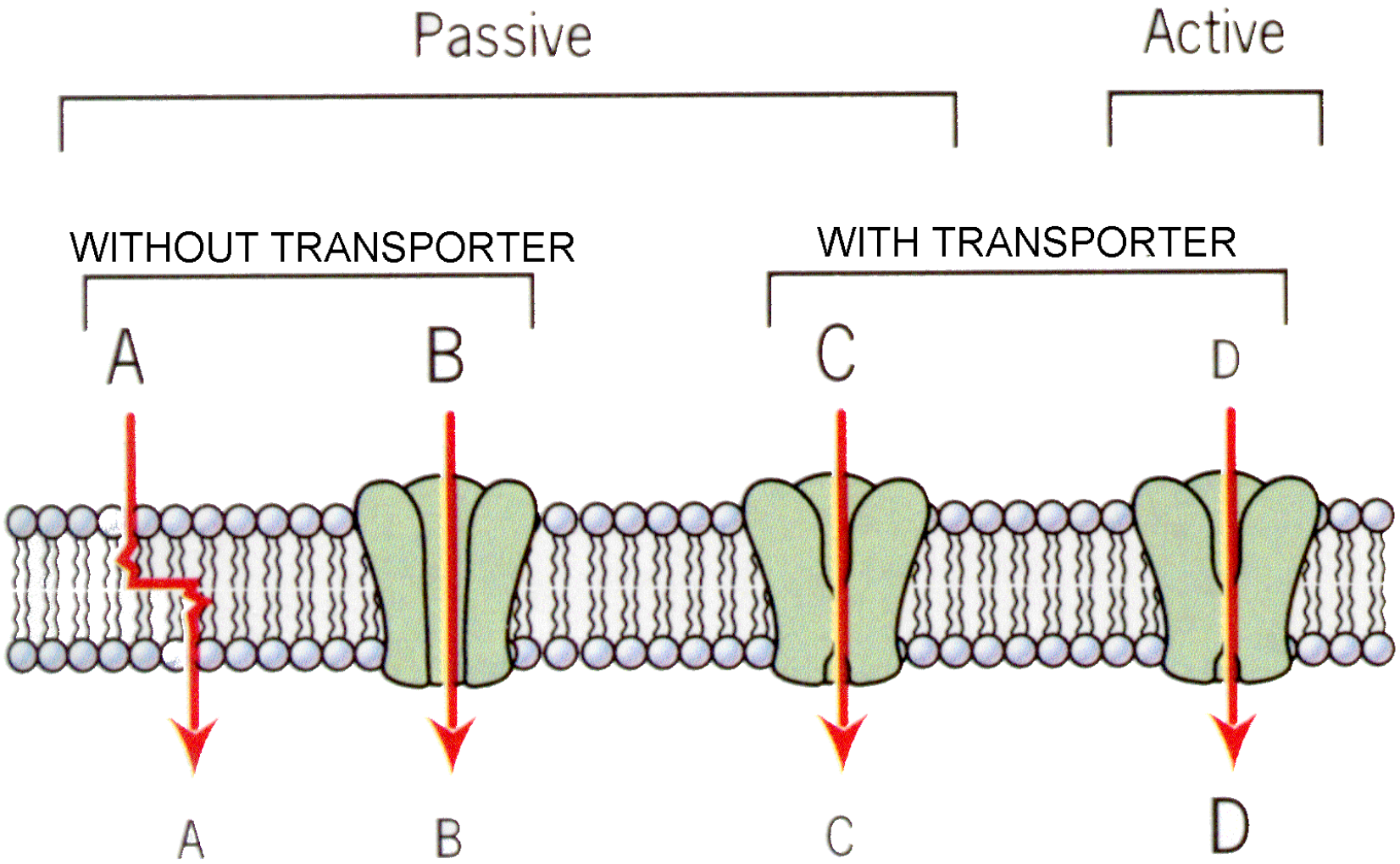
Asian PK/PD Educational Workshop

New developments in PK/PD

- drug transport in eucaryotic cells
- difficult-to-reach compartments
- intracellular infections

This part uses material from presentations made at
- the 4th ISAP Educational Workshop, 2001, Istanbul, Turkey
- the 9th ISAP Symposium (a pre-ICAAC symposium), 2001, Chicago, Ill.
- the 10th ISAP Symposium (a post-ECCMID symposium), 2001, Milan, Italy

How are drugs transported across cell barriers ?



Why do we have drug transporters ?

Drug transporters were originally discovered while studying resistance mechanisms, raising the following questions:

- do cells like to build up specific resistance mechanism ?
- have cells have been exposed to drugs in their history ?
- have drugs special properties ?

There seems to be so many drugs transporters ...

mid-90's :

The genomic analysis of *Saccharomyces cerevisiae* and of *Pseudomonas aeruginosa* reveals a very large number of potential "drug" transporters

Inventory of transporters in the complete genomes of *S. cerevisiae* and *E. coli*

Abundance of proteins of differing predicted membrane topologies

nb TMS	nb proteins		% of total		
	Yeast	*E. coli*	Yeast	*E. coli*	
0	4364	2861	70.8	66.8	soluble proteins
1	937	655	15.3	15.3	signal peptides
2-3	390	220	6.5	5.1	potential transport proteins (14 %)
4-6	185	211	3.1	4.9	
7-9	144	153	2.3	3.6	
> 10	121	82	2.0	4.3	

Paulsen *et al* FEBS Lett (1998) 430:116-125

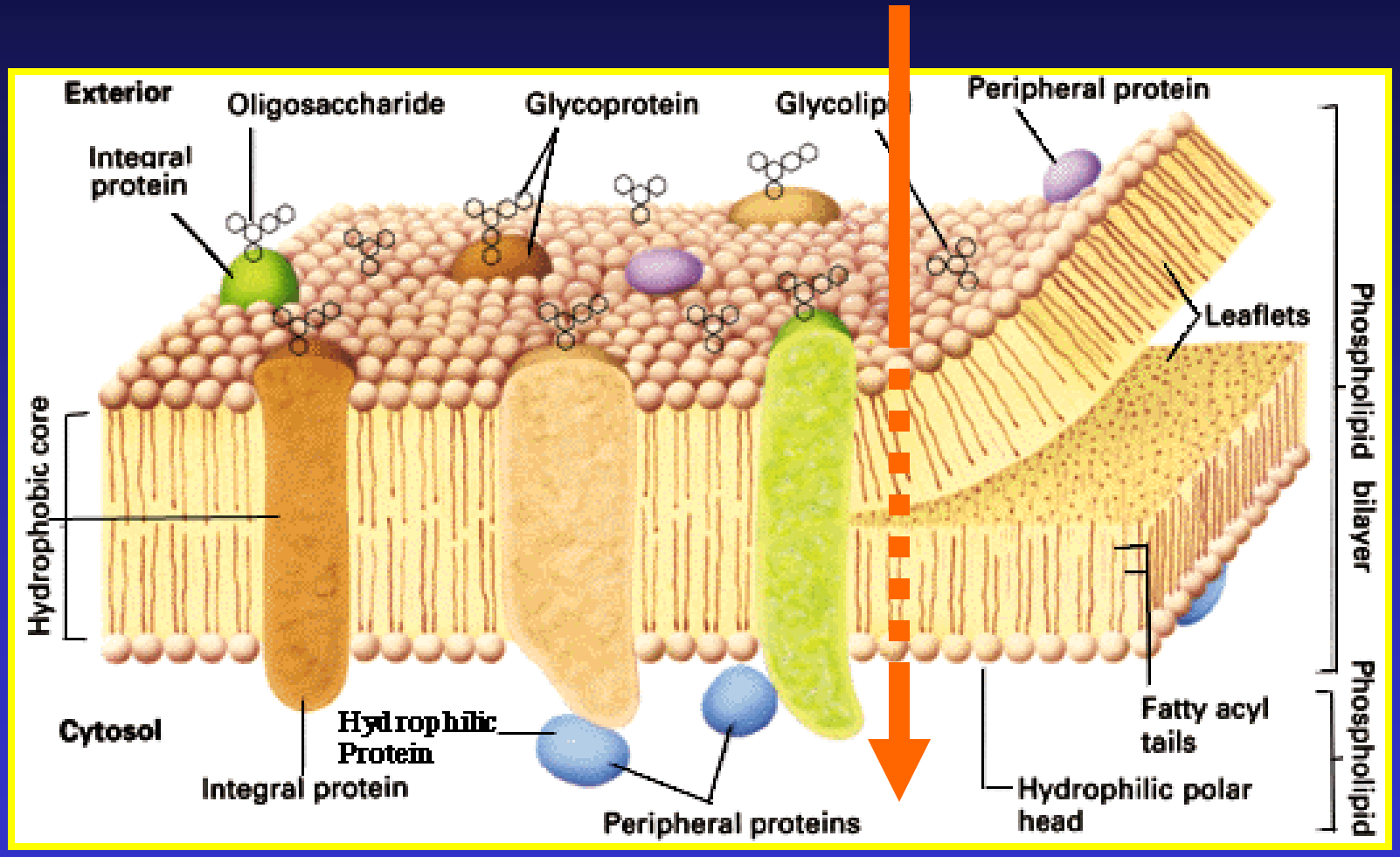
There must be something more than drugs ...

