

**Comparative analysis of the potential of polymyxin B and gentamicin to cause apoptosis and necrosis in cultured renal LLC-PK1 cells: concentration-dependent studies with incubated and electroporated cells.**

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21st  
27th  
**ECCMID**  
**ICC**

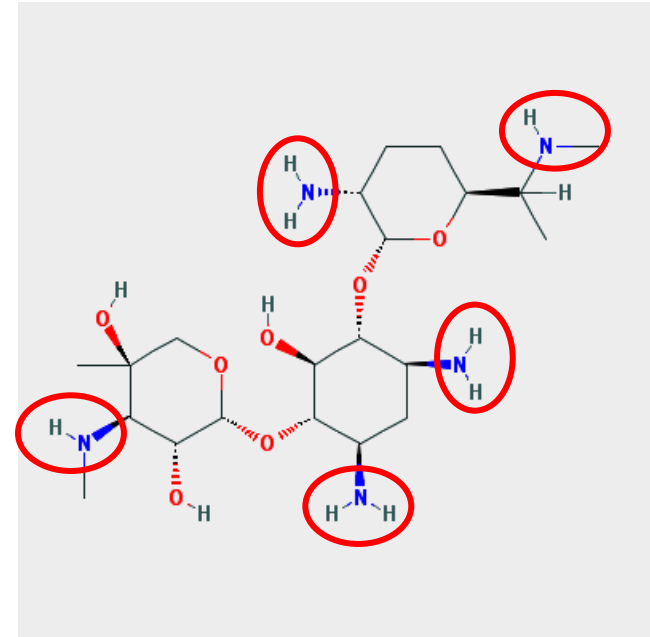
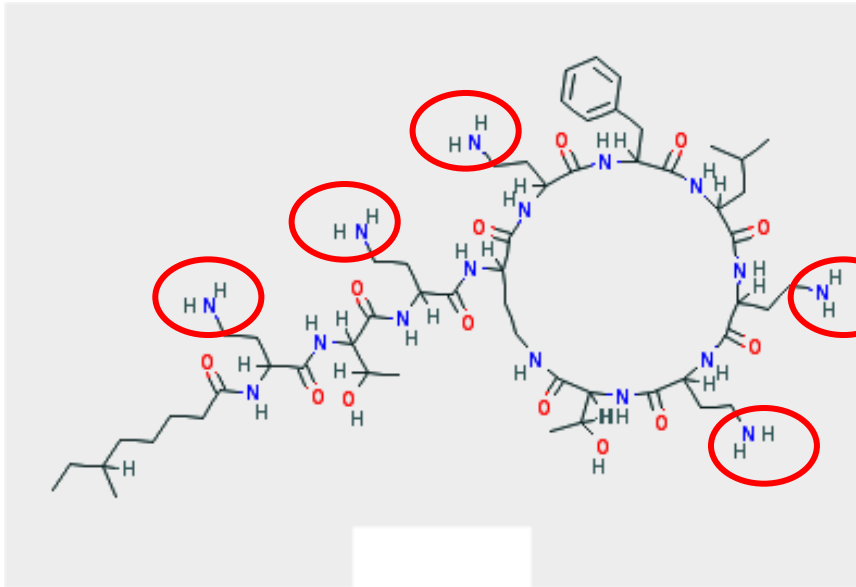
21<sup>st</sup> European Congress of Clinical Microbiology  
and Infectious Diseases (ECCMID) &  
27<sup>th</sup> International Congress of Chemotherapy (ICC)  
Milan, Italy, 7-10 May 2011

# Contents of the presentation

- Why polymyxin B and gentamicin ?
- Why necrosis and apoptosis ?
- Why incubated cells vs electroporated cells ?
- What did we observed ?
- What can we conclude ?

as you can see, many " ? "

# Polmyxin B and gentamicin...



- both are useful against Gram-negative bacteria (with possibility to have derivatives active against multidrug resistant bacteria)...
- both are polycationic ... (5 positive charges)
- both are nephrotoxic ...
- for both, efforts are being made to design less toxic derivatives...

