



Université catholique de Louvain, Brussels
Unité de pharmacologie cellulaire et moléculaire

Biochemical and Biophysical Studies of the Interactions between Aminoglycoside Antibiotics and Lipid Layers :

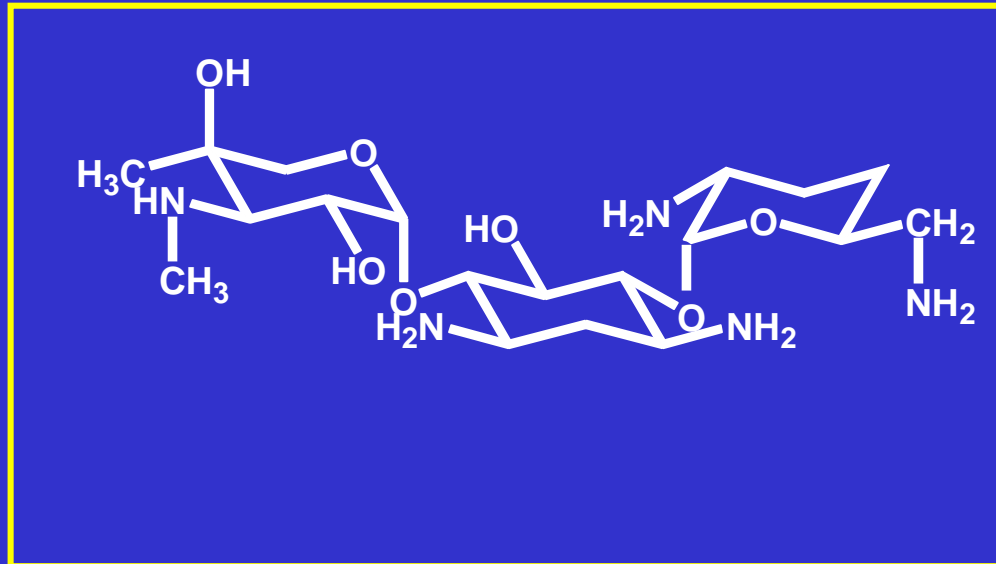
**A molecular approach to the understanding of the cellular toxicity
induced by aminoglycosides**

Thesis presented by
Marie-Paule Mingeot-Leclercq, M Sc., PhD in Pharm.
(Agrégation de l'enseignement supérieur en médecine)

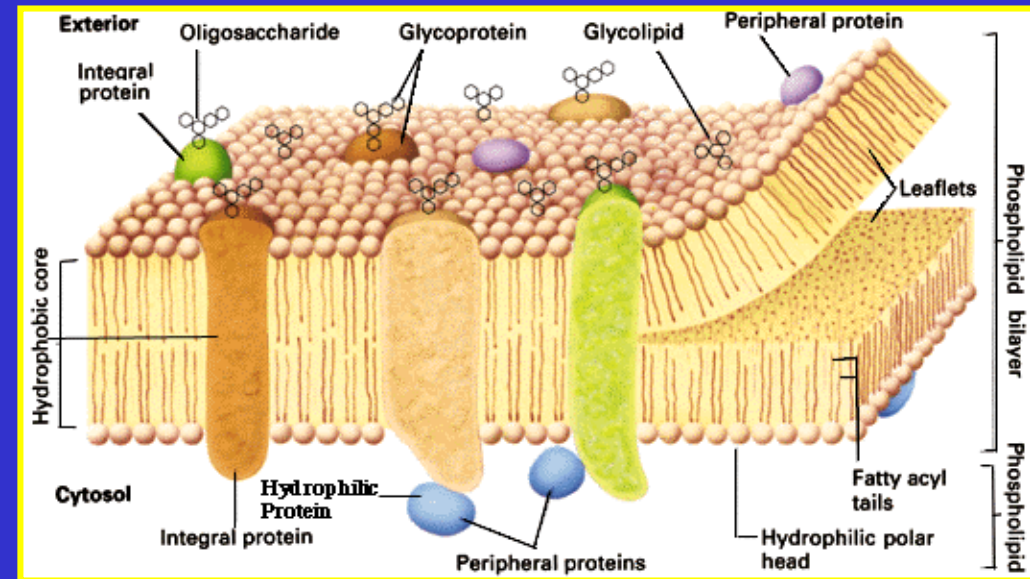
May 5th, 2000

AMINOGLYCOSIDES - LIPIDS INTERACTIONS

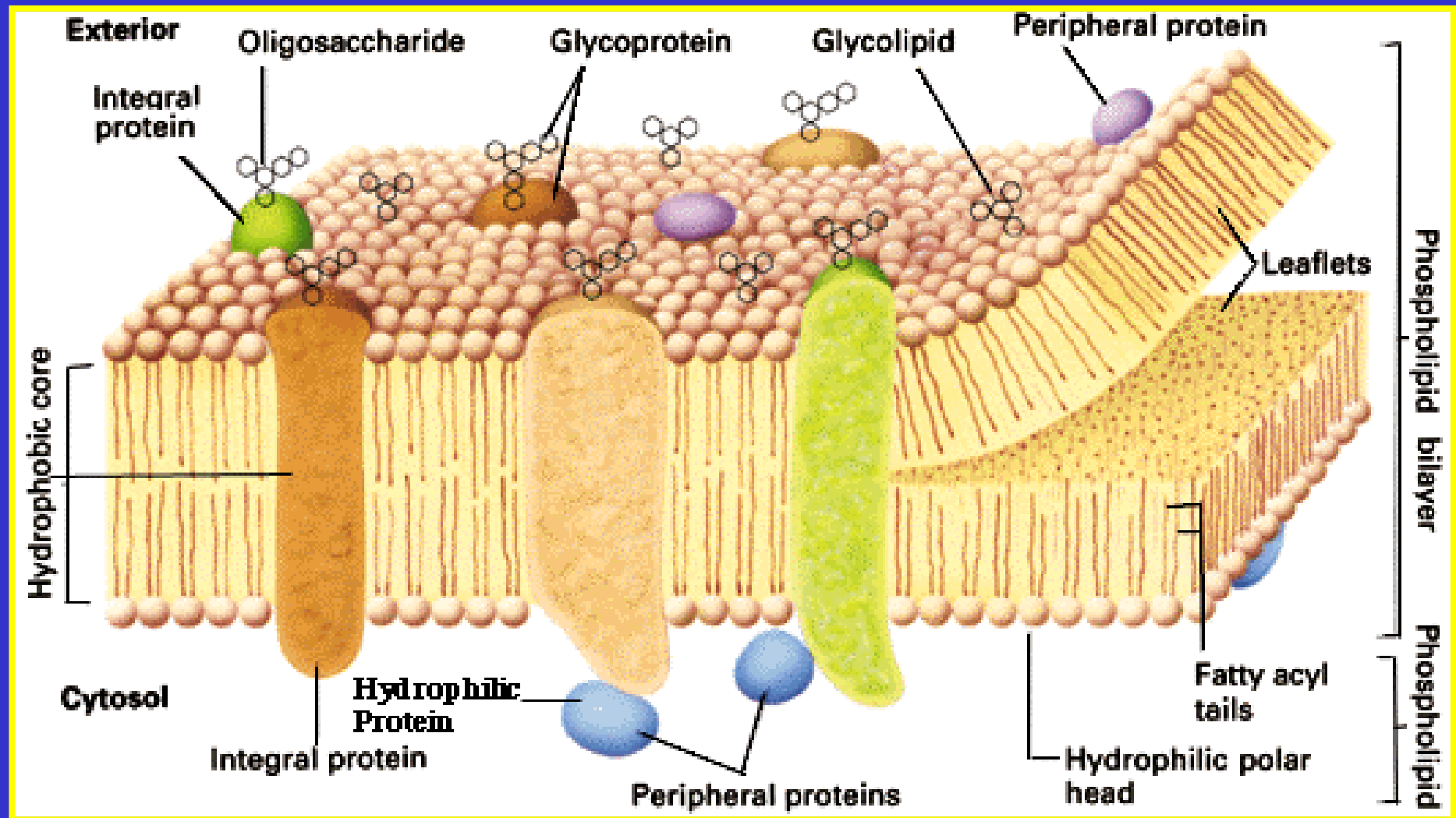
Aminoglycoside
(Gentamicin C_{1a})



Biological
membrane



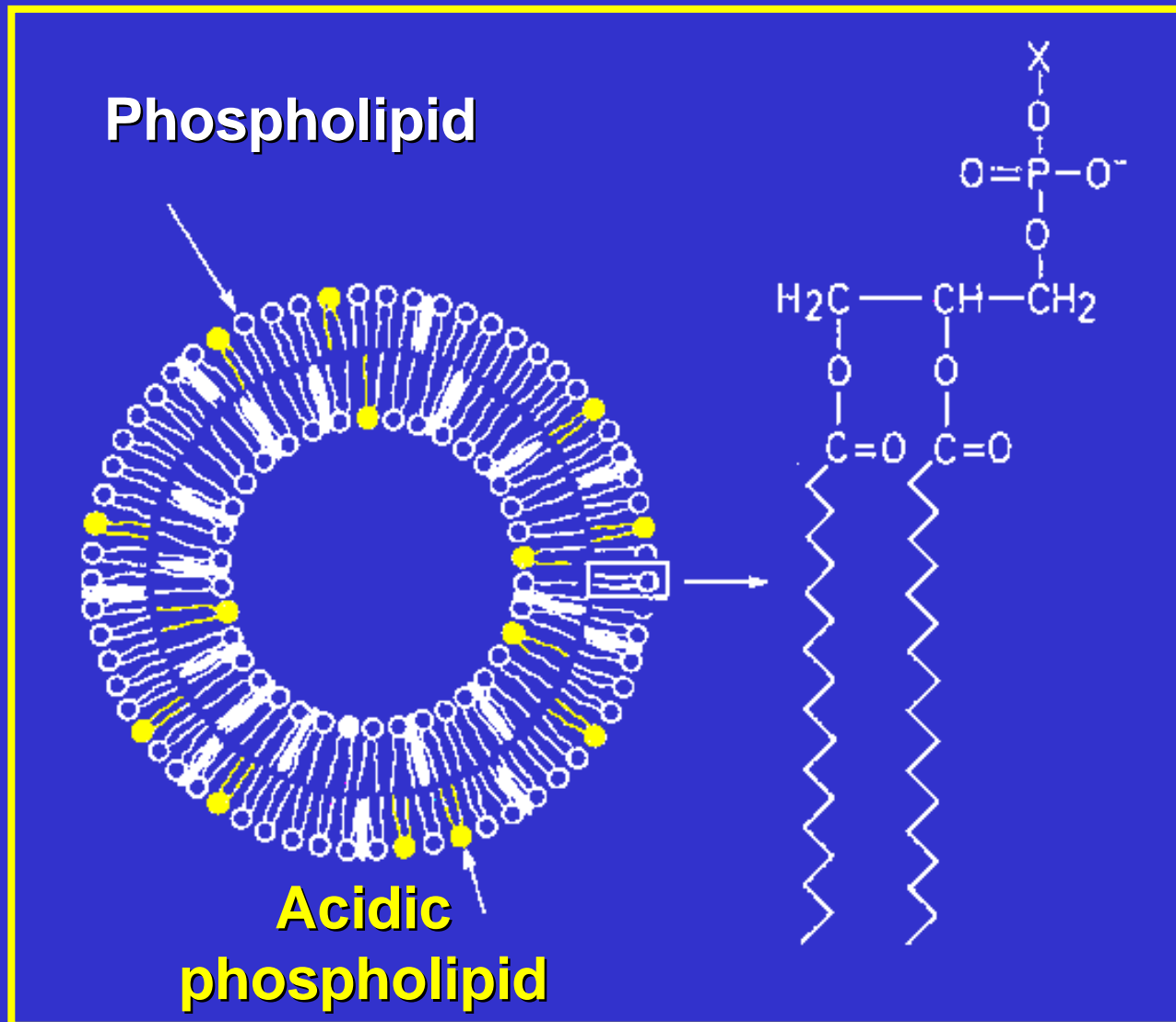
BIOLOGICAL MEMBRANES



MAJOR ROLES PLAYED BY MEMBRANE LIPIDS

CELLULAR	PHARMACOLOGICAL
<ul style="list-style-type: none">• Compartmentation → Endocytosis/exocytosis pathways• Structural support to the activation of signal transduction• Regulation of enzymatic activity	<ul style="list-style-type: none">• Inhibition of the synthesis of the lipidic constituents of membranes• Anchorage of drug inside lipidic compartment• Modulation of cellular process involving membranes

AMINOGLYCOSIDES - LIPIDS INTERACTIONS



Polar domain ^{31}P NMR

Interfacial domain

Fluorescence

depolarization - TMA-DPH

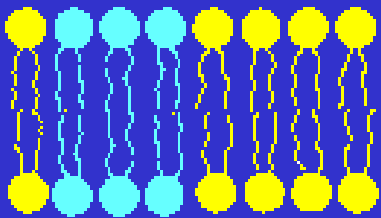
Hydrocarbon domain

Fluorescence

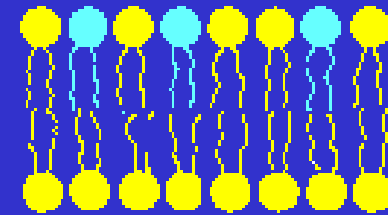
depolarization - DPH

IMPORTANT FEATURES RELATED TO BIOLOGICAL ROLES PLAYED BY MEMBRANES

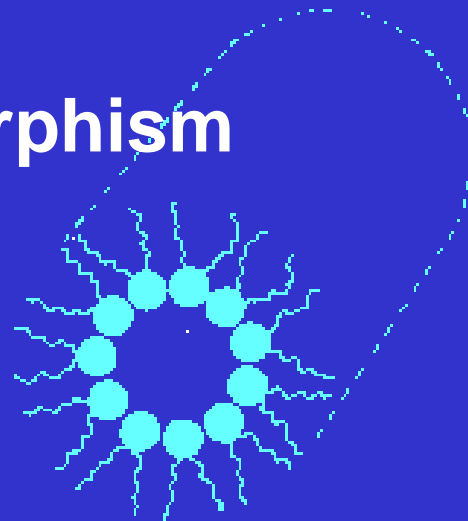
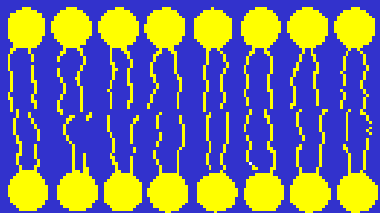
Lateral domains



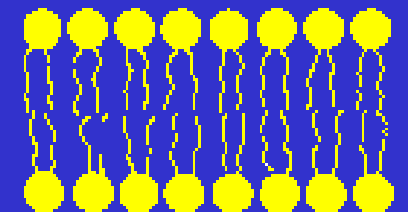
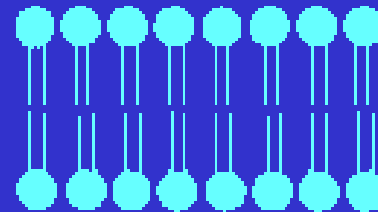
Transversal asymmetry



