

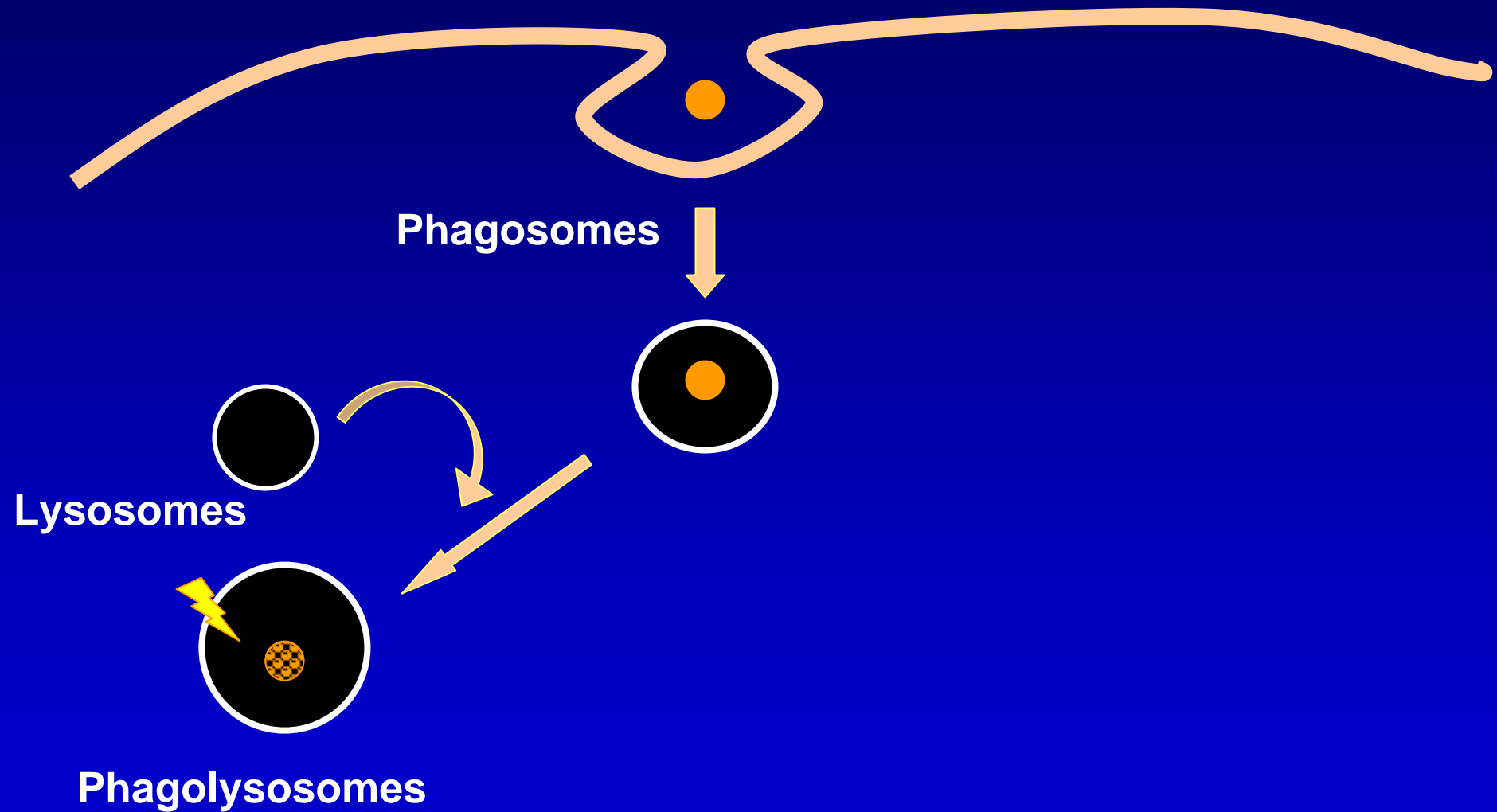


**intracellular *S. aureus* :
what does it mean ?**

F. Van Bambeke

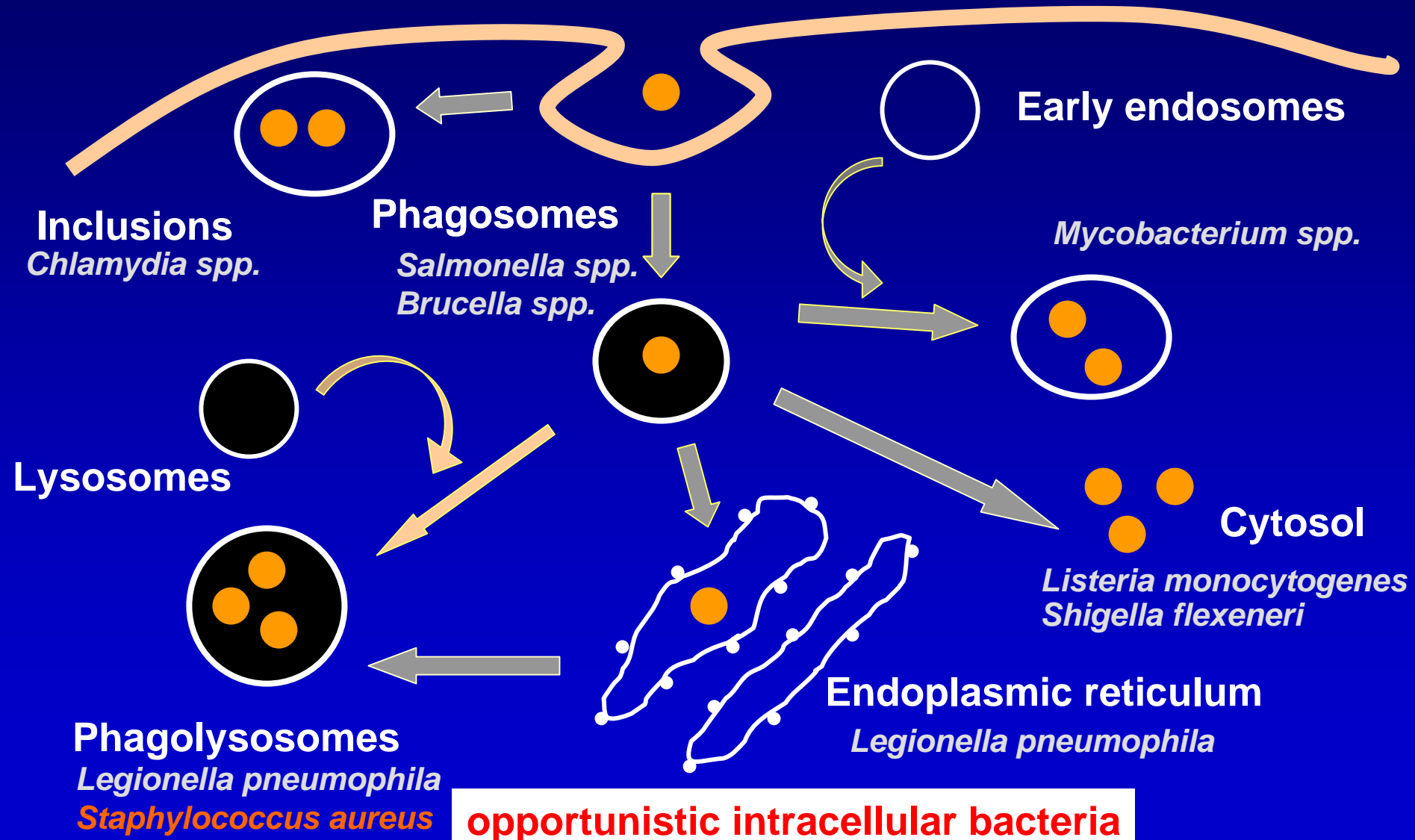
Unité de Pharmacologie cellulaire et moléculaire
Université catholique de Louvain
