Aminoglycoside nephrotoxicity: a paradigm in toxicodynamic research

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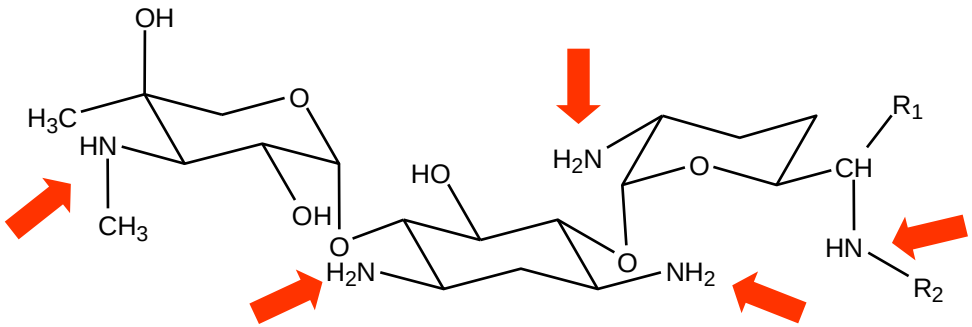
In his book "*The Structure of Scientific Revolutions*", Thomas S. Kuhn (1922-1996) defines a scientific paradigm as:

* what is to be observed and scrutinized
* the kind of questions that are supposed to be asked and probed for answers in relation to this subject
* how these questions are to be structured
* how the results of scientific investigations should be interpreted

From: http://en.wikipedia.org/wiki/Paradigm

But what are aminoglycosides ?
(in one structure [of the lead compound] and 4 lines)



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

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

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

- polyaminated, hydrophilic
- broad spectrum (mostly Gram-negative), highly bactericidal
- predictable pharmacokinetics (no metabolism; renal excretion only)
- resistance remains low in most set-ups
  (many semi-synthetic derivatives with activity against resistance strains)
- parenteral administration only (no gut resorbtion)

You said nephrotoxicity ?

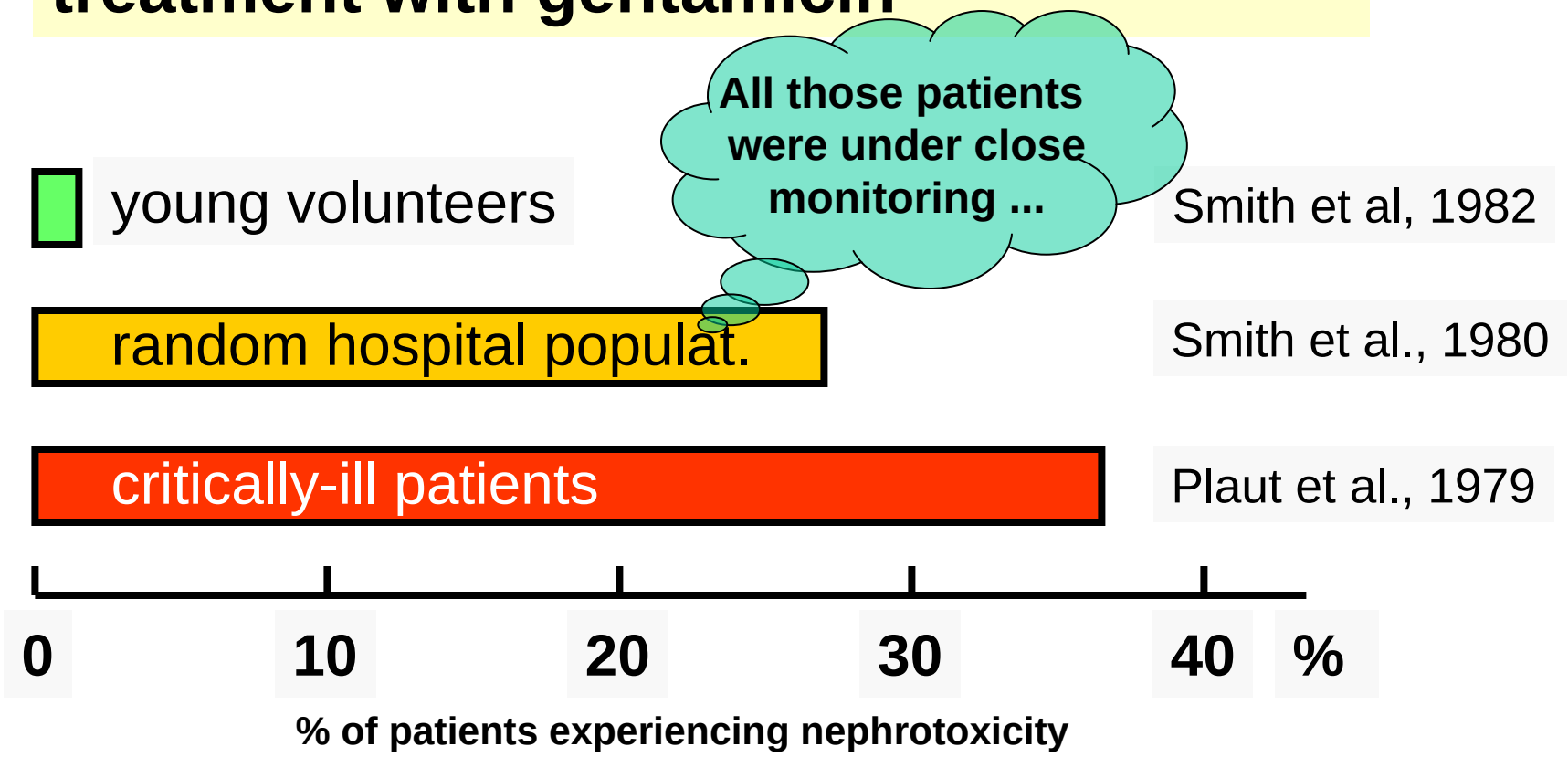
- Typing **"(gentamicin OR aminoglycoside*) AND nephrotoxicity"** on PubMed will yield 1540 papers (among which 229 reviews), with the first one in 1969... (gentamicin was introduced in the clinics in 1967...)

- Controversies were immediate since among the 6 first papers, two say opposite things:
  - Falco et al. **Nephrotoxicity** of aminoglycosides and gentamicin.
    J Infect Dis. 1969 Apr-May;119(4):406-9.
    PMID: 4306975
  - Stille et al. [**Arguments against** the nephrotoxicity of cephalothin and gentamicin] Med Welt. 1972 Oct 28;23(44):1603-5. German. PMID: 5085870

- Perhaps the true was:
  - Schultze et al. **Possible nephrotoxicity** of gentamicin.
    J Infect Dis. 1971 Dec;124 Suppl:S145-7.
    PMID: 5126240

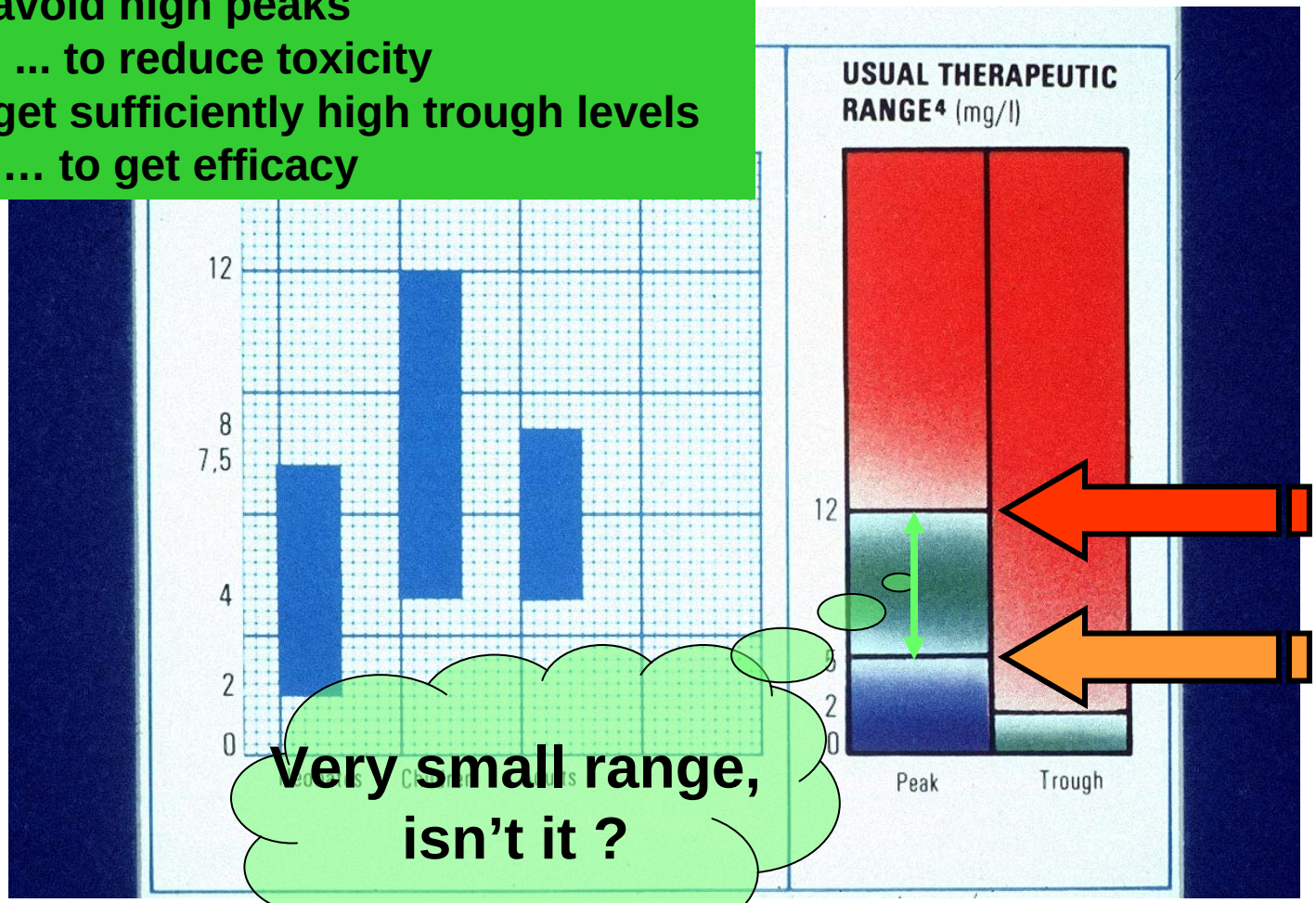
Aminoglycoside nephrotoxicity: its is all how you look at it...

Patients with nephrotoxic reaction after treatment with gentamicin



What was monitoring aminoglycosides on those times ?

- **avoid high peaks**
  ... to reduce toxicity
- **get sufficiently high trough levels**
  … to get efficacy

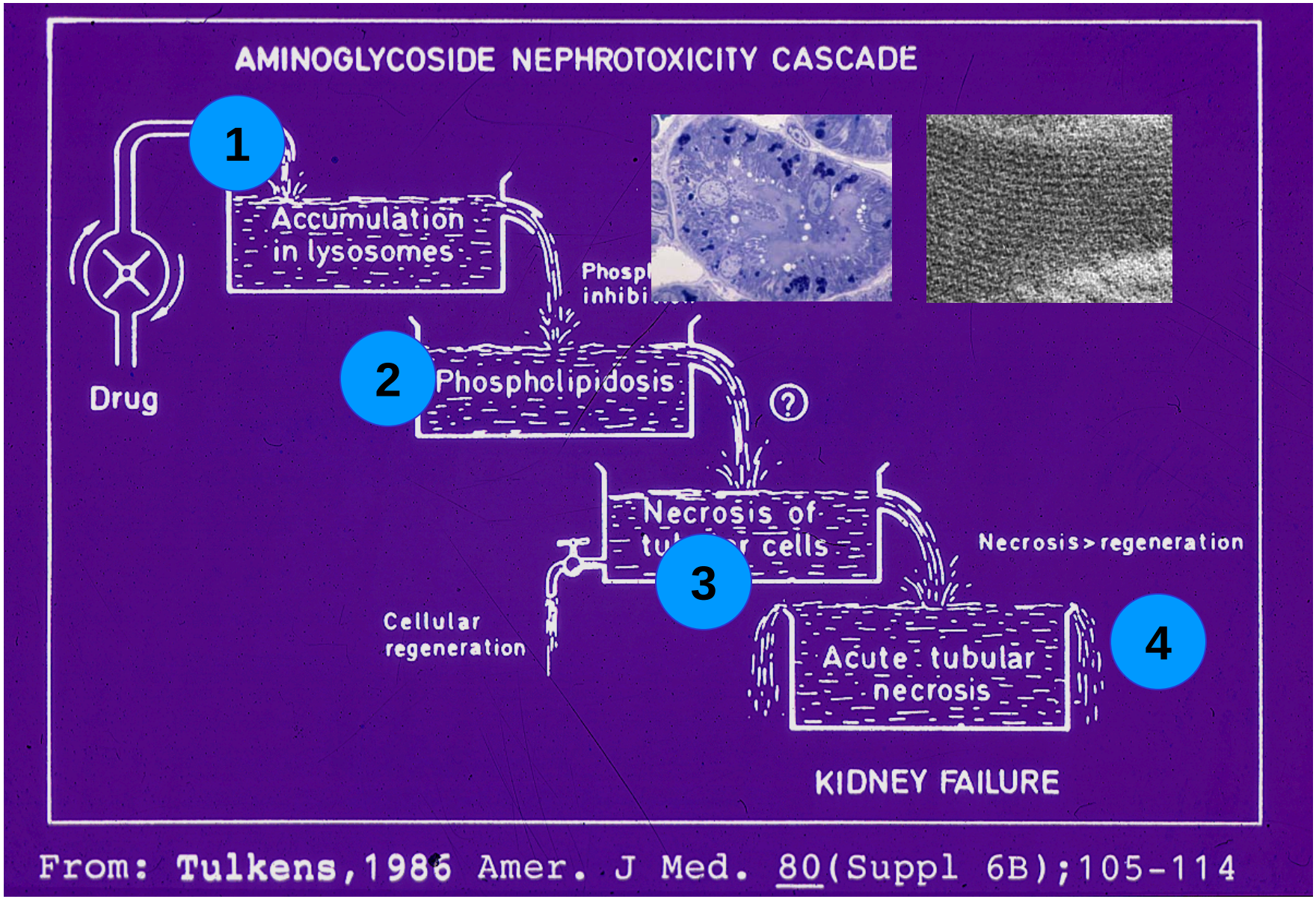


From an "Abbott TdX booklet" (1976) to which I contributed...
Belgian guidelines kept this until 2000...