mid-90's :

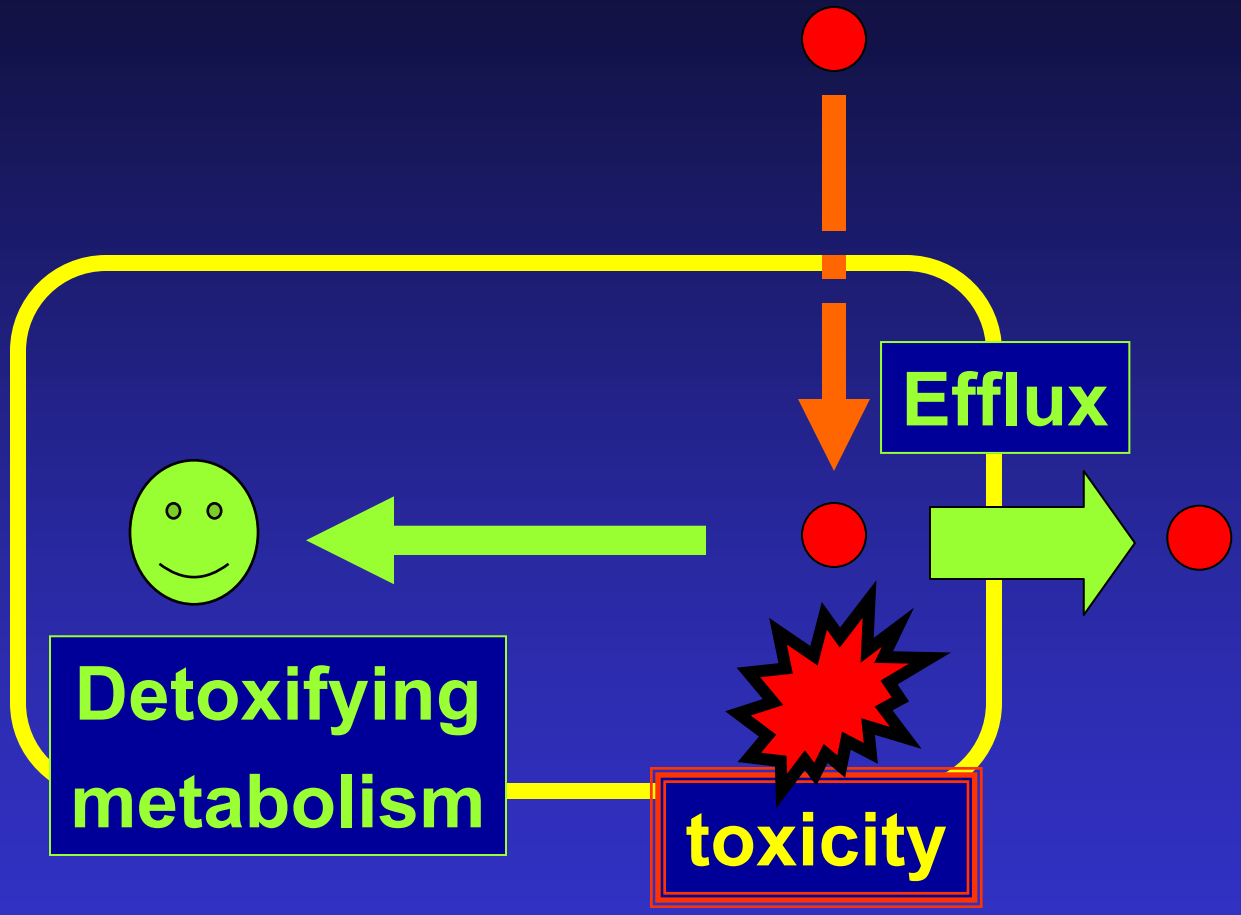
Proteomic and functional analyses reveals that the potential transporters identified by genomics are responsible for the efflux of a very large variety of substances with a common biophysical property:

amphiphilicity...

Amphiphilic substances can easily pass across membrane bilayers ...



Why should cells extrude amphiphilic compounds ?



Concerted barrier against invasion ...

But why drugs ?

Most if not all of our drugs are amphiphilic ...

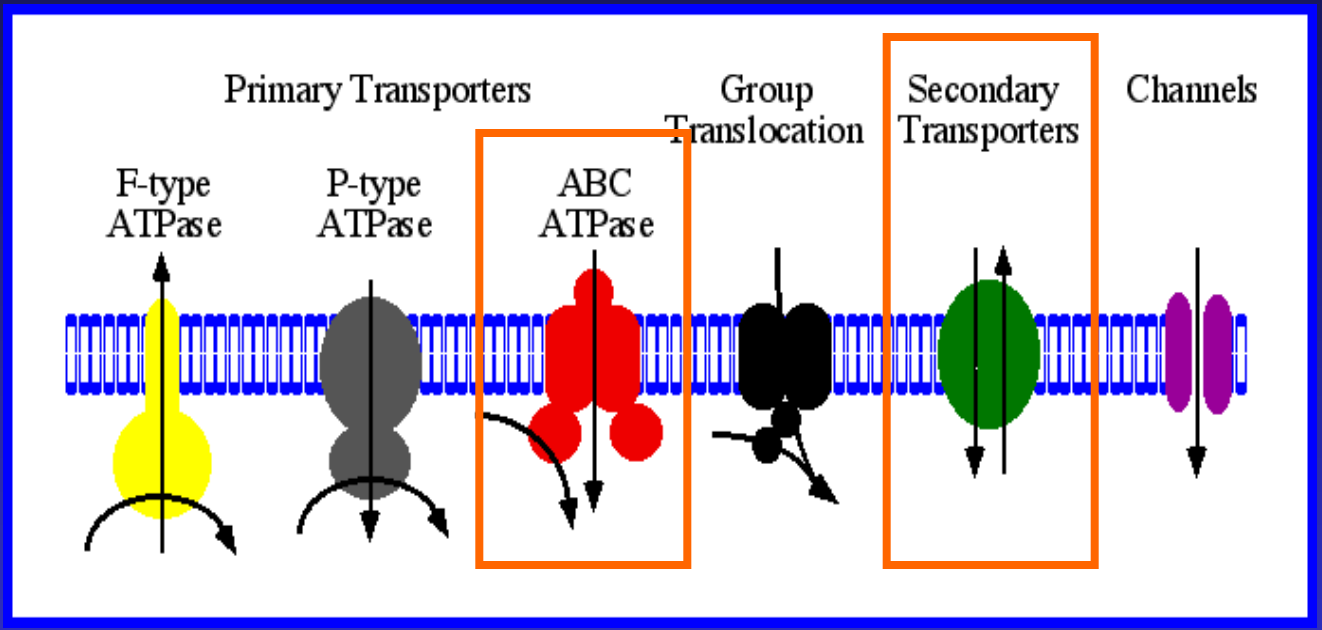
because we selected or designed them to be so….

in order to penetrate cells and tissues ...