Brussels, Belgium

Intracellular killing of bacteria by host cell defense mechanisms



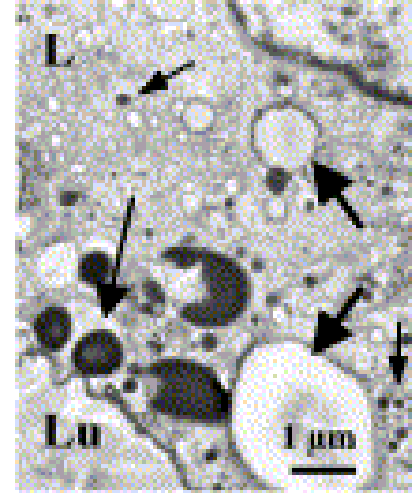
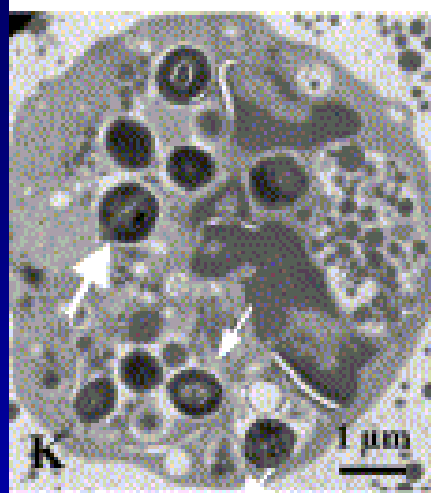
Some bacteria can escape host cell defense mechanisms

