Toxicodynamics…

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AND PUBLIC HEALTH

William C. Craig Symposium

My early steps to toxicodynamics …

Biochemical Pharmacology, Vol. 27, pp. 415–424. Pergamon Press, 1978. Printed in Great Britain.

THE UPTAKE AND INTRACELLULAR ACCUMULATION OF AMINOGLYCOSIDE ANTIBIOTICS IN LYSOSOMES OF CULTURED RAT FIBROBLASTS

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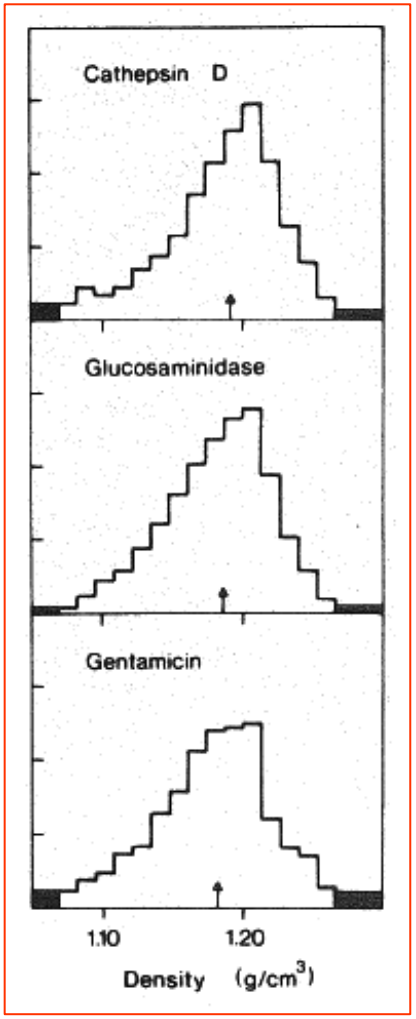
(*Received* 23 *May* 1977; *accepted* 19 *July* 1977)



My early steps to toxicodynamics …

0023-6837/79/4004-0481$02.00/0
LABORATORY INVESTIGATION
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Vol. 40, No. 4, p. 481, 1979
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Gentamicin-Induced Lysosomal Phospholipidosis in Cultured Rat Fibroblasts

Quantitative Ultrastructural and Biochemical Study

GENEVIÈVE AUBERT-TULKENS, M.D., FRANÇOIS VAN HOOF, M.D., PH.D., AND PAUL TULKENS, M.D.

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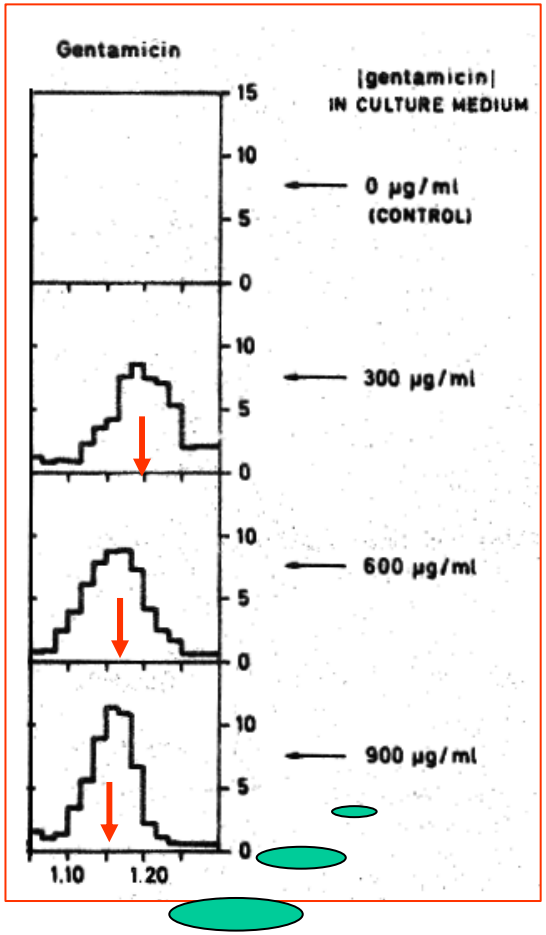


But are those concentrations PK meaningful ?

You said aminoglycoside nephrotoxicity ?