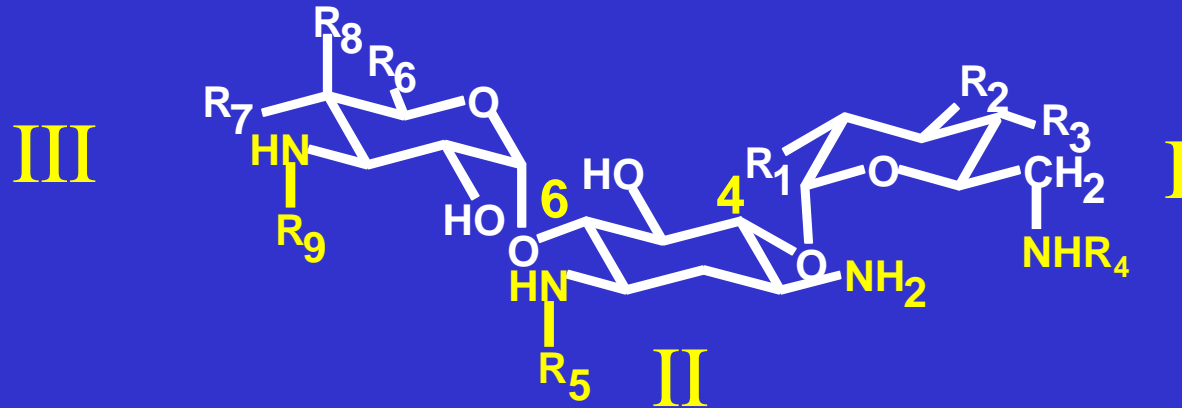
Lipid polymorphism



Fluidity



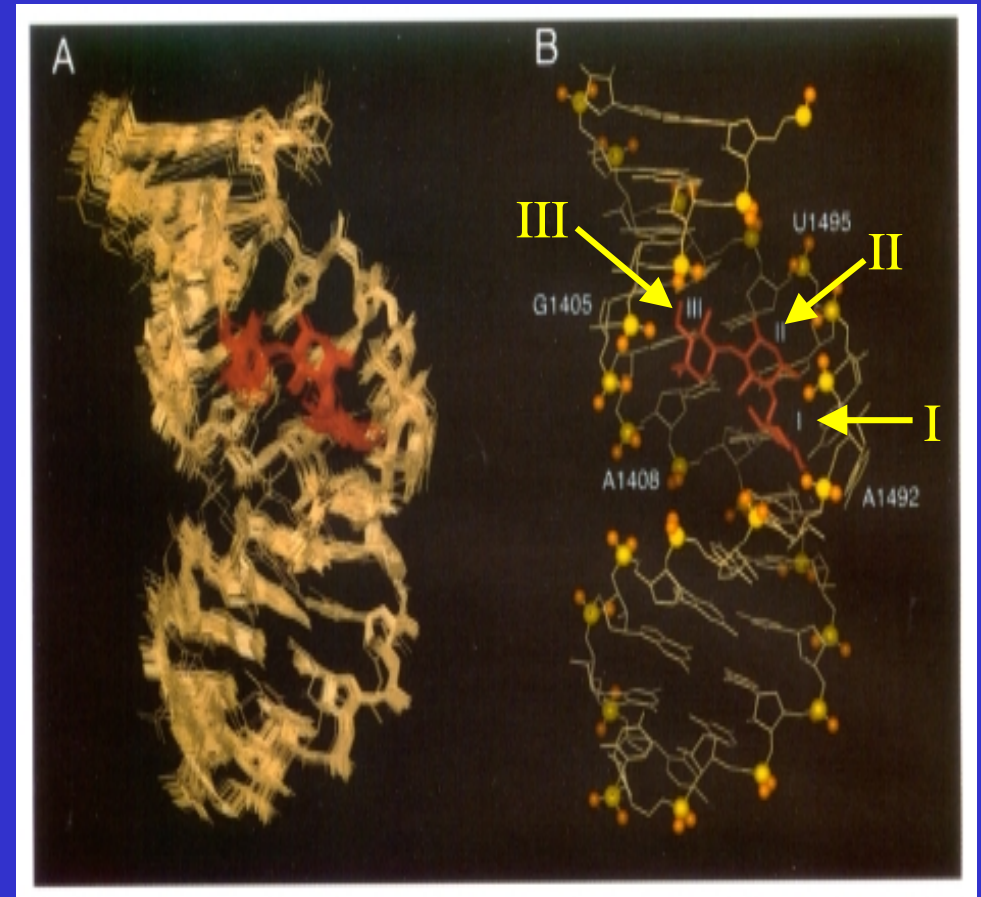
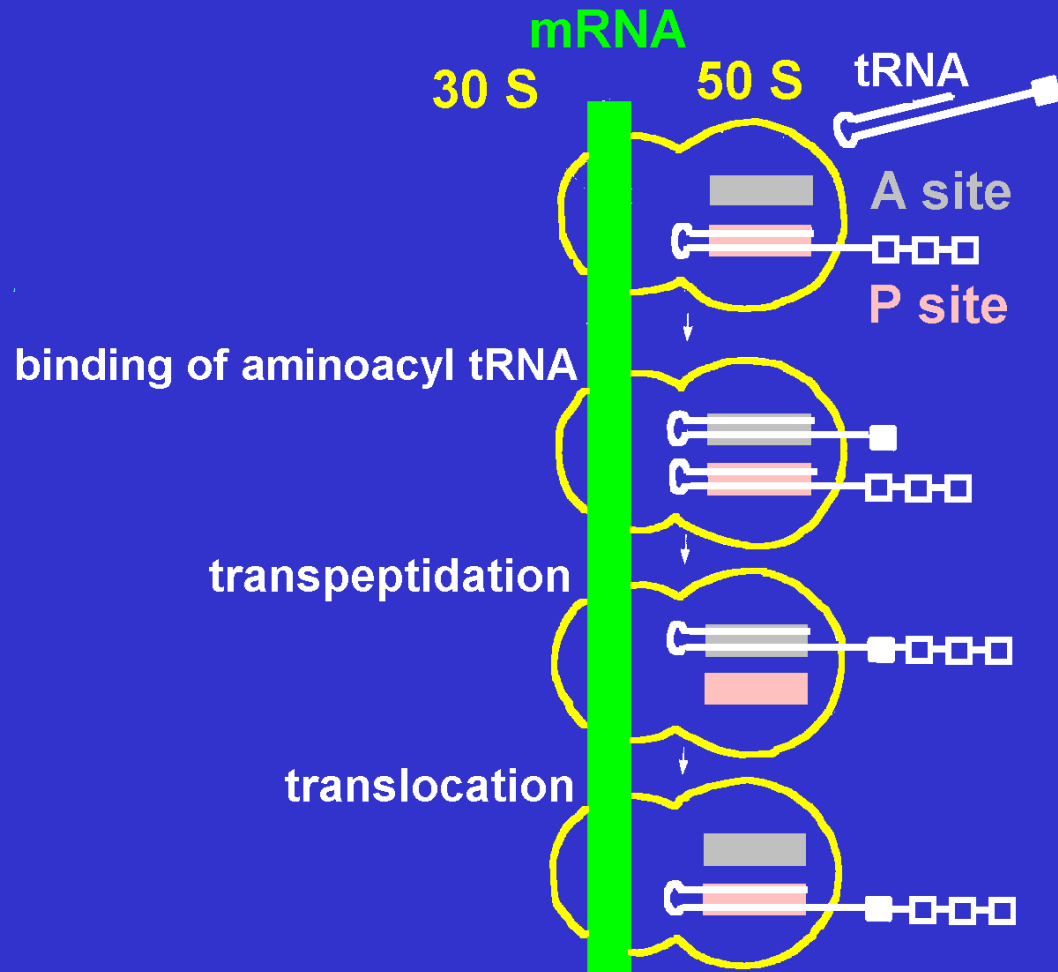
AMINOGLYCOSIDES: CHEMICAL STRUCTURE



Aminoglycoside	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	R ₉
Isepamicin	OH	OH	OH	H	COR	H	CH ₃	OH	CH ₃
Amikacin	OH	OH	OH	H	COR'	CH ₂ OH	OH	H	H
Kanamycin A	OH	OH	OH	H	H	CH ₂ OH	OH	H	H
Kanamycin B	NH ₂	OH	OH	H	H	CH ₂ OH	OH	H	H
Gentamicin C _{1a}	NH ₂	H	H	H	H	H	CH ₃	OH	CH ₃

R = -CHOHCH₂NH₂; R' = -CHOH(CH₂)₂NH₂

AMINOGLYCOSIDES: ANTIMICROBIAL ACTIVITY

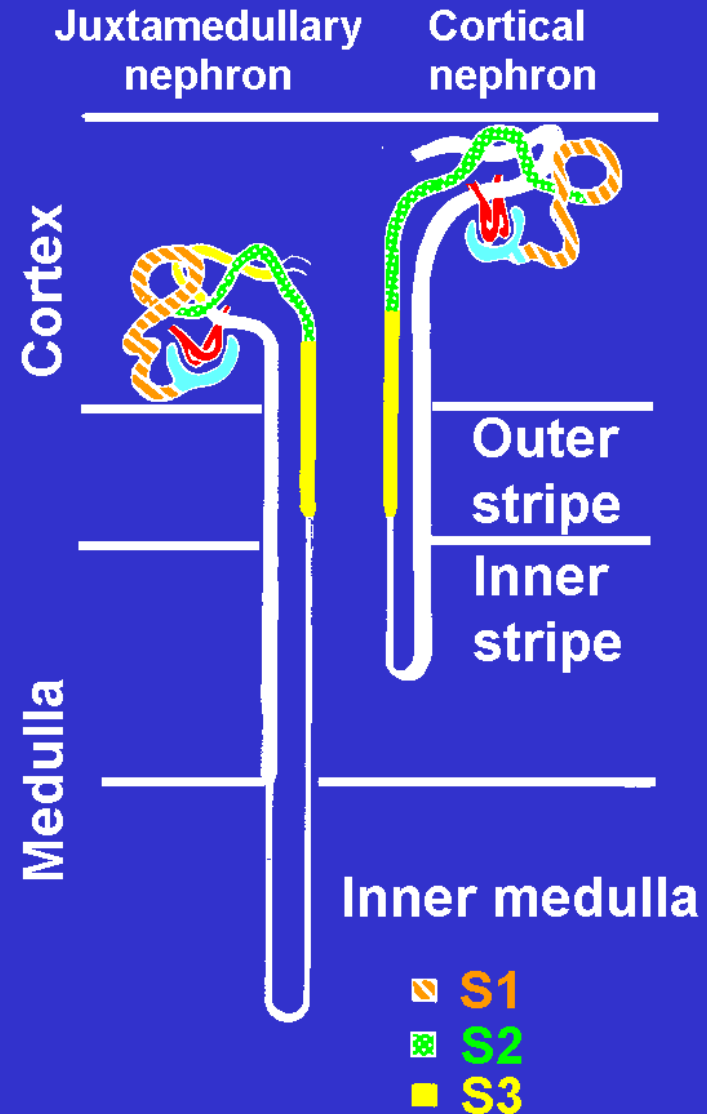


Yoshizawa et al [1998] *EMBO J.* 17:6437-6448
M.P. Mingeot-Leclercq, UCL, Brussels

AMINOGLYCOSIDES: NEPHROTOXICITY

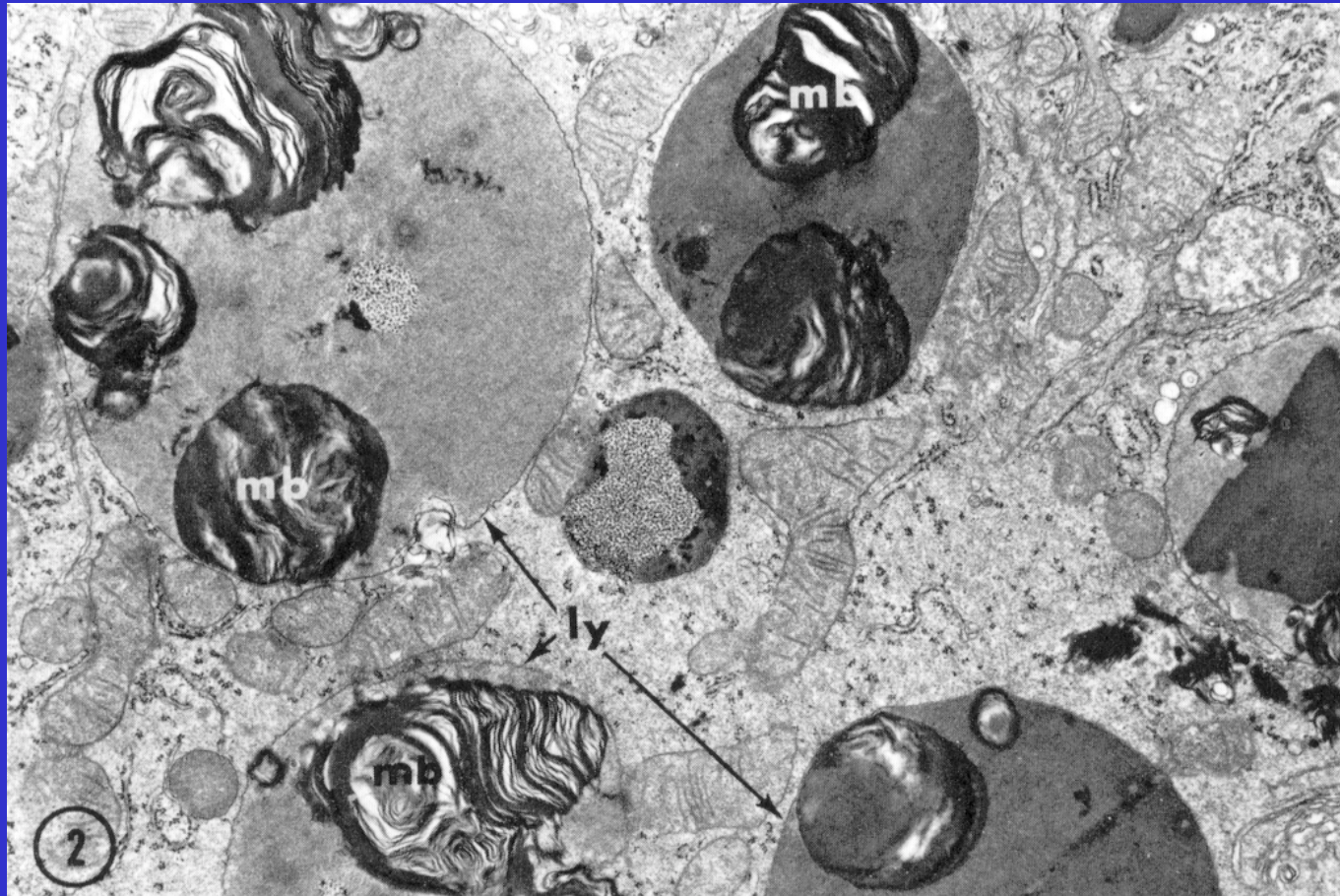
Incidence: 0-50%

- patient factors
- aminoglycoside factors
 - ↑ Gentamicin
 - Netilmicin
 - Isepamicin
 - Amikacin
- schedule of administration
- concomitant drugs