Carryn *et al*, Infect Dis Clin North Am. (2003) 17:615-34

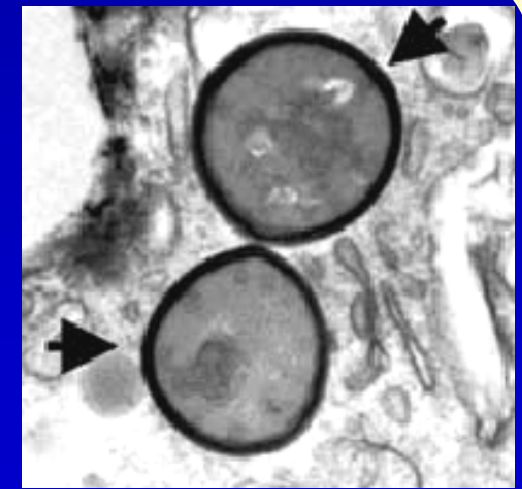
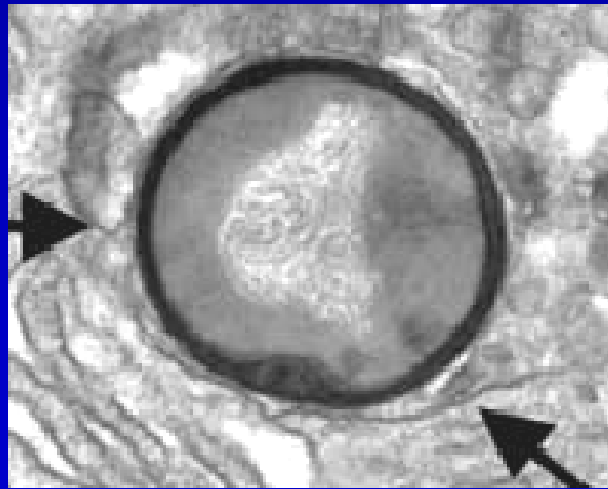
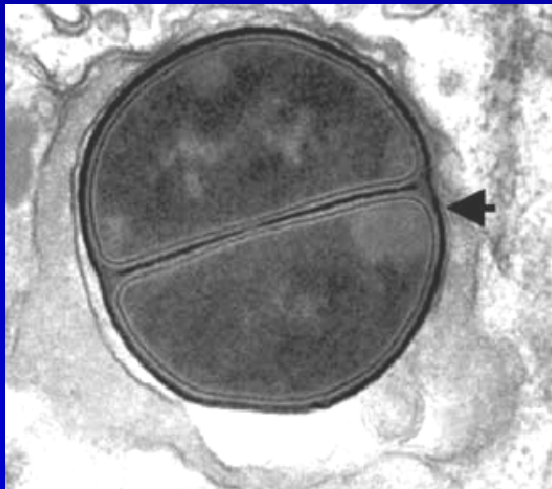