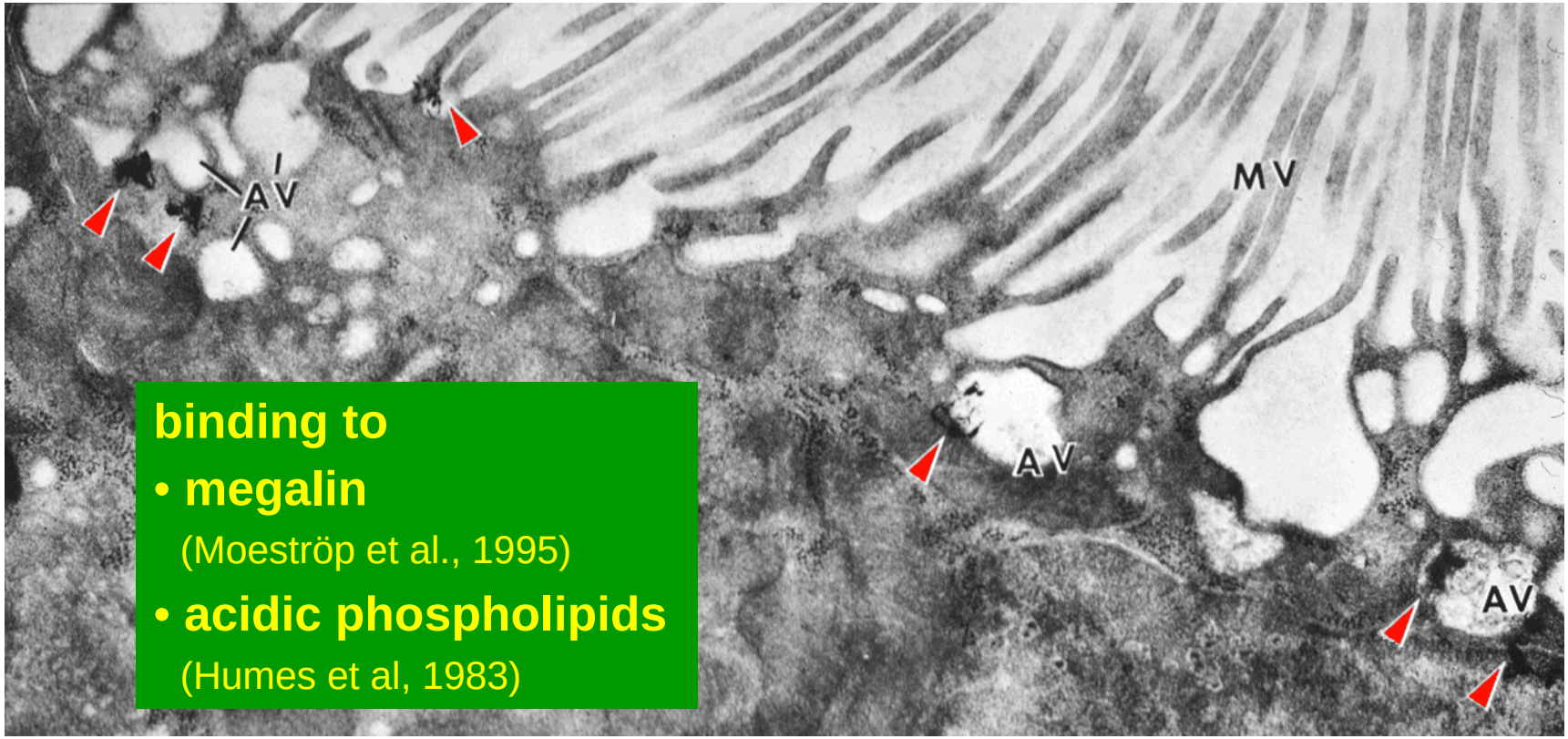
So, now you have two series of questions ...

- Is it toxic or not ? If yes, please
  - explain the mechanism...
  - what are the risk factors ?
- Can we do something to reduce this toxicity ? If yes,
  - can we put in clinical practice ?
  - or, perhaps, in drug development ?

Aminoglycoside toxicity cascade (1st version)…



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

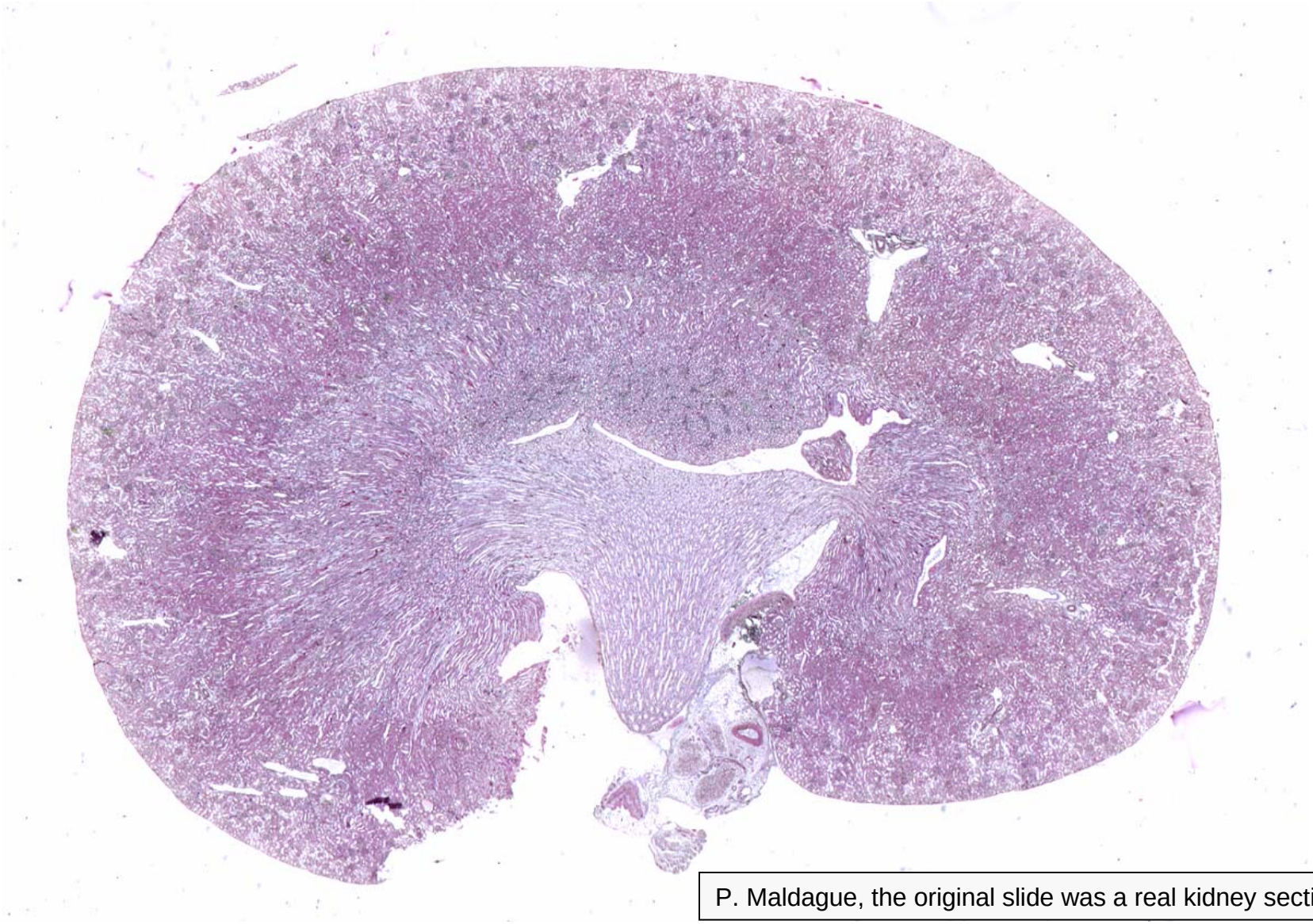


binding to

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

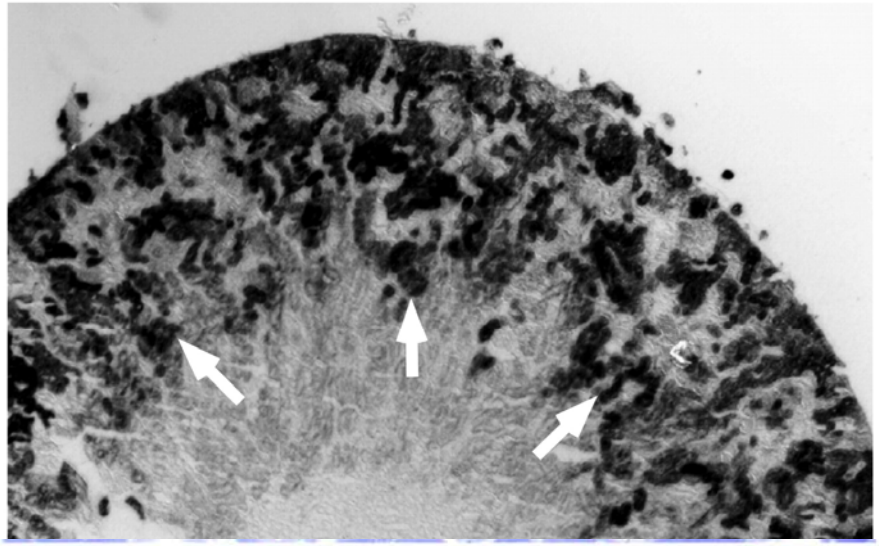
* Just *et al*, Naunym Schmied. Arch. Pharmacol, 1977
Silverblatt & Kuehen, Kidney Intern., 1979

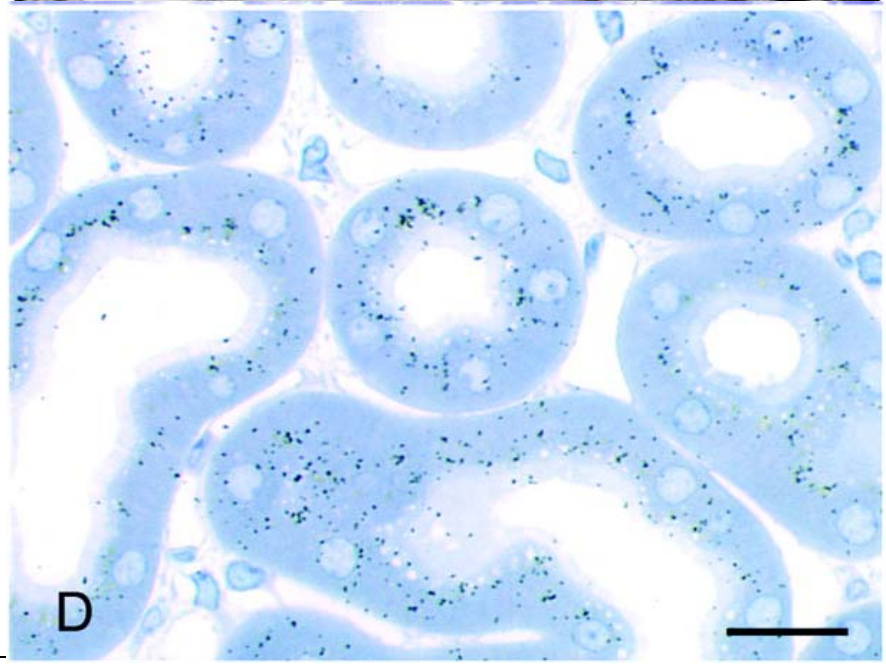
A look in the microscope in a rat treated with low doses … (10mg/kg)