- **Oral bioavailability**
- **tissue distribution**
- **penetration in difficult-to-reach compartments, etc...**

Transporters - data bases

http://www-biology.ucsd.edu/~msaier/transport/titlepage.html



main drug transporters

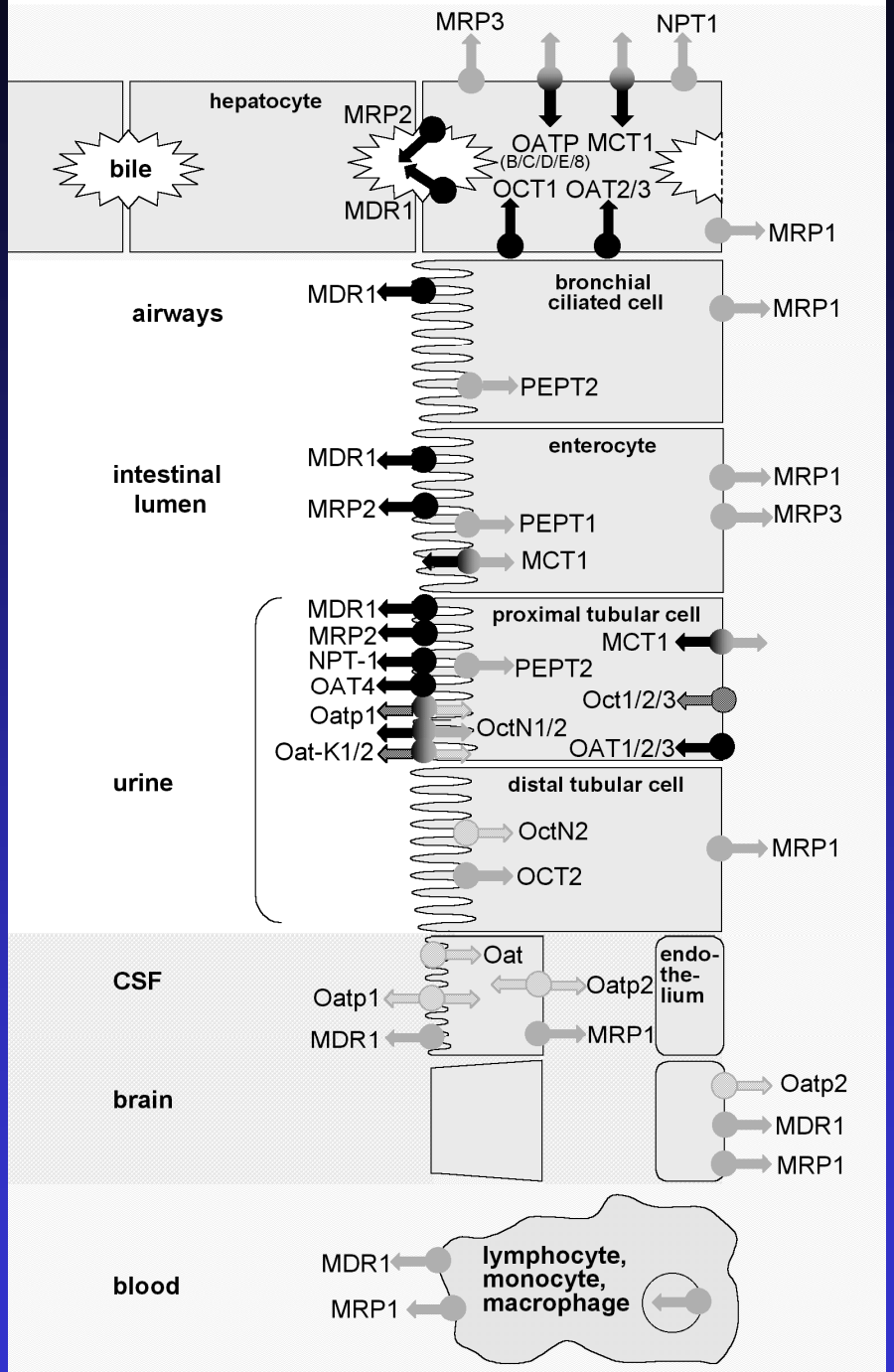
Classification page → combination of phylogenetic and functional information
Transport analysis page → comparison of transporters in complete genomes
Phylogenetic page → phylogenetic trees of transporters families

Antibiotic classes recognized by efflux pumps in different types of organisms

Antibiotic class	bacteria Gram (+)	bacteria Gram(-)	fungi	superior eucaryotes
β-lactams	●	●	●	●
fusidic acid		●		
macrolides	●	●	●	●
streptogramins	●			●
tetracyclines	●	●	●	●
aminoglycosides		●	●	
chloramphenicol	●	●	●	
rifamycins				●
sulfamides			●	
trimethoprim		●		
fluoroquinolones	●	●		●

Drugs and physiological substrates for transporters in eucaryotic cells

superfamily	transporter	physiol. substrates	antibiotics
ABC	MDR1 (= P-gp)	phospholipids	fluoroquinolones macrolides β-lactams tetracyclines streptogramins
	MRP1	phospholipids leukotrienes conjugates	fluroquinolones macrolides rifamycins
	MRP2	conjugates	fluoroquinolones β-lactams
MFS	NPT1	phosphates	β-lactams
OAT	OATP1	bile salts steroids	β-lactams



Main antibiotic transporters known in 2003 ...

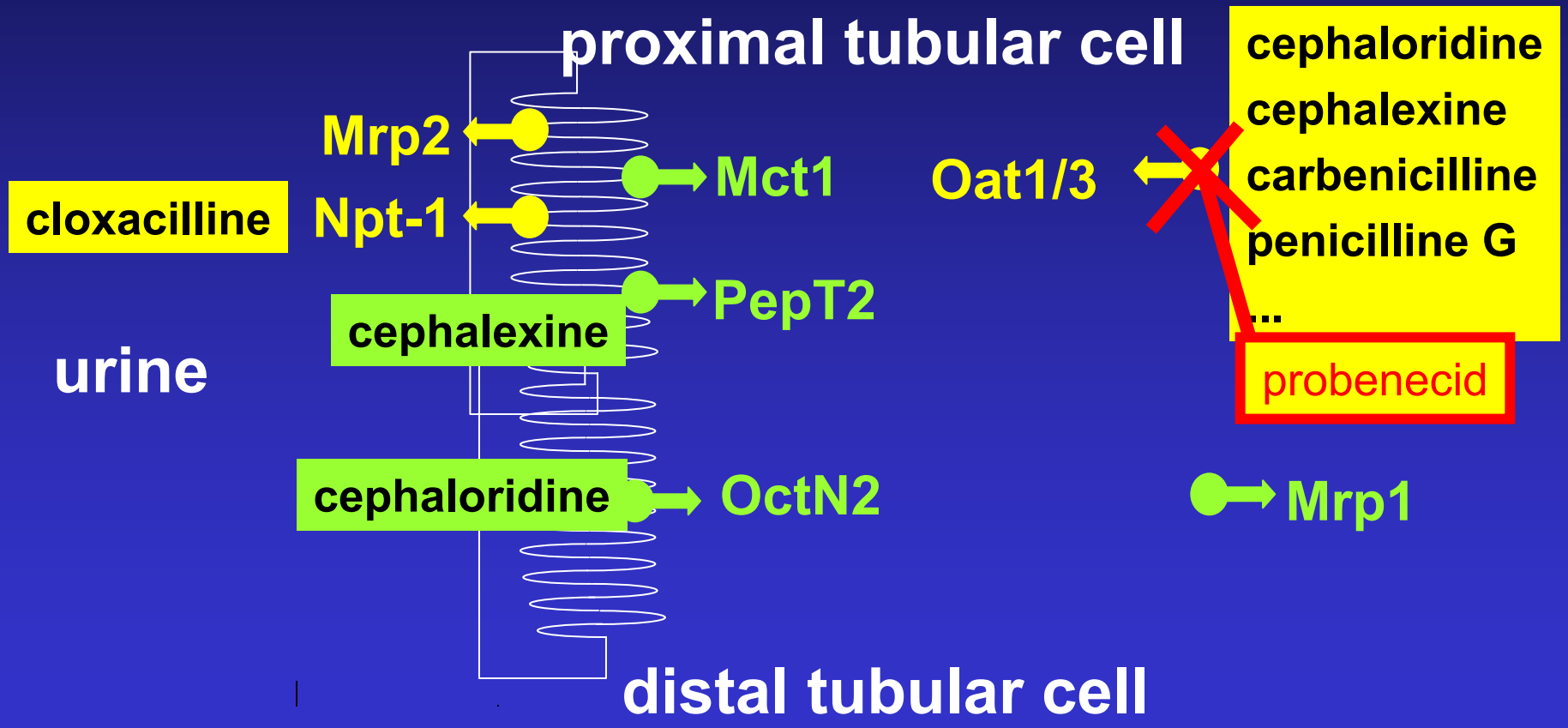
Responsible for modulation of transport and of