# Gentamicin nephrotoxicity

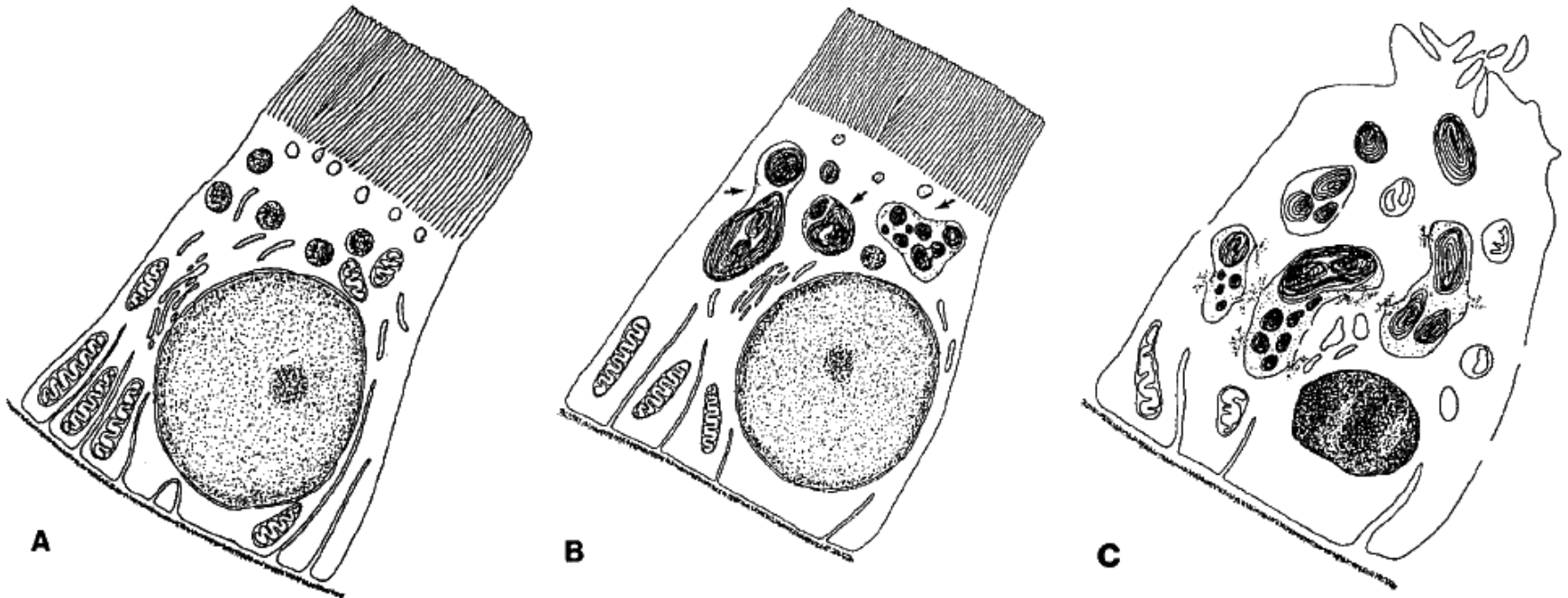
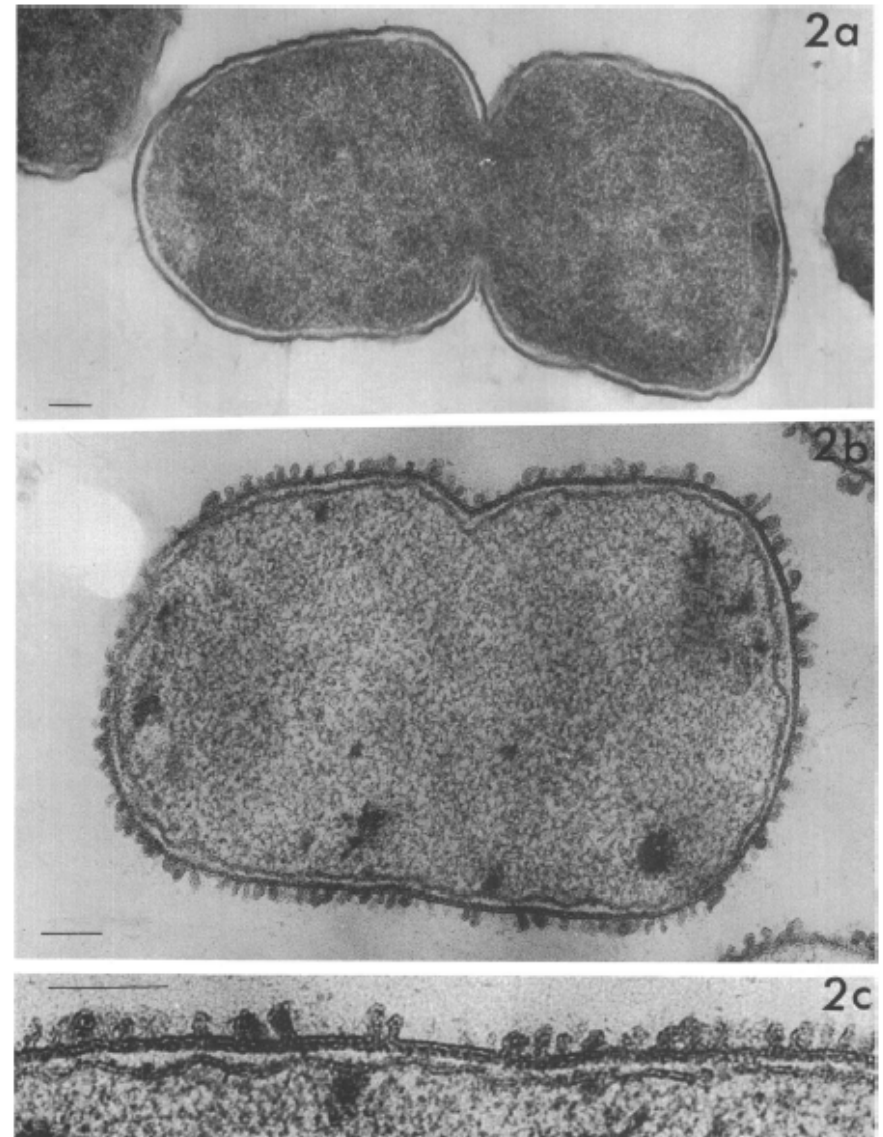
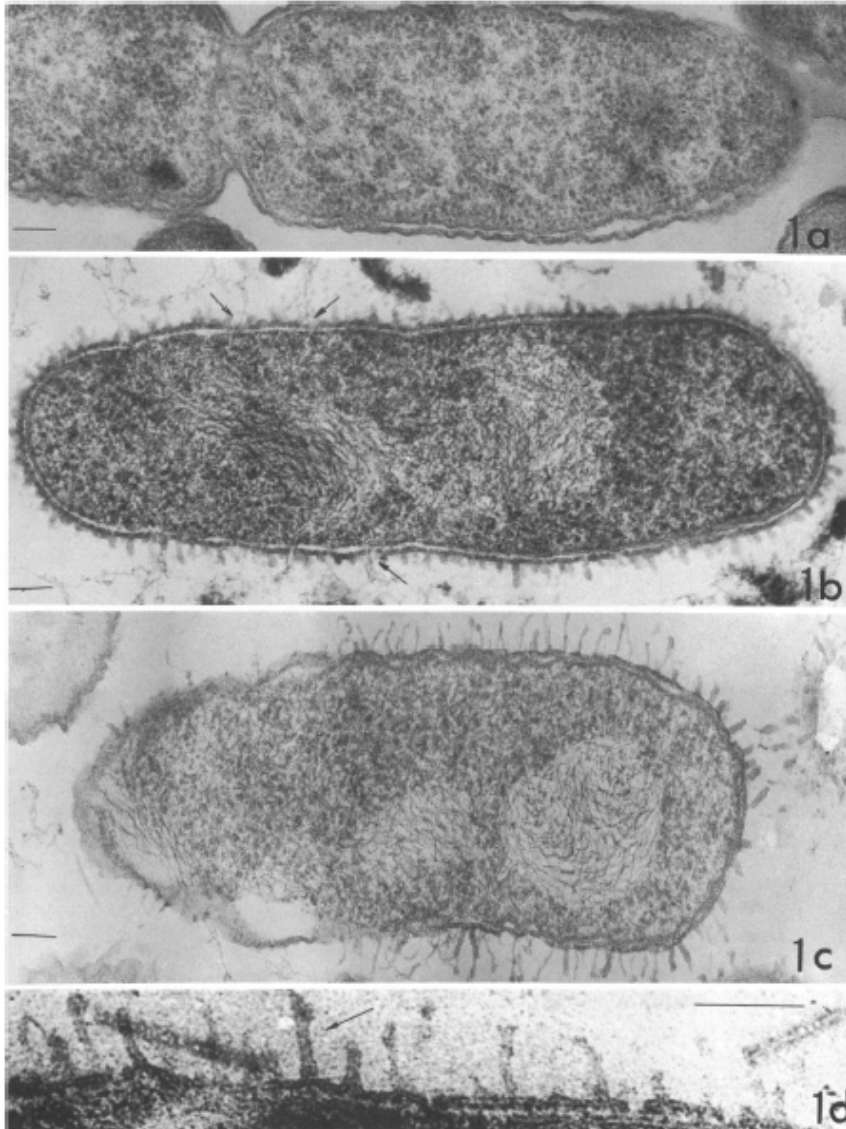


FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive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

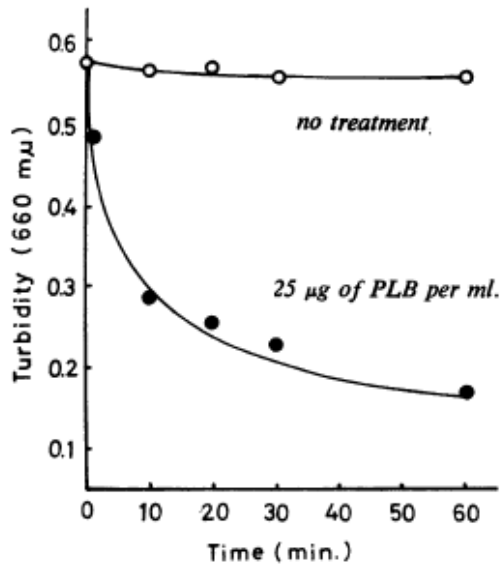
Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. 1999; 43:103-1012