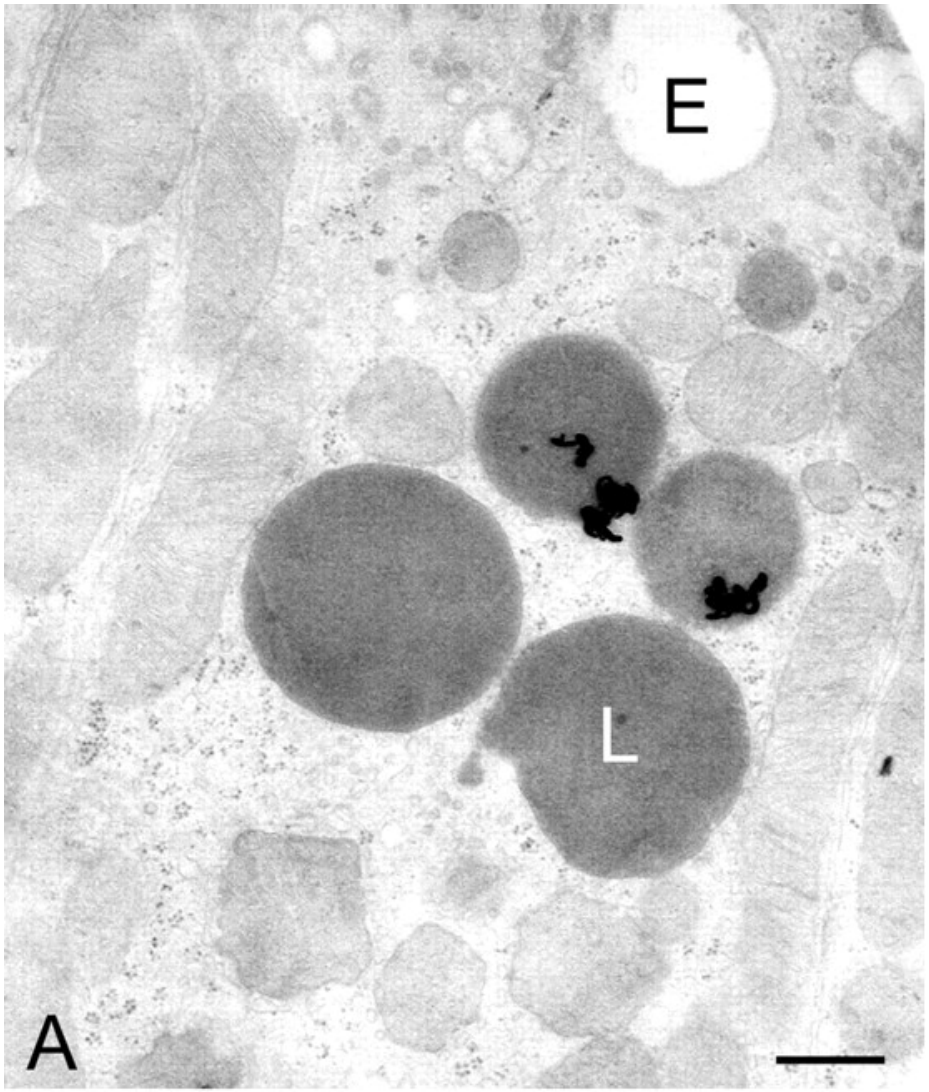


P. Maldague, the original slide was a real kidney section…

Gentamicin accumulates in lysosomes of proximal tubular cells

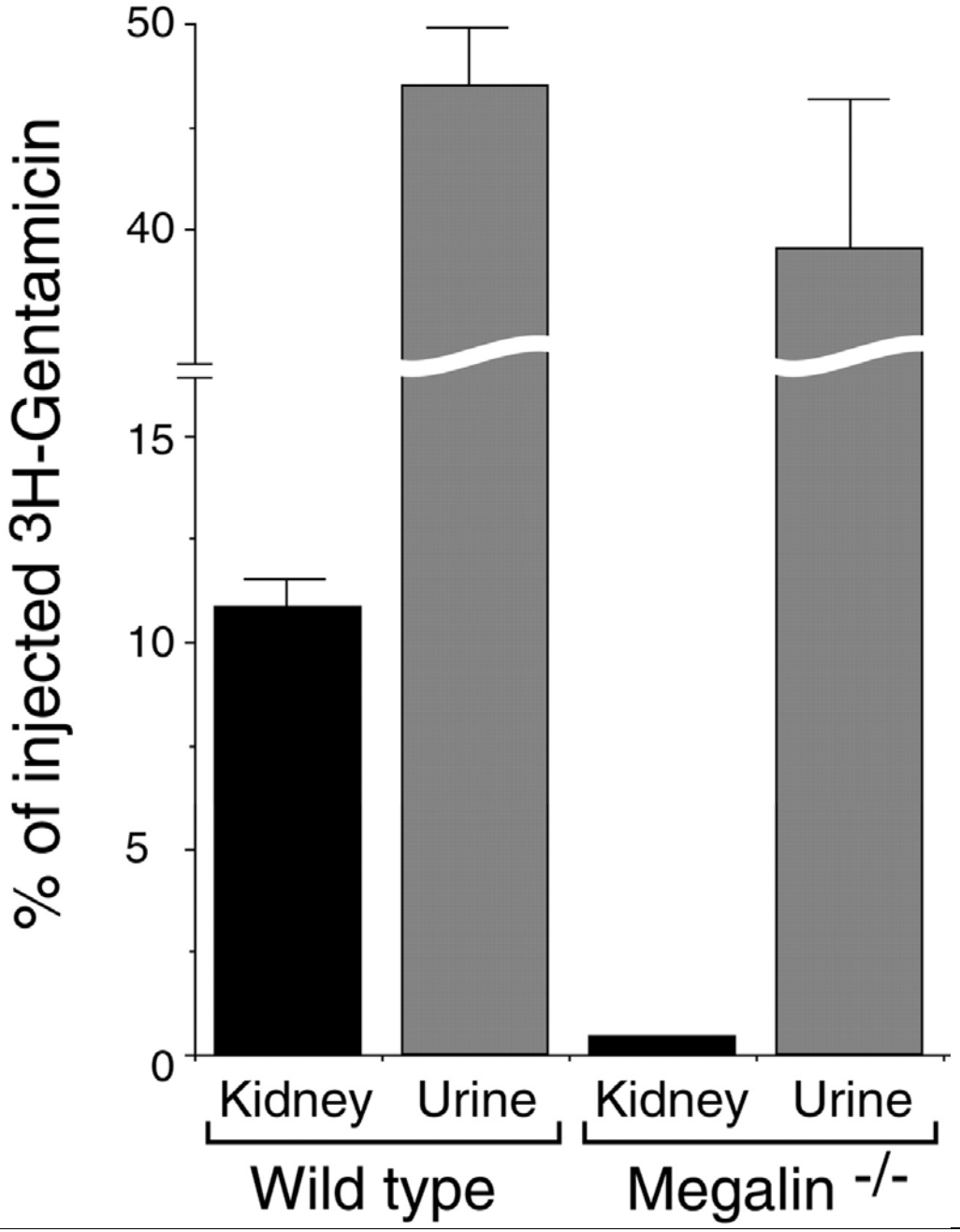






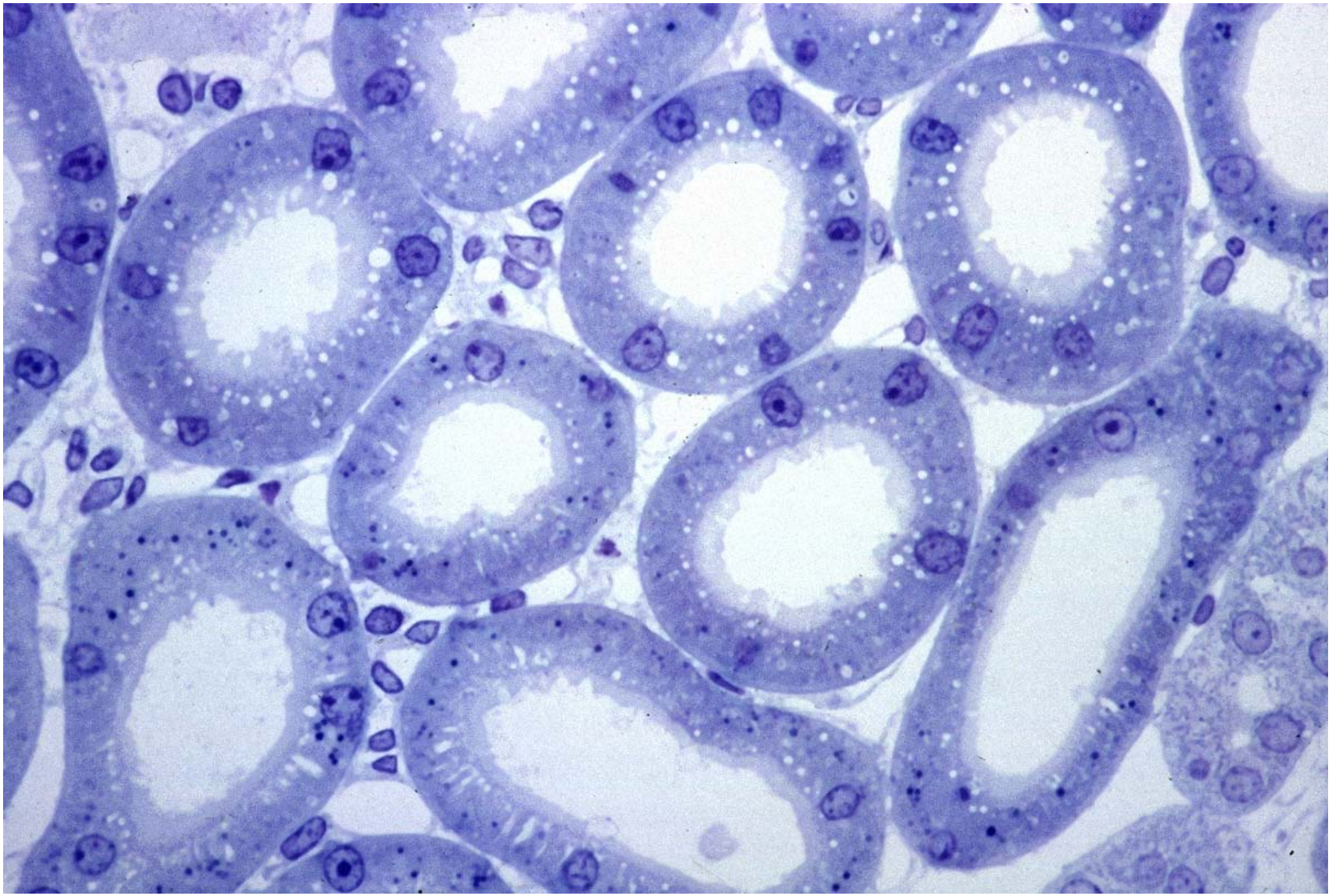
Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Mice deficient in megalin do not accumulate gentamicin in kidney