- elimination
- resorbtion
- penetration

Van Bambeke et al., 2003, submitted

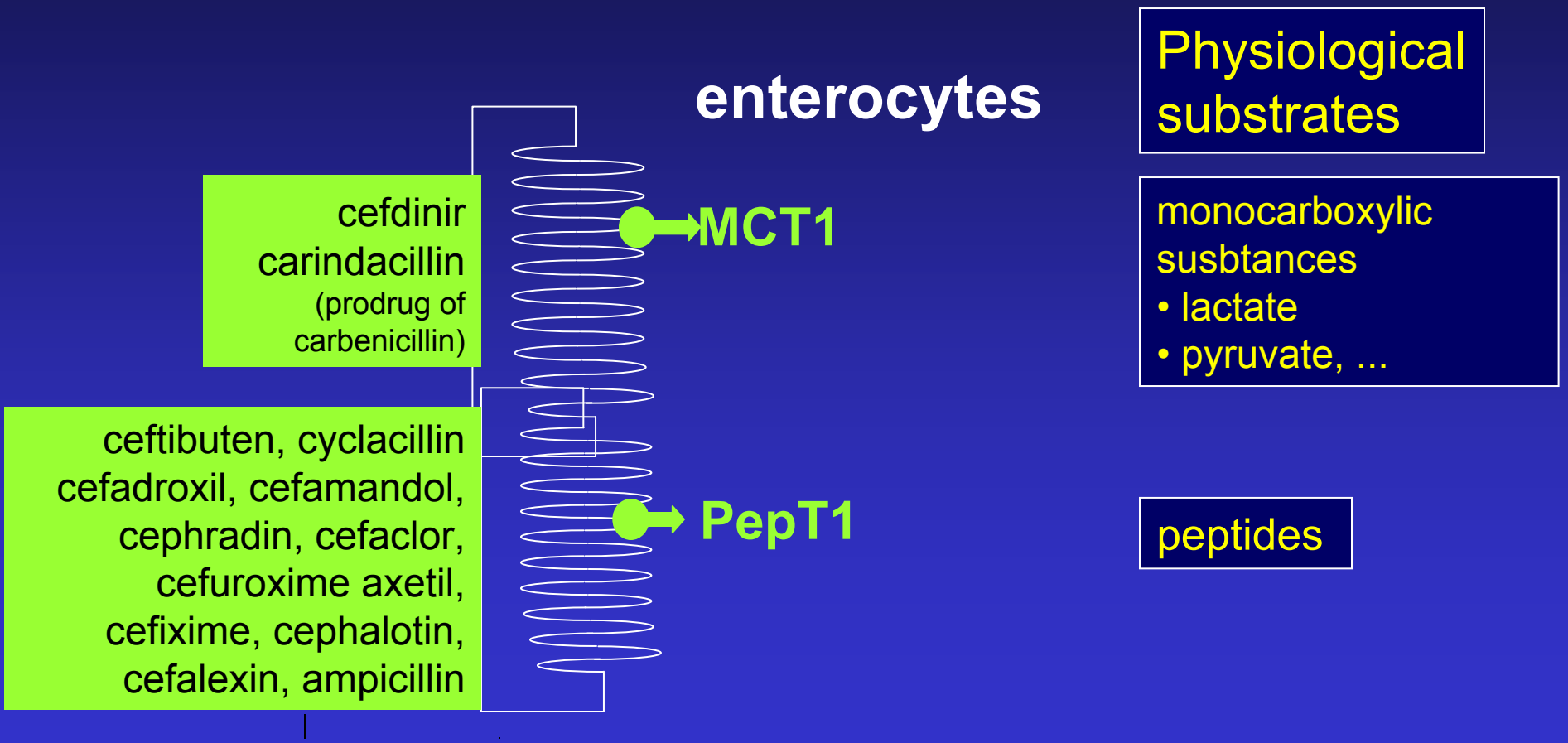
One example of the role of drug transporters for drug elimination

efflux / reuptake of β-lactams in kidney



One example of the role of drug transporters for drug absorbtion

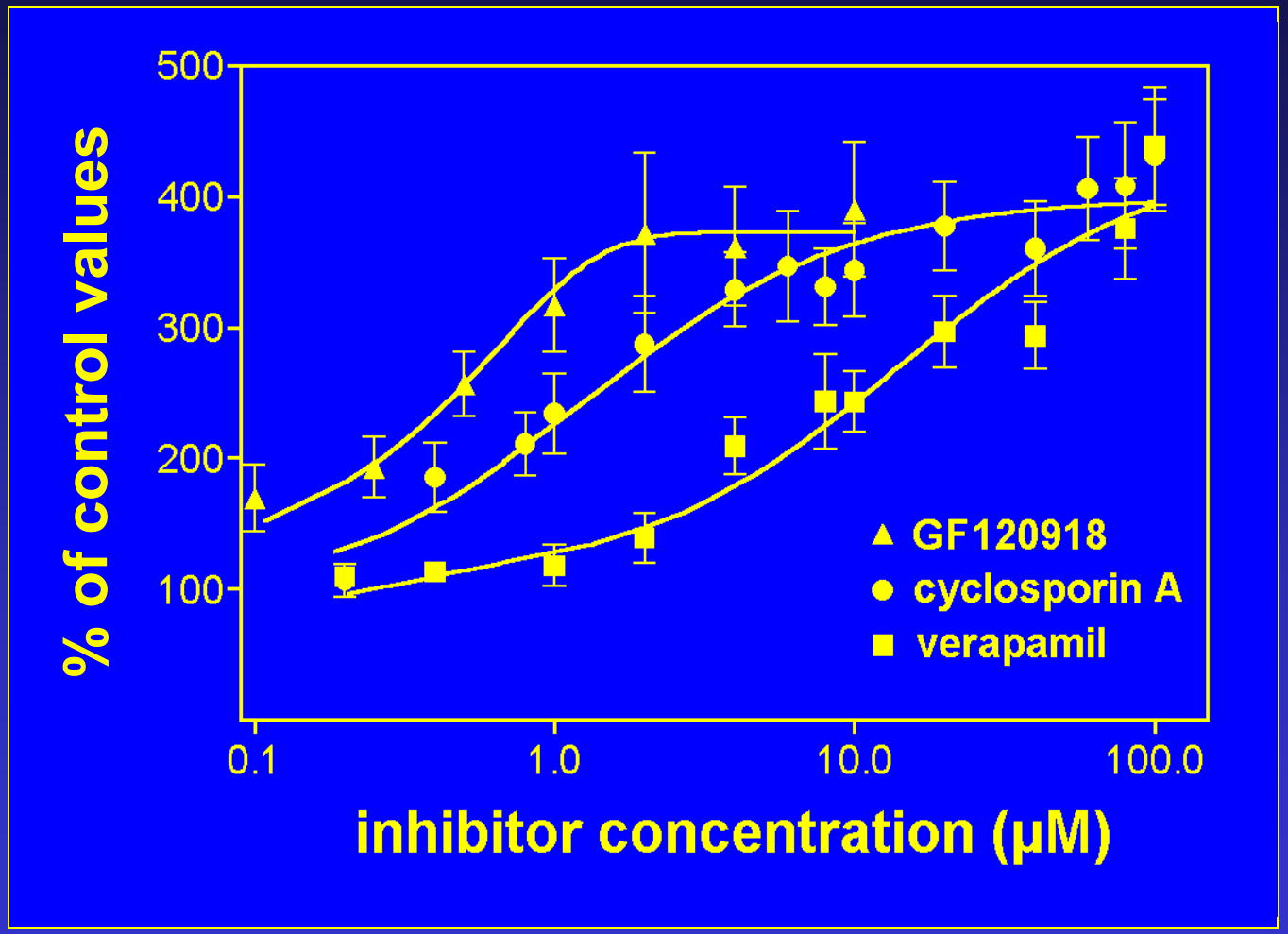
uptake of β-lactams through the intestinal barrier



One example of the role of drug transporters in cellular accumulation of antibiotics

Influence of MDR (P-gp) inhibitors on azithromycin accumulation (5µg/ml; 3 h)