AMINOGLYCOSIDES: NEPHROTOXICITY

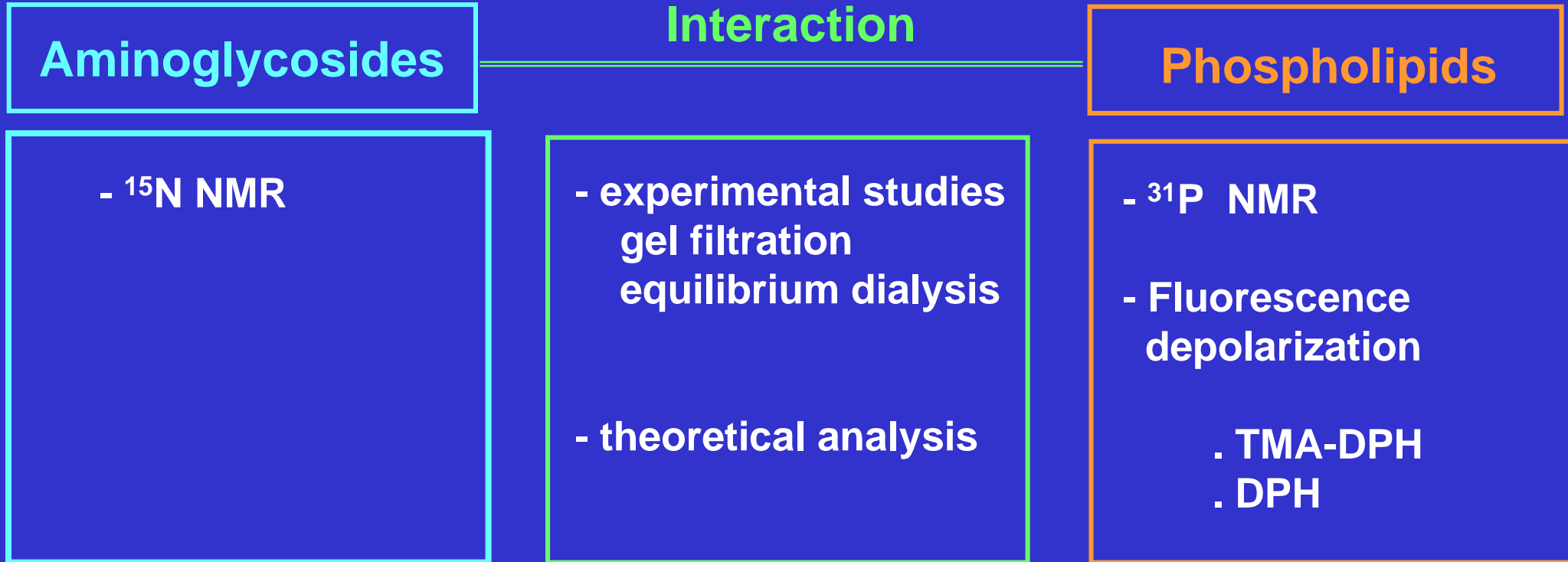
Proximal tubular cell - rat treated with 10 mg/kg.day gentamicin for 7 days



Kosek et al [1974] Lab. Invest 30: 48-57

AMINOGLYCOSIDES - LIPIDS INTERACTIONS

CHARACTERIZATION

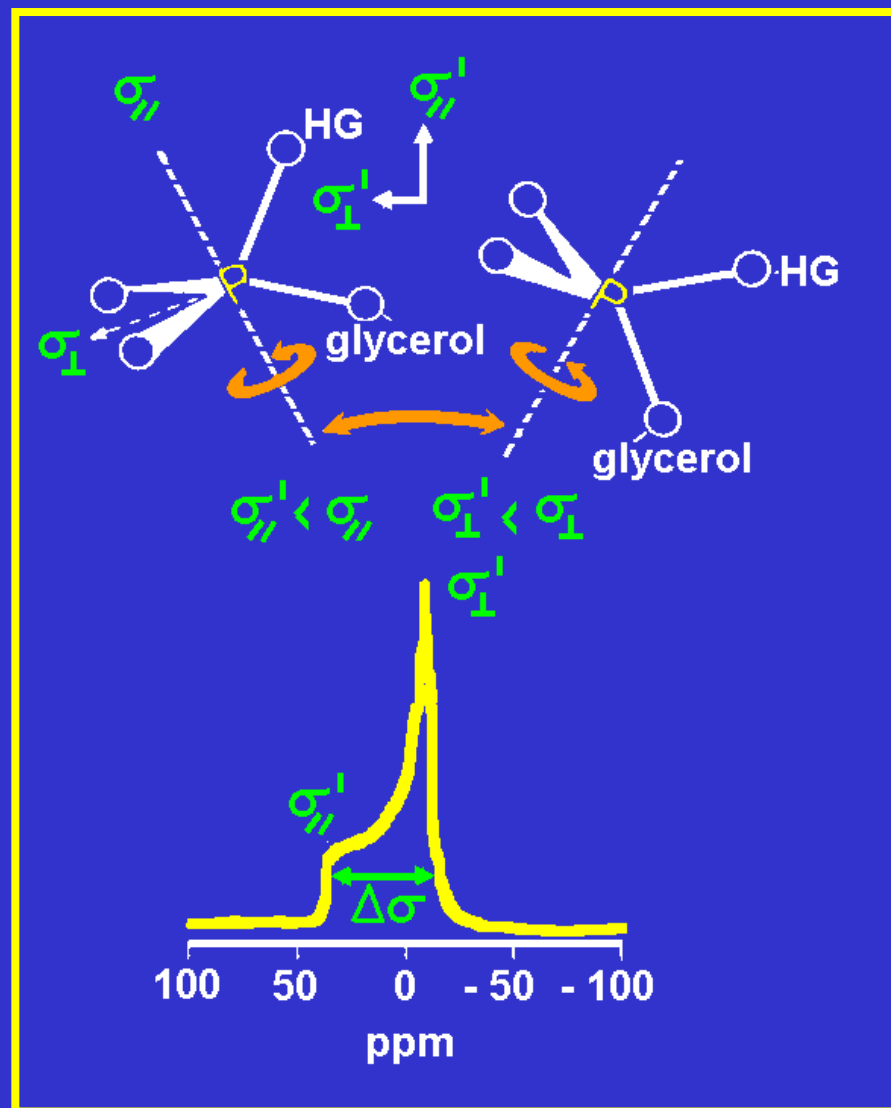


CONSEQUENCES

INTEREST

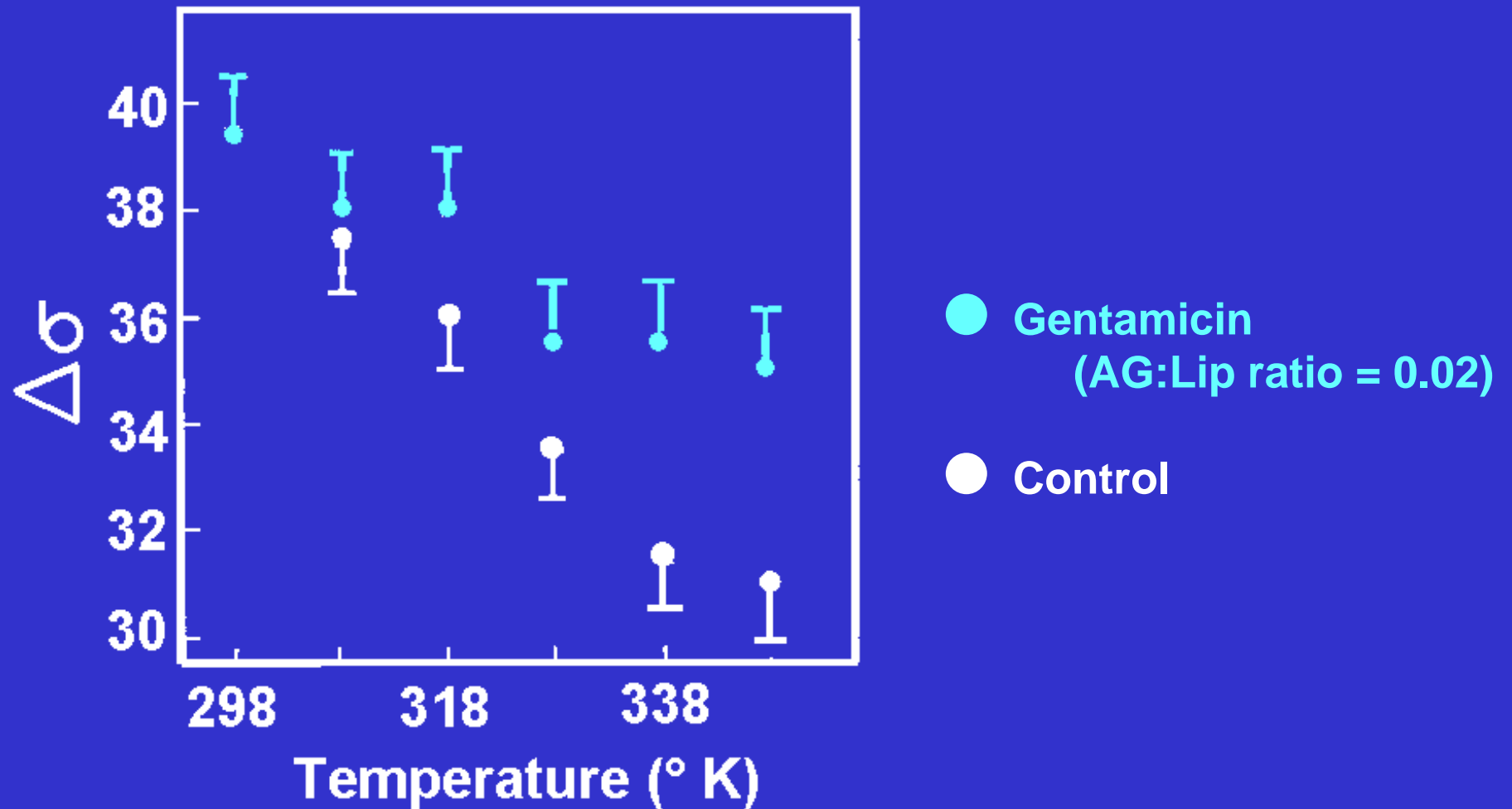
AMINOGLYCOSIDES - LIPIDS INTERACTIONS

^{31}P NMR Studies



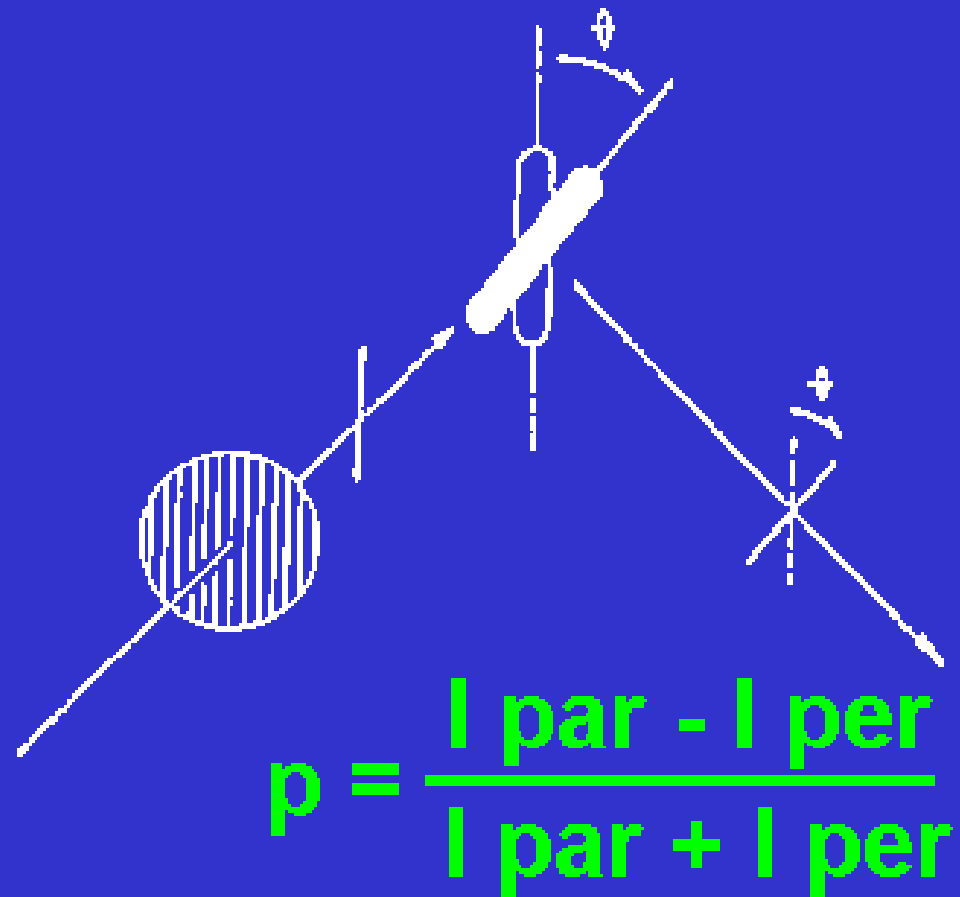
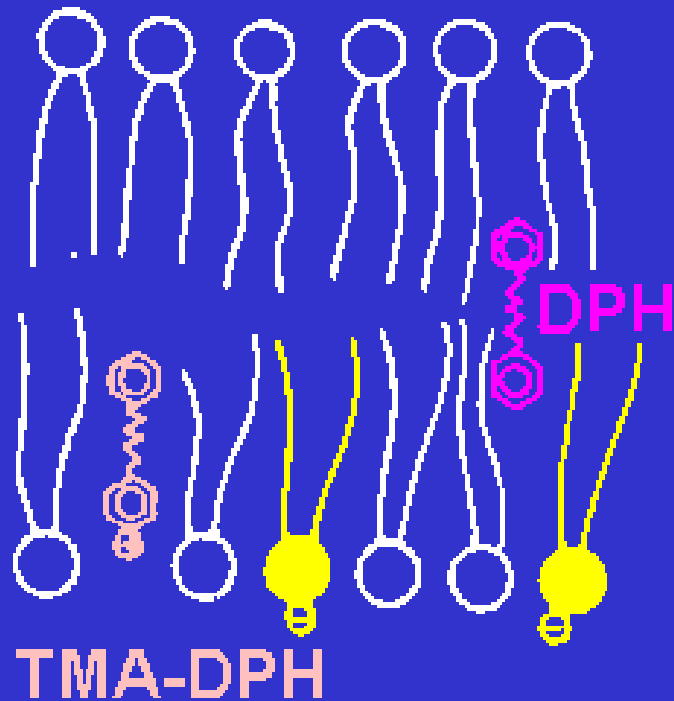
AMINOGLYCOSIDES - LIPIDS INTERACTIONS