# Polmyxins antibacterial activity: morphological aspects

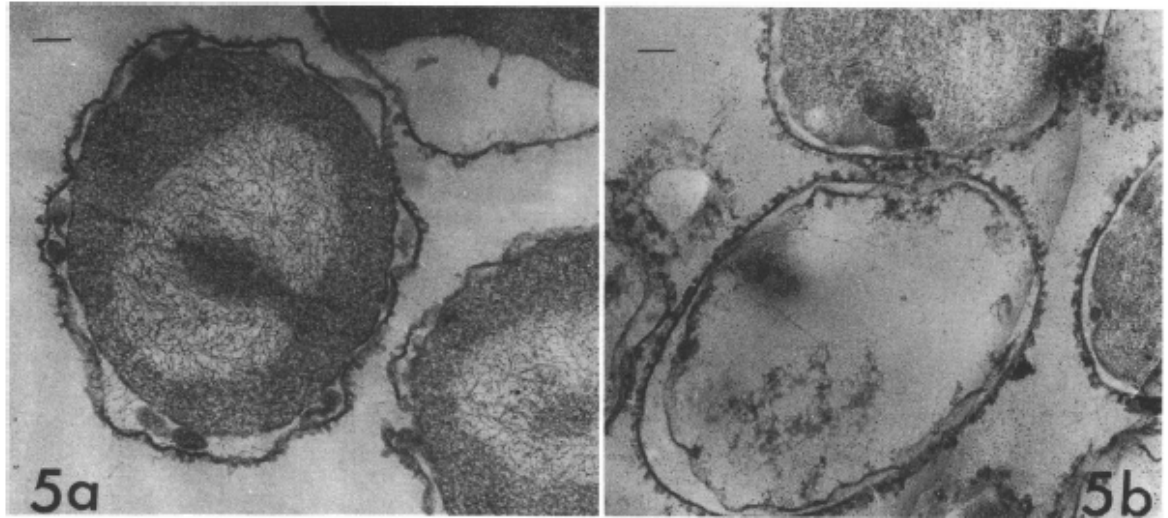


Koike et al. J. Bacteriol. 1969; 97:448-452

# Polymyxins antibacterial activity : lysis of bacteria



*Lysis of the spheroplast of E. coli B*

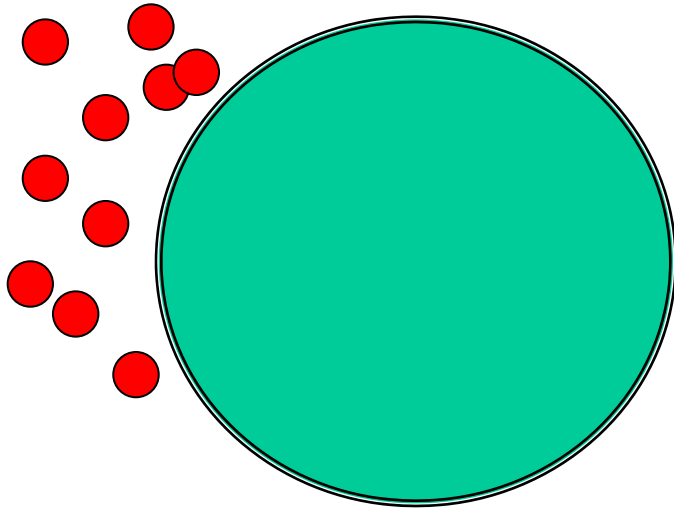


Koike et al. J. Bacteriol. 1969; 97:448-452

# Toxicity of polymyxins

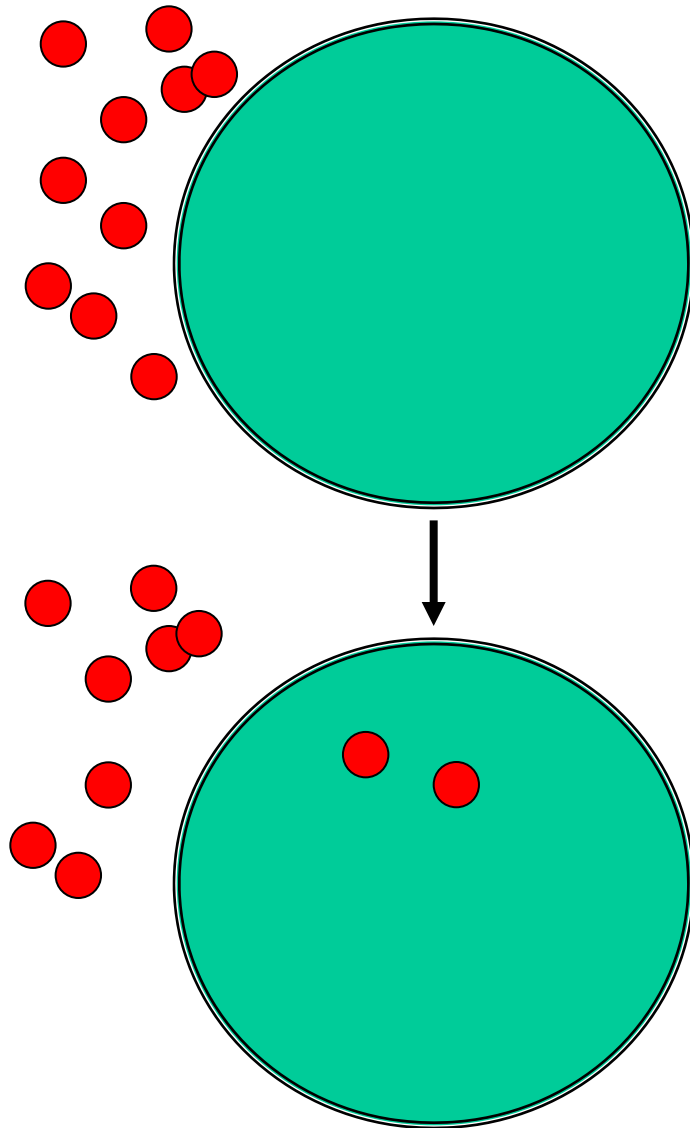
- Renal toxicity
  - Up to 20 % of patients in early trials
  - Occurs after 4 days of treatment
  - Acute tubular necrosis (can progress after drug discontinuation)
  - Related to overdosage (obese ! Oliguric renal failure if if doses higher than recommended are used)
- Neurotoxicity:
  - Giddiness, numbness, paresthesia, peripheral neuropathy
  - Confusion, coma, psychosis at large doses
  - Neuromuscular blockade (paralysis) related to doses but other contributing factors

