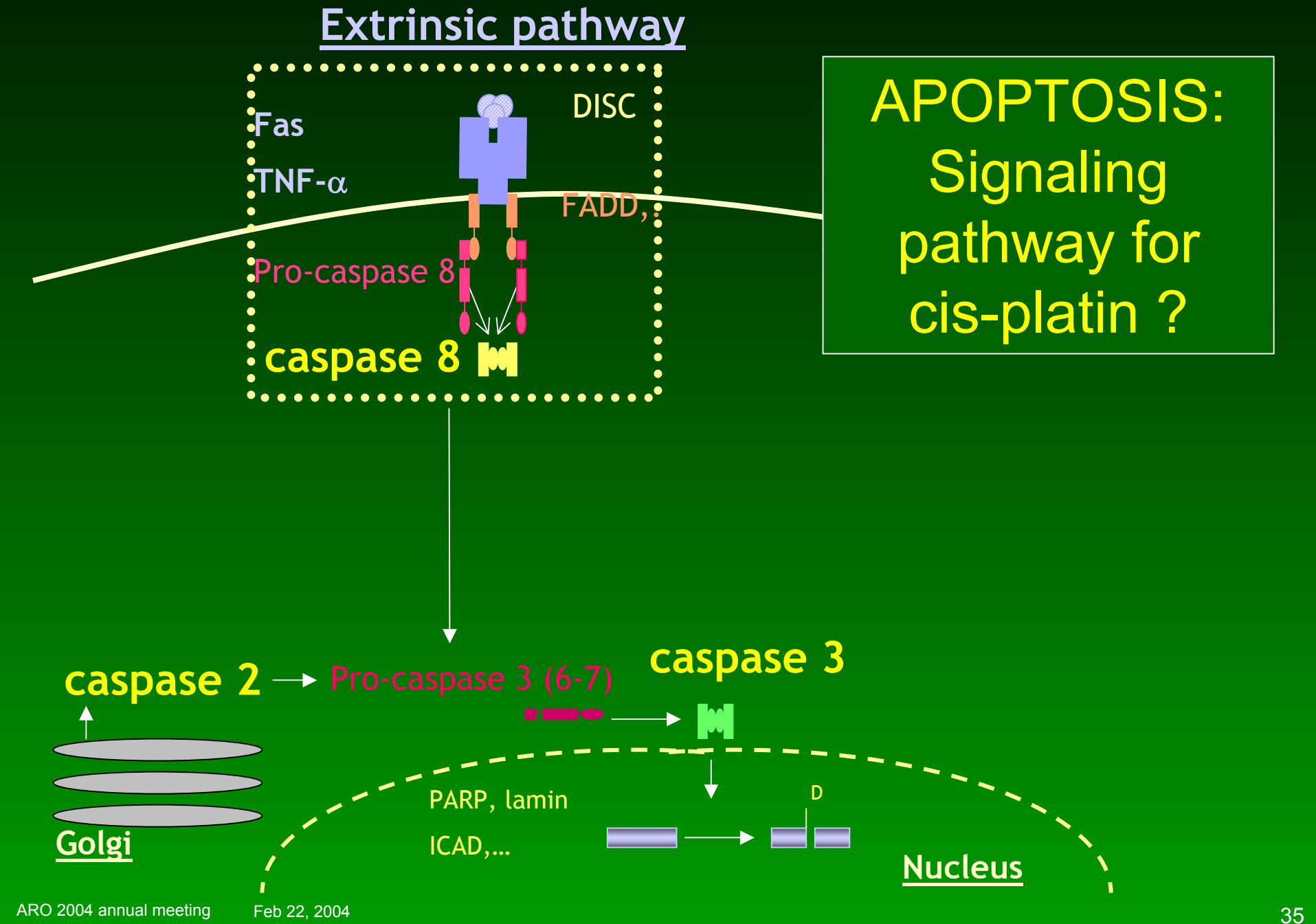
through caspase activation …



Caspase 3 (executioner)



Sheikh-Hamad et al., Arch Toxicol. 2003 Oct 10 [Epub ahead of print]


Extrinsic pathway
DISC
Fas
TNF-α
FADD,
Pro-caspase 8
caspase 8
APOPTOSIS: Signaling pathway for cis-platin ?
caspase 2
Pro-caspase 3 (6-7)
caspase 3
Golgi
PARP, lamin
ICAD,...
D
Nucleus