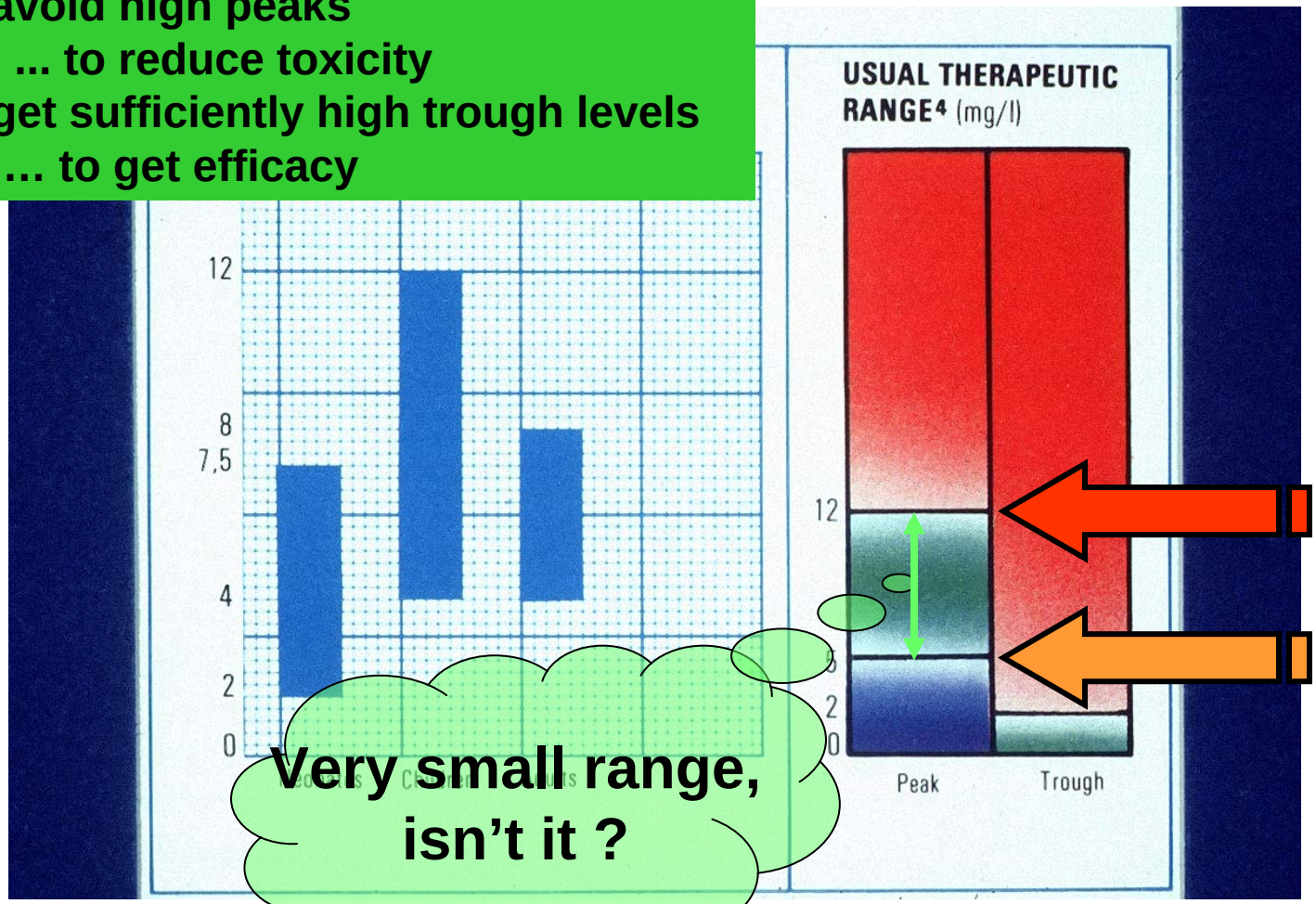
- Typing **"(gentamicin OR aminoglycoside*) AND nephrotoxicity"** on PubMed will yield 1540 papers (among which 229 reviews), with the first one in 1969... (gentamicin was introduced in the clinics in 1967...)

- 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

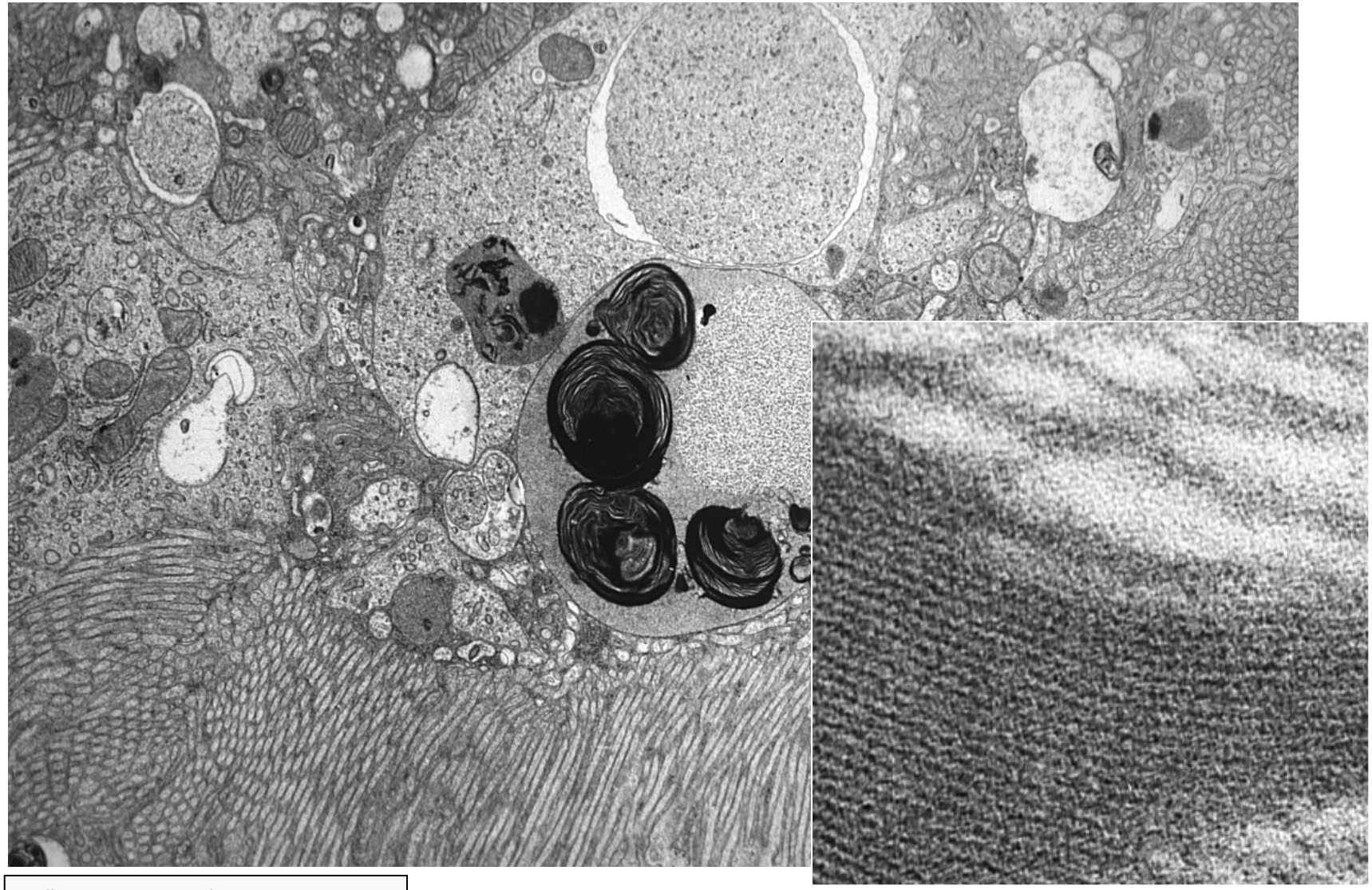
What was monitoring aminoglycosides in the early times of gentamicin ?