^{31}P NMR Studies



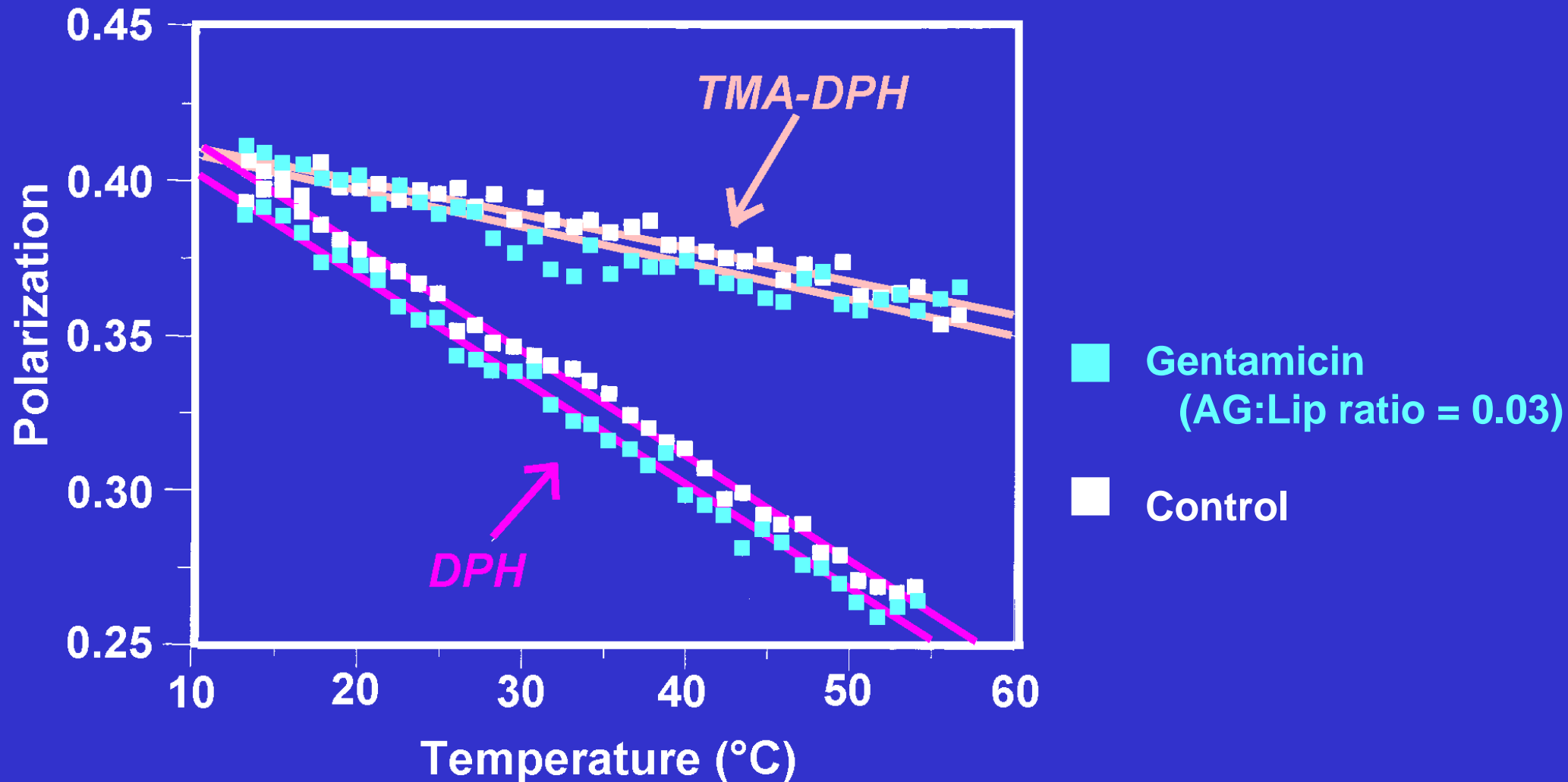
AMINOGLYCOSIDES - LIPIDS INTERACTIONS

Fluorescence depolarization studies



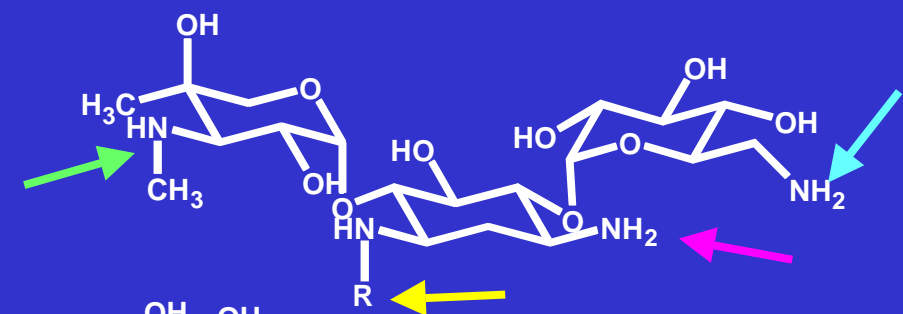
AMINOGLYCOSIDES - LIPIDS INTERACTIONS

Fluorescence depolarization studies

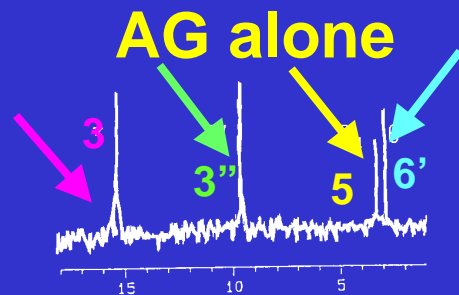


AMINOGLYCOSIDES- LIPIDS INTERACTIONS

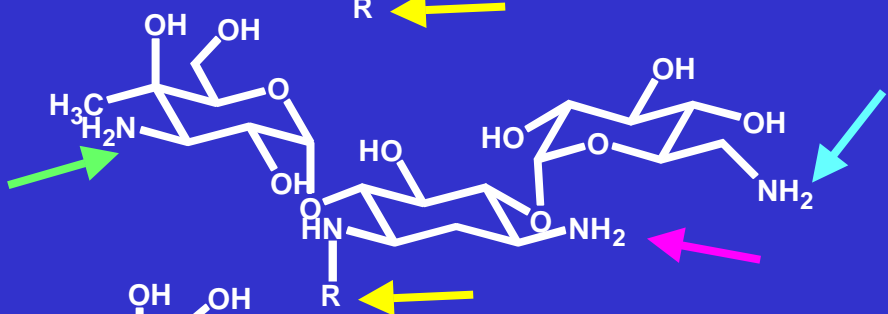
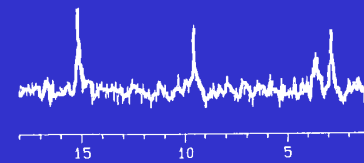
^{15}N NMR Studies



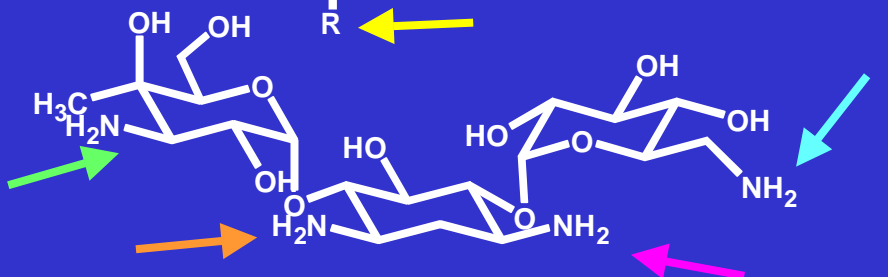
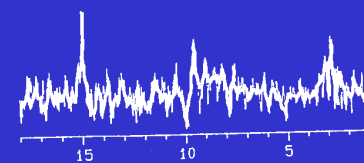
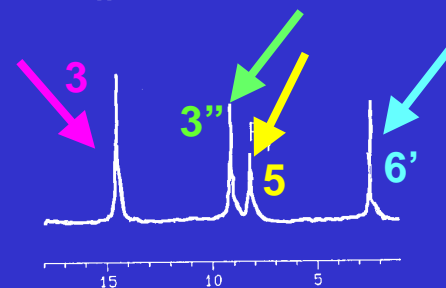
isepa



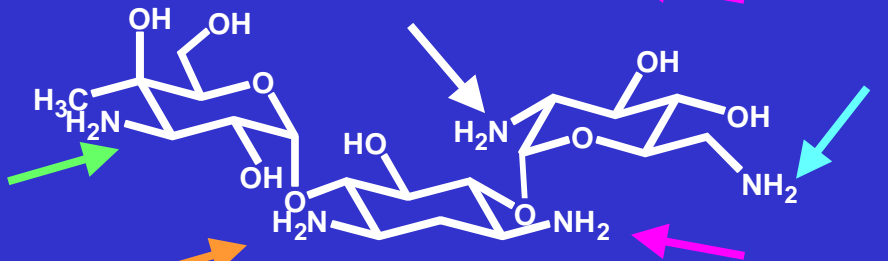
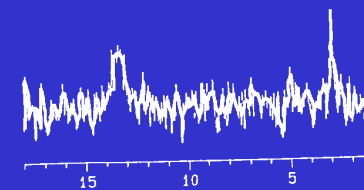
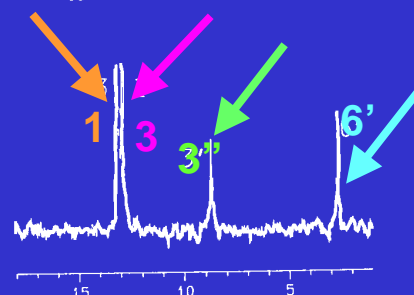
AG + PI



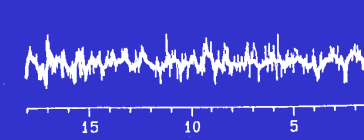
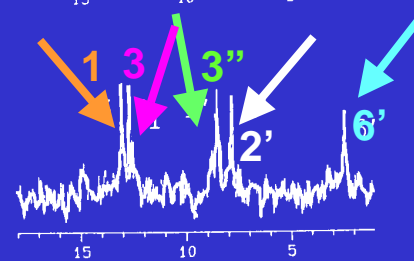
amika



kana A



kana B



AMINOGLYCOSIDE-LIPIDS INTERACTIONS

Correlation between the theoretical and experimental studies

Aminoglycoside	Interaction detected by equilibrium dialysis Kd (μM)	Interaction detected by ^{15}N NMR	Calculated interaction (KJ / mol)
isepamicin	47	weak	- 23.4
amikacin	30	medium	- 35.7
kanamycin A	7	medium	- 46.8
kanamycin B	6	strong	- 48.5

AMINOGLYCOSIDES - LIPIDS INTERACTIONS

CHARACTERIZATION

CONSEQUENCES

- **Lysosomal phospholipase activity**
 - Surface potential
 - Lipidic lateral organization
- **Ability of membrane to fuse or to aggregate**
 - Lipidic polymorphism

INTEREST

AMINOGLYCOSIDES - LIPIDS INTERACTIONS

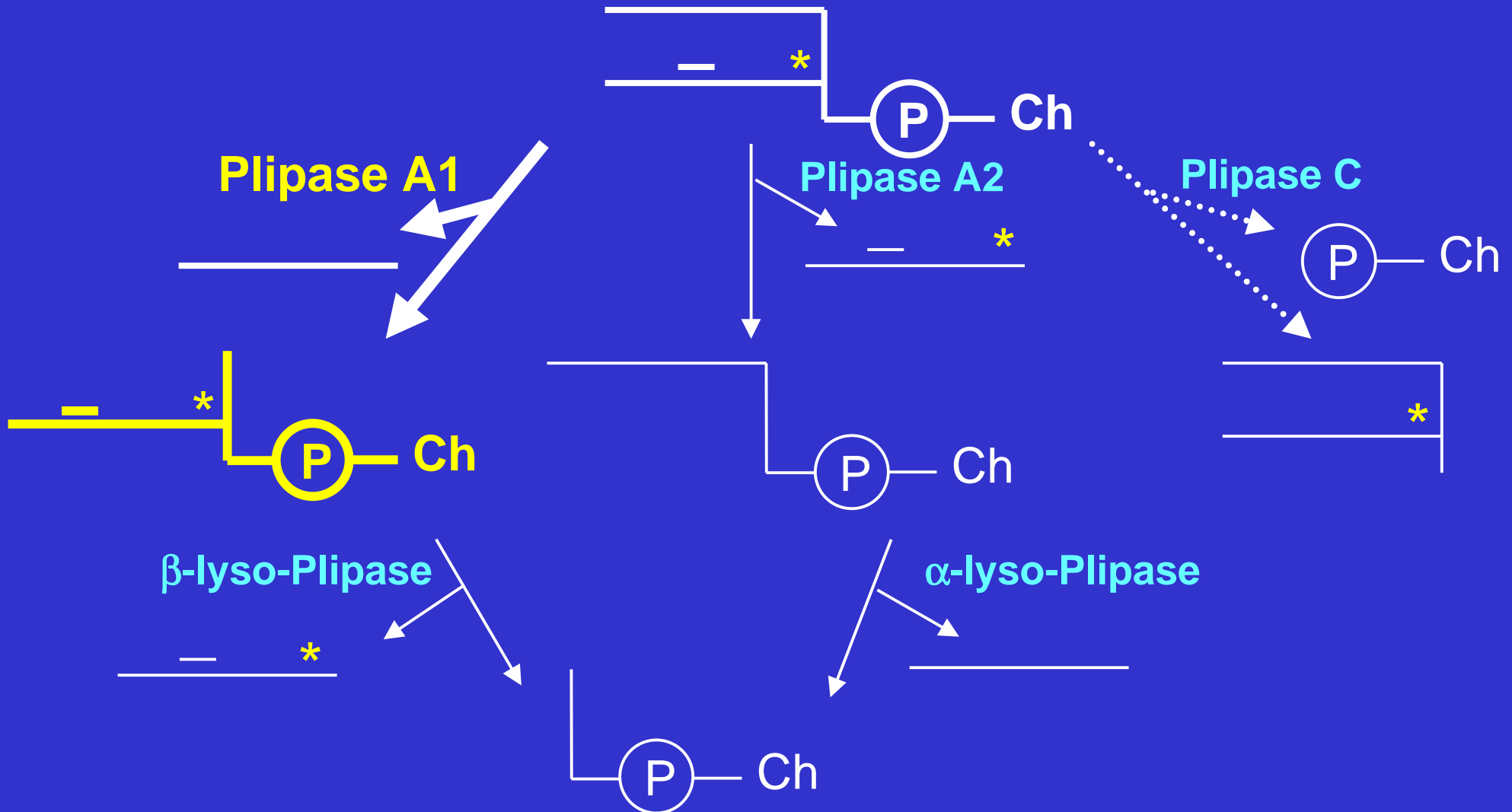
CHARACTERIZATION

CONSEQUENCES

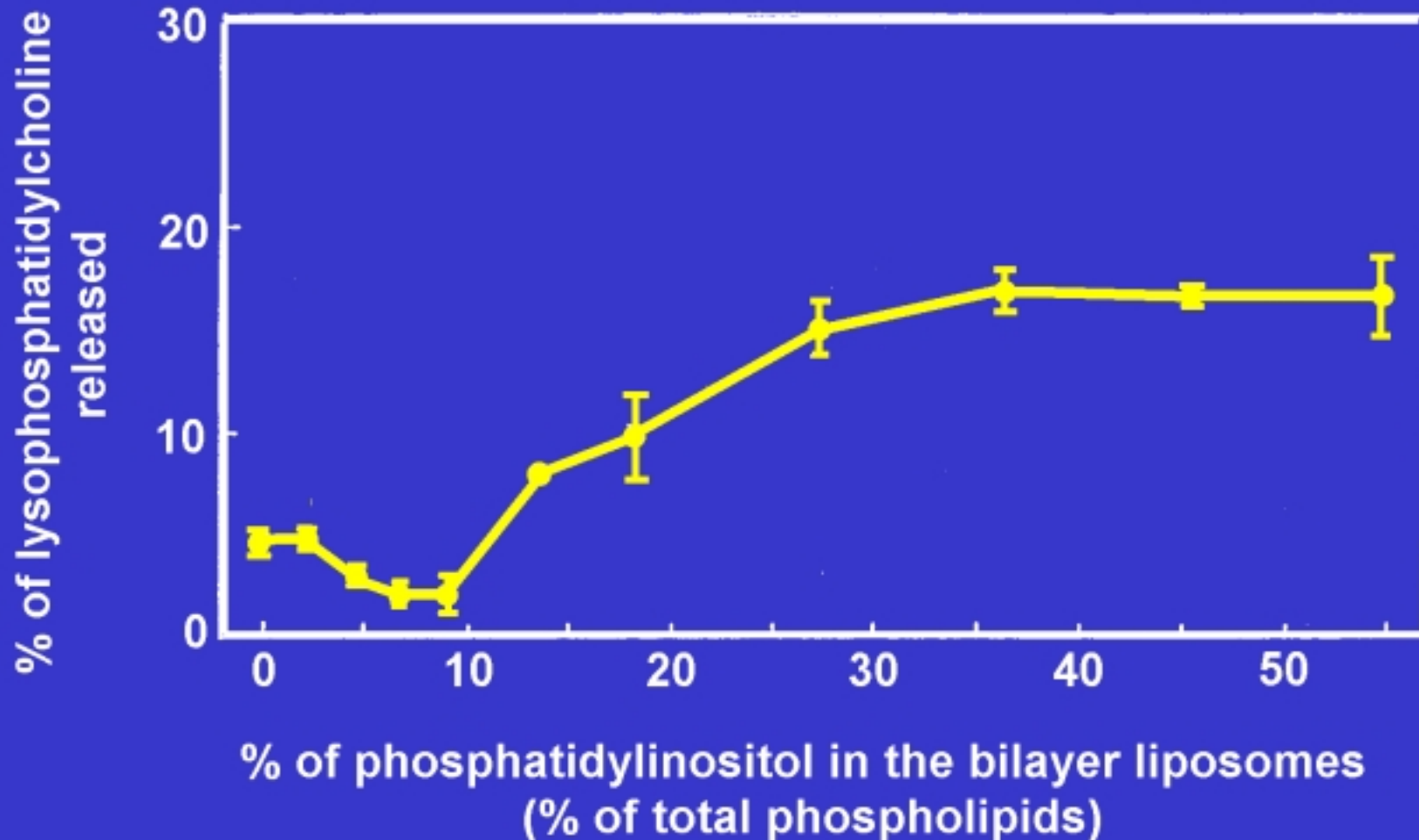
- **Lysosomal phospholipase activity**
 - Surface potential
 - Lipidic lateral organization
- **Ability of membrane to fuse or to aggregate**
 - Lipidic polymorphism