Towards a mechanism of toxicity …

- platinum is accumulated by renal tissue against a concentration gradient.
- it is thought to produce renal damage because of interaction with renal sulfhydryl (SH) groups and ensuing depletion of SH groups (and the same mechanism may be operating in cochlea)

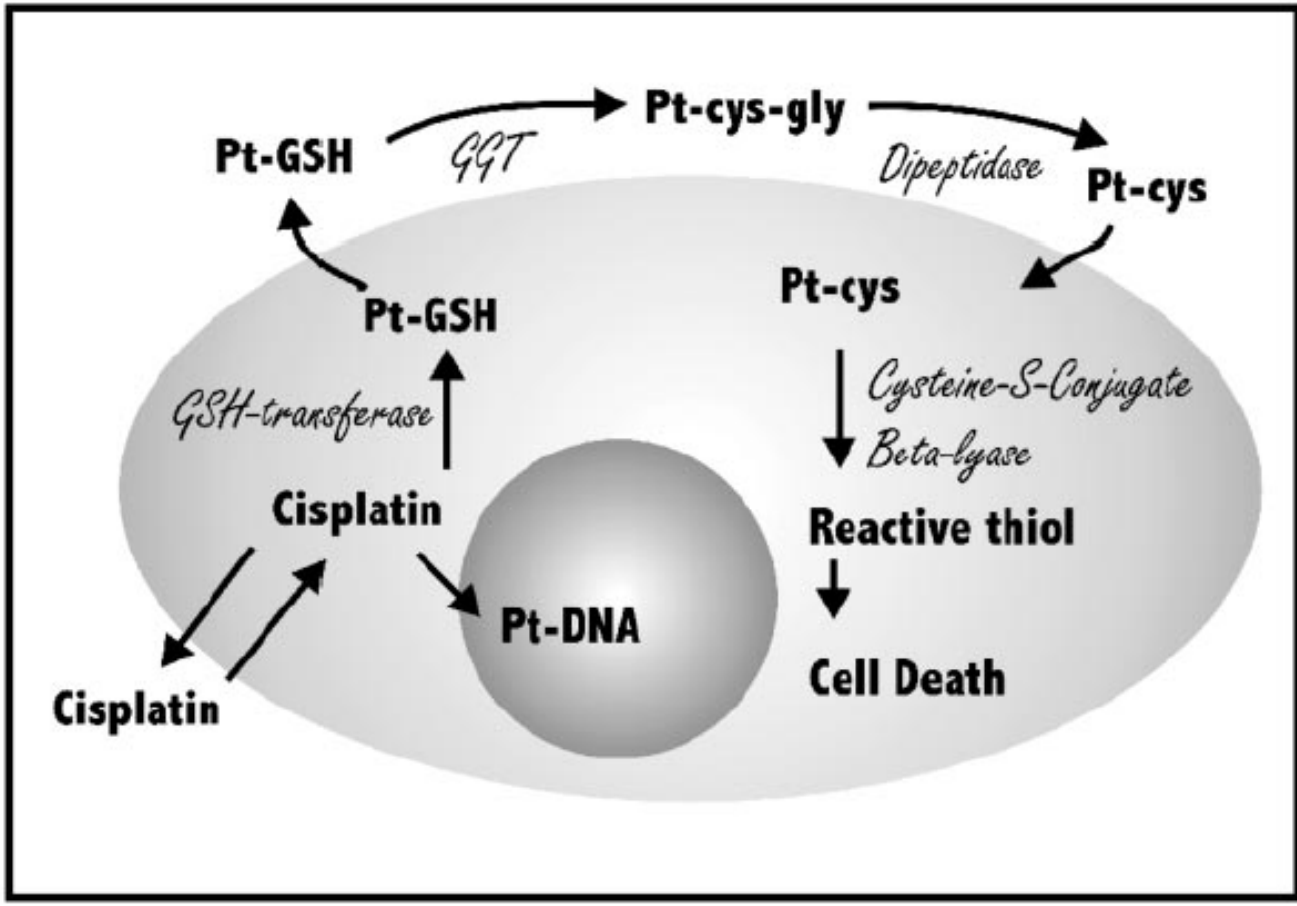
Dobyan et al., 1980; Weiner et al., 1983; Singh et al., 1988; Ravi et al., 1995; Towsend et al., 2003