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



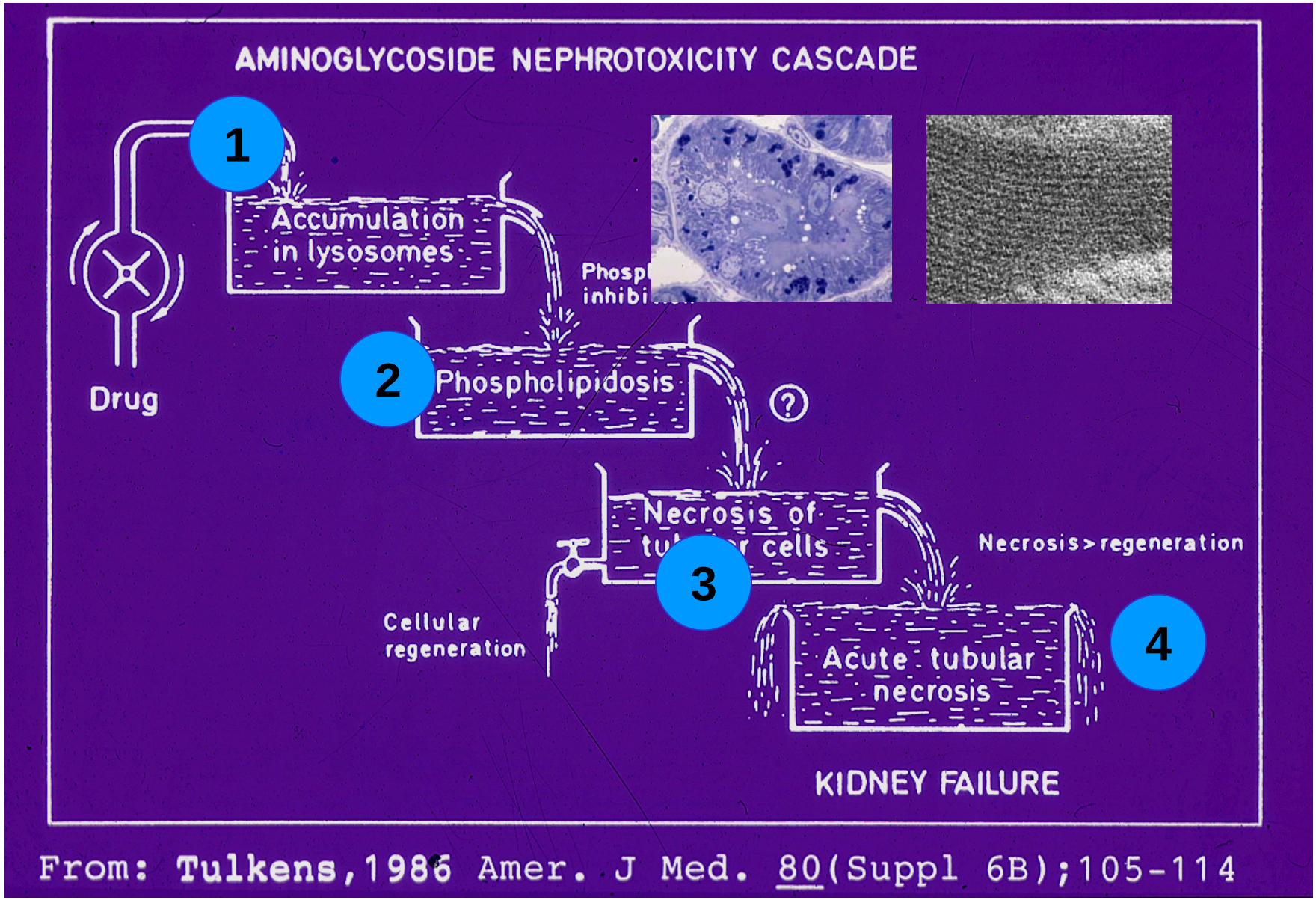
From an "Abbott TdX booklet" (1976) to which I contributed...
Belgian guidelines kept this until 2000...

Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis **at low doses…**

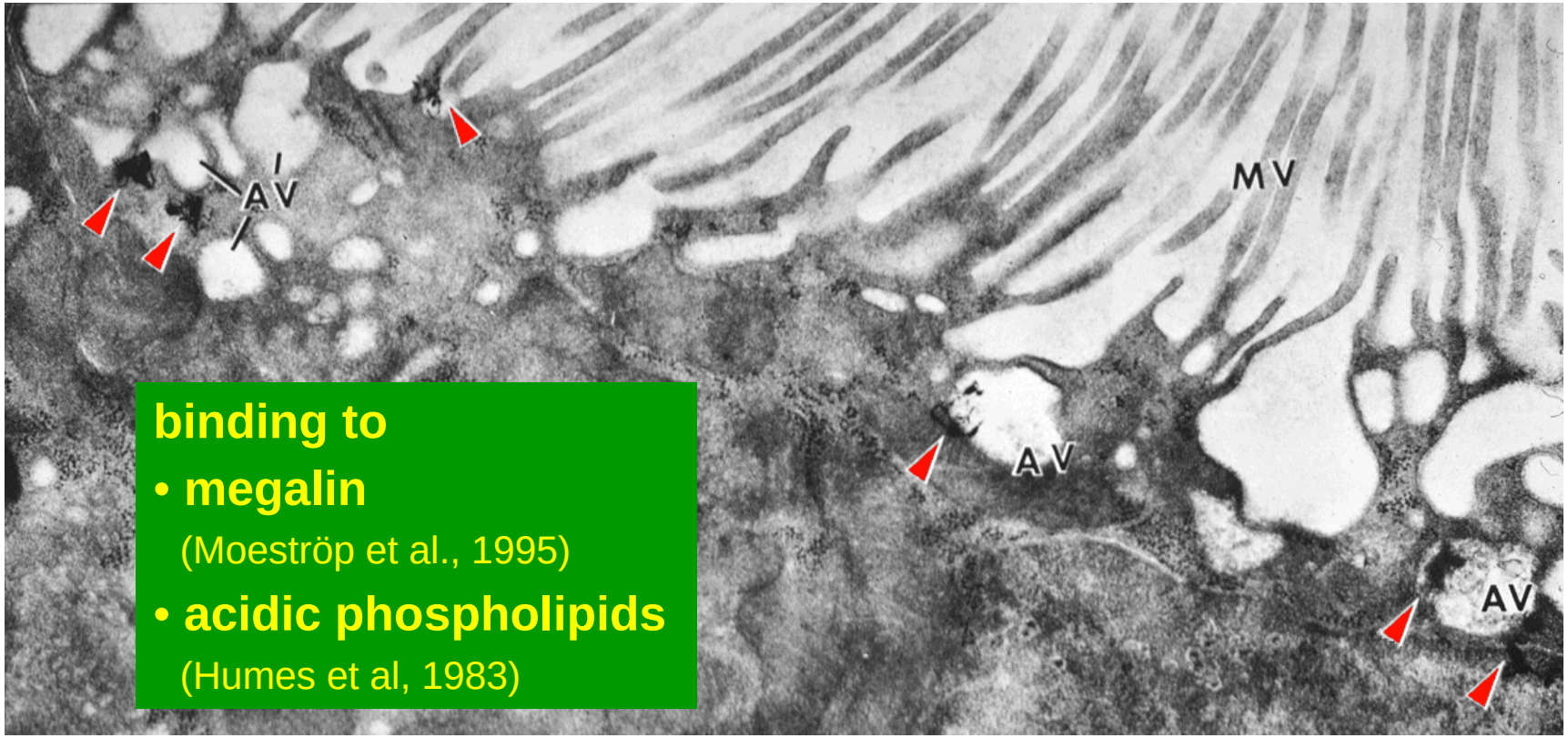


Tulkens, Am. J. Med. 80:105-114, 1986

Aminoglycoside toxicity cascade (1st version)…