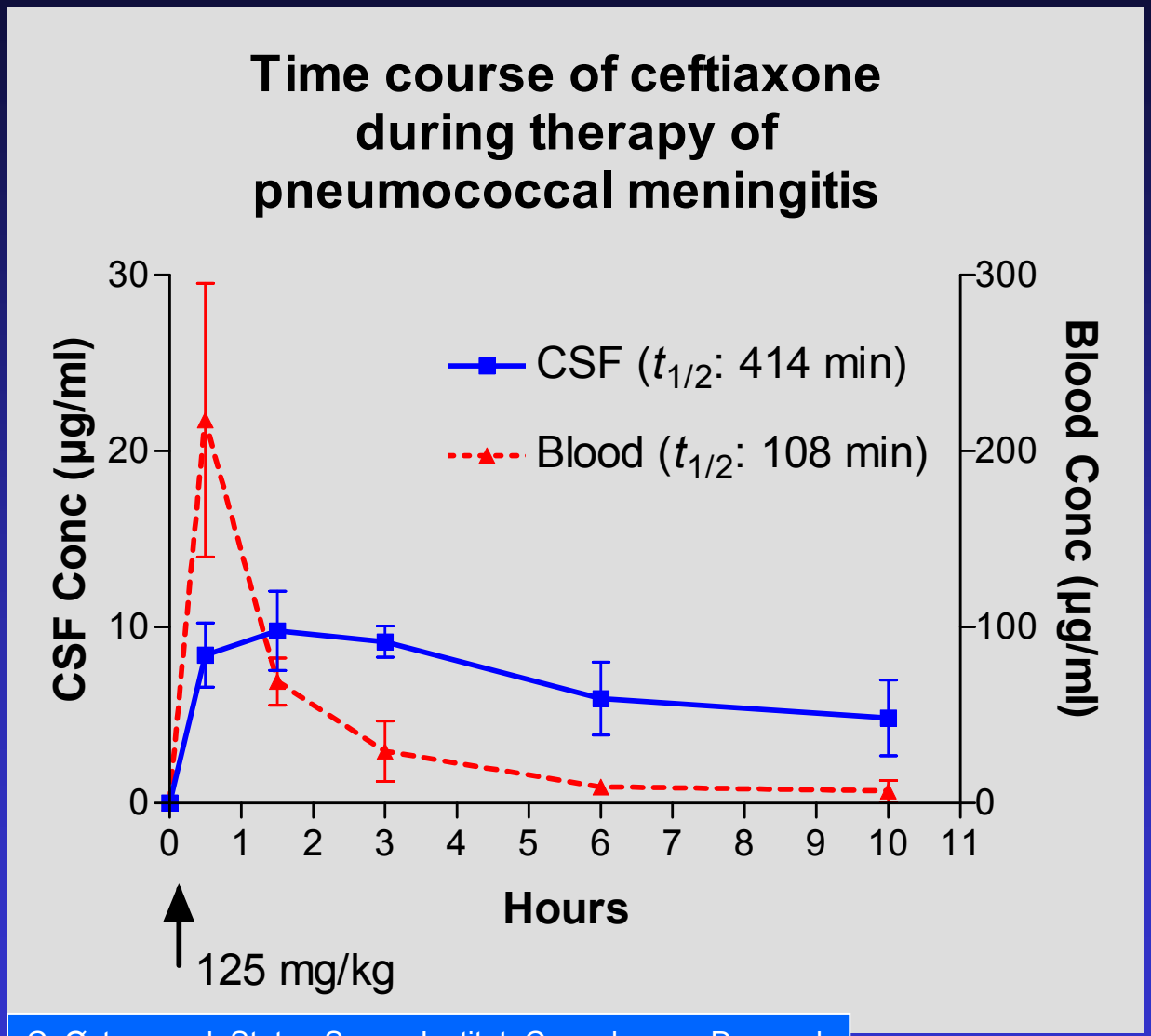
Seral et al., 2003, in press



Difficult-to-reach compartments

- brain and CSF
- abcesses
- ...

Brain and CSF : low penetration ...

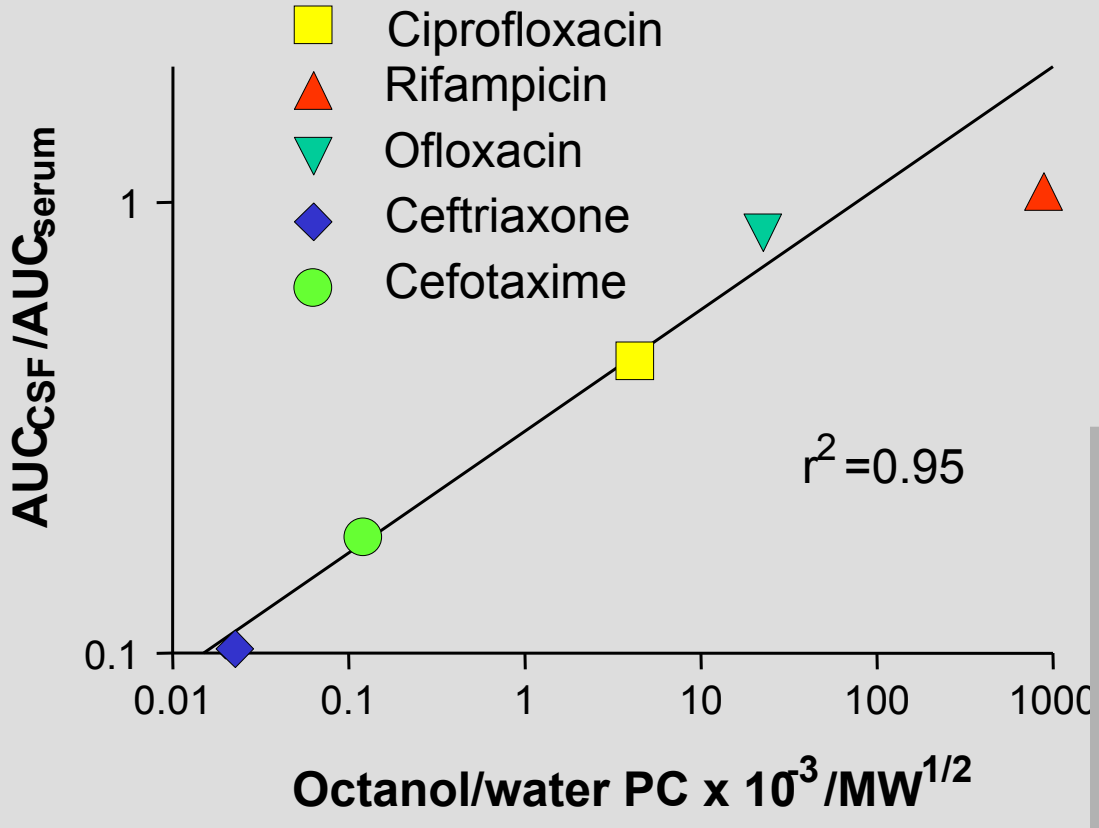


C. Østergaard, Staten Serum Institut, Copenhagen, Denmark
9th ISAP symposium (pre-ICAAC), 2001, Chicaho, Ill.

$Conc_{CSF}/Conc_{blood}$: ~4-71 %

AUC_{CSF}/AUC_{blood}: ~19 %

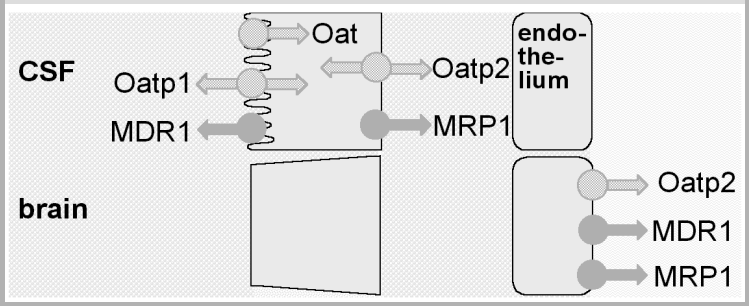
Brain and CSF: parameters governing penetration



C. Østergaard, Staten Serum Institut, Copenhagen, Denmark
9th ISAP symposium (pre-ICAAC), 2001, Chicaho, Ill.

A high lipid solubility or a low molecular weight increase CSF penetration

But transporters are also important ...



Van Bambeke et al., 2003, submitted

PK/PD for experimental bactarial meningitis: some proposals ...