# Incubation vs. electroporation in drug toxicity evaluation

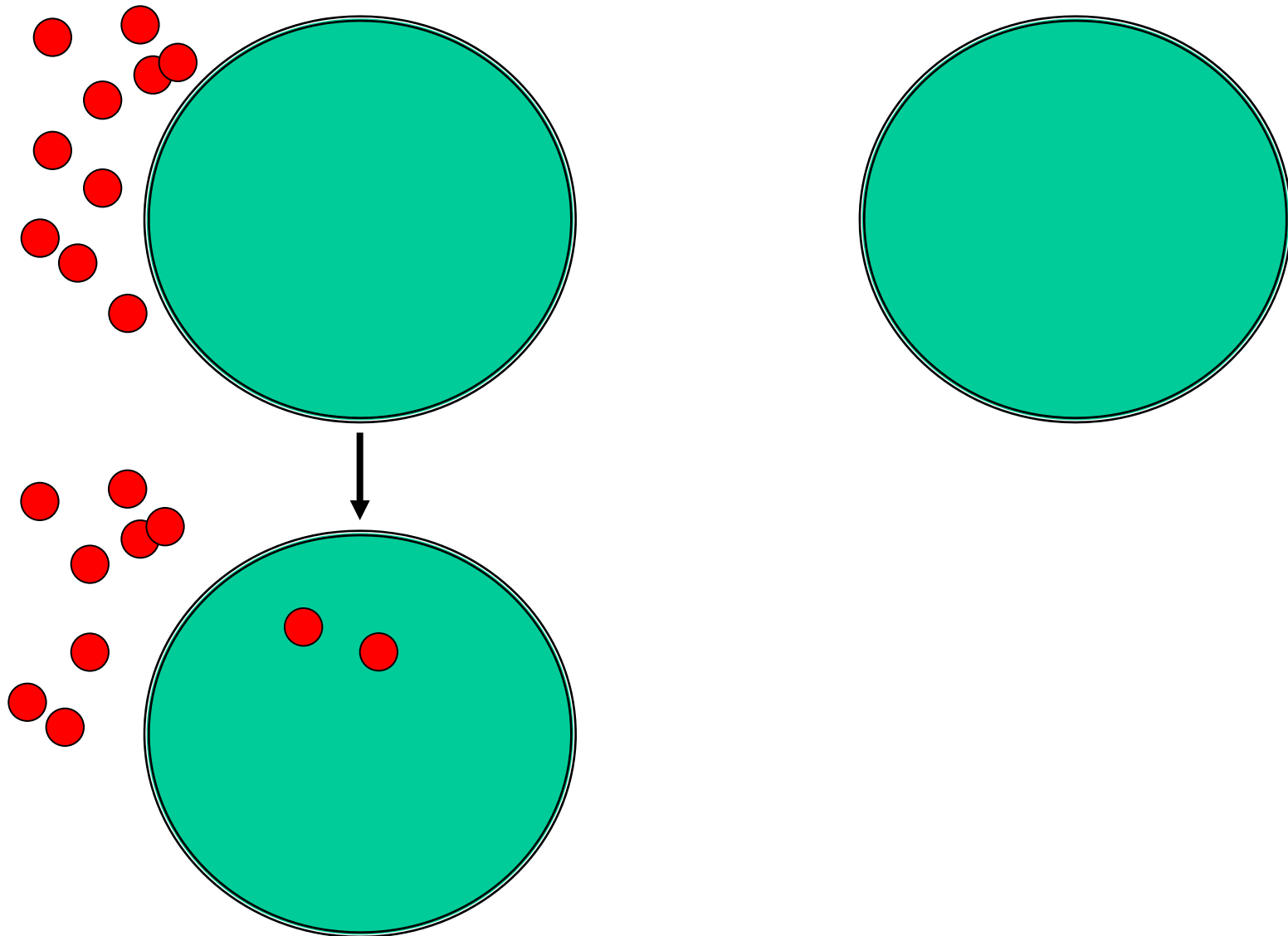




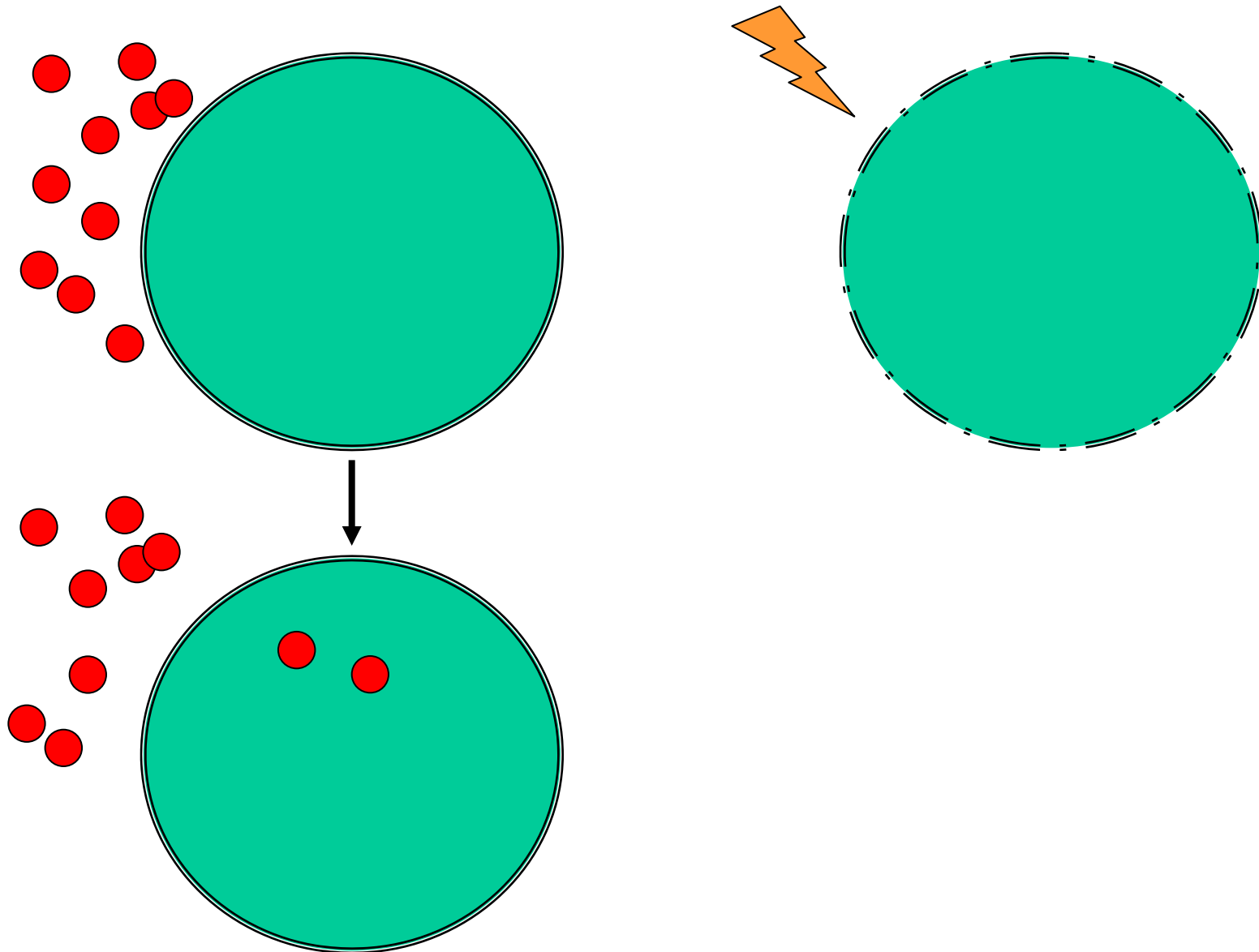
# Incubation vs. electroporation in drug toxicity evaluation