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

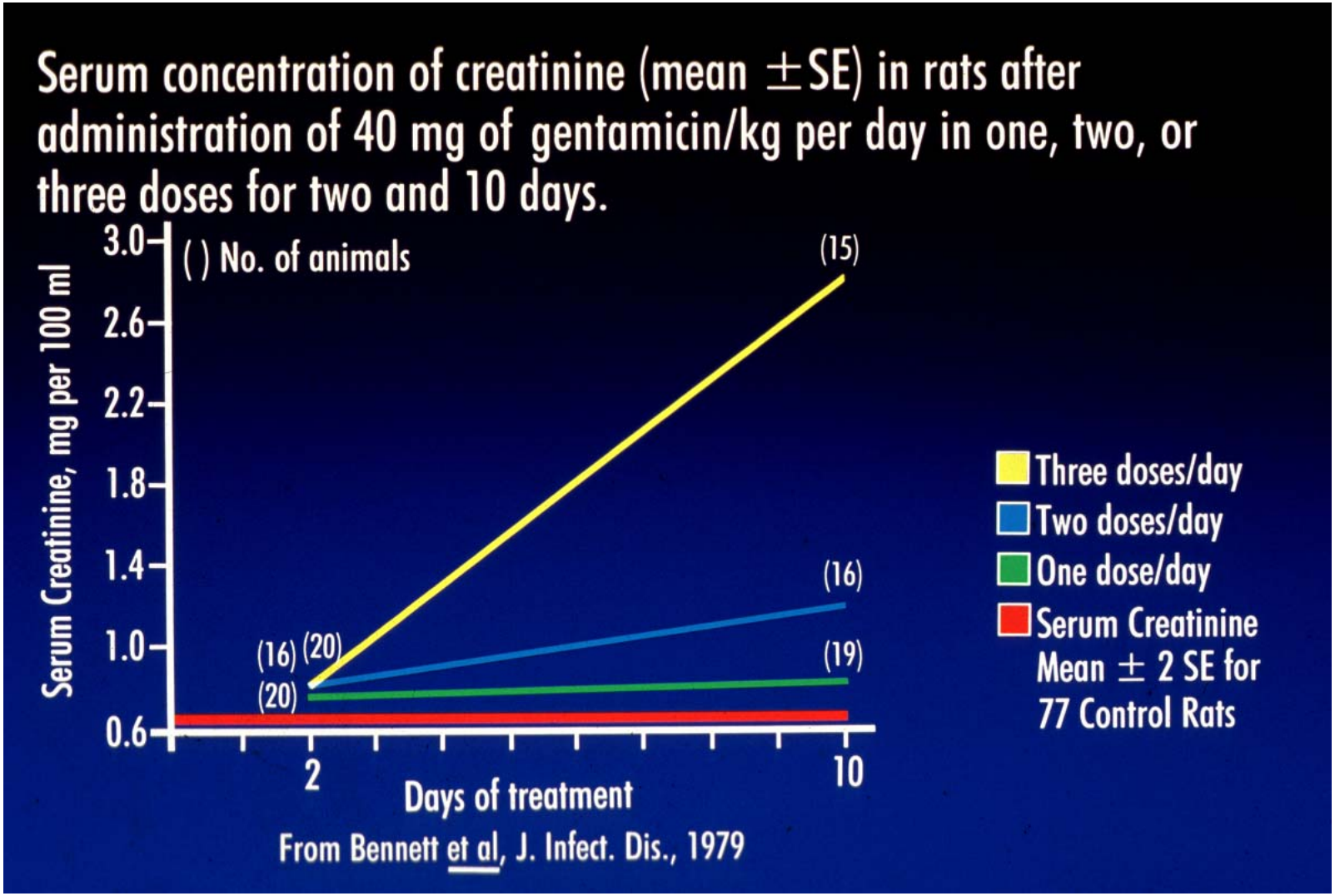


binding to

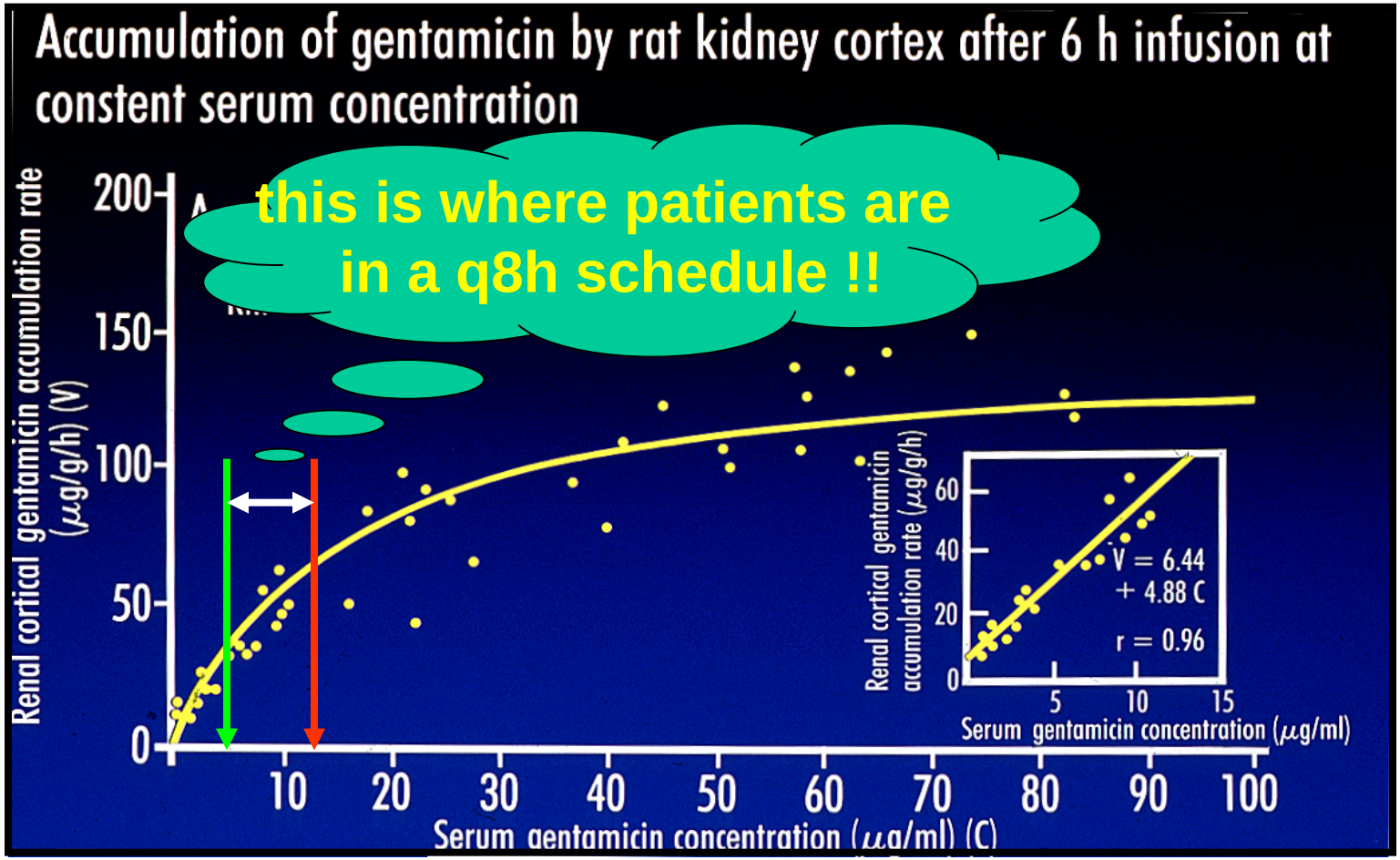
- **megalin**
  (Moeström et al., 1995)
- **acidic phospholipids**
  (Humes et al, 1983)