INTEREST

DEGRADATION PATHWAYS OF PHOSPHOLIPIDS IN LYSOSOMES

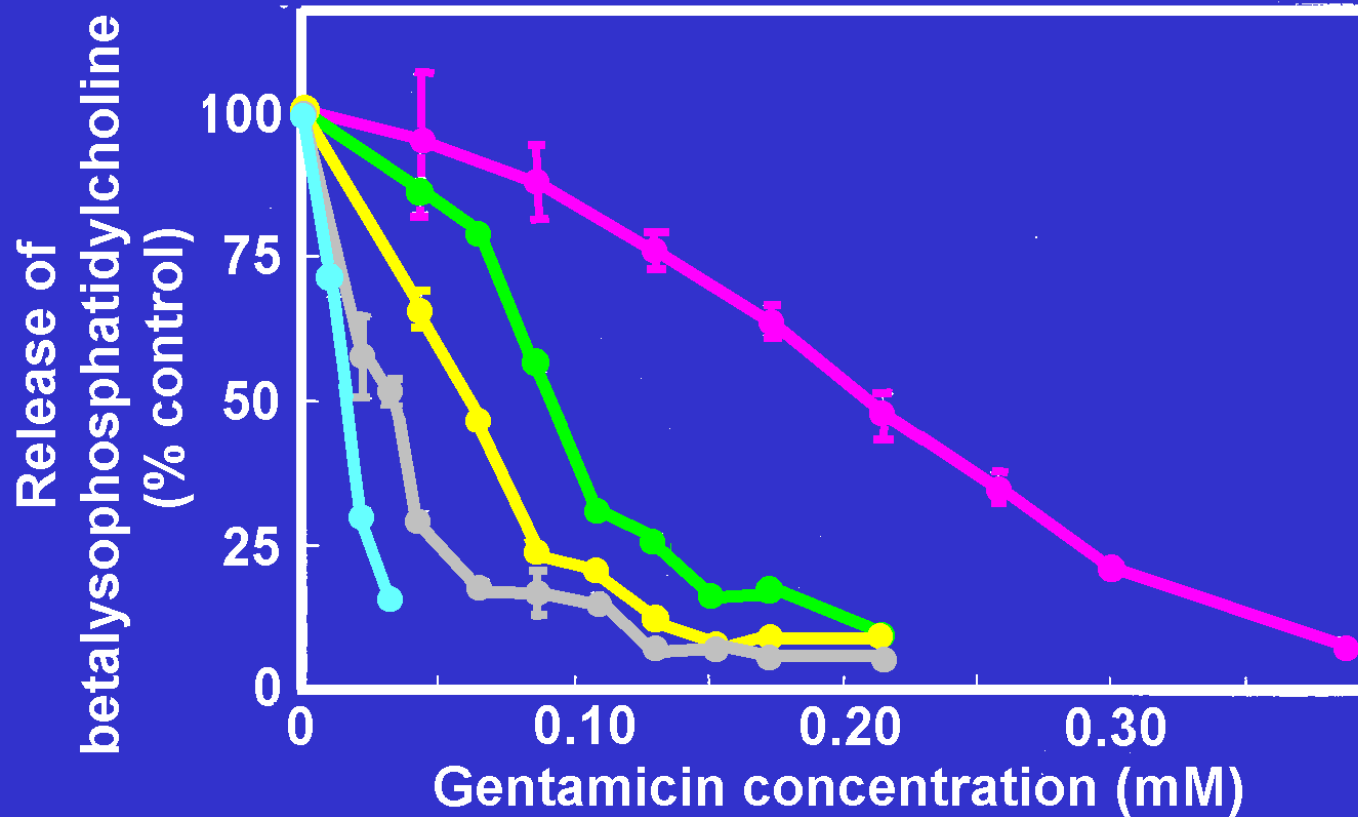


EFFECT OF NEGATIVELY-CHARGED PHOSPHOLIPIDS ON LYSOSOMAL PHOSPHOLIPASE ACTIVITY



AMINOGLYCOSIDES- LIPIDS INTERACTIONS

Inhibition of lysosomal phospholipase activity



Phosphatidylcholine content:

13.6 %

18.2 %

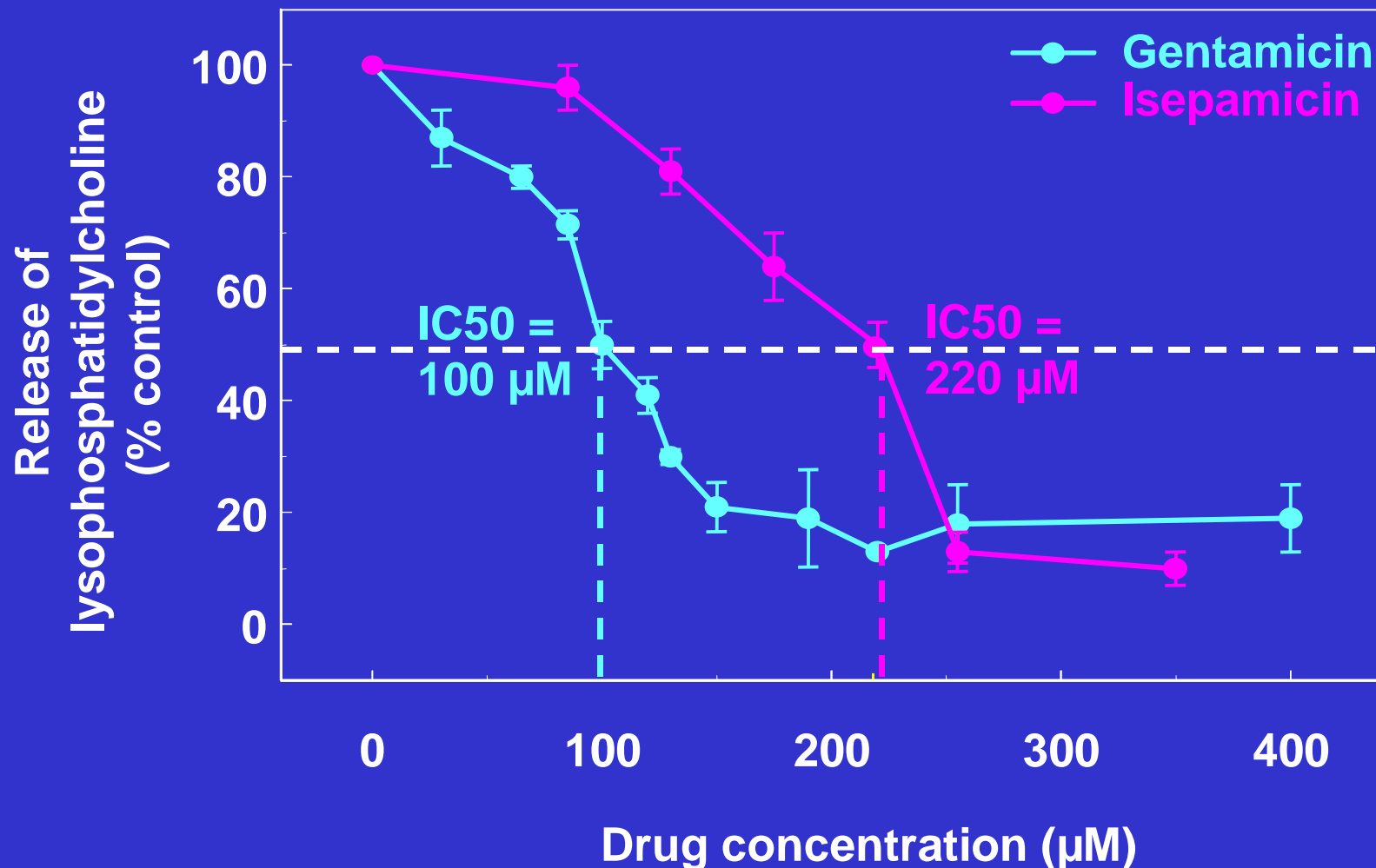
27.3 %

36.4 %

54.5 %

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

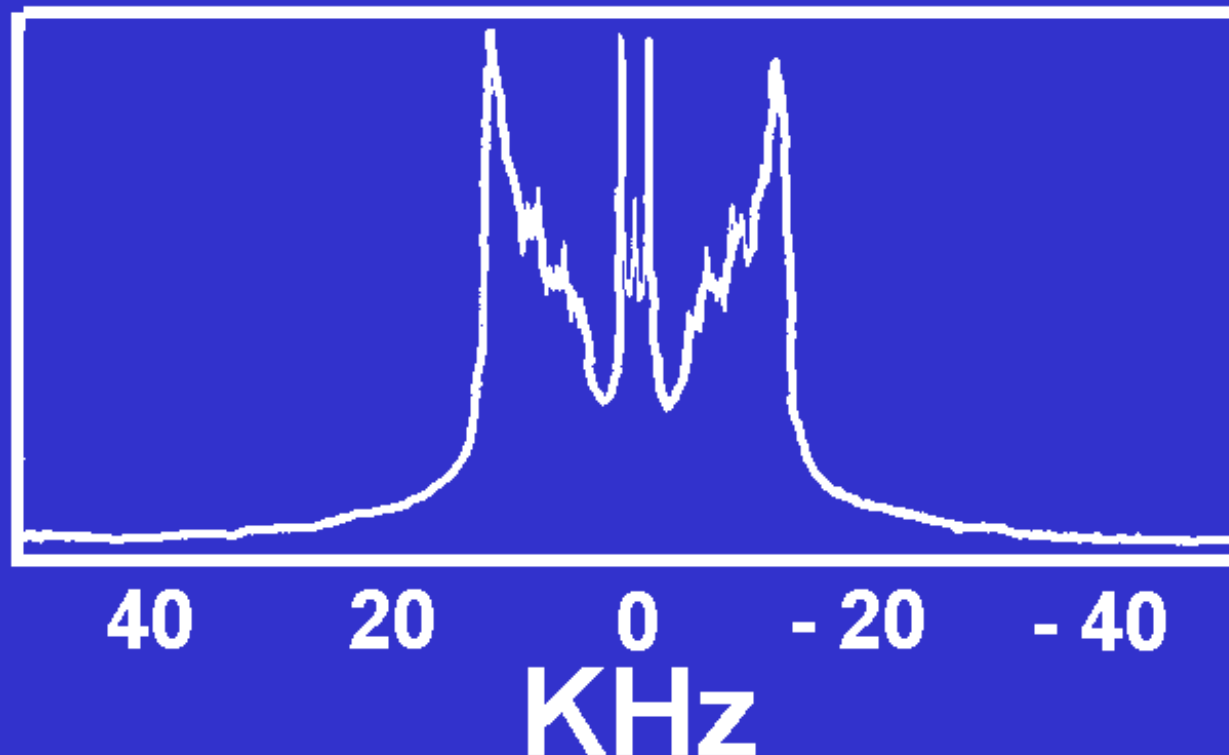
Inhibition of lysosomal phospholipase activity



INTERACTIONS AMINOGLYCOSIDES - LIPIDS

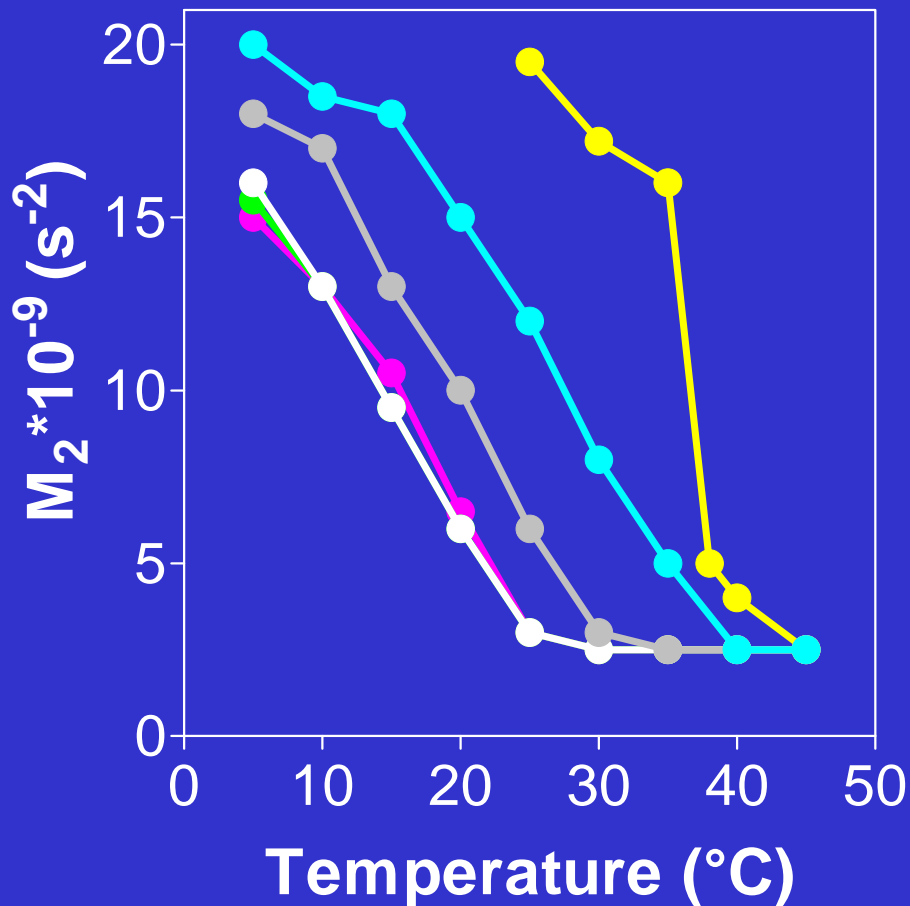
Effect on lysosomal phospholipase activity and domains formation?

^2H NMR spectrum of DPPC- d_{62}



INTERACTIONS AMINOGLYCOSIDES - LIPIDS

Effect on lysosomal phospholipase activity and domains formation?