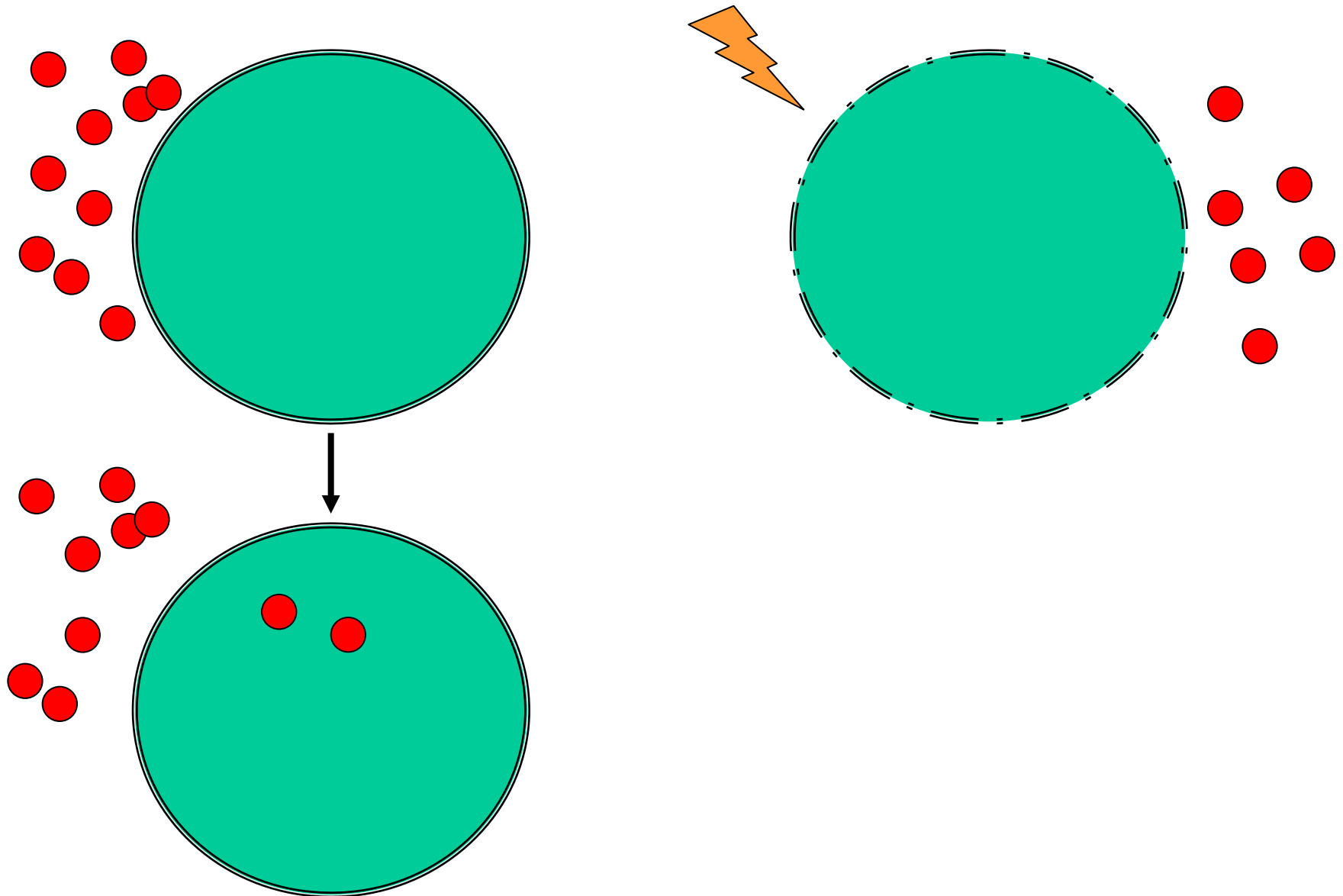
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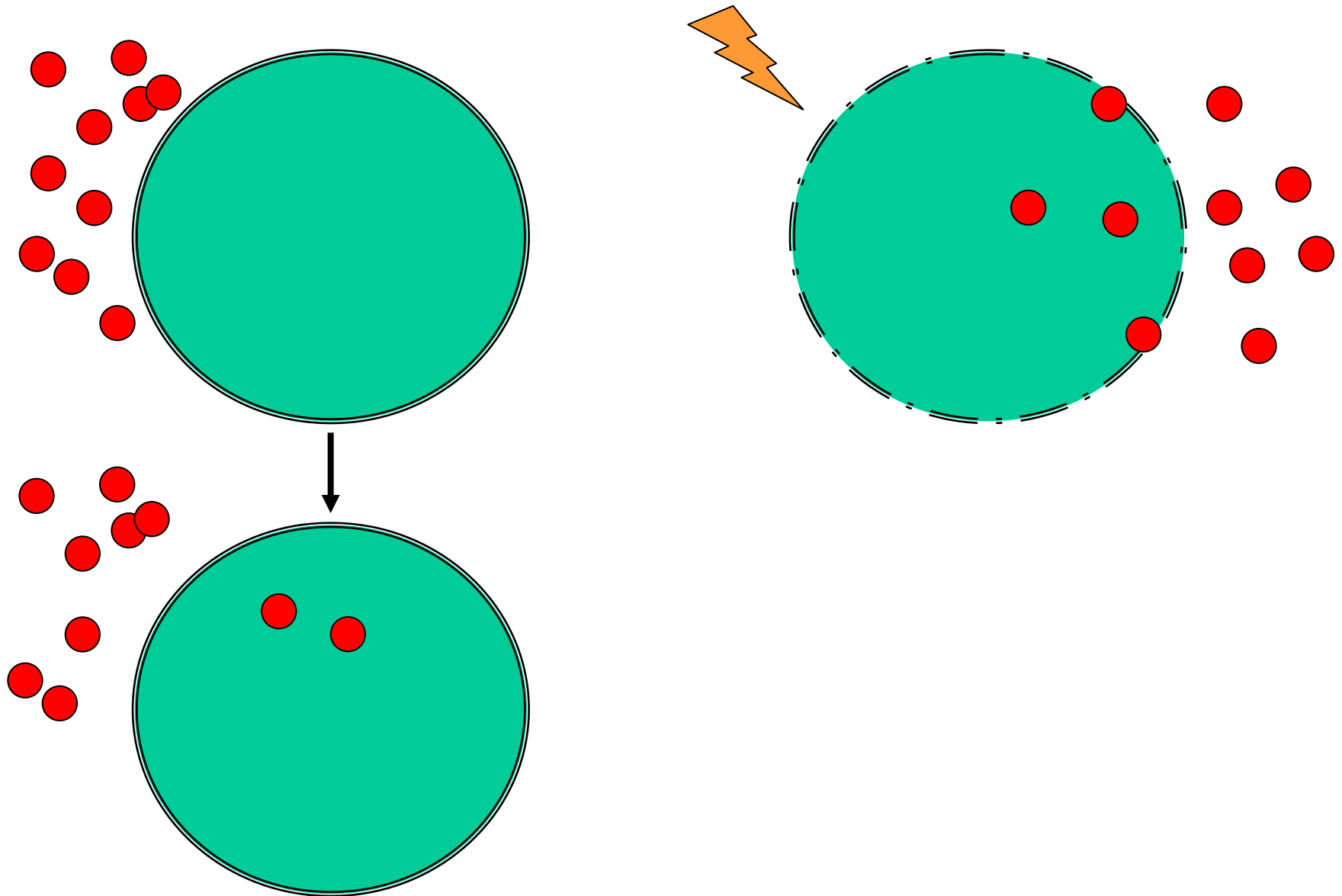
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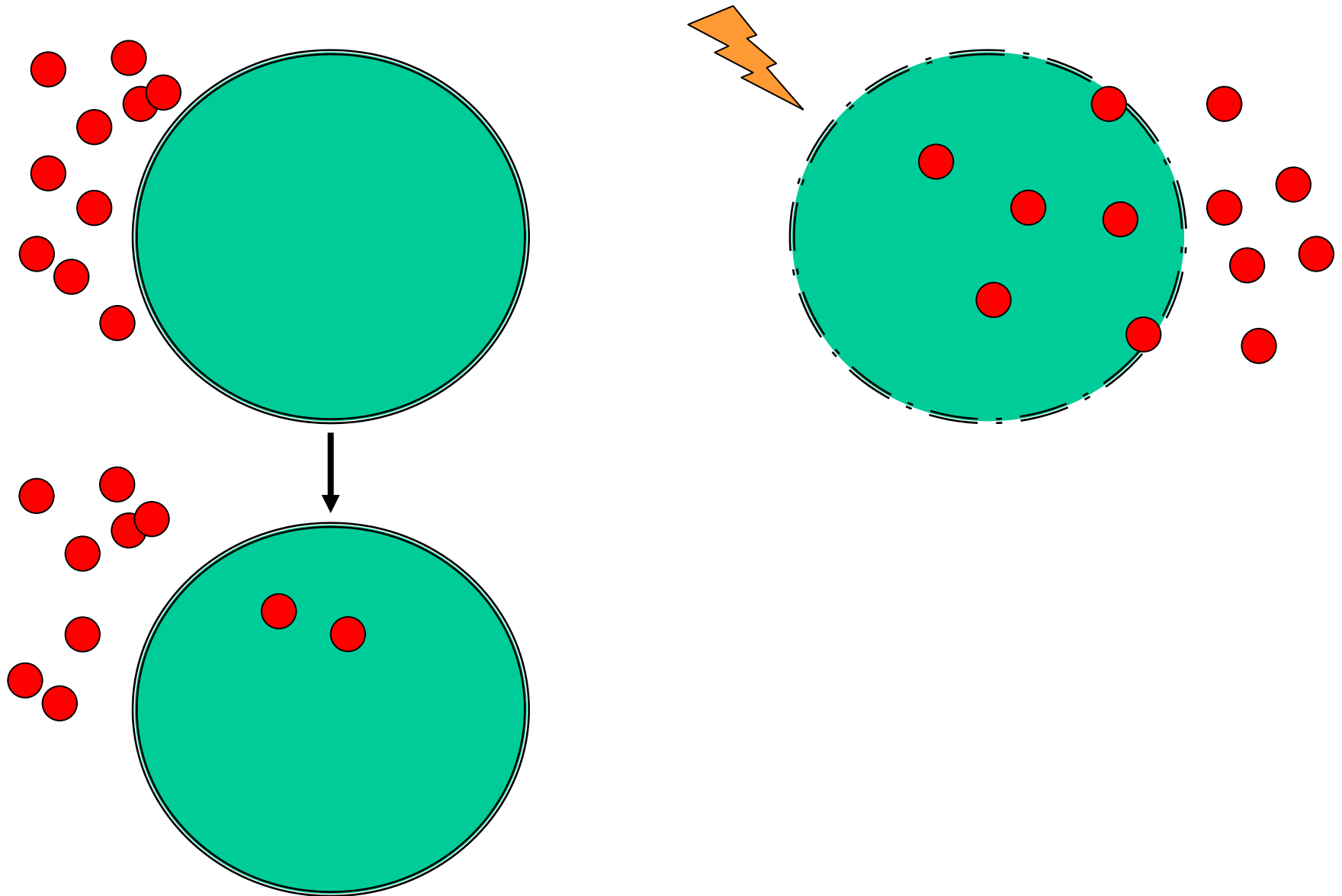
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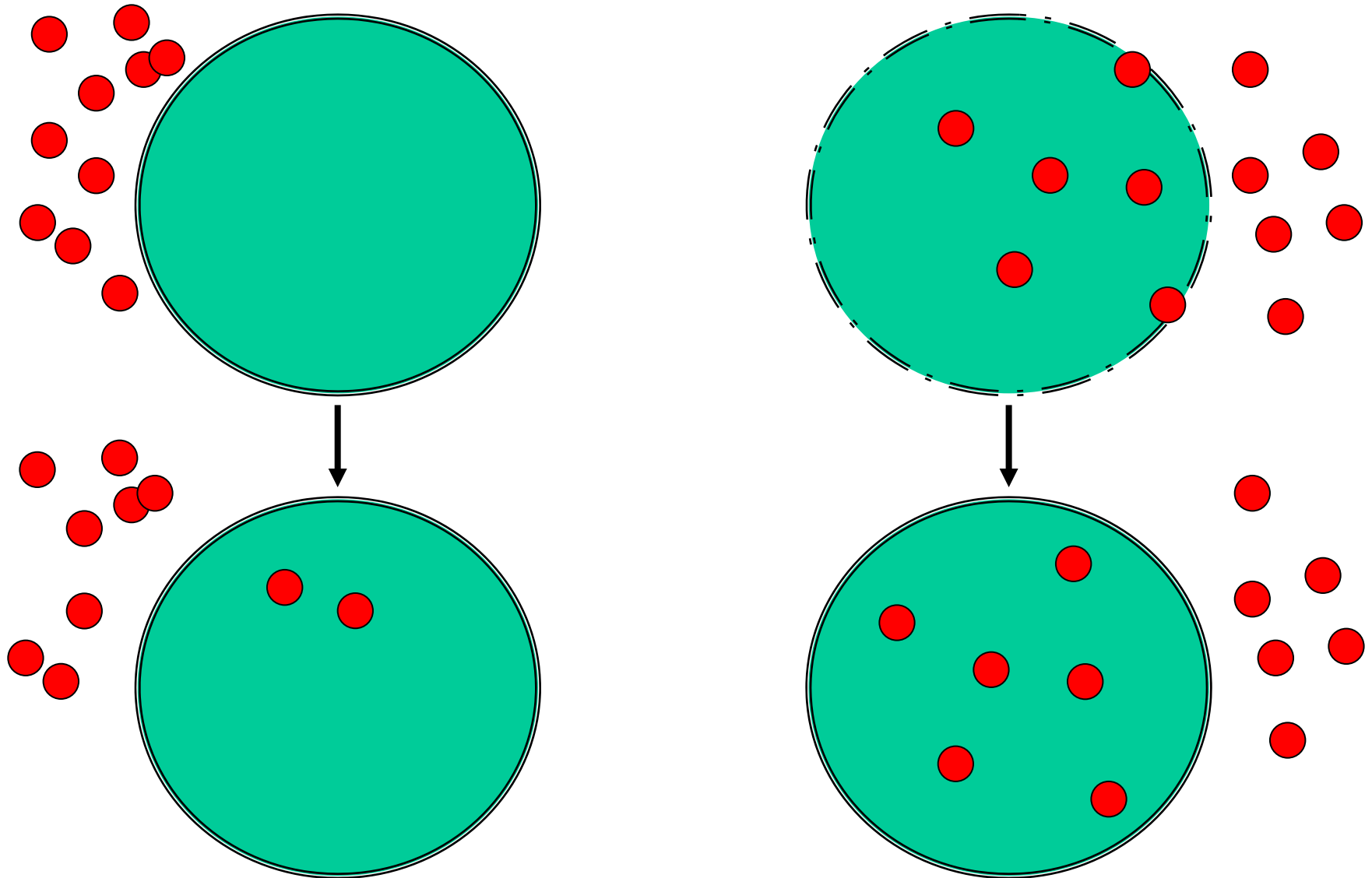
# Incubation vs. electroporation in drug toxicity evaluation