Evidence for intracellular *S. aureus* in vitro and in vivo – model of mastitis

phagocytic cells
PMN, →
macrophages



non phagocytic
cells

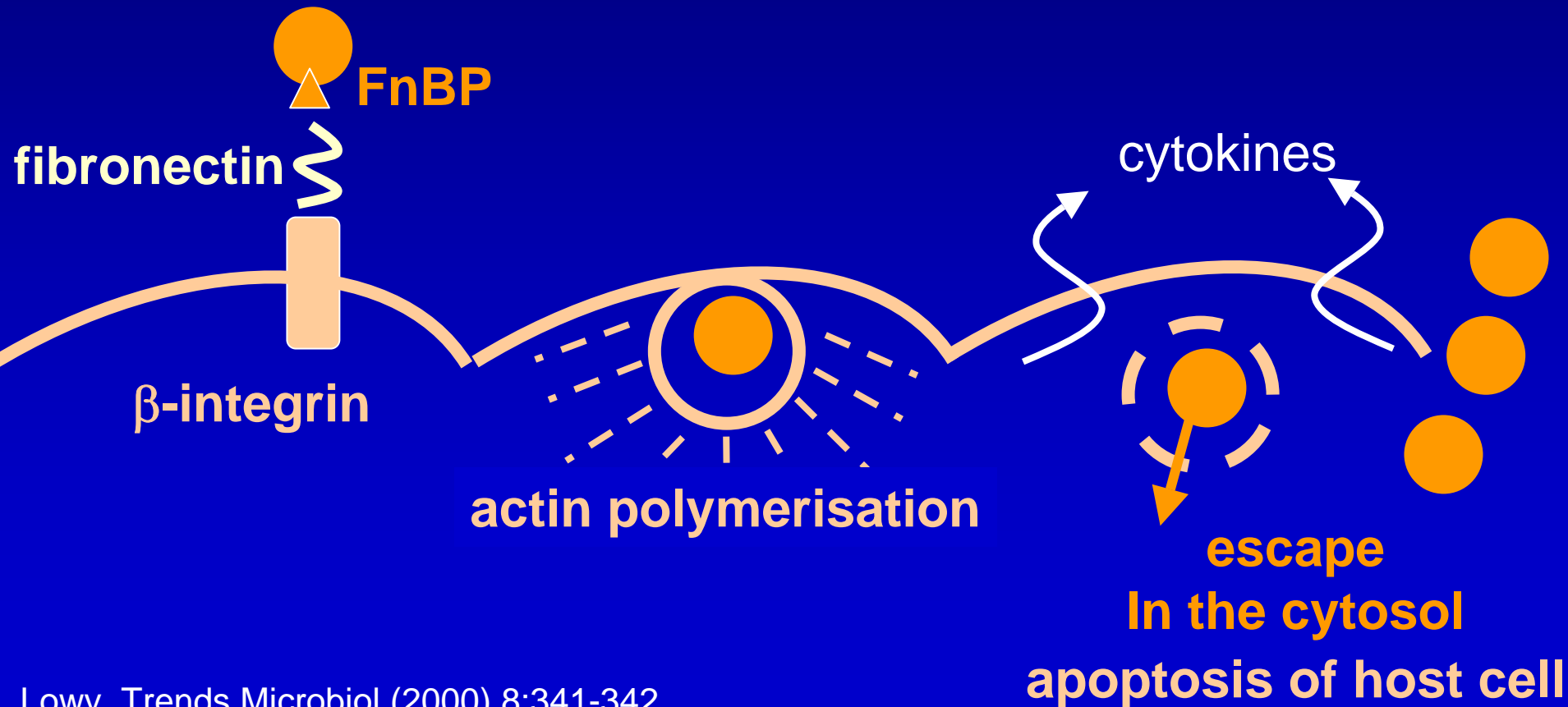


Intracellular cycle of *S. aureus*

adhesion

internalisation

cytotoxicity
and
cellular response

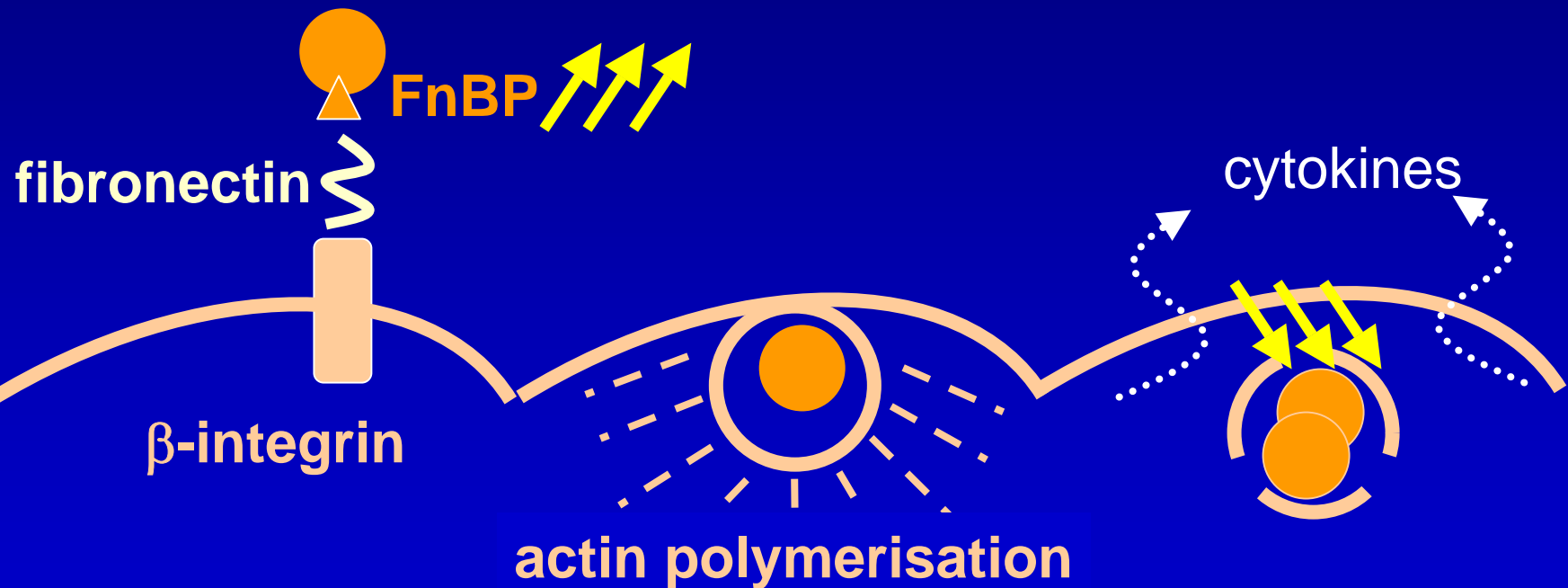


Intracellular survival of *S. aureus*

adhesion

internalisation

cytotoxicity
and
cellular response



**In vivo implication remains however
a matter of debate**

Is *Staphylococcus aureus* an
intracellular pathogen?