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

Aminoglycoside toxicity is not linked to peak (alone)



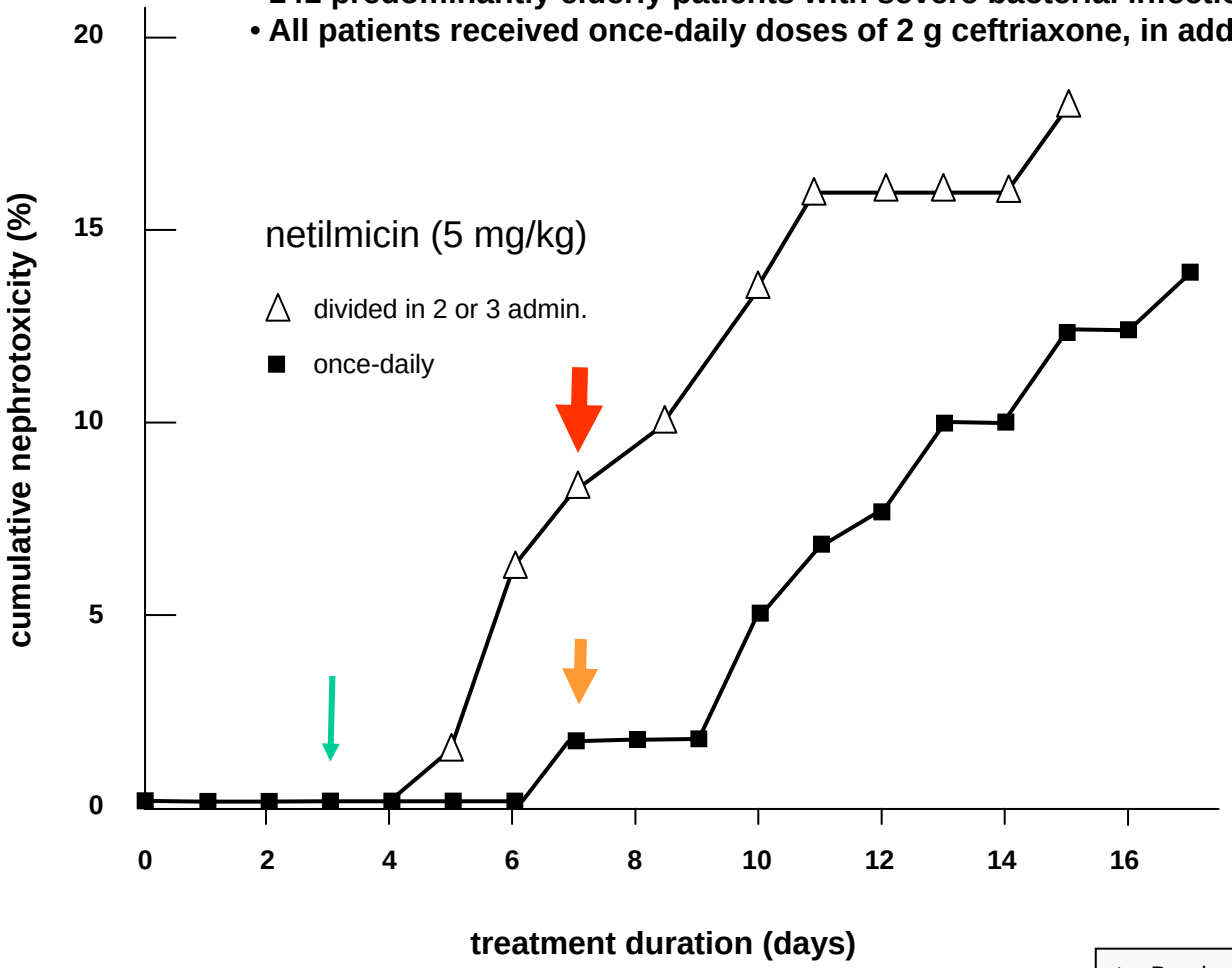
Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...



* 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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* a direct application of PK/TD principles…

Can we further prevent aminoglycoside toxicity ?