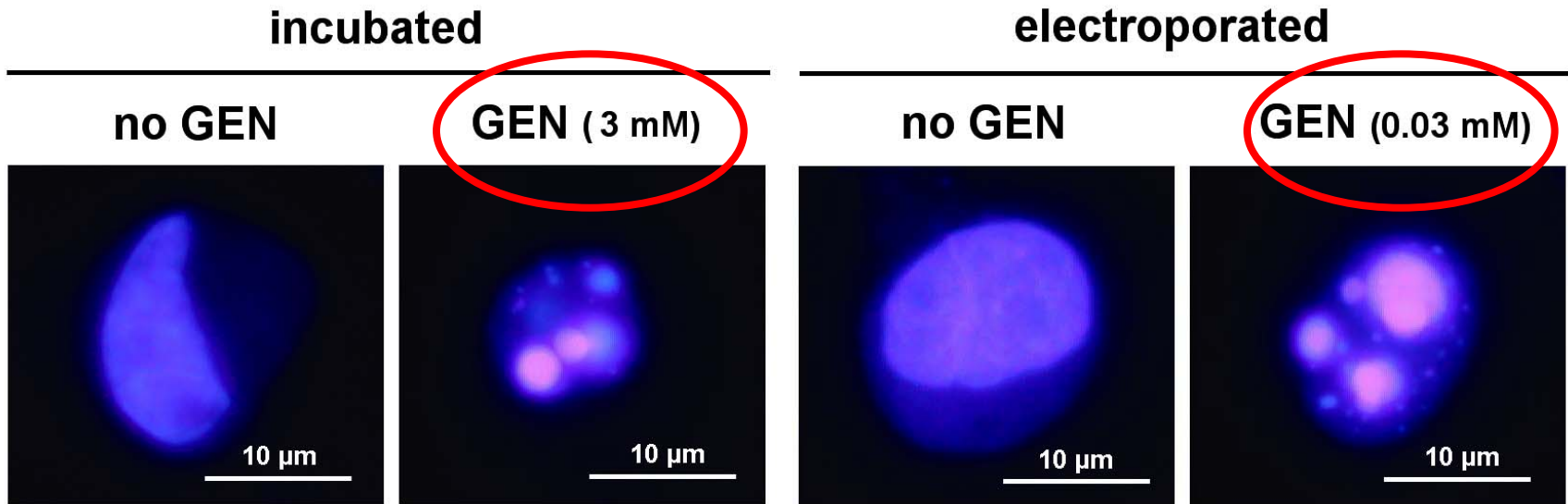
# Incubation vs. electroporation in drug toxicity evaluation