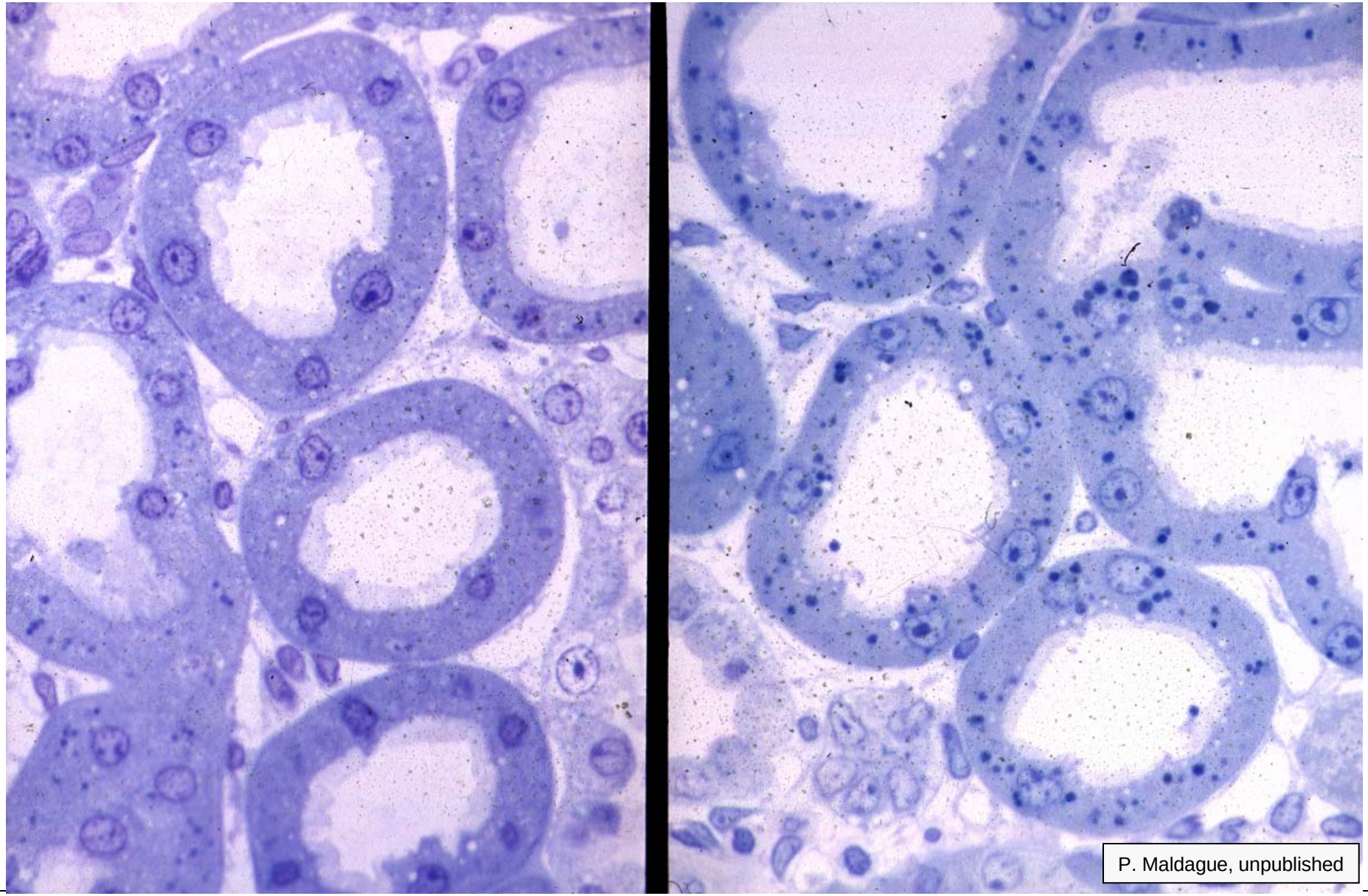


Schmitz et al., J. Biol. Chem. 277:618-622, 2002

Somewhat closer in the control …

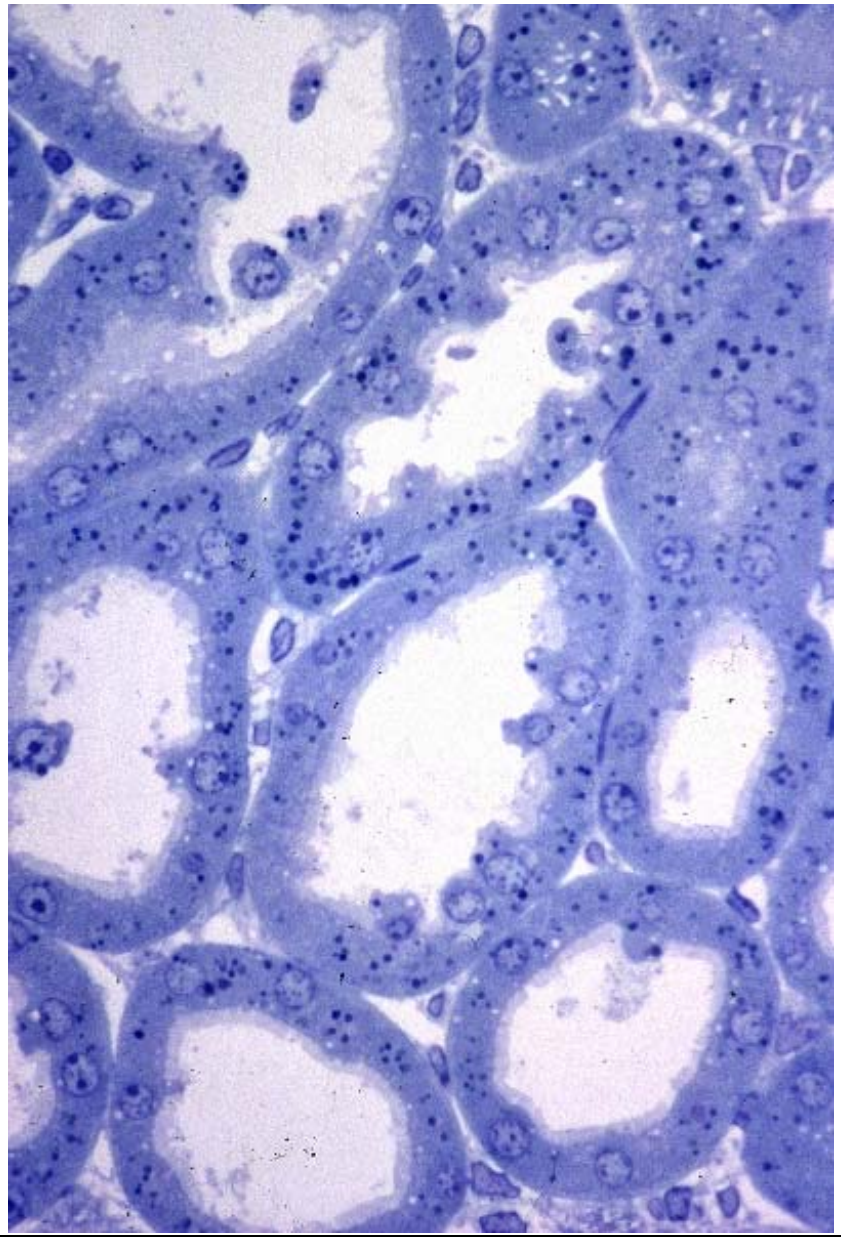


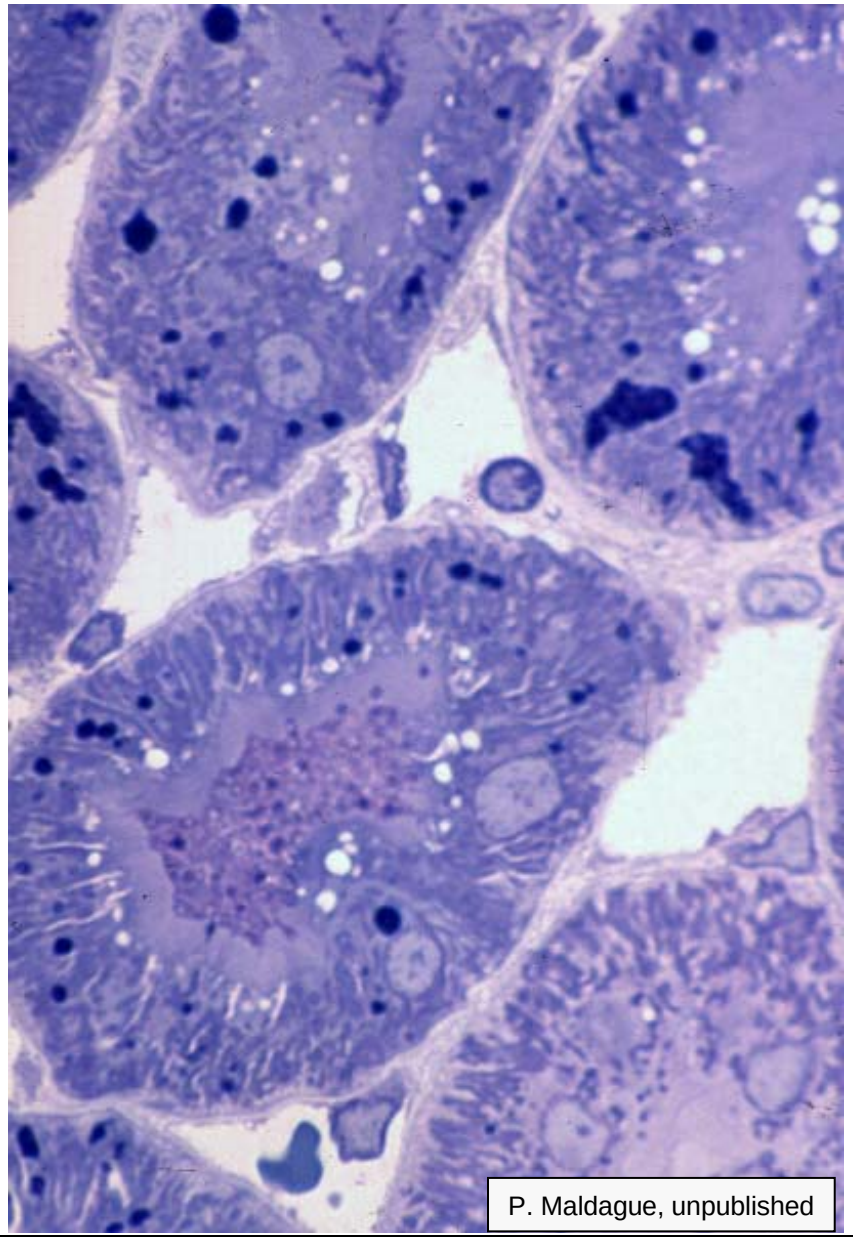
Compare ...