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Protection against Gentamicin-Induced Early Renal Alterations (Phospholipidosis and Increased DNA Synthesis) By Coadministration of Poly-L-Aspartic Acid[1]

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Laboratoire de Chimie Physiologique and International Institute of Cellular and Molecular Pathology (D.B., G.L., B.K.K., P.M.T.) and Laboratoire de Cytologie et de Pathologie Expérimentales (P.M., S.A.), Université Catholique de Louvain, Bruxelles, Belgium

Accepted for publication July 13, 1990

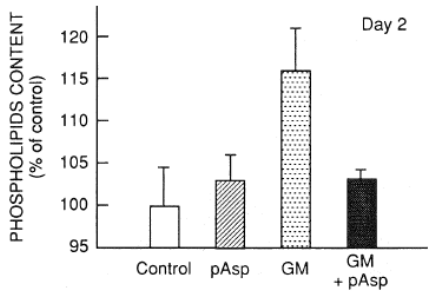

Fig. 2. Activity of sphingomyelinase and phospholipid content of kidney cortex expressed as percentage of control values. Control, animals infused with saline (groups 1a and 1b); polyaspartic acid (pAsp), animals infused with pAsp (250 mg/kg) (groups 3a and 3b); GM, animals infused with GM (100 mg/kg) (groups 2a and 2b); GM + pAsp, animals infused with GM (100 mg/kg) and pAsp (250 mg/kg) (groups 4a and 4b). See table 1 for definition of the experimental protocols and for number of animals in each group.

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Mechanism of Protection Afforded by Polyaspartic Acid against Gentamicin-Induced Phospholipidosis. II. Comparative *in Vitro* and *in Vivo* Studies with Poly-L-Aspartic, Poly-L-Glutamic and Poly-D-Glutamic Acids[1]

B. K. KISHORE,[2] P. LAMBRICHT, G. LAURENT,[3] P. MALDAGUE, R. WAGNER[4] and P. M. TULKENS

Laboratoire de Chimie Physiologique and International Institute of Cellular and Molecular Pathology (B.K.K., P.L., G.L., R.W., P.M.T.) and Unité de Pathologie et de Cytologie Expérimentales (P.M.), Université Catholique de Louvain, Bruxelles, Belgium

Accepted for publication July 13, 1990

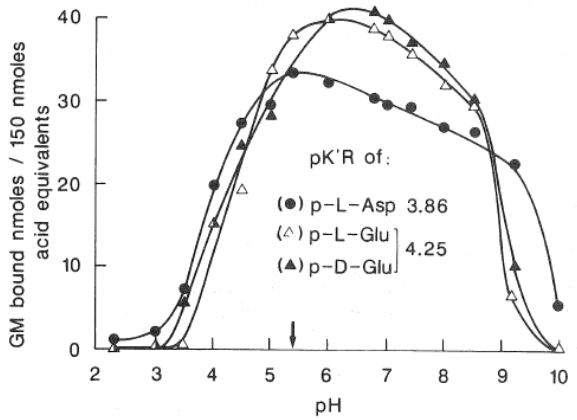
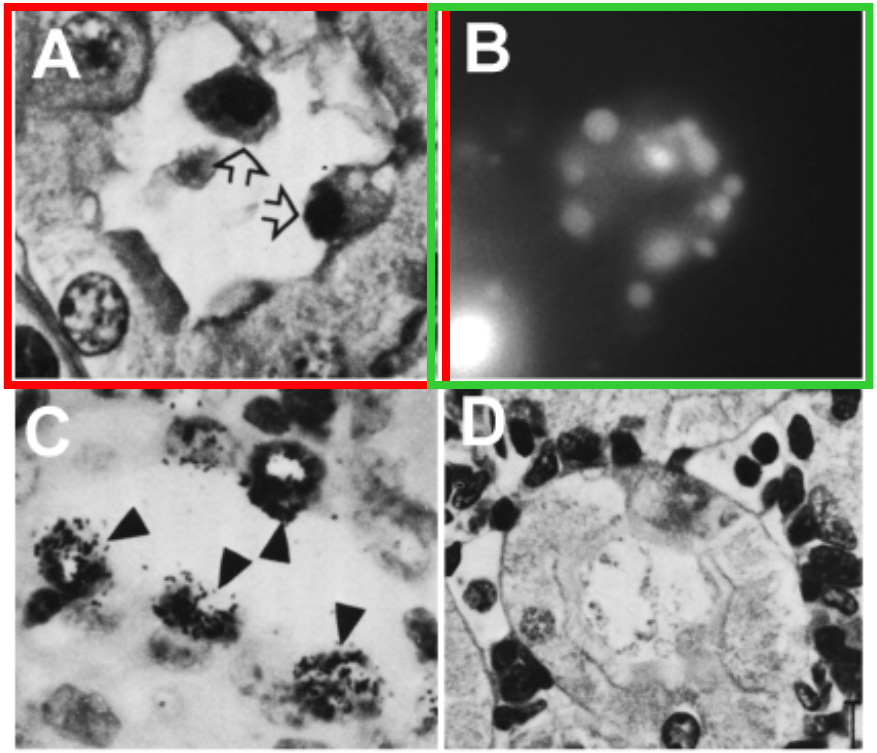


Fig. 1. Results of a typical experiment showing the binding of gentamicin to polyanionic peptides as a function of pH at 4 mM ionic strength. Results obtained by dialyzing 100 nmol of gentamicin against 150 nmol of acid equivalents of polyanions at 37°C for 3 hr. The buffers used were HCl-KCl (pH 2–3.5), Na acetate (pH 4–5.4), phosphate (pH 6–7.4), Tris-HCl (pH 8–8.5) and carbonate-bicarbonate (pH 9–10). B_{max} occurs between pH 5 to 6 in case of poly-L-Asp (p-L-Asp) and around pH 6 in case of poly-L-Glu (p-L-Glu) and poly-D-Glu (p-D-Glu).