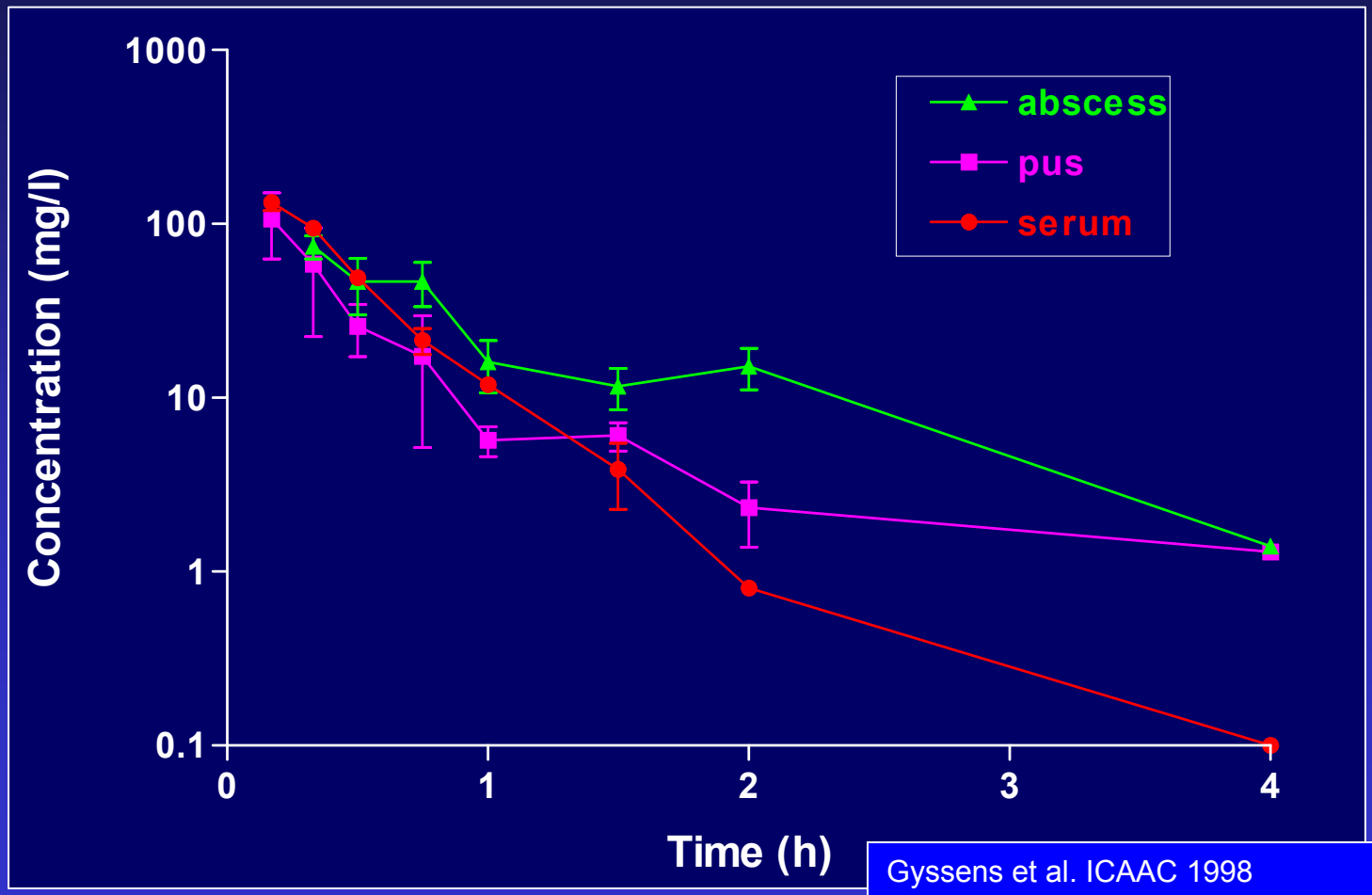
- **Concentration dependent killing**
  - **AUC/MBC is the most important pk/pd parameter for aminoglycosides and quinolones**
  - **Short persistent effects for quinolones, but not for aminoglycosides**
- **Concentration independent killing**
  - **Concentration dependent killing at 10-30 × MBC for β-lactams and glycopeptides**
  - **$T_{>MBC}$ is most important pk/pd parameter**
  - **Short persistent effects**

Higher loading dose and less frequent dosing are necessary

C. Østergaard, Staten Serum Institut, Copenhagen, Denmark
9th ISAP symposium (pre-ICAAC), 2001, Chicaho, Ill.

Abcesses ...

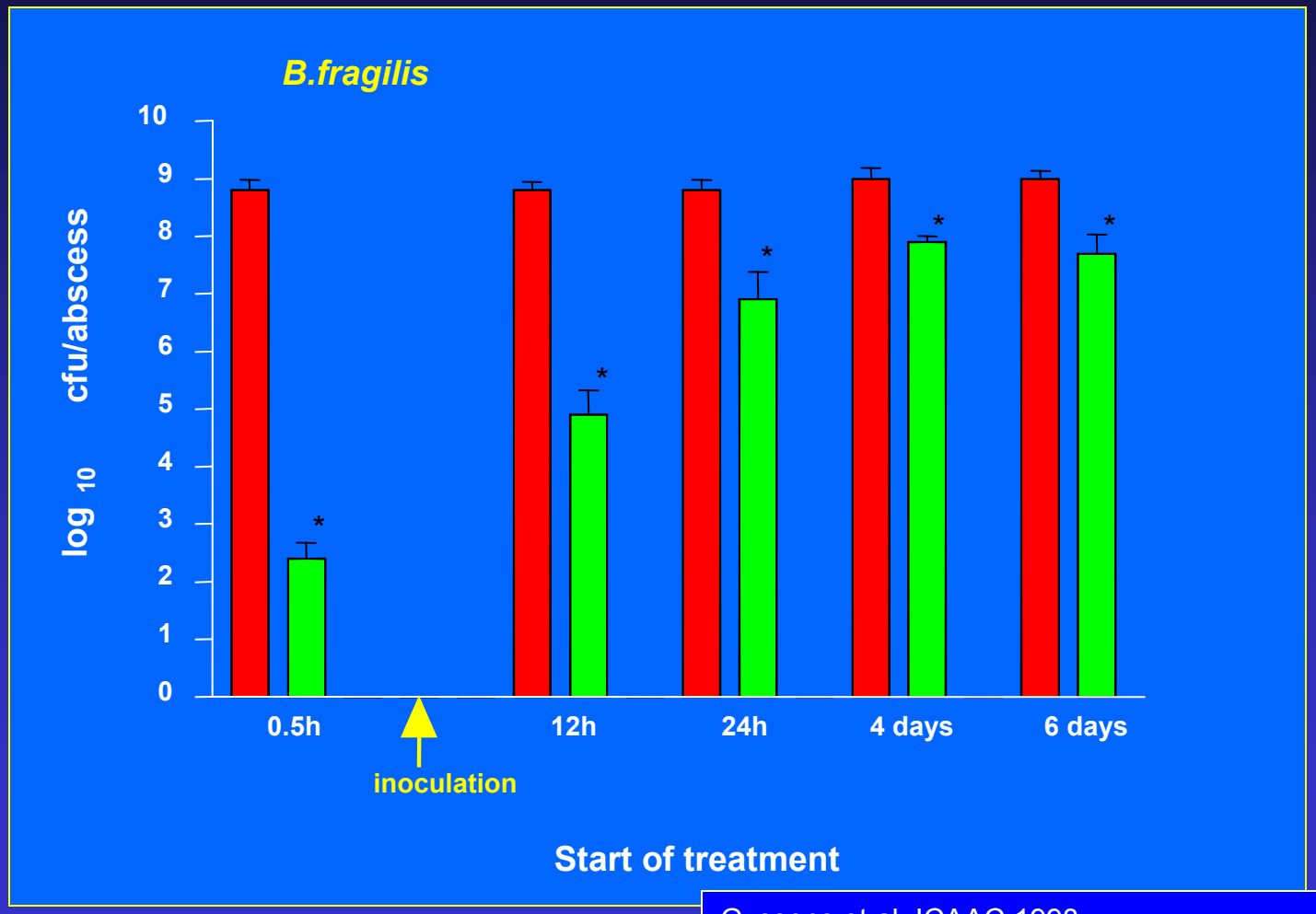
Comparative pharmacokinetics of imipenem in serum in abscess and in pus (single dose of 128 mg/kg)



Gyssens et al. ICAAC 1998
and 9th ISAP symposium (pre-ICAAC), 2001, Chicago, Ill.

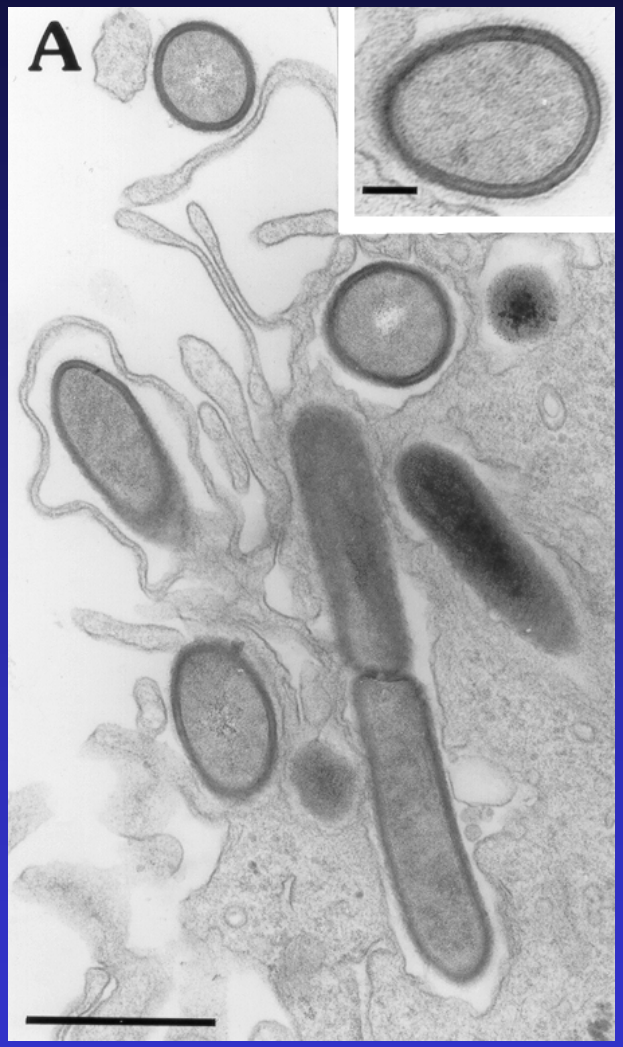
Abcesses ...