P. Maldague, unpublished

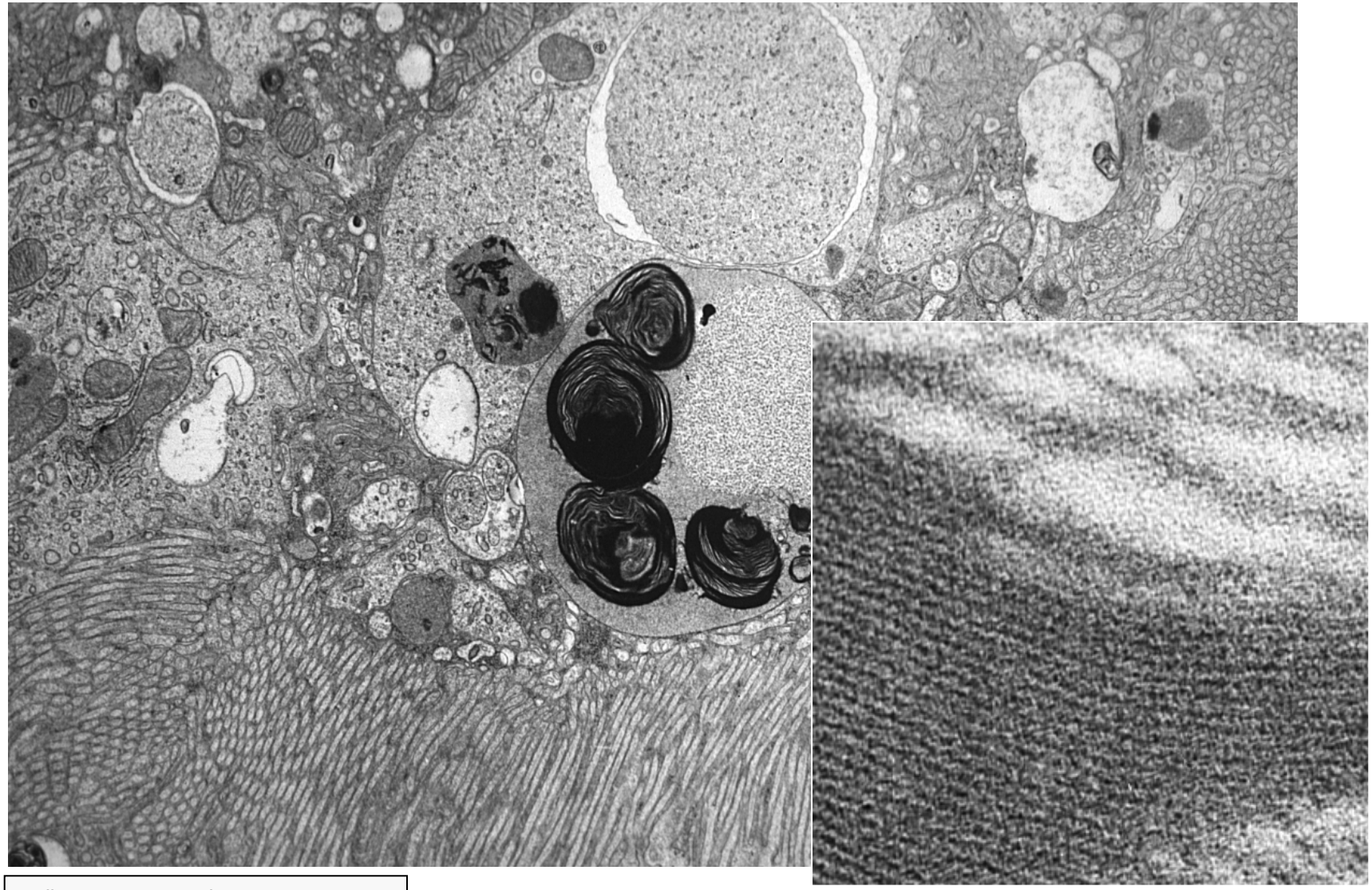
And examine …





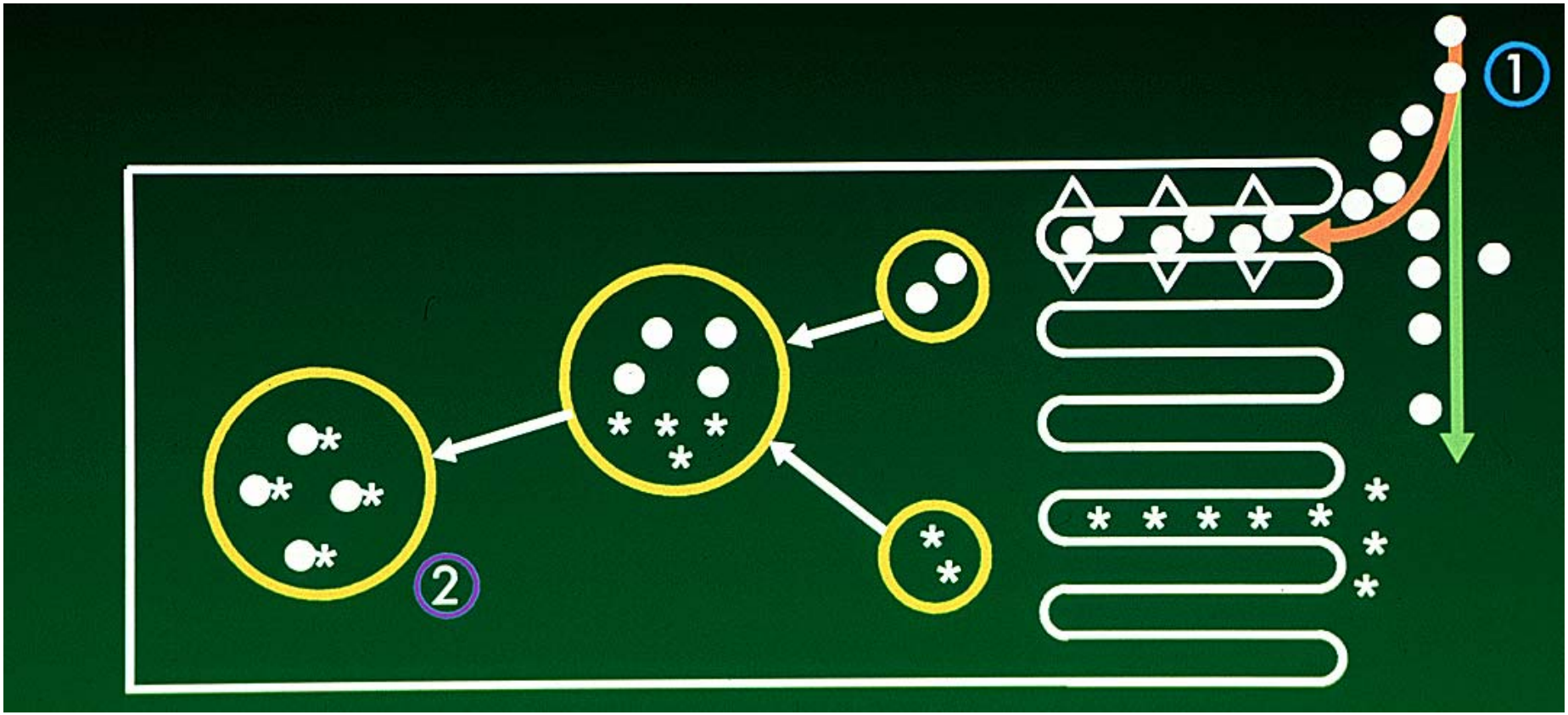
P. Maldague, unpublished

Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis



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

Towards a mechanism …*



1. **binding to brush border (via megalin / ac. phospholipids)**
2. **accumulation in lysosomes and phospholidosis / phospholipiduria**

* what is to be observed and scrutinized

Aminoglycoside toxicity: a 2^{d} view…

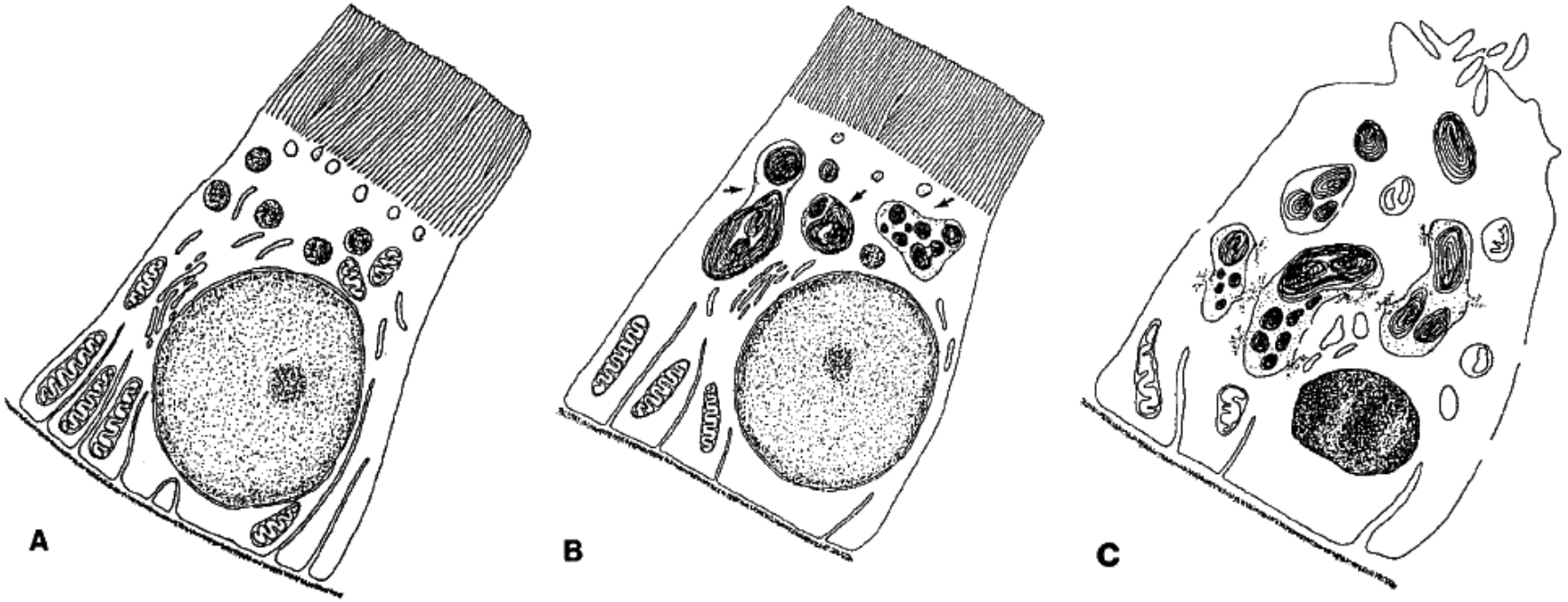
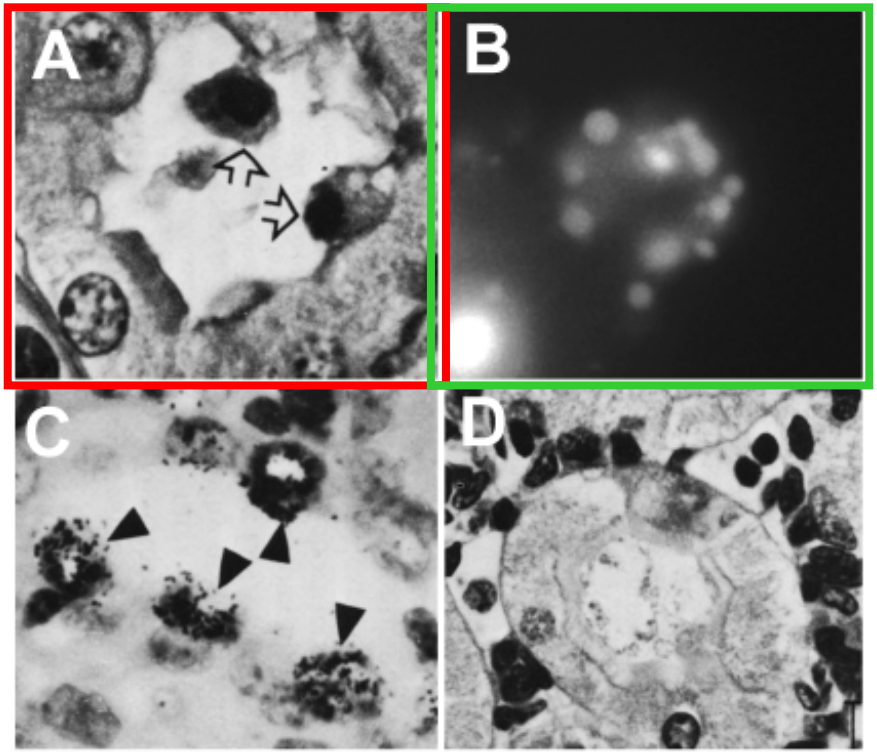


FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive 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; 43:103-1012

Apoptosis in kidney and renal cells as first sign of toxicity…

rat cortex **LLC-PK1 cells**



Morphological changes in rat renal cortex (A,C,D) upon treatment with gentamicin at low doses (10 mg/kg; 10 days) and in cultured LCC-PK1 renal cells (B) upon incubation with gentamicin (under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated in A, B, and C [approx. 10 µg/g;

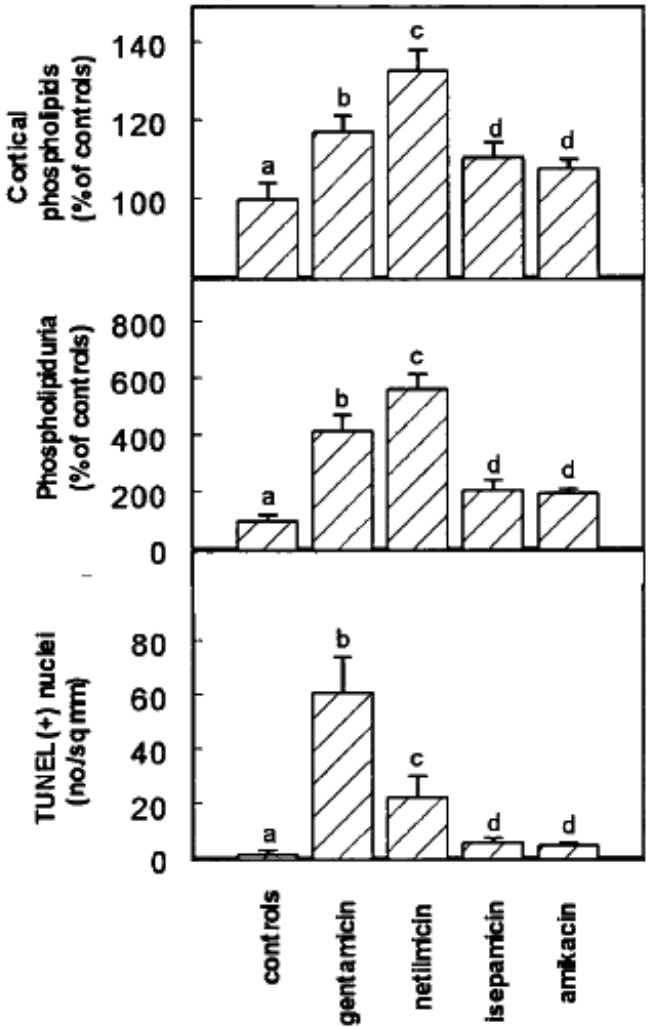
Laurent et. al Antimicrob Agents Chemother (1983) 24:586-593.
Servais et al. In: Toxicology of the Kidney (Target Organ Toxicology Series), 2004, chap. 16, pp 635-685,

Apoptosis and phospholipidosis in kidney cortex: an early sign of toxicity ?

rats treated with therapeutically-relevant doses of aminoglycosides (*)

phospholipids

apoptosis



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

What do you mean by therapeutically-relevant doses of aminoglycosides ?

rats treated with therapeutically-relevant doses of aminoglycosides (*)

TABLE 1. Experimental groups, conditions of treatments, and relevance to the clinical use of aminoglycosides

Drug	Dose (mg/kg)[a]	Duration (days)	Fold increase over:	
			Clinical dose[b]	Clinical daily drug exposure[c]
Gentamicin	10	4–10	~2	~0.5
	20	4–10	~4	~1
Netilmicin	10	4–10	~1.7	~0.4
	20	4–10	~3.3	~0.8
Amikacin	40	10	~2.7	~0.7
Isepamicin	40	10	~2.7	~0.7

[a] Twice-a-day schedule (daily dose split into two administrations at 12-h intervals). This schedule (or even a three-times-a-day schedule) was long considered mandatory for aminoglycosides but is known to increase toxicities at both low and high doses in animals (38, 52). Data for patients are less definite, even though a trend toward less toxicity is commonly observed with a once-a-day schedule (21, 48).

[b] Suggested maintenance doses for an adult patient with an estimated creatinine clearance of 90 ml/min (20) (gentamicin, 5.1; netilmicin, 6; and amikacin, 15 mg/kg, respectively) or based on the registered dosage in Belgium and many other countries for isepamicin.

[c] Based on estimated ratio of areas under the serum concentration-time curve, AUC ratio, using the dose ratio defined in footnote *b* and assuming apparent half-lives of ~30 min in rats and ~120 min in humans (β-elimination phases).