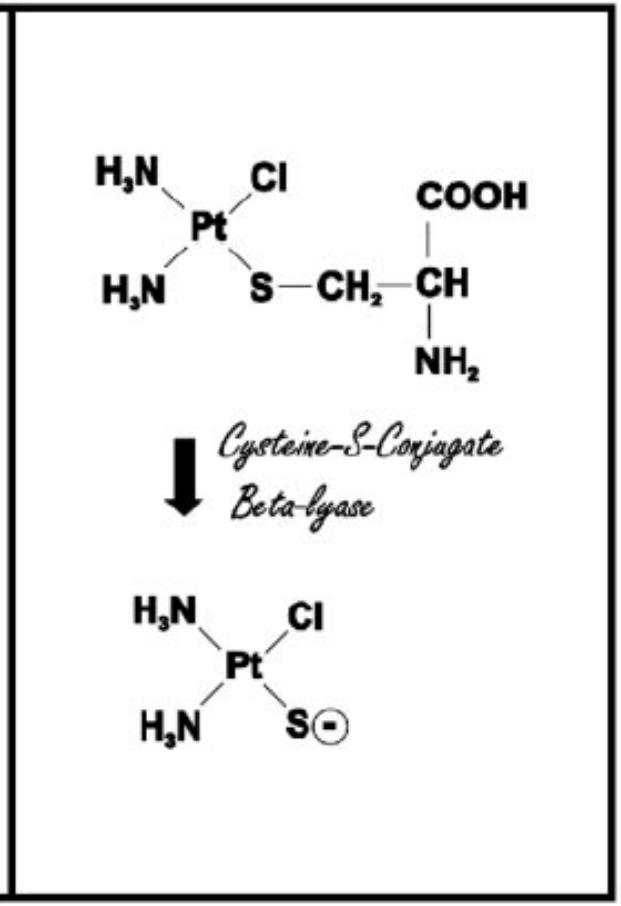
Towards a mechanism of toxicity …

- the steroisomer trans-platin is not toxic …
- cisplatin may be activated in the kidney to a toxic metabolite through the same pathway that has been shown to activate the halogenated alkenes.
- Inhibition of cysteine-S-conjugate beta-lyase reduces toxicity in cultured cells

Dobyan et al., 1980; Weiner et al., 1983; Singh et al., 1988; Ravi et al., 1995; Towsend et al., 2003