Franklin D. Lowy

TRENDS IN MICROBIOLOGY

AUGUST 2000

Clearly established in *in vitro* models

Few reports *in vivo*

- low density of intracellular foci
- destructive nature of *S. aureus* infection
- multiple mechanisms of adherence and invasion

***S. aureus* can survive and multiply in phagocytes and several other cell types**

Infect Immun. 2001 May;69(5):2872-7.

Staphylococcus aureus fibronectin binding proteins are essential for internalization by osteoblasts but do not account for differences in intracellular levels of bacteria.

Ahmed S, Meghji S, Williams RJ, Henderson B, Brock JH, Nair SP.

Cellular Microbiology Research Group, Eastman Dental Institute, University College London, United Kingdom.

Br J Dermatol. 2002 Jun;146(6):943-51.

Invasion of human keratinocytes by Staphylococcus aureus and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death.

Mempel M, Schnopp C, Hojka M, Fesq H, Weidinger S, Schaller M, Korting HC, Ring J, Abeck D.

Department of Dermatology and Allergy Biederstein, Technical University Munich, Biedersteiner Str. 29, D-80802 Munich, Germany.
m.mempel@lrz.tum.de

Infect Immun. 1987 Sep;55(9):2155-63.

Ingestion of Staphylococcus aureus by bovine endothelial cells results in time- and inoculum-dependent damage to endothelial cell monolayers.

Vann JM, Proctor RA.

J Dairy Sci. 1996 Jun;79(6):1021-6.

Staphylococcus aureus invasion of bovine mammary epithelial cells.

Almeida RA, Matthews KR, Cifrian E, Guidry AJ, Oliver SP.

Department of Animal Science, University of Tennessee, Knoxville 37901-1071, USA.

But is this associated with pathology ?

J Bone Joint Surg Br. 2003 Aug;85(6):918-21.

Intracellular Staphylococcus aureus. A mechanism for the indolence of osteomyelitis.

Ellington JK, Harris M, Webb L, Smith B, Smith T, Tan K, Hudson M.



Dep Clin Infect Dis. 2001 Jun 1;32(11):1643-7. Epub 2001 Apr 30.

Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.

von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G.



Institu Infect Immun. 1986 Dec;54(3):833-6.

Phagocytosis of Staphylococcus aureus by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.

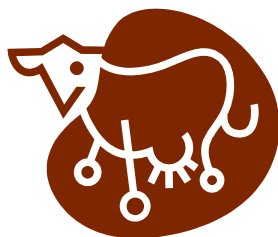


FEMS Microbiol Lett. 2000 Dec 1;193(1):57-62.

Demonstration of intracellular Staphylococcus aureus in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk.

Hebert A, Sayasith K, Senechal S, Dubreuil P, Lagace J.

Department of Microbiology and Immunology, Faculty of Medicine, University of Montreal, Centre-Ville, Montreal, Que., Canada.