Efficacy of imipenem treatment (384 mg/kg/day; q4h; 3 days) started before or at increasing times after inoculation ...



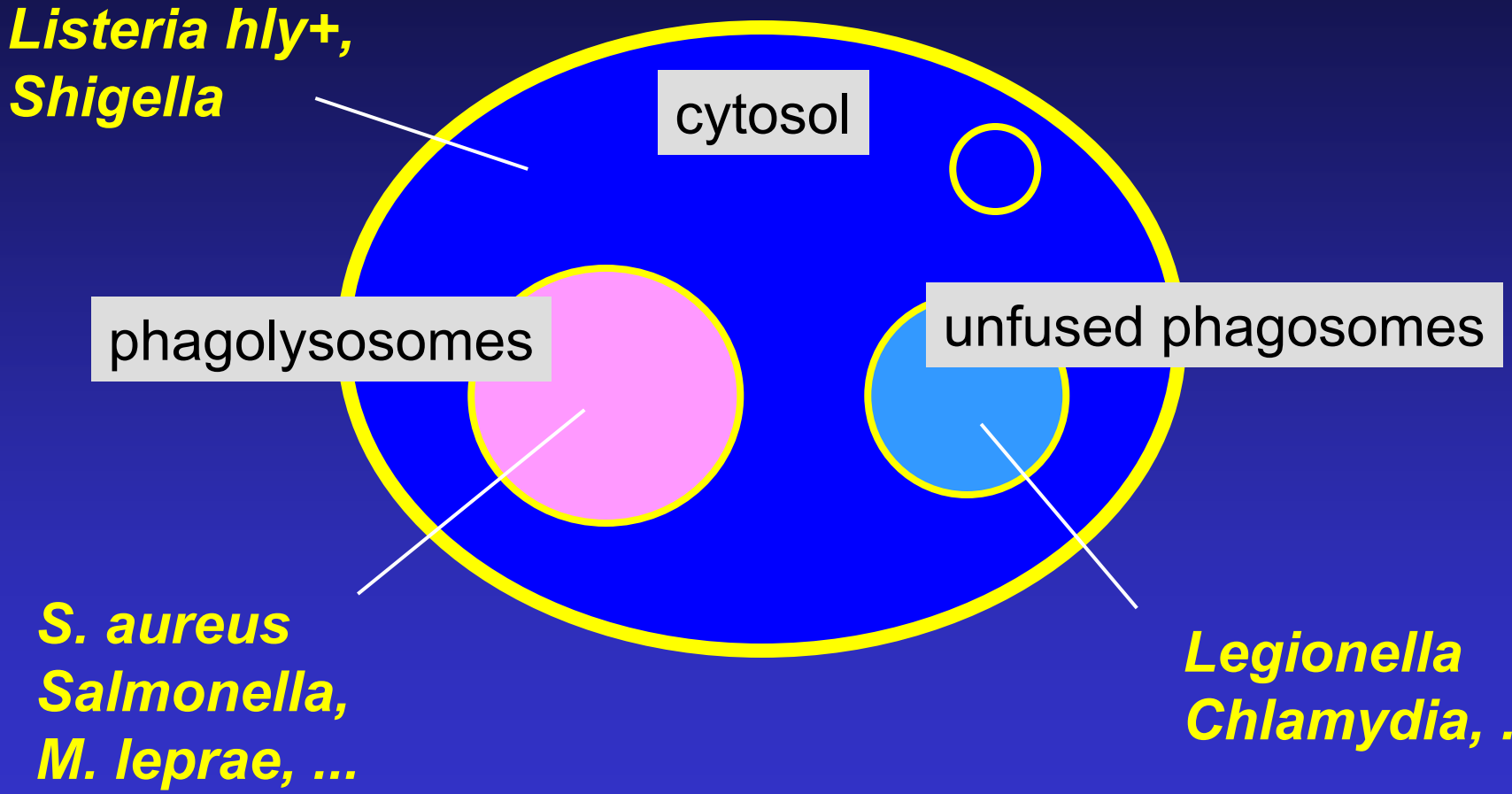
Gyssens et al. ICAAC 1998
and 9th ISAP symposium (pre-ICAAC), 2001, Chicago, Ill.

Intracellular infection : the questions ...



- Where are the bacteria
- Which antibiotic accumulate in cells and where are they localized ?
- Are intracellular antibiotics active ?

Where are the bacteria ?



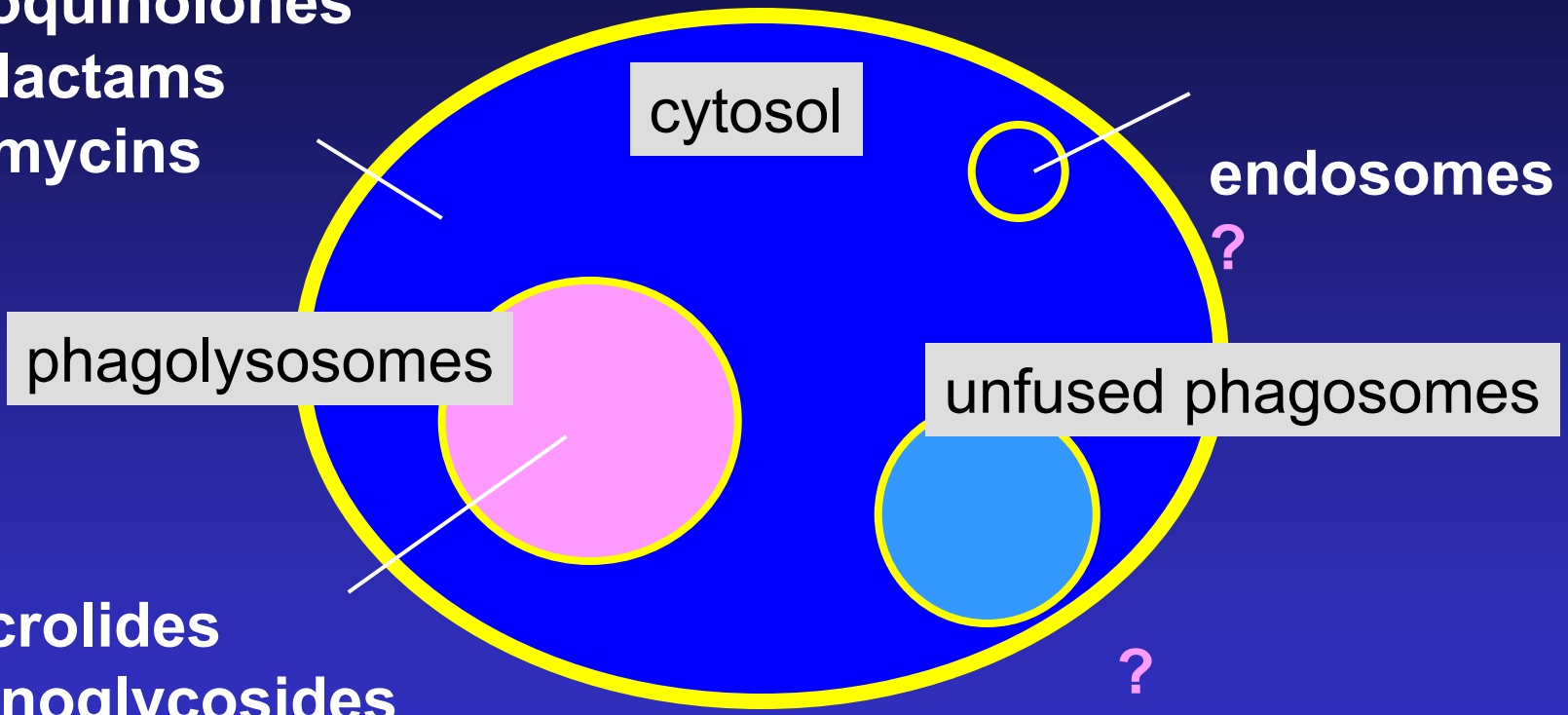
Which antibiotics accumulate in cells ?