DPPC_{d62}

control

DPPC_{d62}:PI 1:1

control

+ Kanamycin B

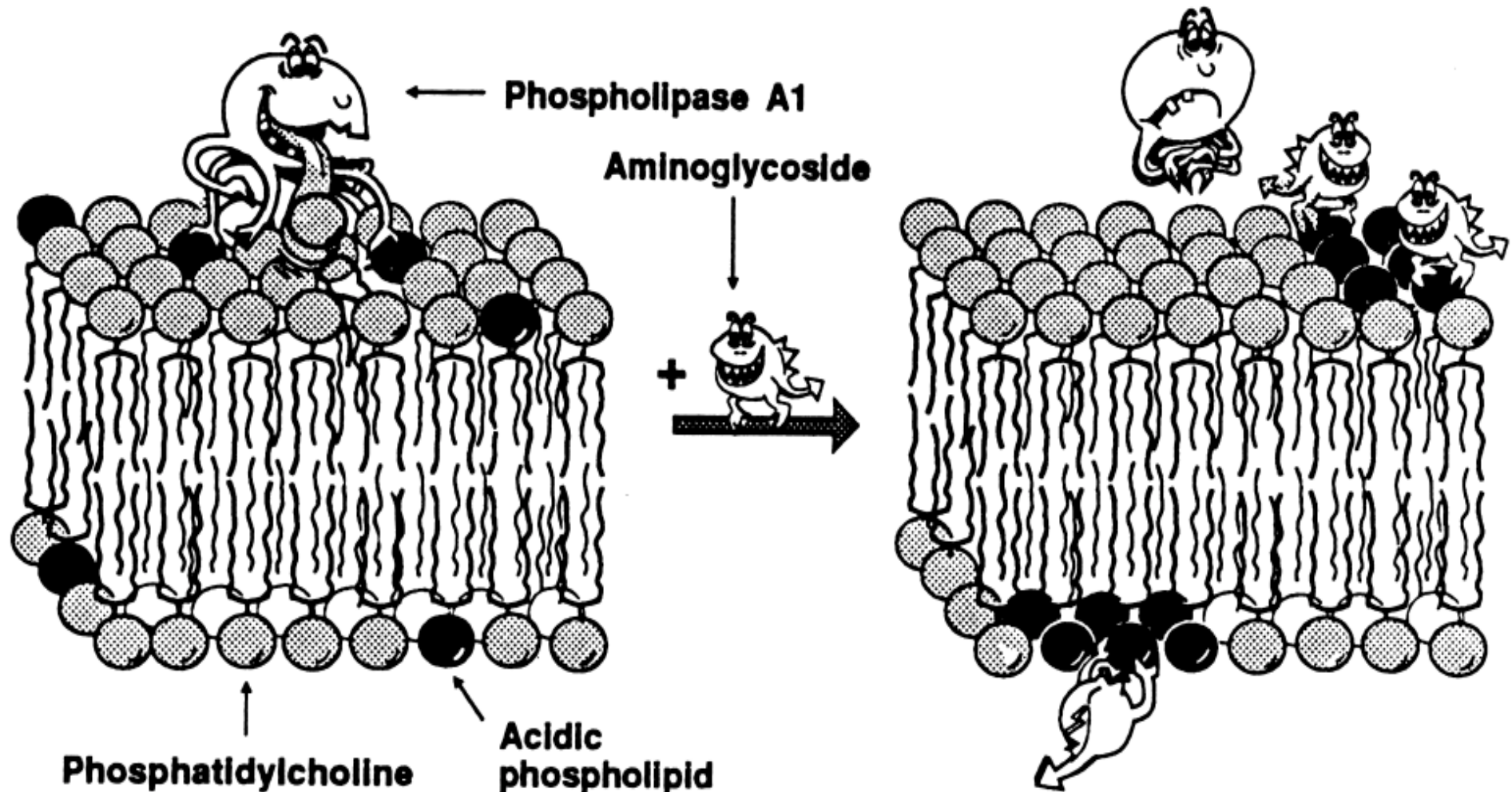
+ Kanamycin A

+ Amikacin

+ Isepamicin

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

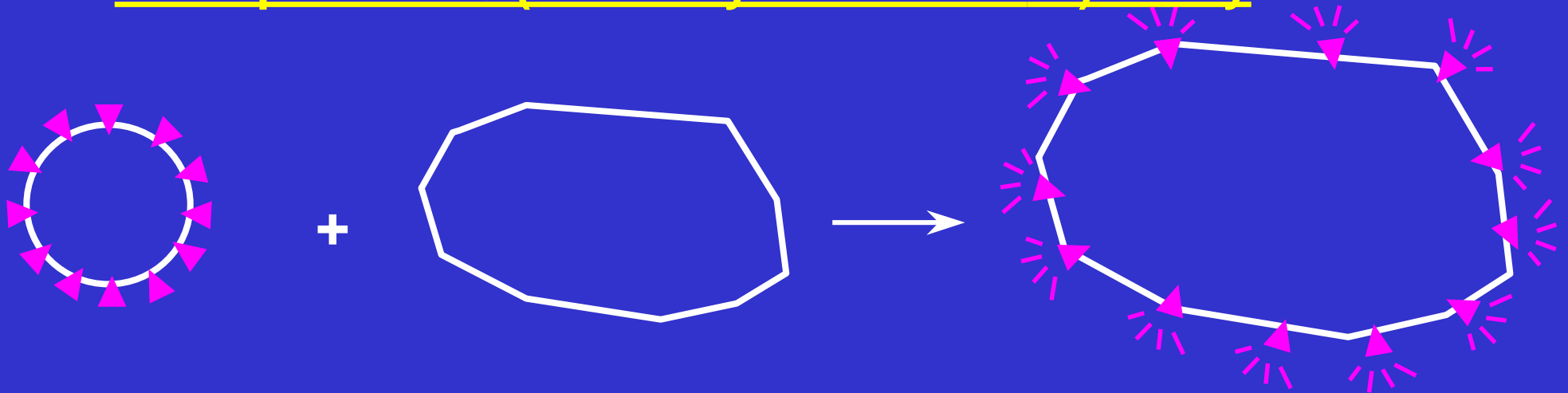
Inhibition of lysosomal phospholipase activity



INTERACTIONS AMINOGLYCOSIDES - LIPIDS

Effect on membrane fusion or aggregation

Principle of R18 (octadecylrhodamine B) assay



membrane - labeling
selfquenching
concentration

target membrane

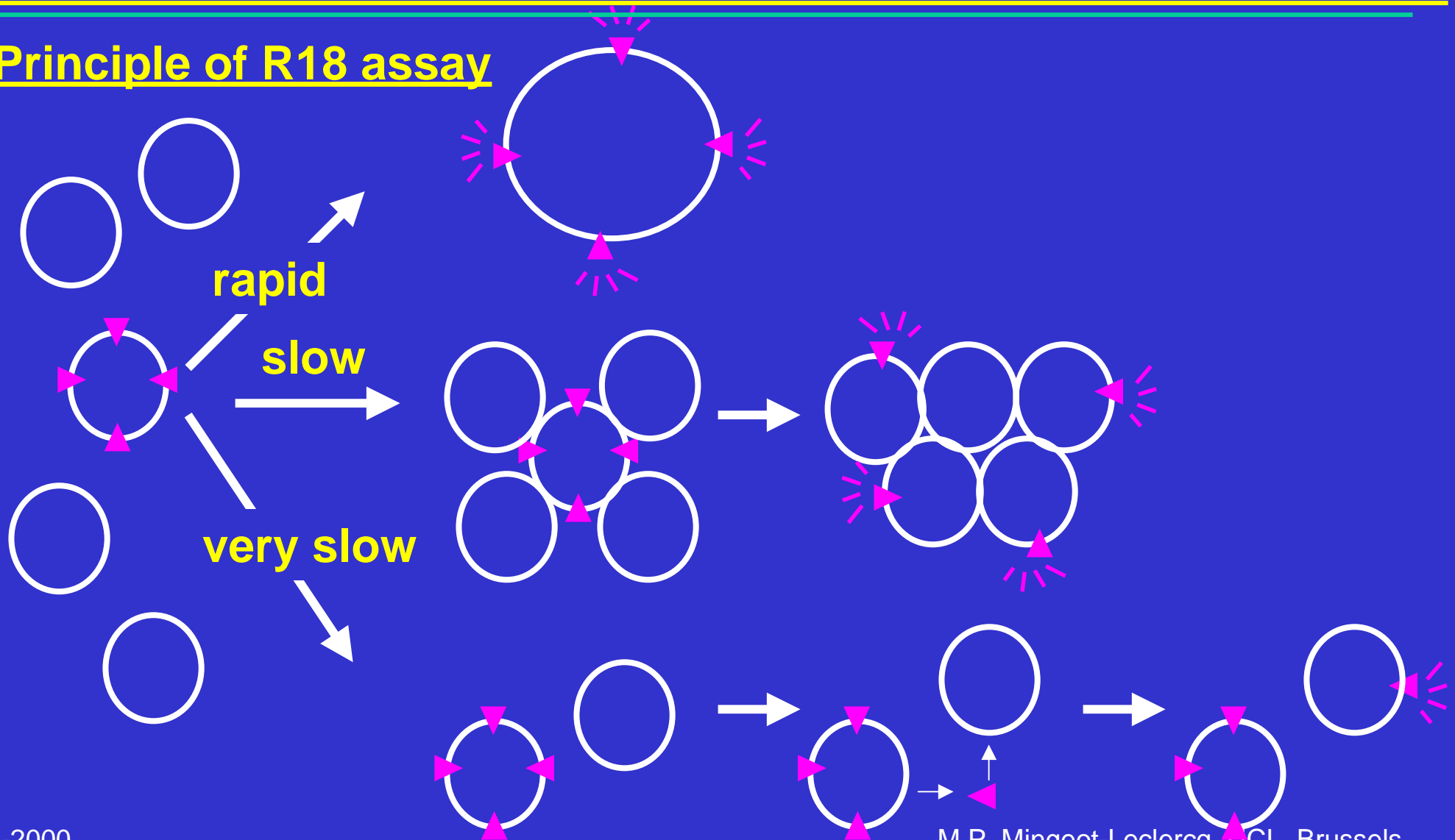
Lipid dilution : relief
of fluorescence
selfquenching

Hoekstra et al [1984] Biochemistry, 23, 5675-5681
Hoekstra [1990] Hepatology, 12, 61S - 66S

INTERACTIONS AMINOGLYCOSIDES - LIPIDS

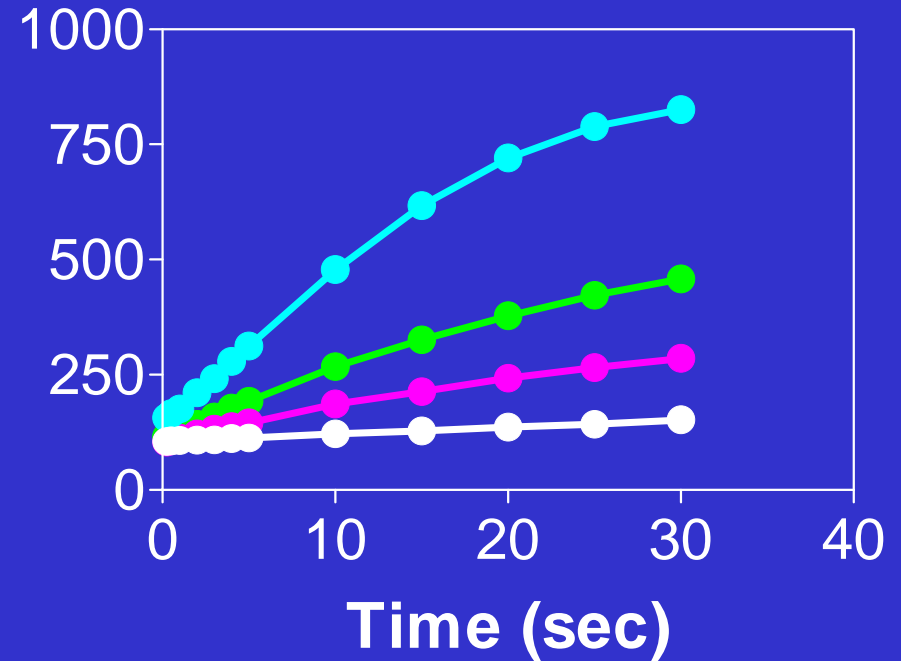
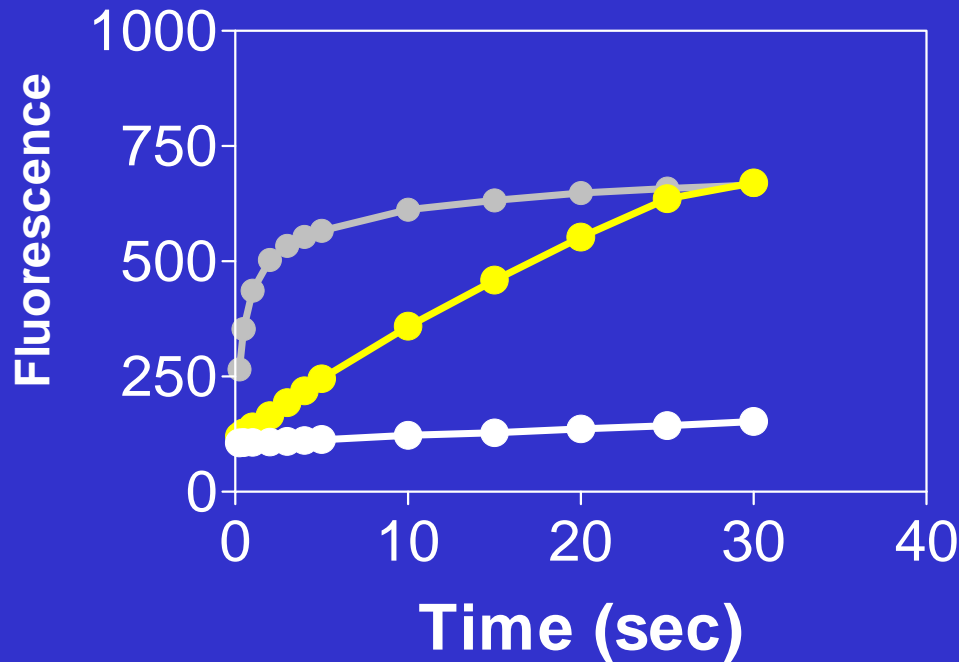
Effect on membrane fusion or aggregation

Principle of R18 assay



INTERACTIONS AMINOGLYCOSIDES - LIPIDS

Effect on membrane fusion or aggregation



Control
(agent:Lip ratio)

Spermine
156

Melittin
0.127

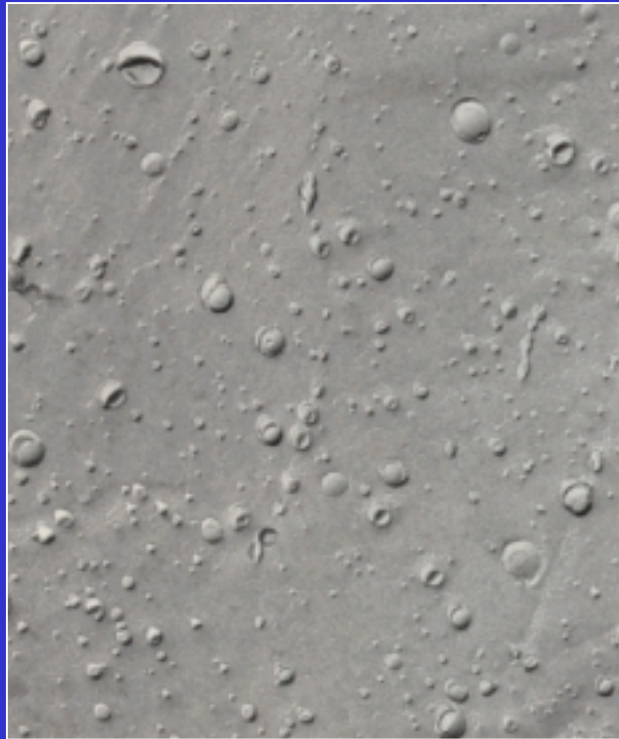
Gentamicin
125

Amikacin
125

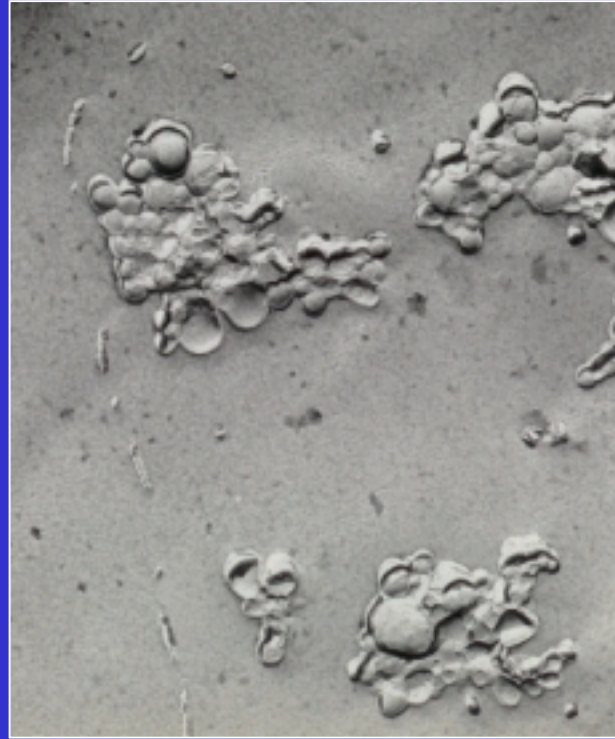
Isepamicin
125

INTERACTIONS AMINOGLYCOSIDES - LIPIDS

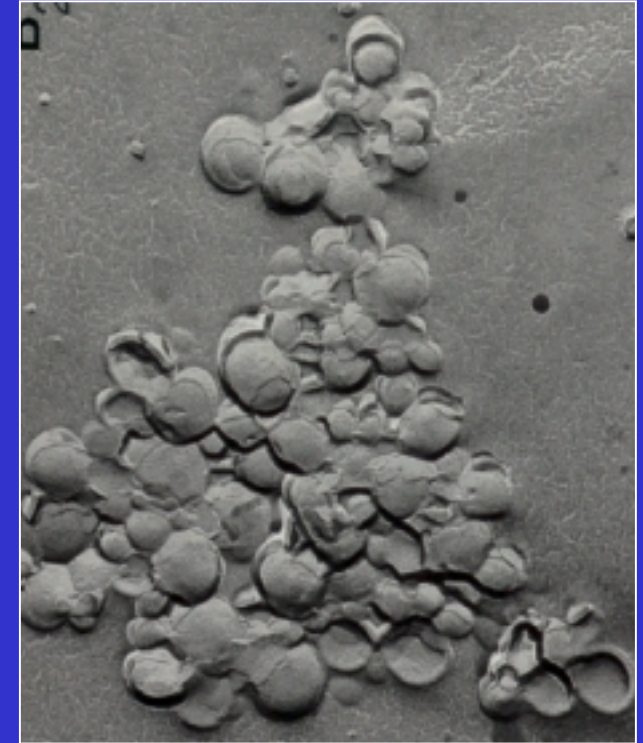
Effect on membrane fusion or aggregation