# Incubation vs. electroporation in drug toxicity evaluation



# Incubation vs. electroporation in drug toxicity evaluation: Application to gentamicin



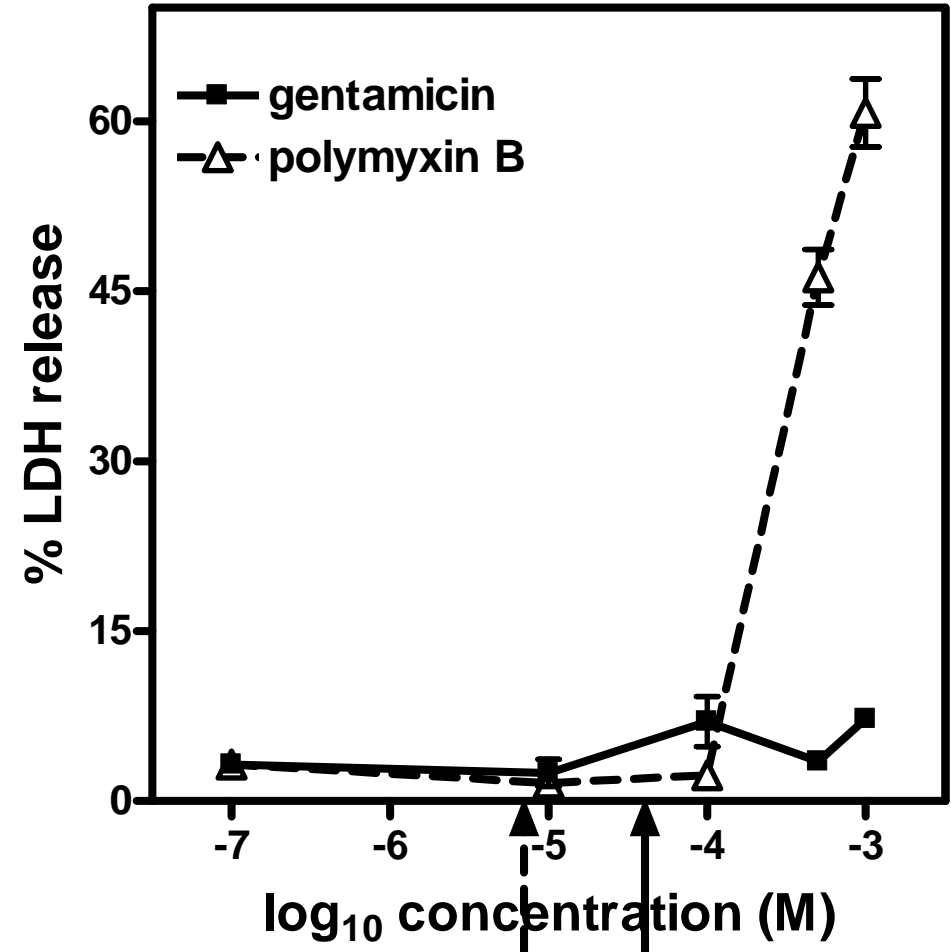
**Figure 1:** Staining of nuclei of LLC-PK<sub>1</sub> cells by 4',6'-diamidino-2'-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated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