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,

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

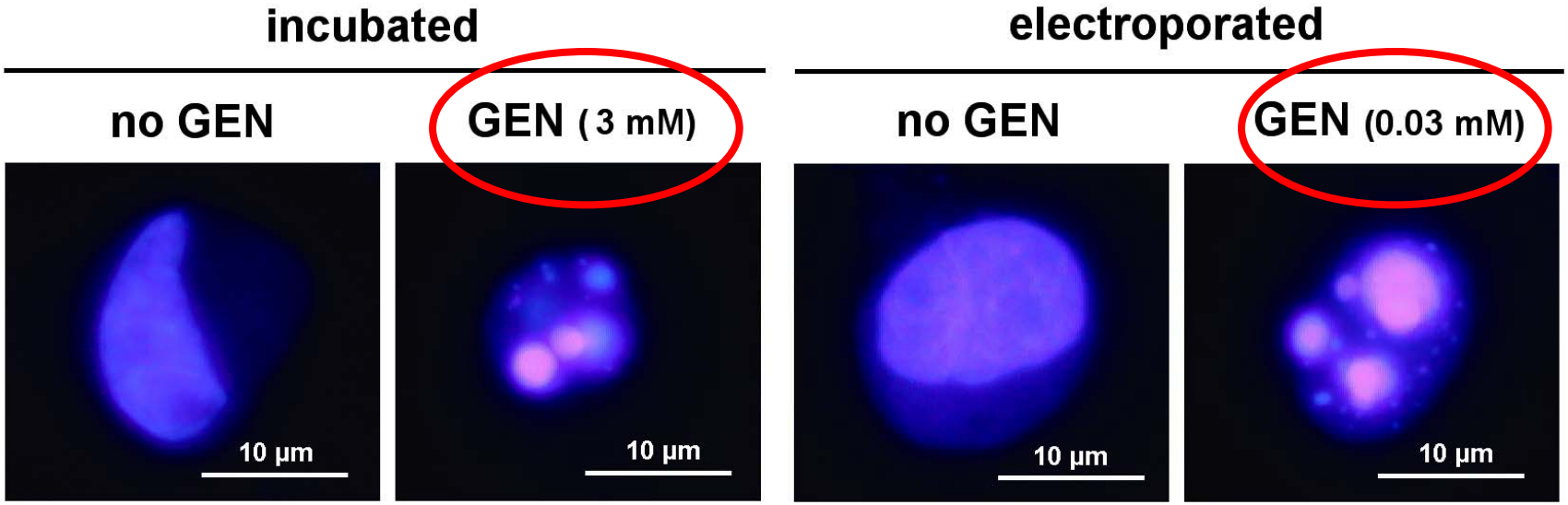


Figure 1: Staining of nuclei of LLC-PK_1 cells by 4',6'-diamidine-2'-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the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

Apoptosis in electroporated cells as a means to test for toxicity

Reconciling PK and cell culture models

11-54 mg/L

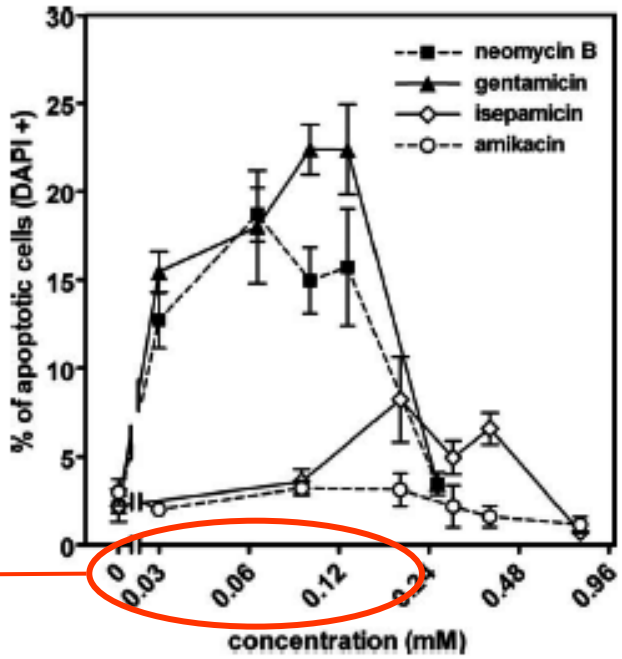


FIG. 2. Apoptosis in electroporated cells. Cells were electroporated in the absence (controls) or in the presence of neomycin B, gentamicin, isepamicin, or amikacin and returned to aminoglycoside-free medium, and apoptotic nuclei were enumerated 24 h later. Values are means ± standard deviations ($n = 3$). 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

Could aminoglycoside toxicity be a paradigm ?

European Journal of Pharmacology 314 (1996) 203–214

Interaction of the macrolide azithromycin with phospholipids. I. Inhibition of lysosomal phospholipase A_1 activity

Françoise Van Bambeke [a,*], Jean-Pierre Montenez [a], Jocelyne Piret [a], Paul M. Tulkens [a], Pierre J. Courtoy [b], Marie-Paule Mingeot-Leclercq [a]

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Large concentratrations only … probably not PK relevant

PK relevance under study …

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2005, p. 1695–1700 Vol. 49, No. 5
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.5.1695–1700.2005

Mixed-Lipid Storage Disorder Induced in Macrophages and Fibroblasts by Oritavancin (LY333328), a New Glycopeptide Antibiotic with Exceptional Cellular Accumulation

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But old cars can still drive you far …

You are never old when you do good research …

You are never old when you do good research …