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

Apoptosis is probably induced by disruption of gentamicin-loaded lysosomes

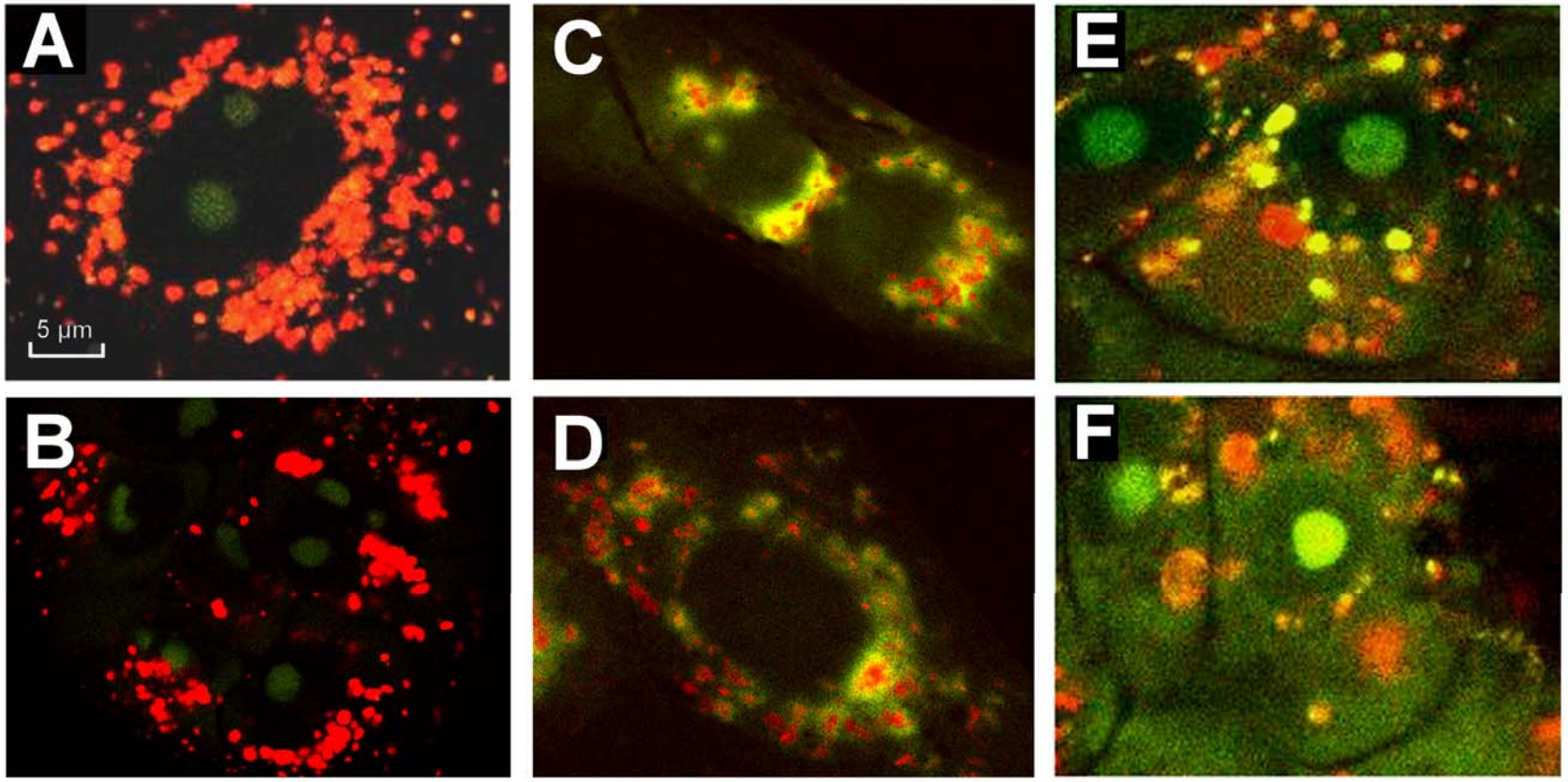


Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 µg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 µM, 3 h).

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

Electroporation allows to by-pass lysosomes and increases cell-susceptibility to gentamicin-induced apoptosis in cultured cells

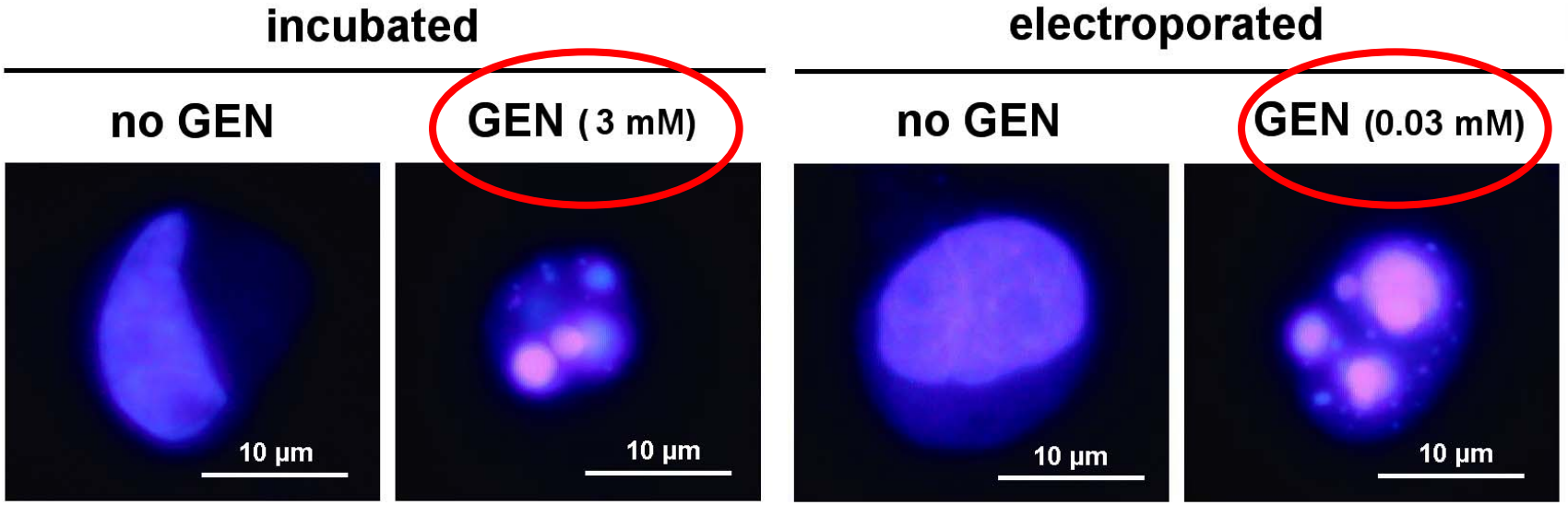
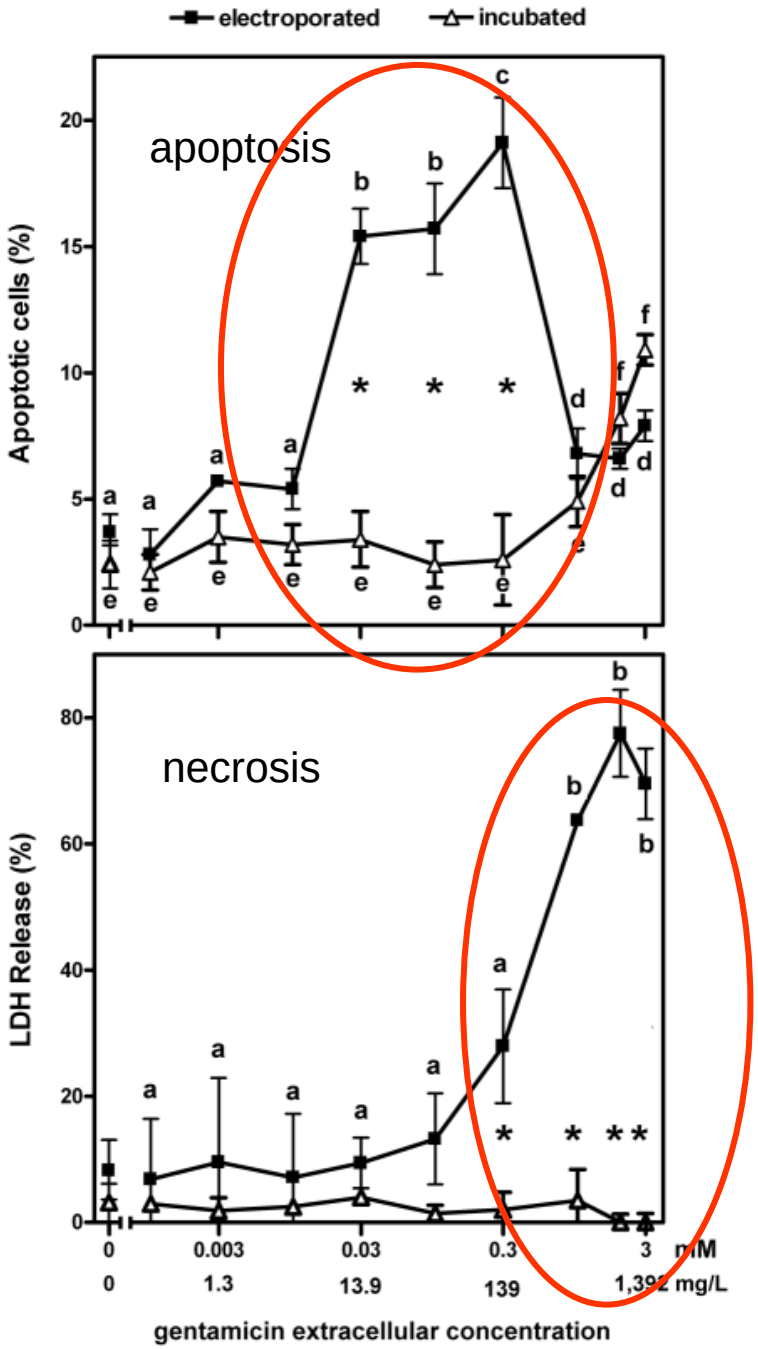


Figure 1: Staining of nuclei of LLC-PK_1 cells by 4',6'-diamidine-2'-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the 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. (2006) 50:1213-1221

Bypassing lysosomes in cultured cells …



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

Apoptosis in electroporated cells as a means to test for toxicity

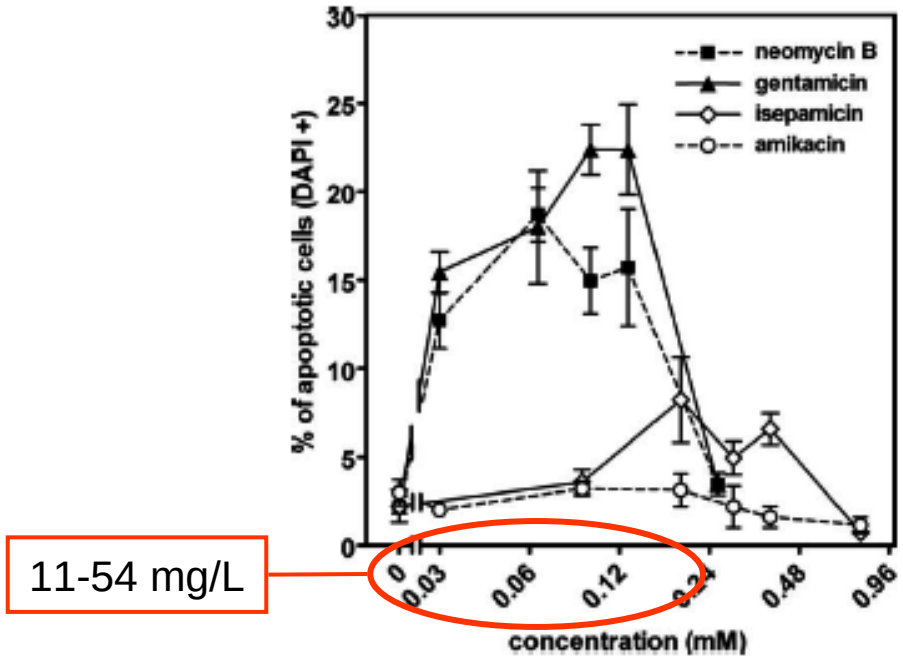
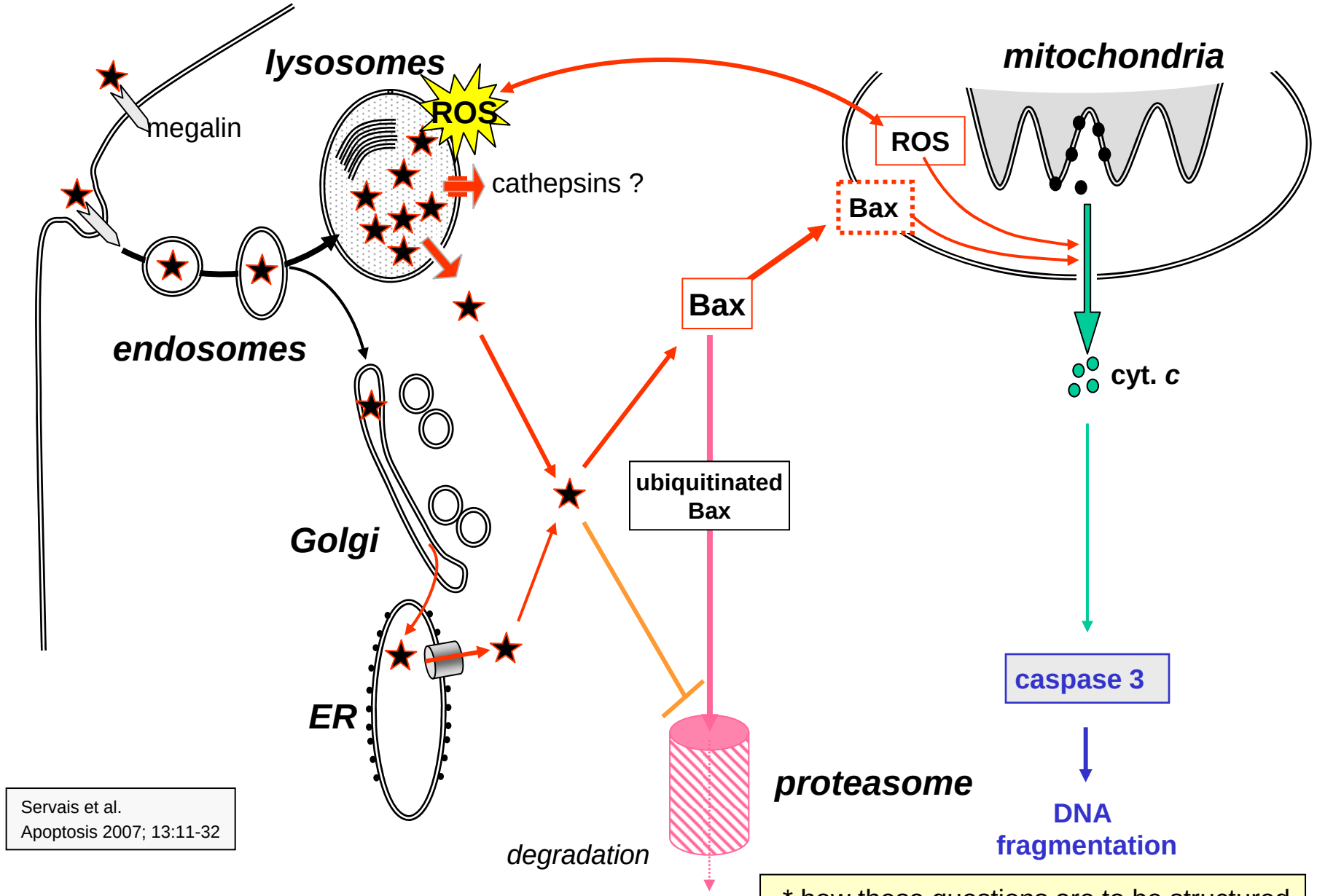