- **beta-lactams: ≤ 1x**
- **aminoglycosides: <1 to 2 x**
- **ansamycins: 2-3 x**
- **tetracyclines: 2-4 x**
- **fluoroquinolones: 5 - 20 x**
- **macrolides: 4 to > 100 x ***
- **glycopeptides: 1 to 400 x !! ****

*** azithromycin, ketolides**
**** LY 333328 (oritavancin)**

Where are antibiotics located in cells ?

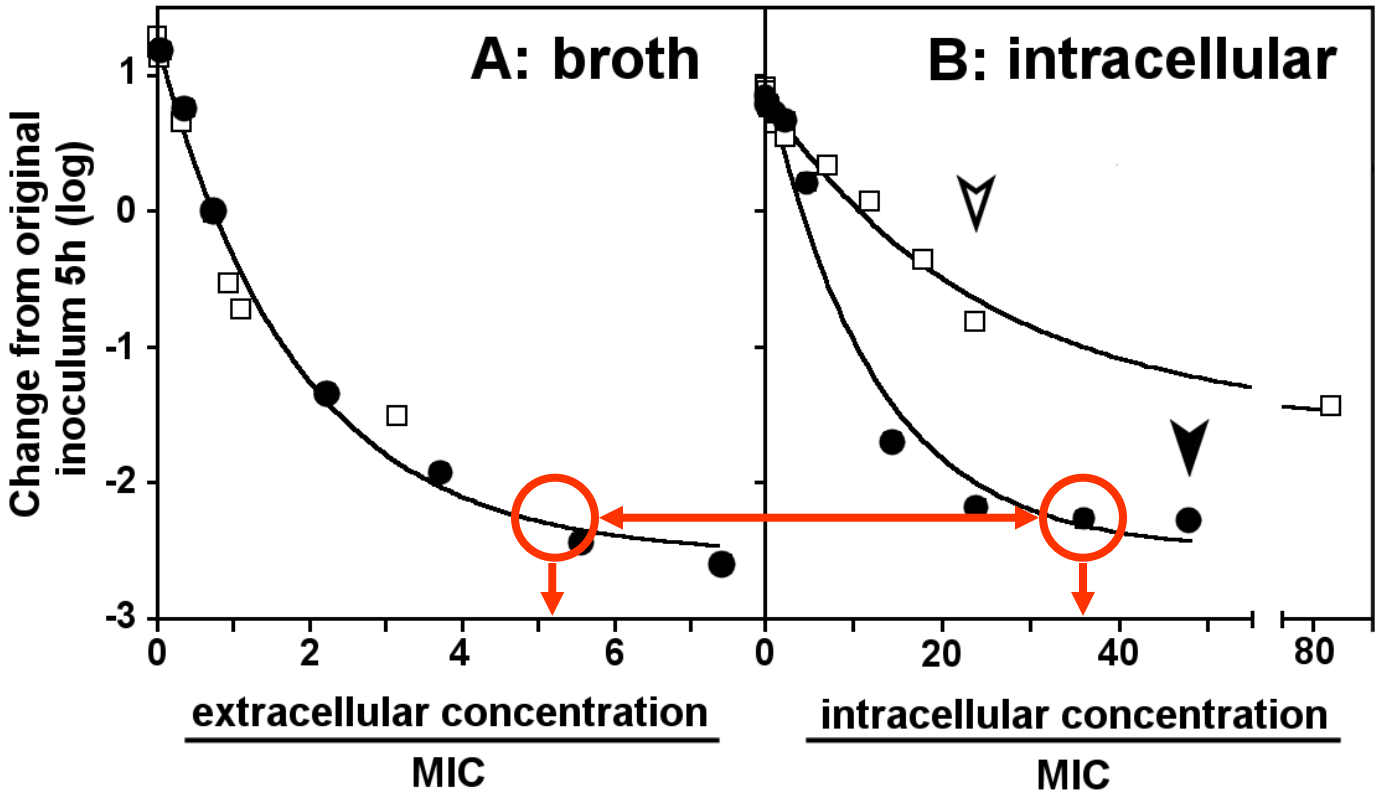
- **fluoroquinolones**
- **beta-lactams**
- **ansamycins**



- **macrolides**
- **aminoglycosides**

How and to what extent are intracellular antibiotics active ? An example with Listeria ...

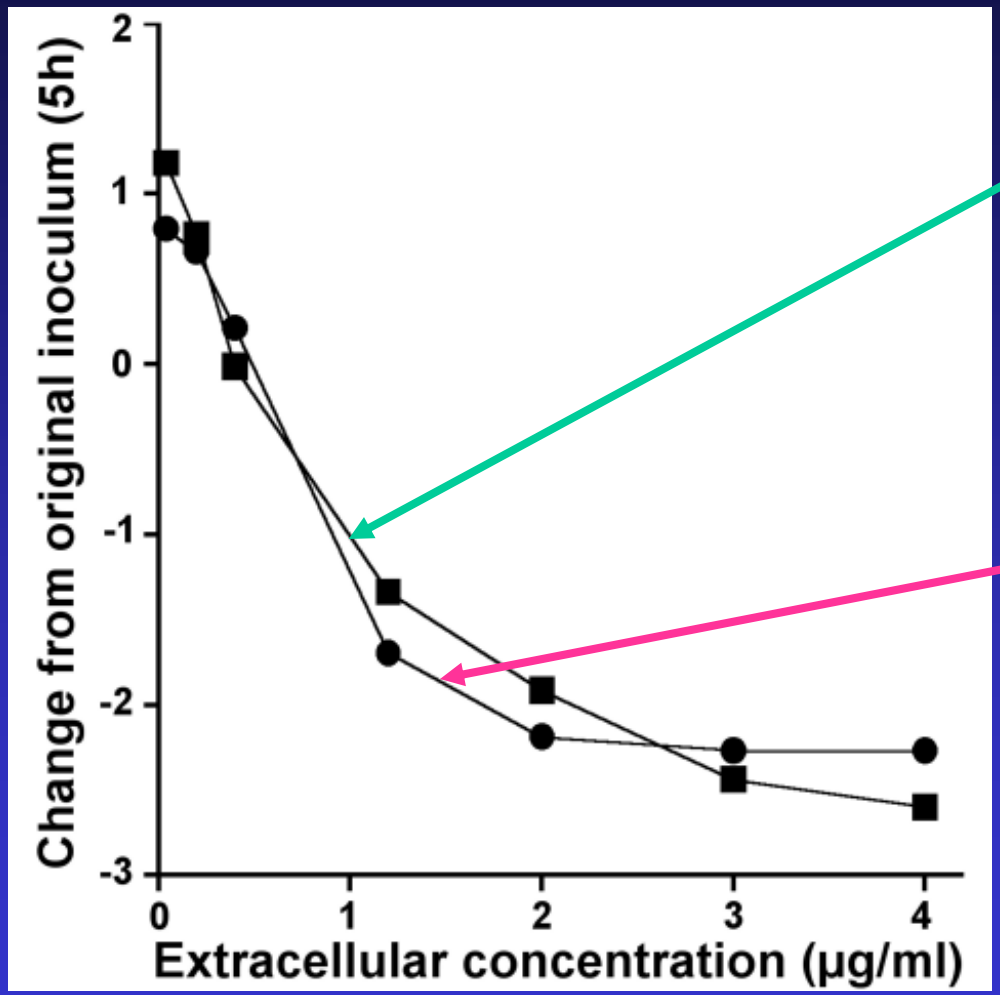
□ ciprofloxacin ● moxifloxacin



Carryn et al., AAC 46:2095-103, 2001

You need a C/MIC ratio 7x fold larger intracellularly

Moxifloxacin accumulates about 7 fold in cells, but is also about 7-fold less active intracellularly ...



extracellular activity

intracellular activity even though the drug shows a 7-fold intracellular accumulation and is localized in tne cytosol where Listeria is actively multiplying ...

Intracellular antibiotics (in a nutshell)

- β-lactams and aminoglycosides accumulate poorly (and slowly for AG)
  - ➔ β-lactams will only be active at very large extracelllar concentrations and will require prolonged exposures (t>MIC)
  - ➔ aminoglycosides will require long exposure times to show activity
- the activity of macrolides (and of aminoglycosides) is severely defeated by the intracellular milieu
  - ➔ the high tissular accumulation of macrolides is not conducting to large activities (mostly a bacteriostatic effect)
  - ➔ aminoglycosides will fail with rapidly developping bacteria
- fluoroquinolones are active intracellularly and remain concentration-dependent, but not to the extent anticipated by their accumulation level

This is where we are now ...

These relations can be modulated

Dosage

Serum concen-tration varying over time

Concentration at the site of infection

Concentration in non-target tissues

Therapeutic effects

But there is still much to do ...