# Incubated cells: necrosis

necrosis



Point of comparison:  
serum concentration

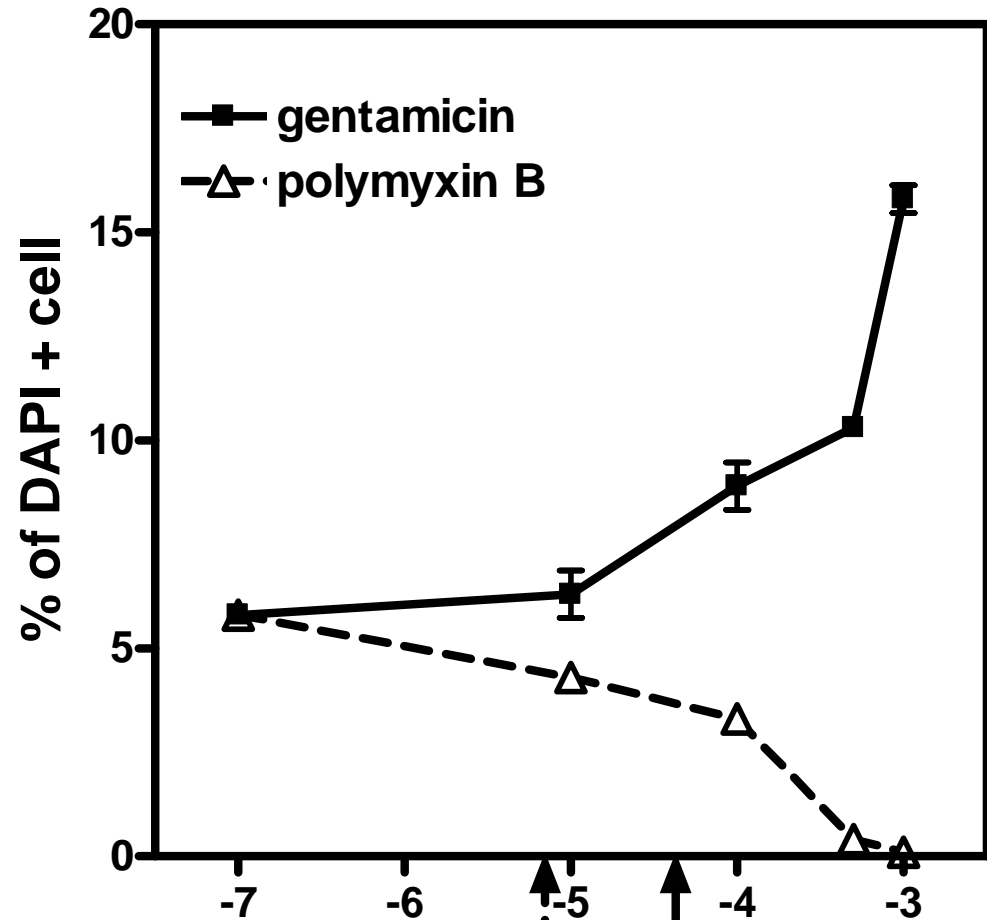


POB C<sub>max</sub>

GEN C<sub>max</sub>

# Incubated cells: apoptosis

## apoptosis



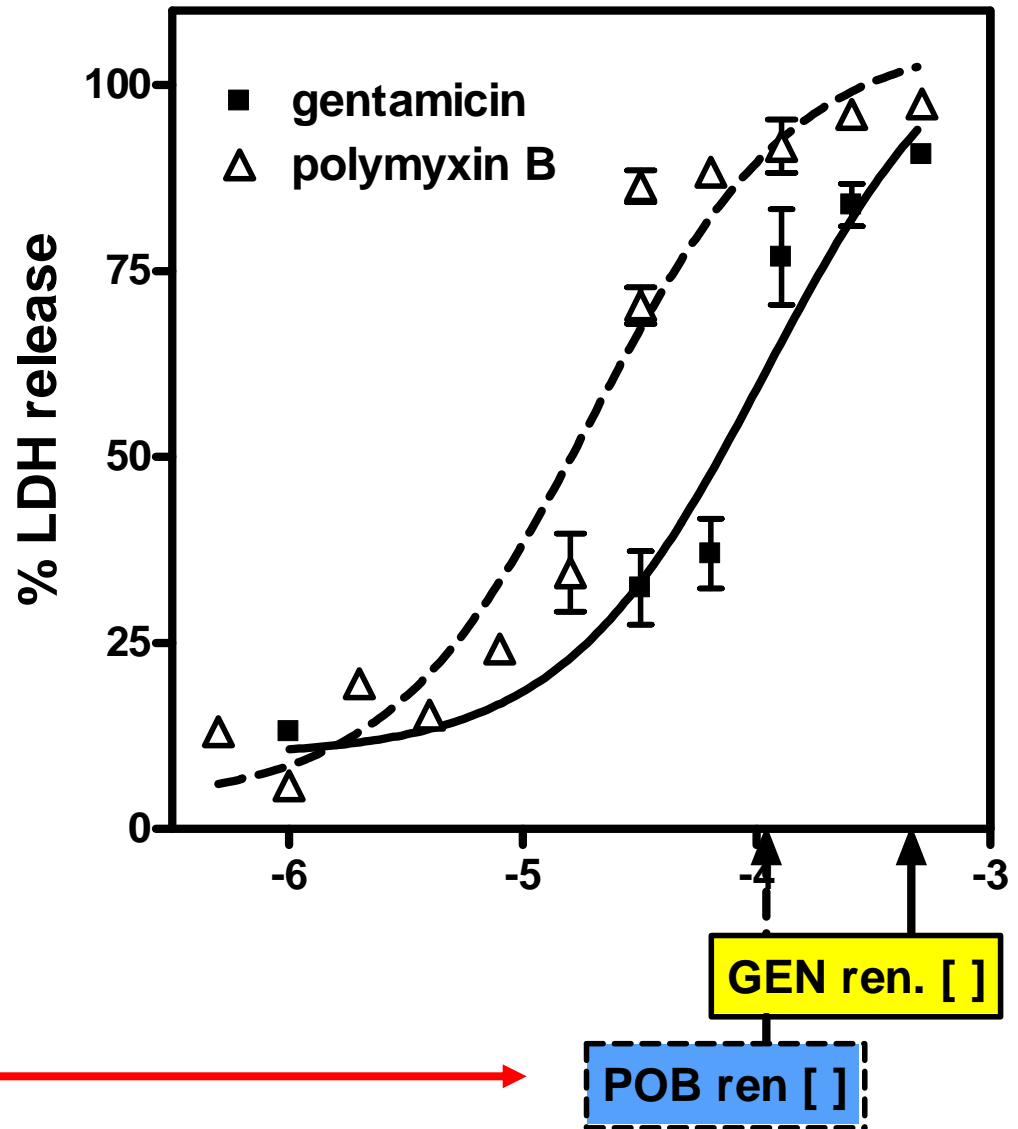
Point of comparison:  
serum concentration

POB  $C_{max}$

GEN  $C_{max}$

# Electroporated cells: necrosis

necrosis

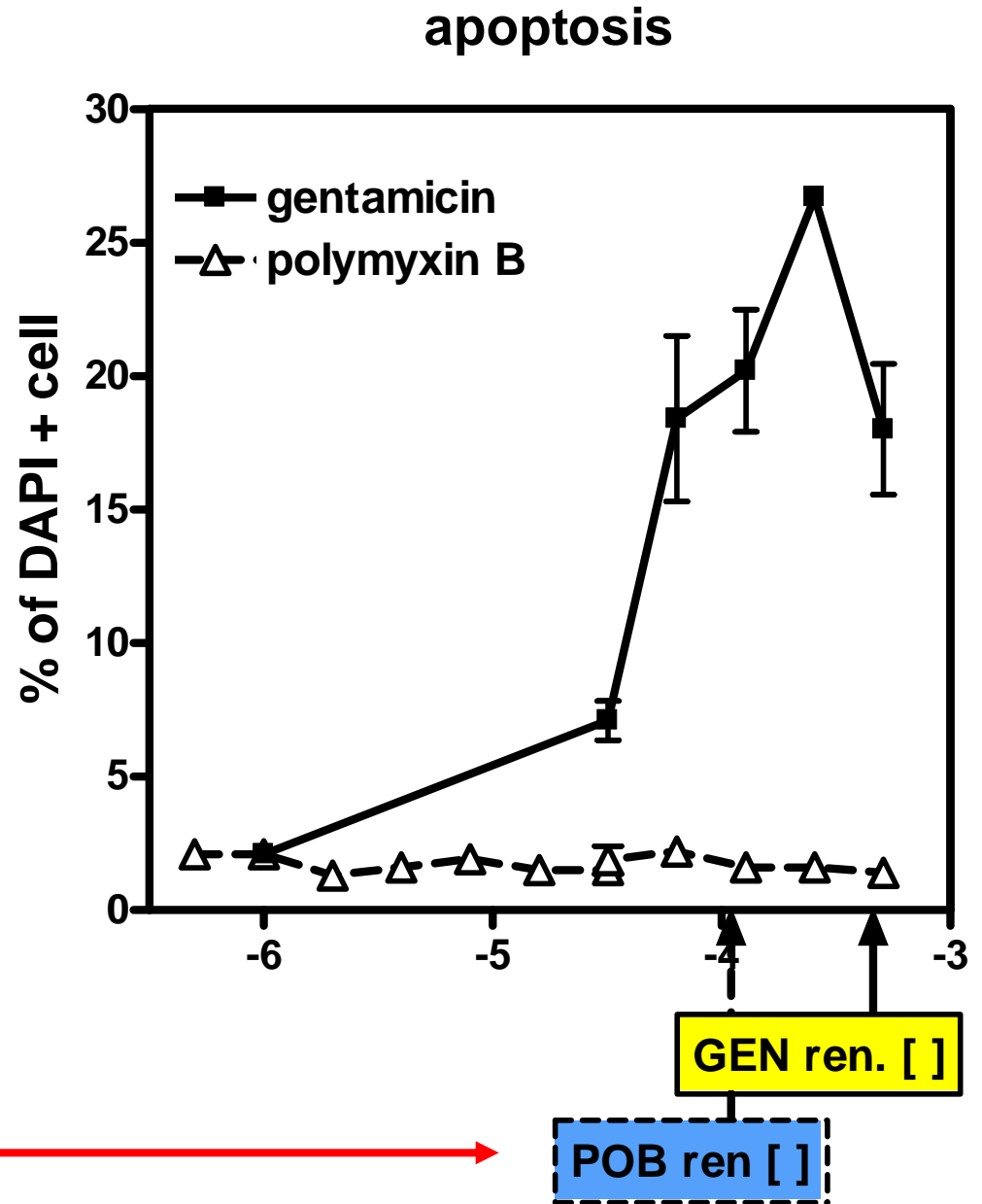


Point of comparison:  
tissue concentration

GEN ren. [ ]

POB ren [ ]

# Electroporated cells: apoptosis



# Conclusions

- Polymyxin B and gentamicin markedly differ in their ability to cause apoptosis, suggesting different intracellular handling and interactions with the cellular components involved in the triggering of apoptosis \*
- Both agents cause necrosis when delivered into the cytosol, suggesting unspecific binding to essential constituents, perhaps related to their polycationic character.
- With incubated cells, polymyxin B show clear dose-dependence for development of necrosis
- The models presented could now be used to study other polymyxins (colistin, e.g.) and novel derivatives from both colistin and gentamicin.

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\* reasonably known for gentamicin (Servais et al., Apoptosis (2008) 13:11-32)

# Disclosures

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