FIG. 2. Apoptosis in electroporated cells. Cells were electroporated in the absence (controls) or in the presence of neomycin B, gentamicin, isepamicin, or amikacin and returned to aminoglycoside-free medium, and apoptotic nuclei were enumerated 24 h later. Values are means ± standard deviations ($n = 3$). Statistical analysis was performed by two-tailed analysis of variance ($P < 0.01$). All values for neomycin B and gentamicin, except those observed for the largest concentration tested (0.256 mM), are significantly different from those of the controls; isepamicin values observed for 0.192, 0.288, and 0.384 mM concentrations are significantly different from those of controls; amikacin values did not differ from control values. The 0.12 mM concentration corresponds to approximately 74 mg/liter for neomycin B, 56 mg/liter for gentamicin (taking into account the respective contents of the commercial gentamicin in C1, C1a, and C2 components), 68 mg/liter for isepamicin, and 70 mg/liter for amikacin. See the supplemental material for structures of tested compounds.

Denamur *et al*.
Antimicrob. Agents Chemother.
2008; 52:2236-2238

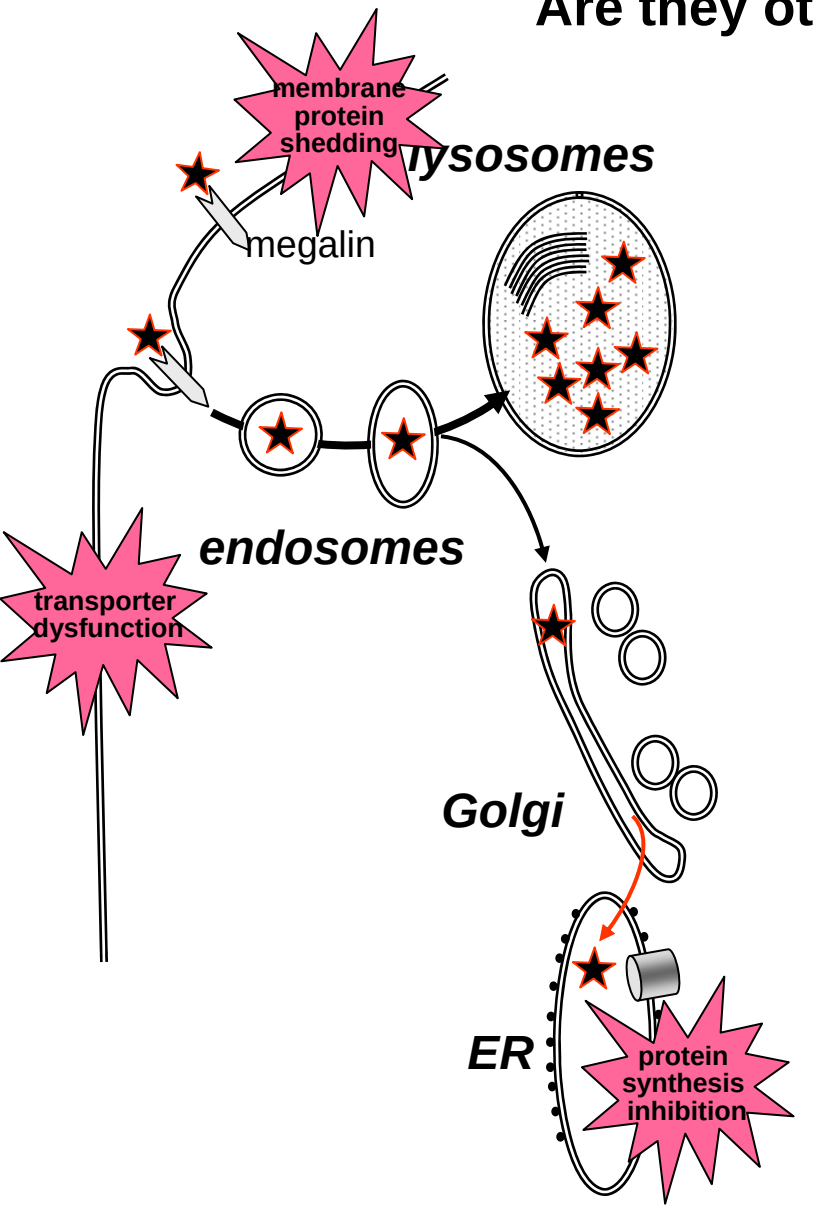
Gentamicin and apoptosis: an overview

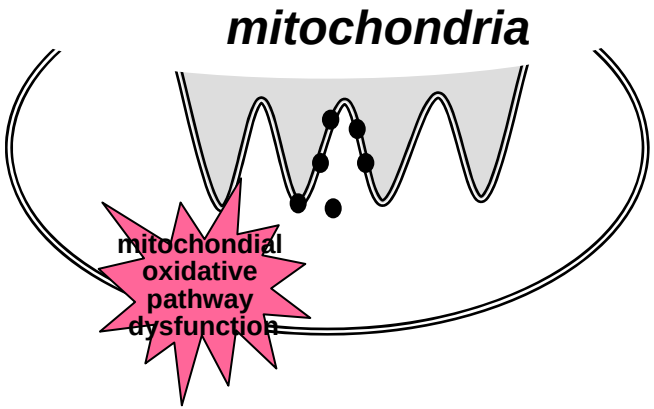


Servais et al.
Apoptosis 2007; 13:11-32

* how these questions are to be structured

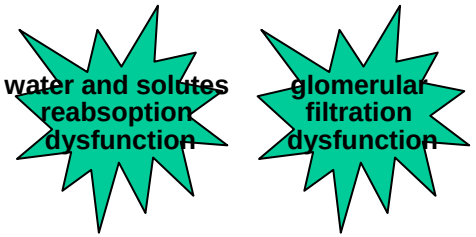
Are they other mechanisms proposed ?





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)



* how these questions are to be structured

So, now you have two series of questions ...

- Is it toxic or not ? If yes, please
  - explain the mechanism...
  - what are the risk factors ?
- Can we do something to reduce this toxicity ? If yes,
  - can we put in clinical practice ?
  - or, perhaps, in drug development ?

Risk factors in a nutshell…

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

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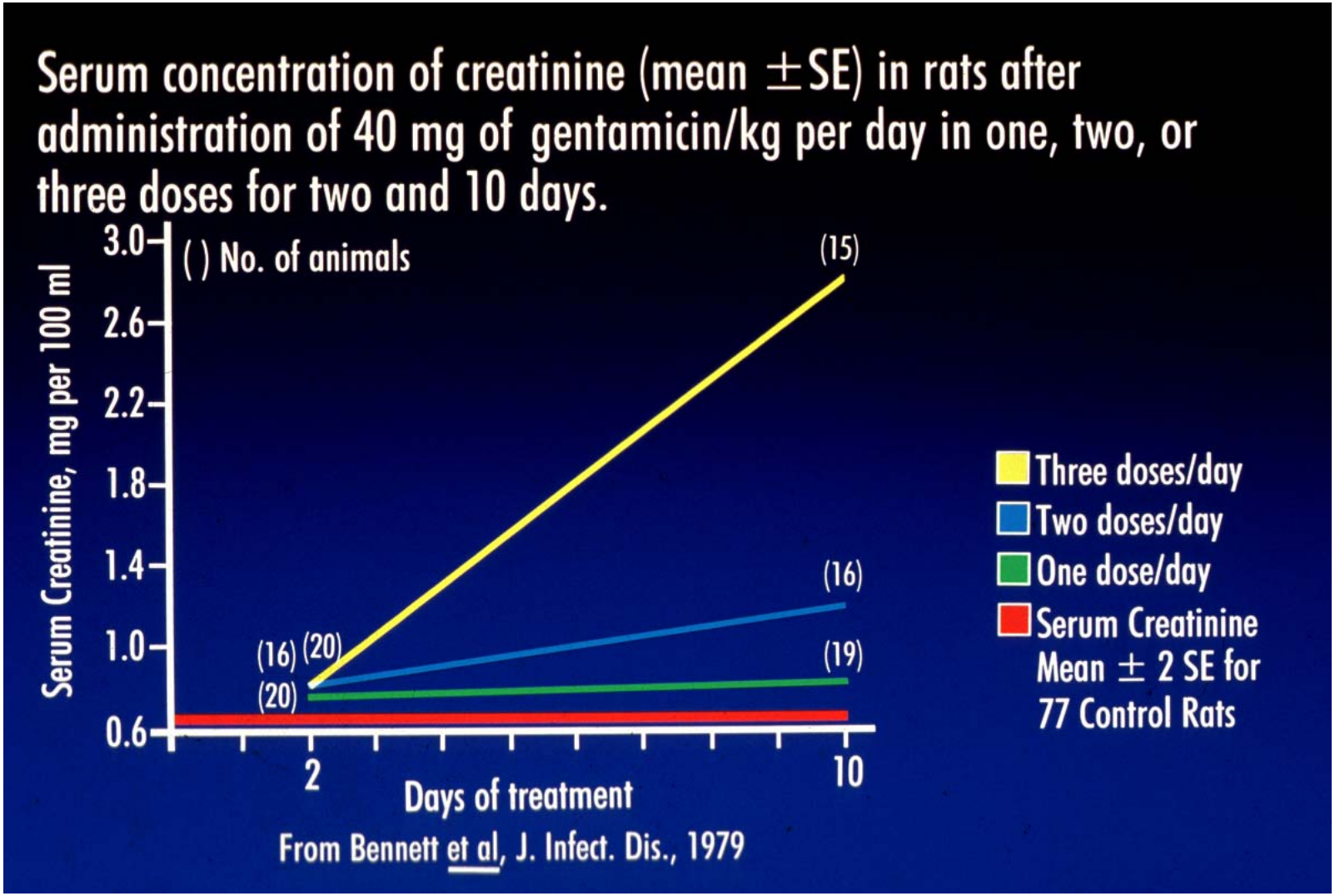
Aminoglycoside prevention of toxicity …

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

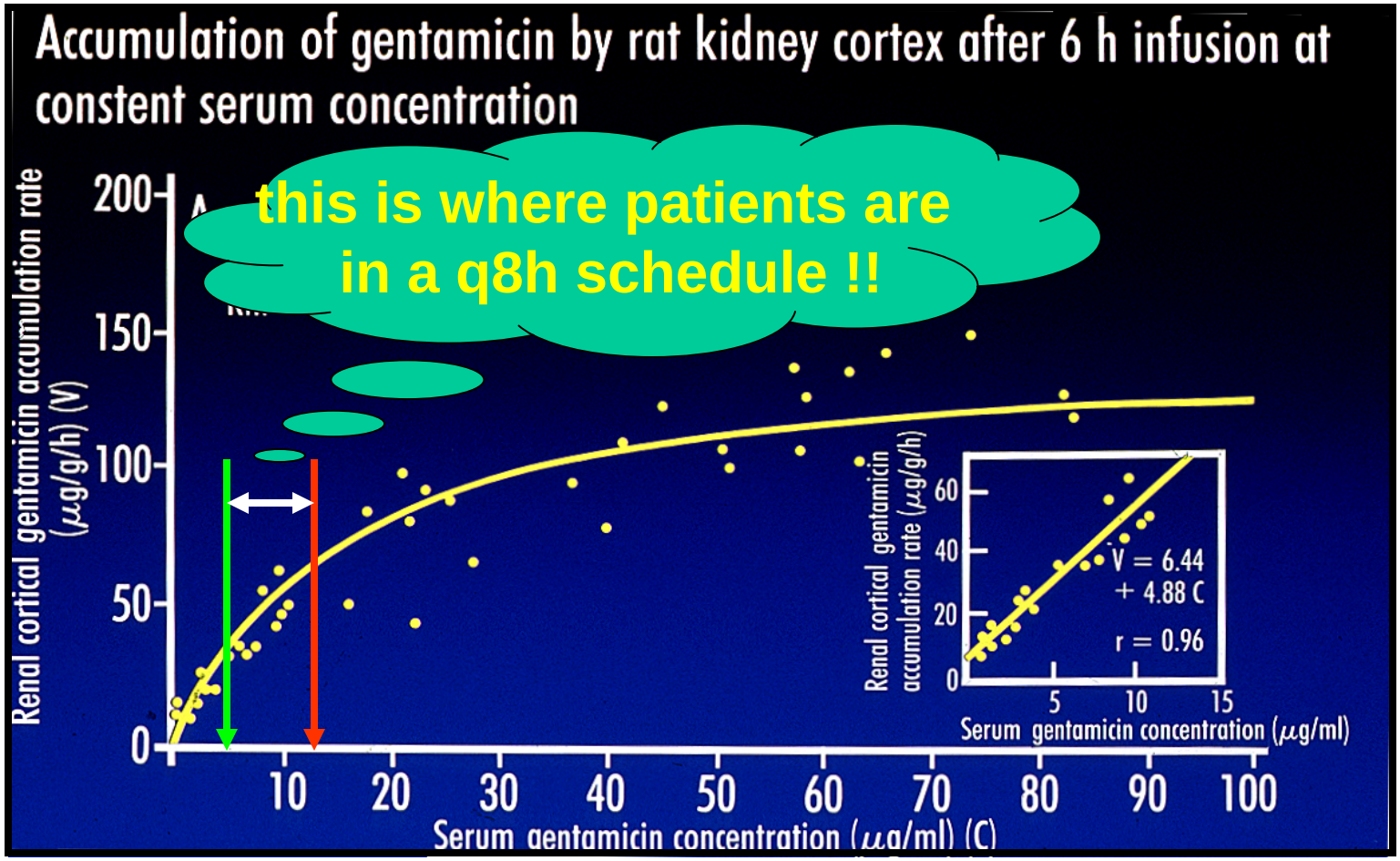
Mechanism	Compound
I. Decrease or prevention of drug accumulation by kidneys	
Intracellular complexation of aminoglycosides	
Competition with or decrease in aminoglycoside binding to brush border membrane	
Competitors	Ca^{2+} (diet supplementation [51] or vitamin D-induced hypercalcemia [21]) Lysine (81) Aminoglycosides (as their own competitors) (39)
II. Prevention or decrease of lysosomal phospholipase inhibition	
Coadministration of agent preventing intralysosomal phospholipidosis	
Intralysosomal sequestration of aminoglycosides	Polyaspartic acid (55, 62)