Mechanism …



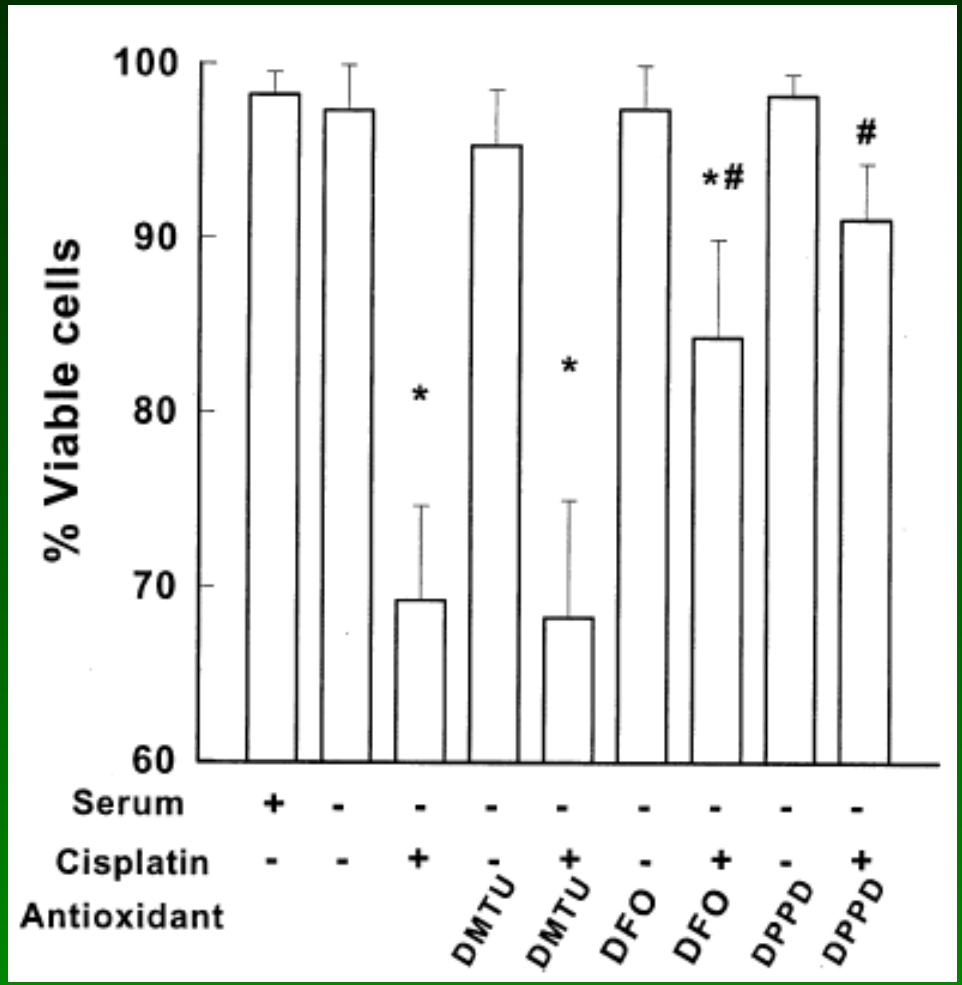


Proposed pathway based on the metabolism of the glutathione-conjugates of the halogenated-alkenes to nephrotoxins (Anders and Dekant, 1998). This mechanism distinguishes tocicity towards dividing cells (anticancer effect) and quiescent cells (nephrotoxicity). The key and final event is the conversion to a highly reactive thiol by cysteine S-conjugate β-lyase.
From Zhang & Hanigan, J. Pharmacol. Exp. Ther. 306:988-994, 2003

Towards a protection by antioxidants…

Effects of antioxidants on cisplatin-induced cytotoxicity in M-1 cells (treated for 2 hr with 0.5 mM cisplatin) and with:

- 30 µM DMTU (dimethylthiourea)
- 50 µM DFO (deferoxamine)
- 10 µM DPPD (diphenyl-p-phenylene-diamine)