**In vivo implication remains however
a matter of debate**

*Is Staphylococcus aureus an
intracellular pathogen?*

Franklin D. Lowy

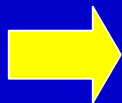
TRENDS IN MICROBIOLOGY

AUGUST 2000

Clearly established in *in vitro* models

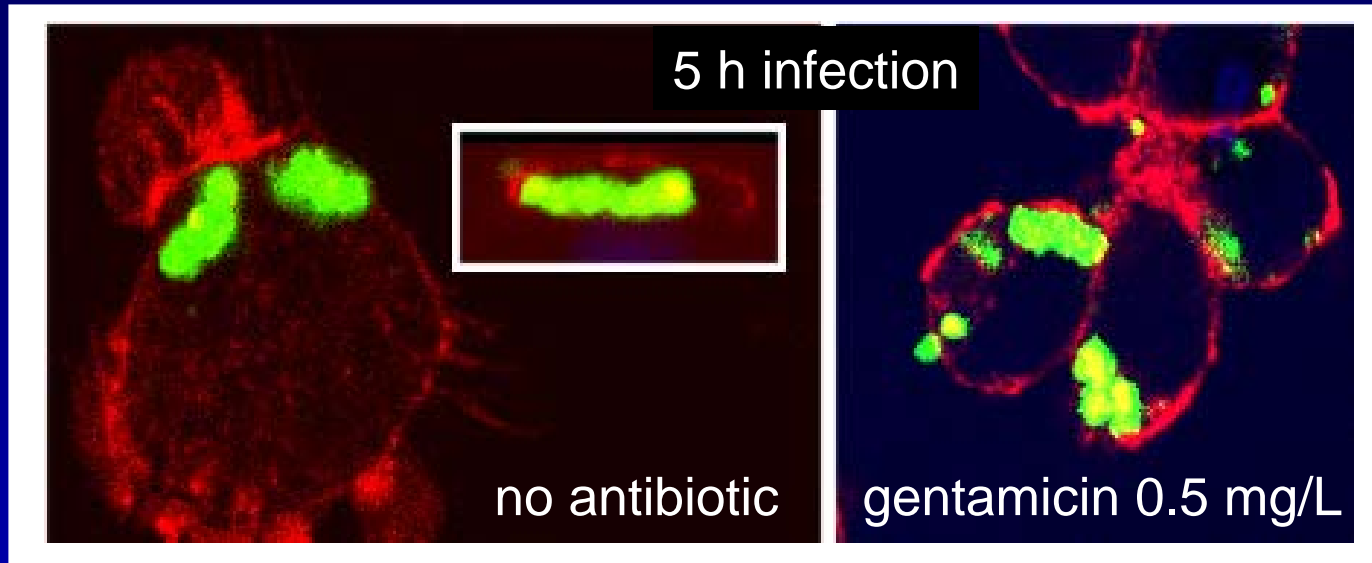
Few reports *in vivo*

- low density of intracellular foci
- destructive nature of *S. aureus* infection
- multiple mechanisms of adherence and invasion



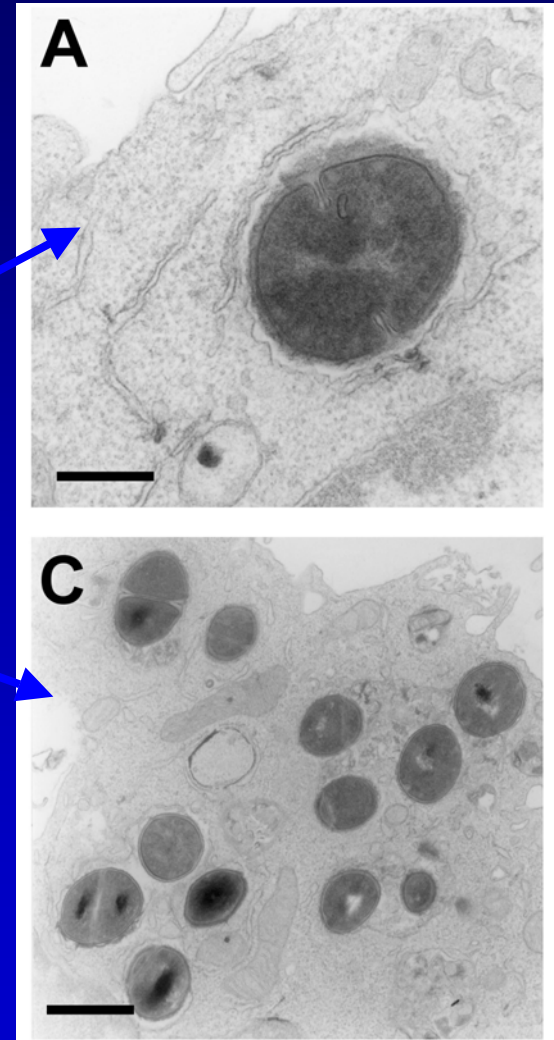
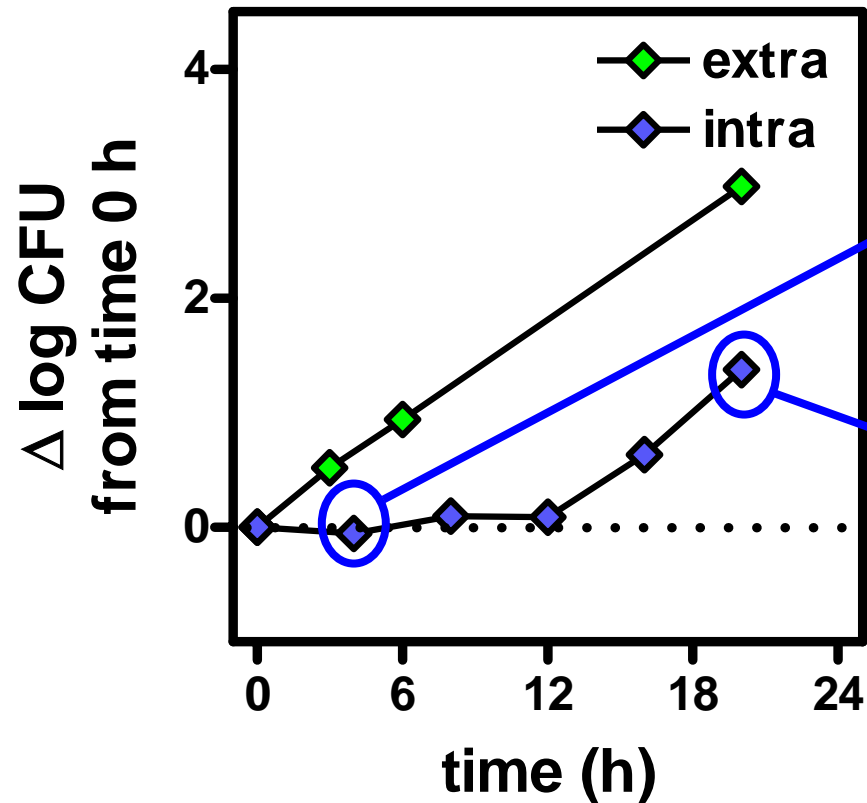
**Useful to study to understand pathogenesis
and explore therapeutic options**