Excerpt from Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. 1999; 43:103-1012

Aminoglycoside toxicity is not linked to peak (alone)



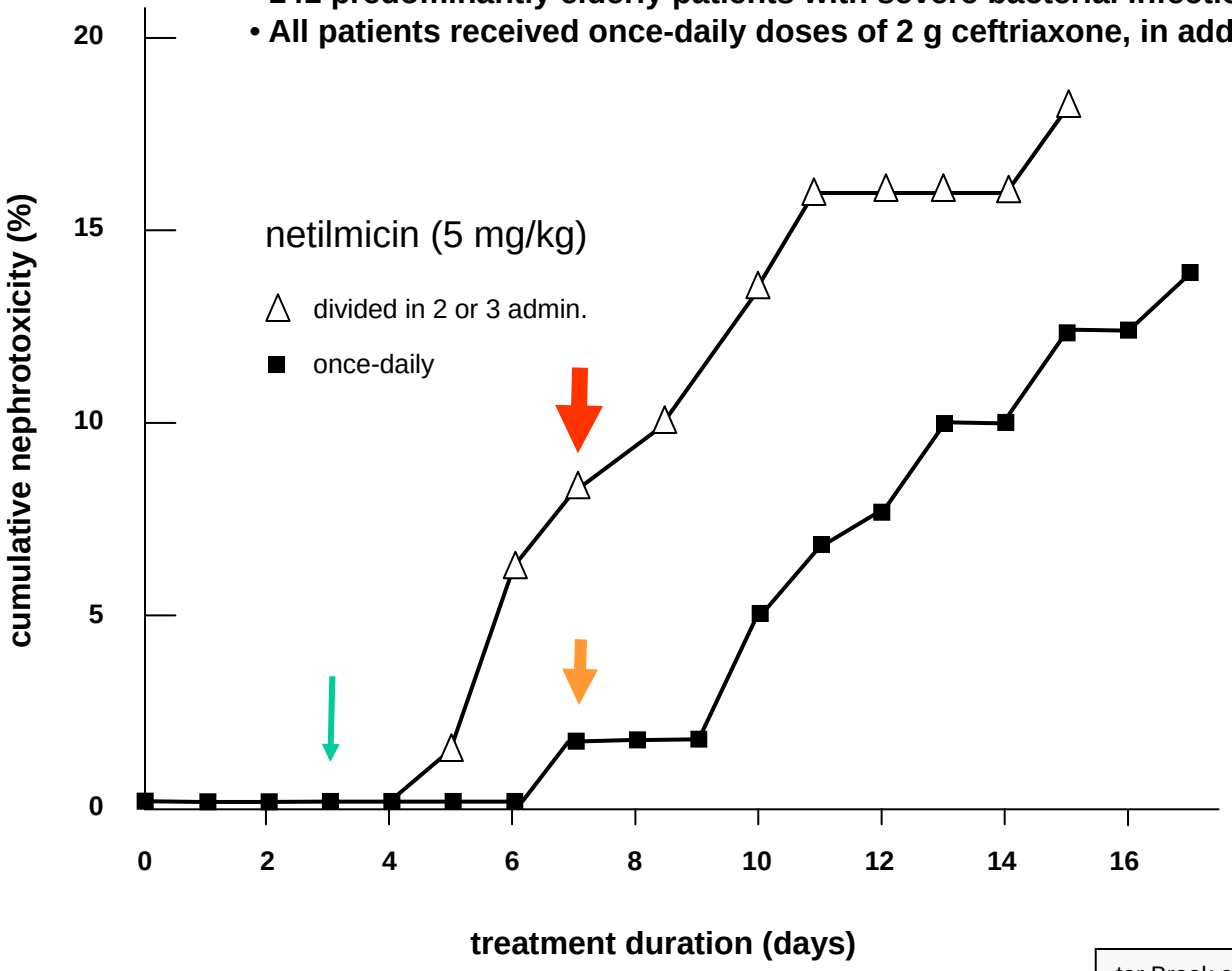
Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...



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

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)**

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Excerpt from Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. 1999; 43:103-1012

1: Gilbert *et al.* Polyaspartic acid prevents experimental aminoglycoside nephrotoxicity. J Infect Dis. 1989 May;159(5):945-53.

2: Kishore *et al.* Mechanism of protection afforded by polyaspartic acid against gentamicin-induced phospholipidosis. I. Polyaspartic acid binds gentamicin and displaces it from negatively charged phospholipid layers in vitro. J Pharmacol Exp Ther. 1990 Nov;255(2):867-74.

** how the results of scientific investigations should be interpreted*

Conclusions *

- Aminoglycosides remain, and may become again 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 should 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 ... and renal uptake (antagonists ?) …
- 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, ...)

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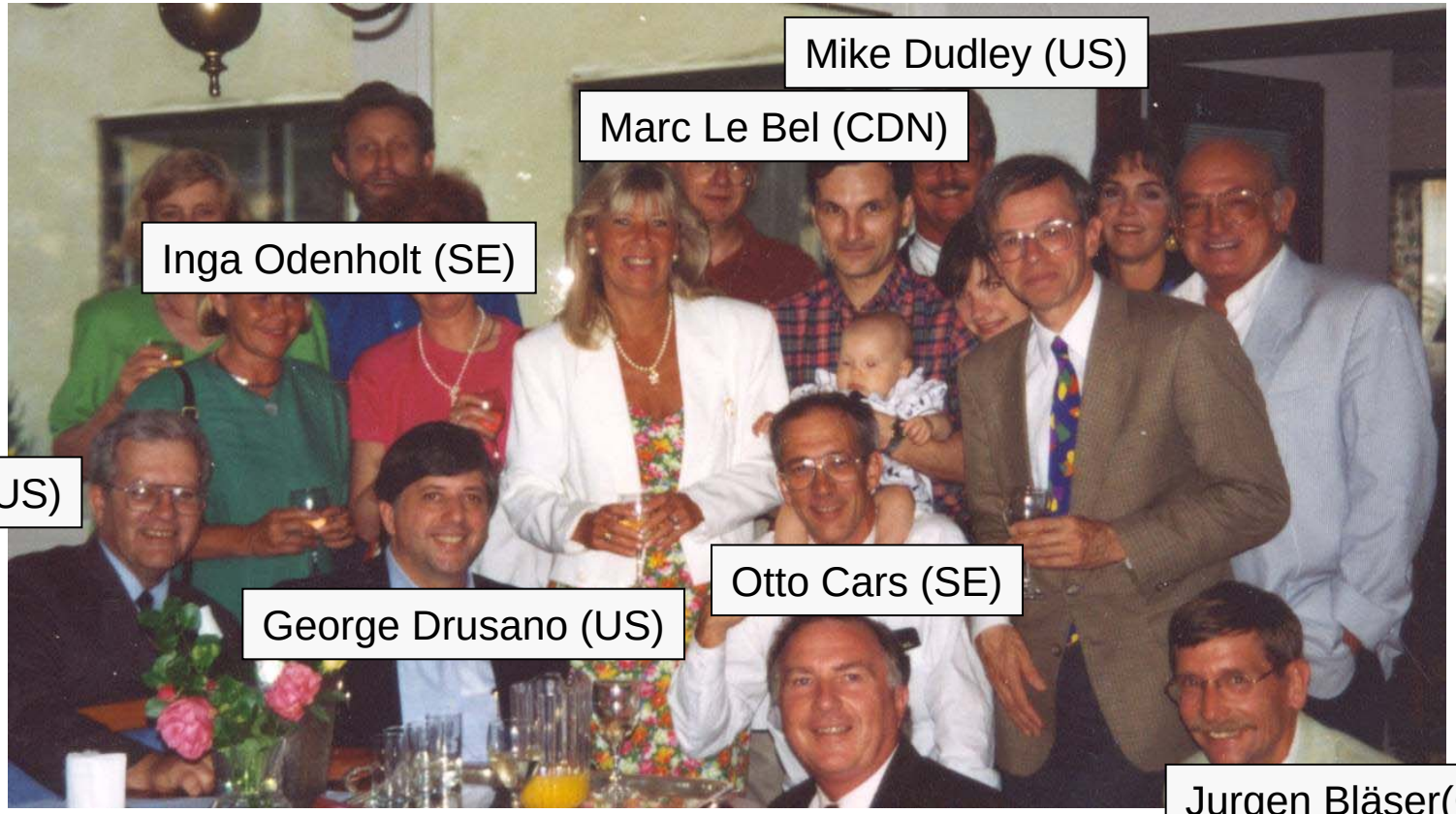
* not all based on what I said, but I can expand if you wish …

So, why not new aminogycosides ? *

* the kind of questions that are supposed to be asked and probed for answers in relation to this subject

You can join the team …



It all started only a few years ago …

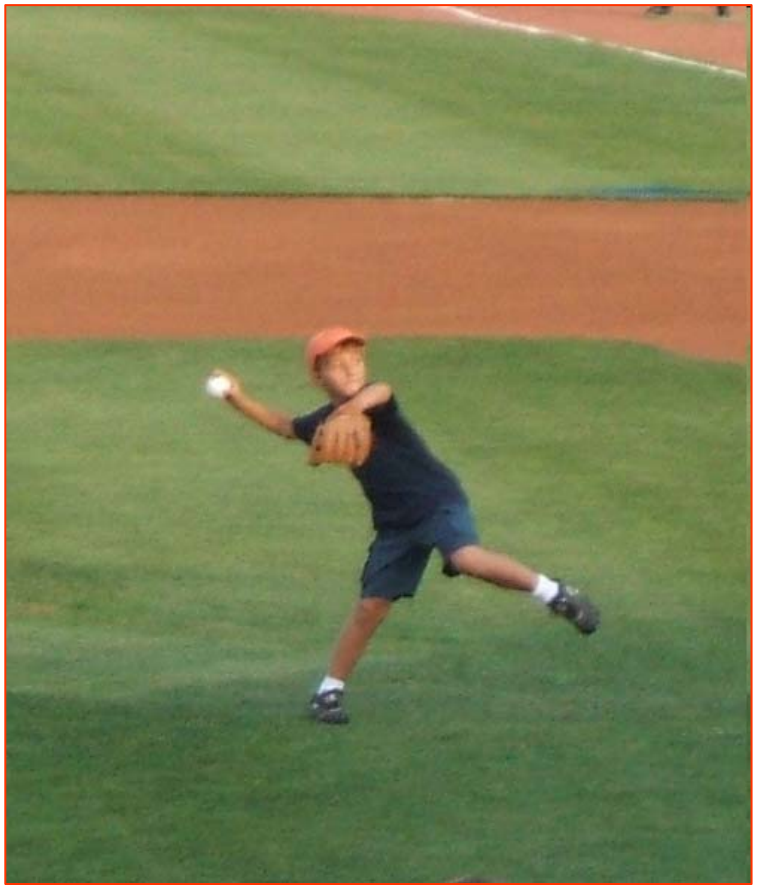
ISAP

After all, he did not hesitate to throw balls …

Paul Ehrlich (1854-1915)

He devoted himself to chemotherapy, basing his work on the idea that the chemical constitution of drugs must be studied in relation to their mode of action and their affinity for the cells of the organisms against which they were directed. These would be, as Ehrlich expressed it, «magic bullets» which would go straight to the organisms at which they were aimed.

From *Nobel Lectures, Physiology or Medicine 1901-1921*, Elsevier Publishing Company, Amsterdam, 1967



Young Ehrlich throwing the magic ball