R.H. Lee et al., Biochem. Pharmacol. 62:1013-1023, 2001

and also in animals …

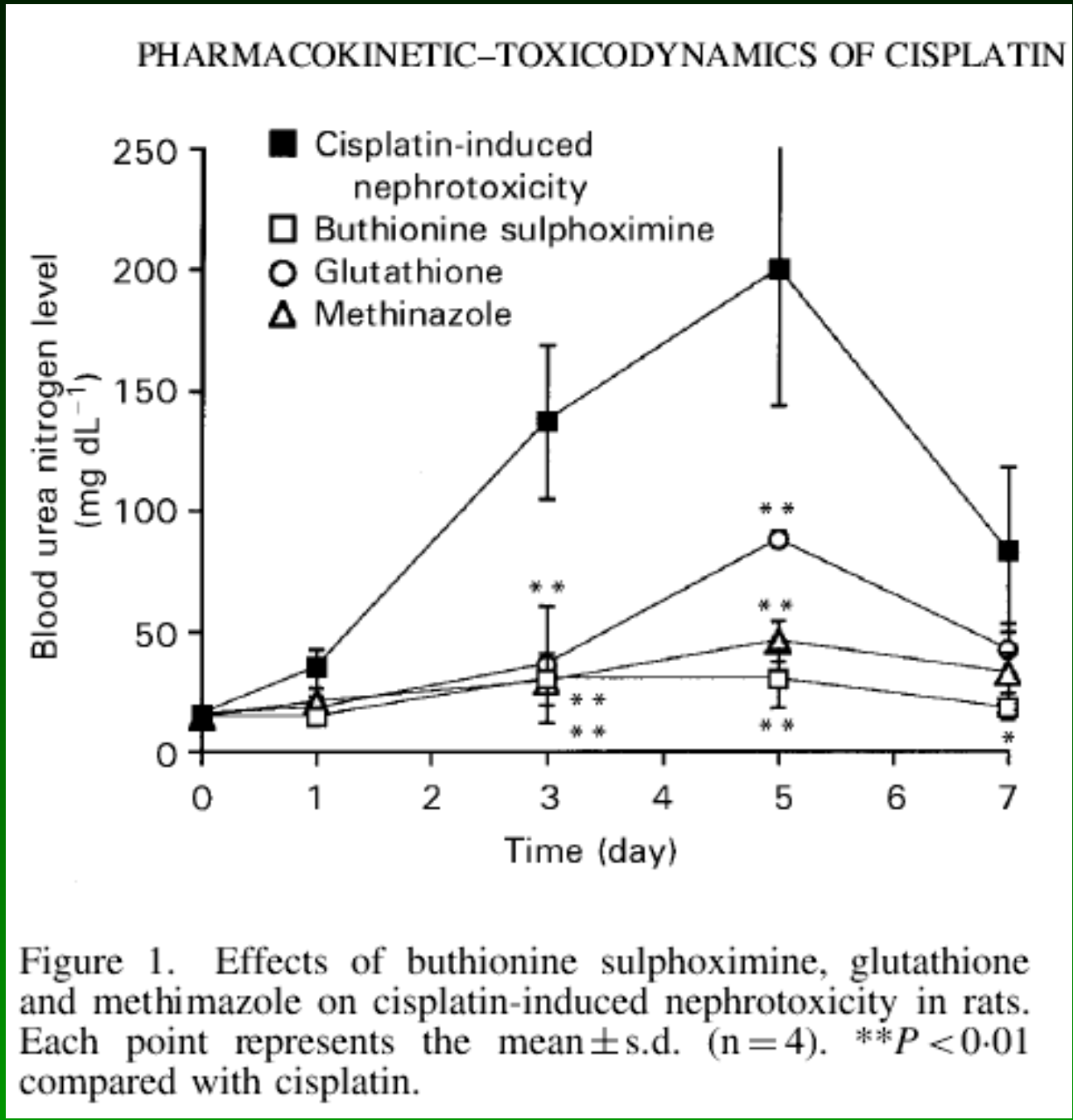
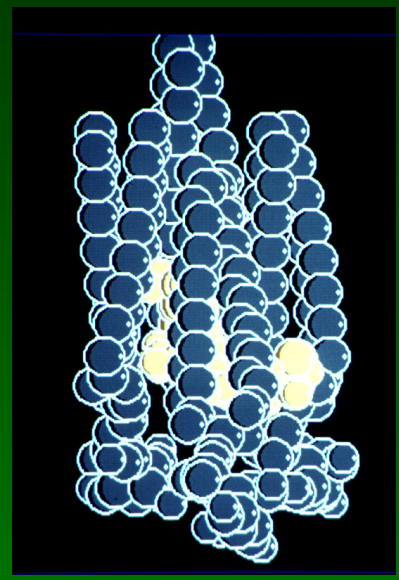


Figure 1. Effects of buthionine sulphoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity in rats. Each point represents the mean ± s.d. (n = 4). $^{**}P < 0.01$ compared with cisplatin.

K. Hanada et al.
J. Pharm. Pharmacol.
2000, 52:1483-90

Thanking people for work on aminoglycosides... and a bit on cis-platin …

Starting very basically in the early 80's ...



G. Laurent
M.B. Carlier
R. Brasseur
J.M. Ruysschaert

Aminoglycosides...

The once-a-day story...
with also a lot of histopathology, rat killing and urine collection …

In the late 80's ...

S. Ibrahim
P. Maldague
L. Giurgea
F. Renoird
M.C. Cambier
G. Laurent
D. Beauchamp

and

F. Clerckx-Braun (FATC)
J. Donnez (St Luc)
M.P. Mingeot
P. Lambricht
R. Wagner
B. Rollmann (CHAM)
P. Herman (SP-Belg.)
M.E. De Broe (UZ-UIA)
G. Verpooten (UZ-UIA)
A. Giuliano (UZ-UIA)
B. Kaufman (VUB)
B. Derde (VUB)

And here is a next generation, and they work(ed) on a lot of things …

M.P. Mingeot
F. Van Bambeke

phospholipidosis

polyaspartic acid

B.K. Kishore
Z. Kallay

M. El Mouedden
H. Servais

apoptosis