Setting up a model of intracellular infection over a 24 h period of time



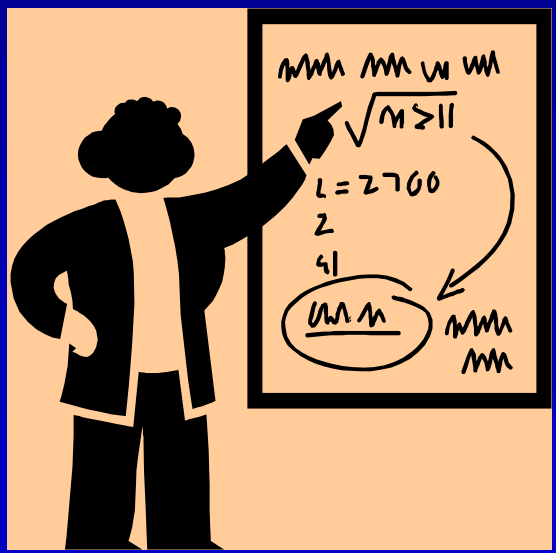
- **infection of macrophages** (with opsonized bacteria)
 - Mouse (J774; 5 bact/cell)
 - Human (THP-1; 4 bact/cell)
- **washing with GEN 50 µg/ml** to eliminate extracellular bacteria
- **incubation** for up to 24 h with
 - GEN 0.5 µg/ml (MIC)
 - antibiotic under study

Description of the model : how does *S. aureus* grow intracellularly ?



intracellular vs extracellular activity of antibiotics : is it easily predictable ?

something very simple ?