Control
37,500X



+ Gentamicin
37,500X

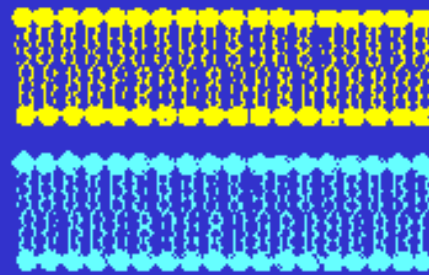


+ Gentamicin
85,000X

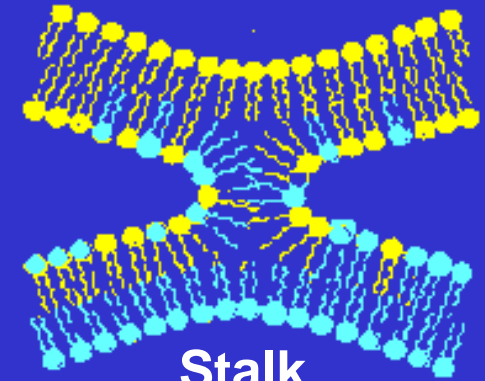
Possible mechanisms of membrane fusion involving

Inverted micellar intermediates

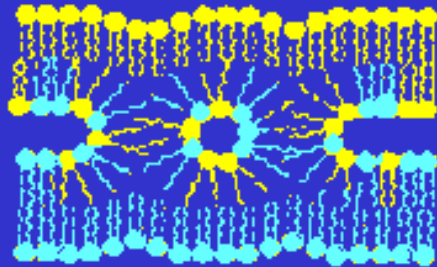
Stalk intermediates



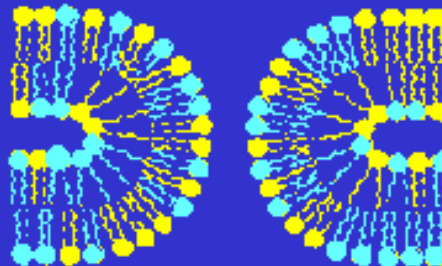
Apposed bilayers



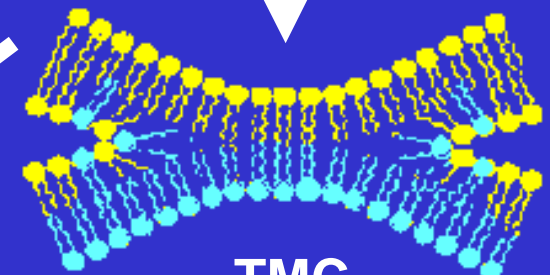
Stalk



IMI



Fusion pore



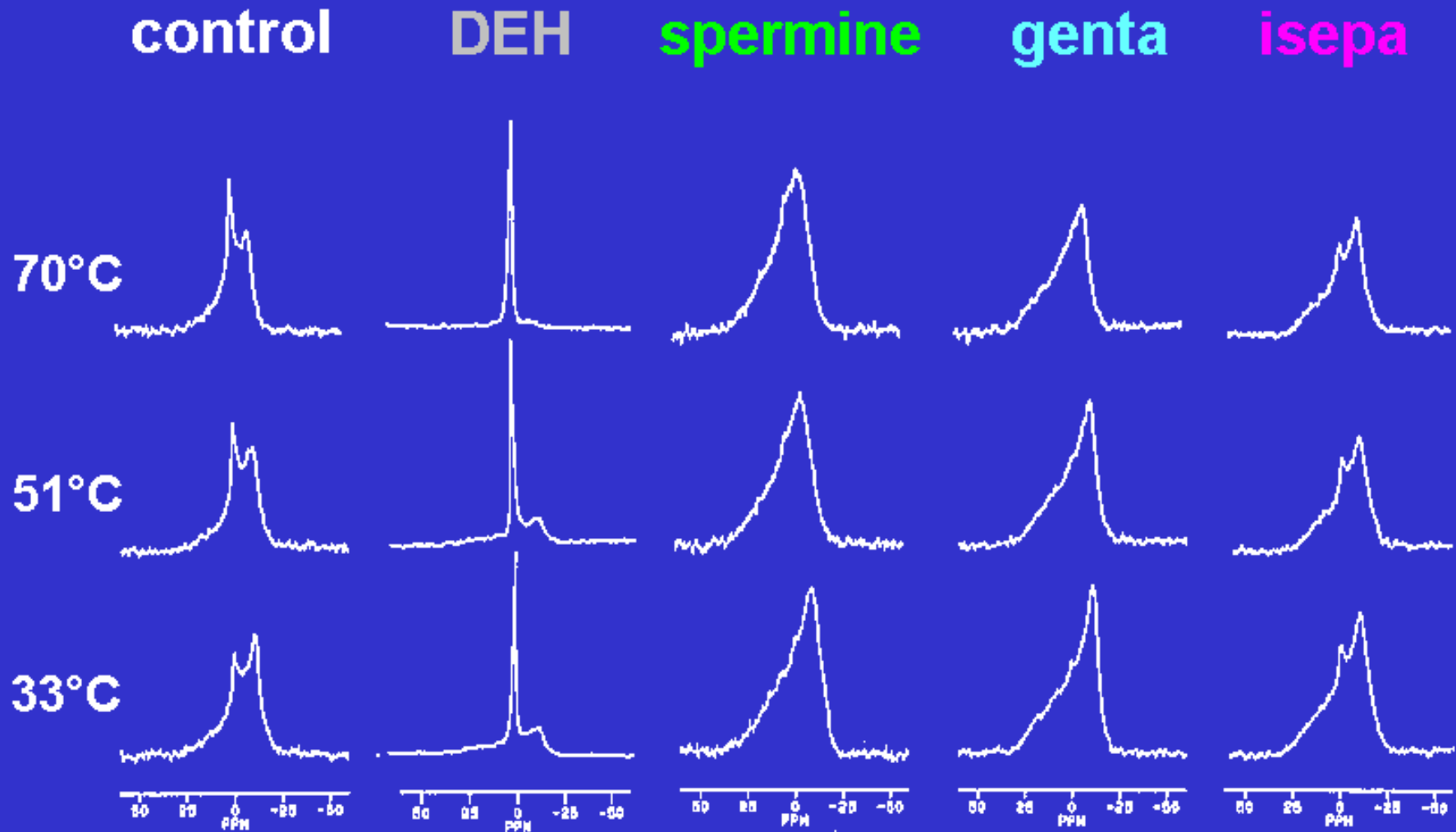
TMC

*Siegel [1993]
Biophys. J., 65, 2124-2140*

05-05-2000

INTERACTIONS AMINOGLYCOSIDES - LIPIDS

Effect on membrane fusion or aggregation



Agent : Lip ratio 0.05

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

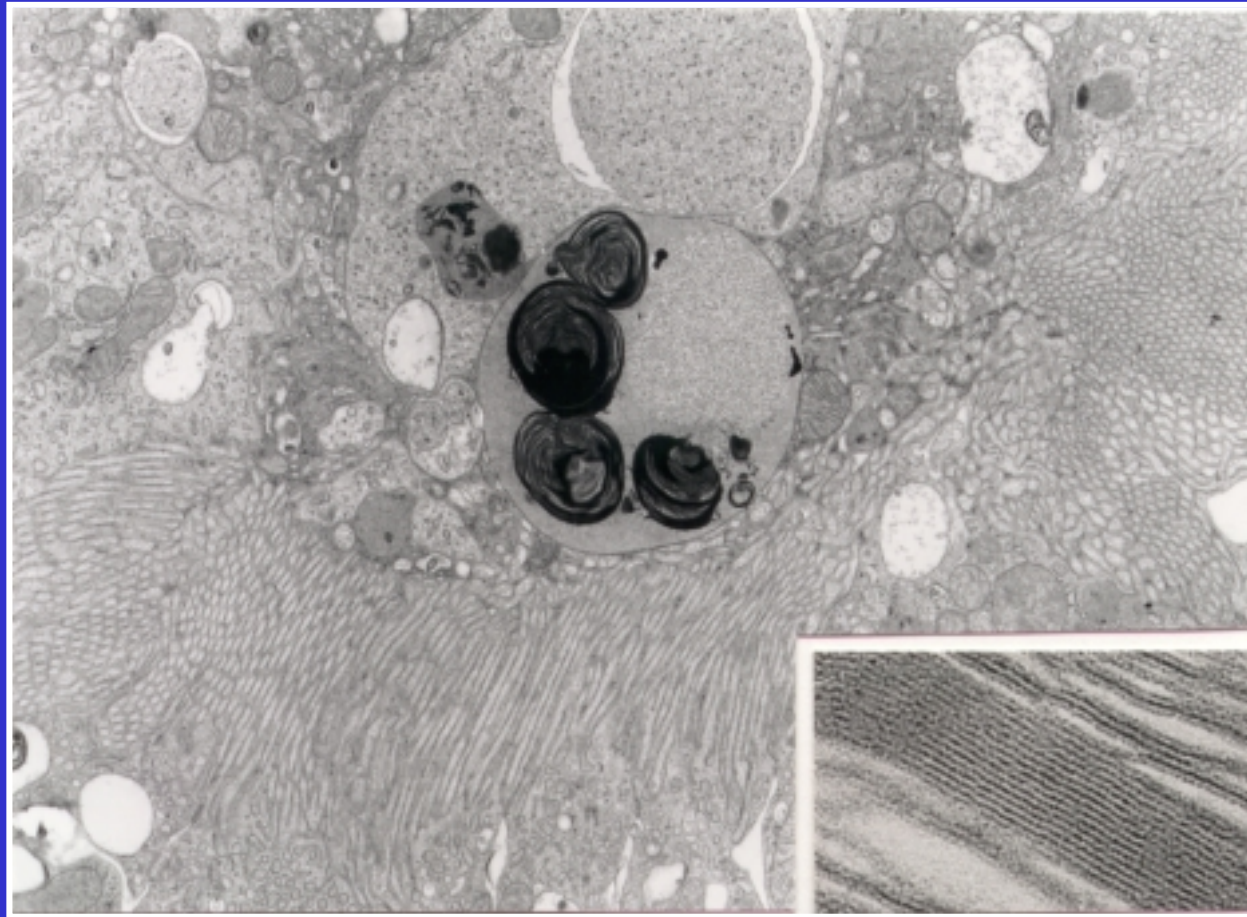
CHARACTERIZATION

CONSEQUENCES

INTEREST

- **Origin of myeloid bodies observed in lysosomes after aminoglycosides treatment \approx nephrotoxicity?**
 - Development of nephroprotectants
 - Rational synthesis of new, less toxic, aminoglycosides
 - Phospholipidosis can also be induced by several cationic amphiphilic drugs
- Potential relation with the impairment of lysosomes - pinocytic vesicles in rat kidney proximal tubules after treatment with gentamicin

INTRALYSOSOMAL PHOSPHOLIPIDOSIS INDUCED BY AMINOGLYCOSIDES



Pictures from P.M. Tulkens & M.B. Carlier

Magnifications: X 75,000 & 200,000

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

CHARACTERIZATION

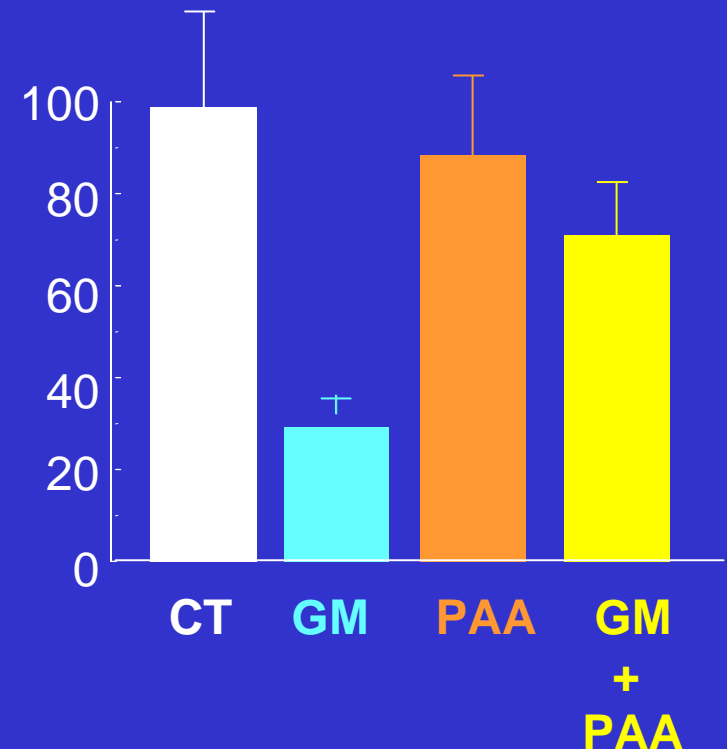
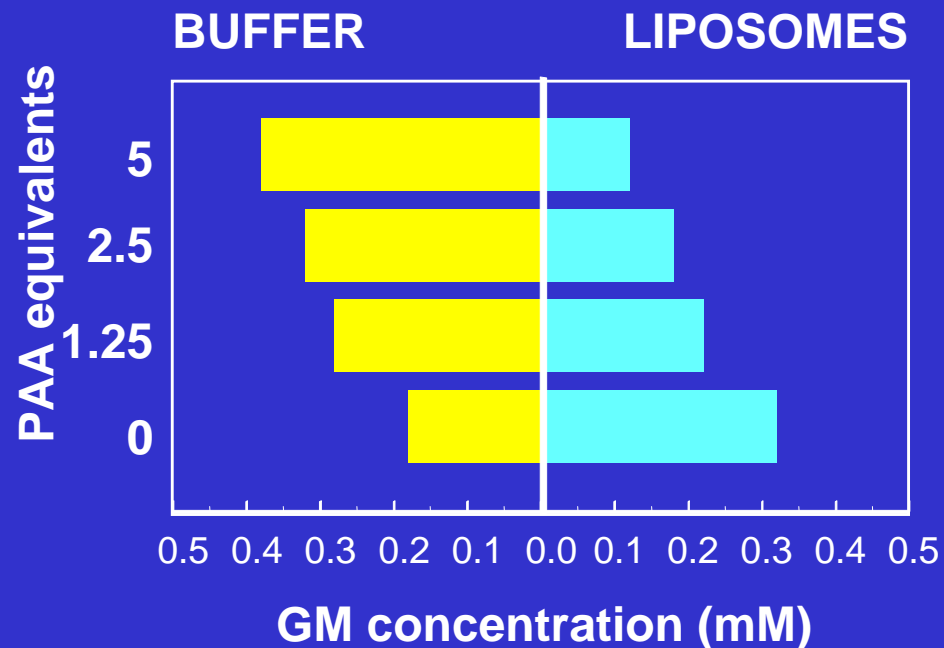
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DEVELOPMENT OF NEPHROPROTECTANT: Polyaspartic acid - *in vitro* studies

Lysoderivative release
(% of base line)



Kishore et al [1990] *J. Pharmacol Exp Ther* 255:867-874

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

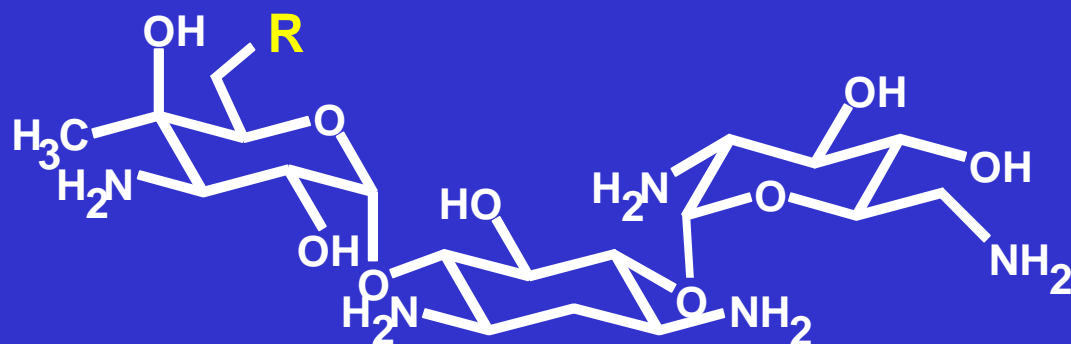
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RATIONAL BASIS FOR SYNTHESIS OF NEW - LESS TOXIC- AMINOGLYCOSIDES



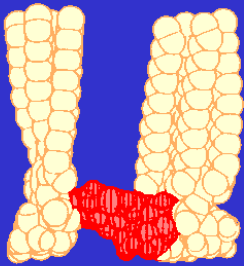
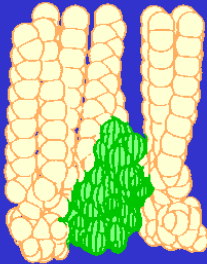
Kanamycin B

- R =**
- OH
 - O- Alkyl
 - O- Aryl
 - X
 - N(H,CH₃, Bu) - CO(O) - Alkyl
 - R- N- Alkyl
 - R- S- Alkyl
 - R - SO - Alkyl
 - R - SO₂ - Alkyl

RATIONAL BASIS FOR SYNTHESIS OF NEW - LESS TOXIC- AMINOGLYCOSIDES

Aminoglycoside	IC50 ($\mu\text{g/ml}$)	Calculated interaction (kJ / mol)	Orientation towards lipid / water interface
Isepamicin Amikacin	125 ± 7 85 ± 4	- 23.4 - 35.7	
Kanamycin A Kanamycin B	69 ± 11 54 ± 4	- 46.8 - 48.5	

RATIONAL BASIS FOR SYNTHESIS OF NEW - LESS TOXIC- AMINOGLYCOSIDES

Drug	Energy of interaction (KJ/mol)	Orientation towards lipid/water interface	IC50 (μM)
Kanamycin B	- 48.5		110 ± 11
6''-deoxy-6''-(N-acetyl-N-methylamino)kanamycin B	- 24.4		134 ± 7

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

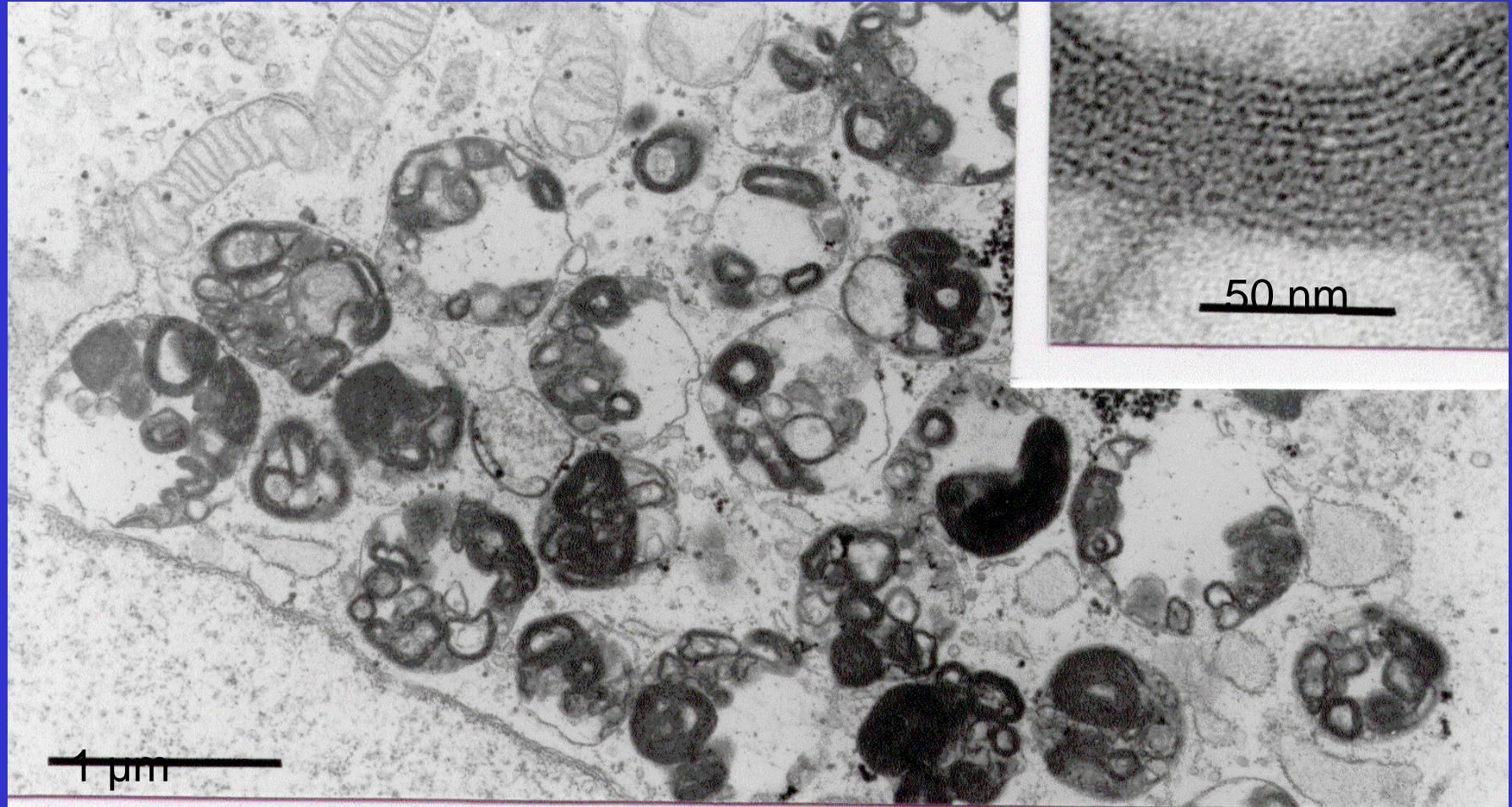
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INTRALYSOSOMAL PHOSPHOLIPIDOSIS INDUCED BY MACROLIDES



Van Bambeke et al [1996] Eur. J. Pharmacol. 314:203-214

AMINOGLYCOSIDES- LIPIDS INTERACTIONS

CHARACTERIZATION

CONSEQUENCES

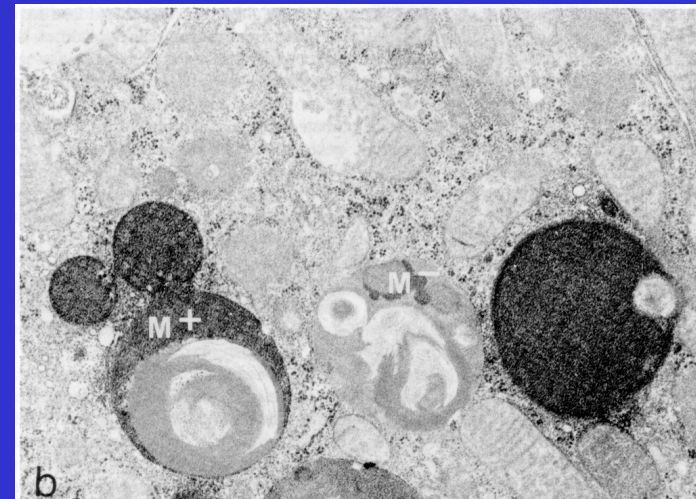
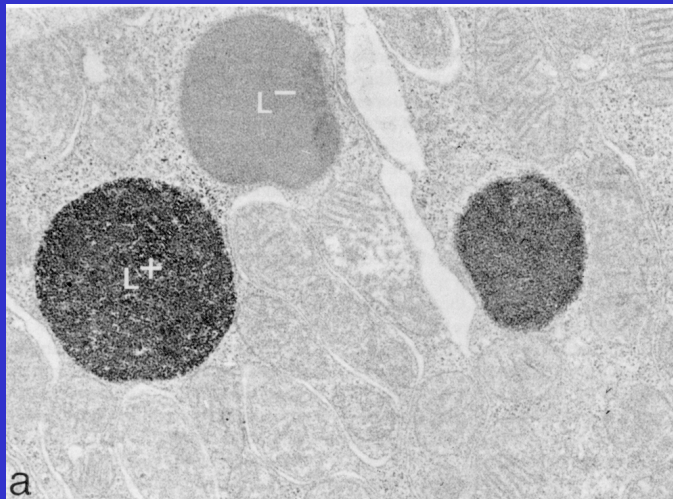
INTEREST

- Origin of myeloid bodies observed in lysosomes after aminoglycosides treatment \approx nephrotoxicity?
 - Development of nephroprotectants
 - Rational synthesis of new, less toxic, aminoglycosides
 - Phospholipidosis can also be induced by several cationic amphiphilic drugs
- Potential relation with the impairment of lysosomes - pinocytic vesicles in rat kidney proximal tubules after treatment with gentamicin

IMPAIRMENT OF LYSOSOME - PINOCYTOTIC VESICLE FUSION

Morphometry of lysosomes in PTC after cytochemical demonstration of HRP

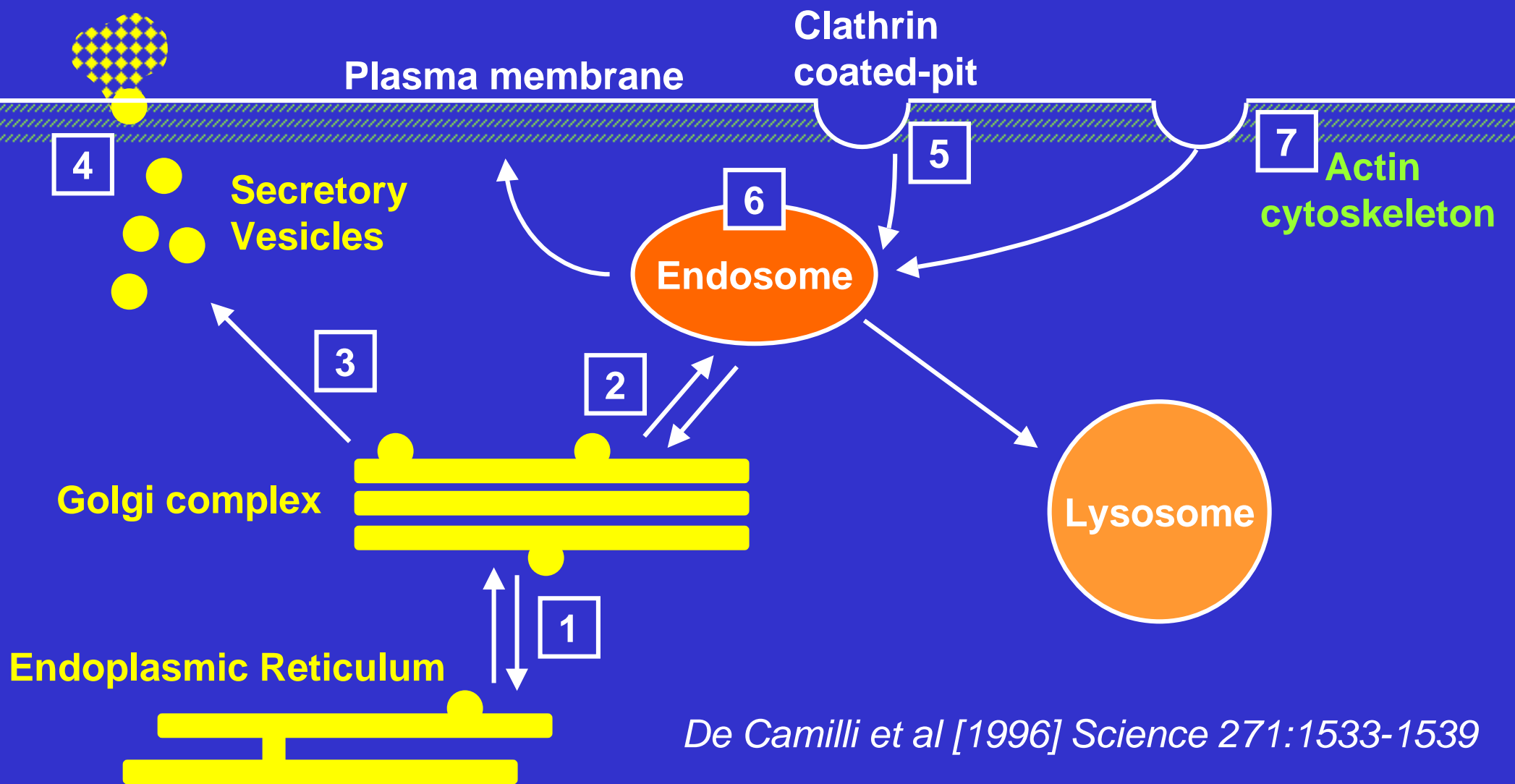
Treatment (9 days)	% cytoplasmic volume			
	L +	L -	M +	M -
(a) control	2.77 ± 0.58	2.03 ± 0.47	-	-
(b) Genta (10 mg/kg.day)	0.34 ± 0.17	1.16 ± 0.47	0.11	4.16 ± 0.32



X 21,500

From Giurgea-Marion et al, *Tox. Appl. Pharmacol.* 86: 271-285 (1986)

ROLE OF PHOSPHATIDYLINOSITOL DERIVATIVES IN VESICULAR TRAFFIC



De Camilli et al [1996] Science 271:1533-1539

AMINOGLYCOSIDES - MEMBRANE INTERACTIONS