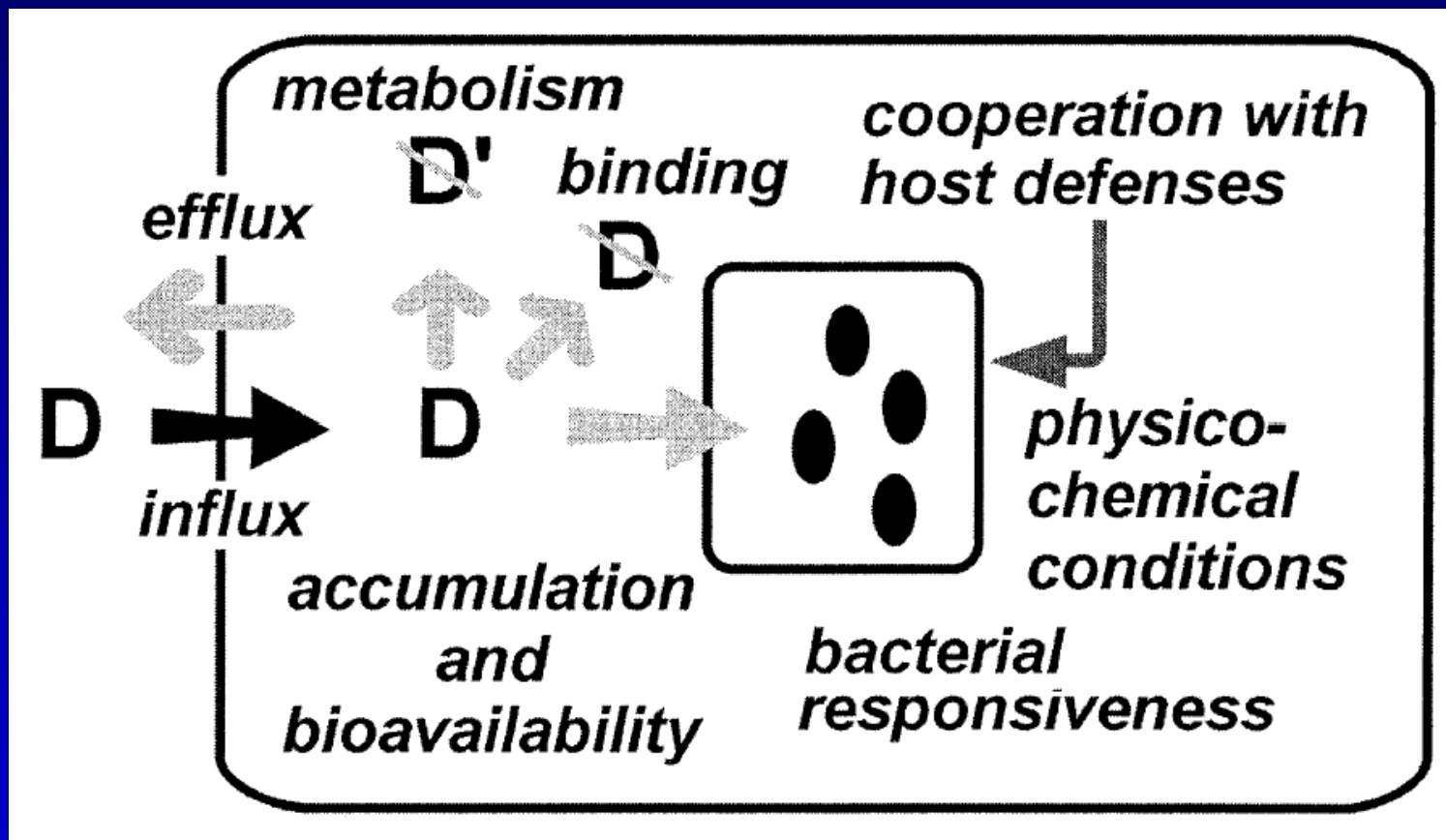


Intracellular activity
=
MIC x accumulation

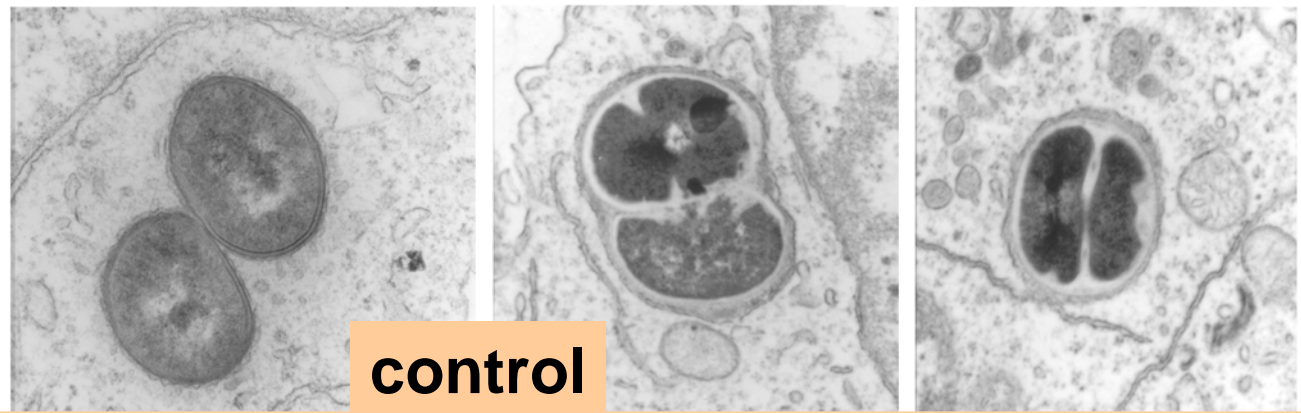


or very complicated ?

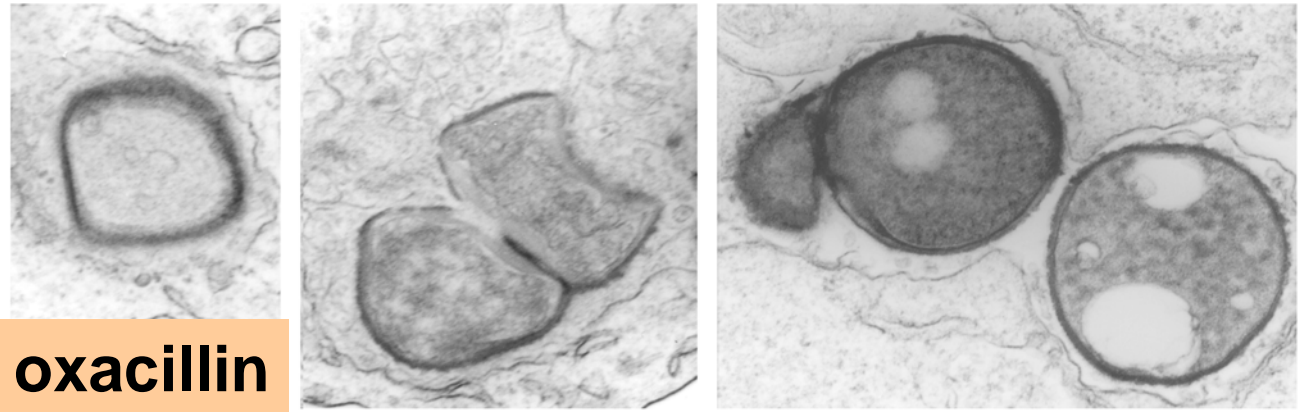
intracellular vs extracellular activity of antibiotics : PK – PD in action



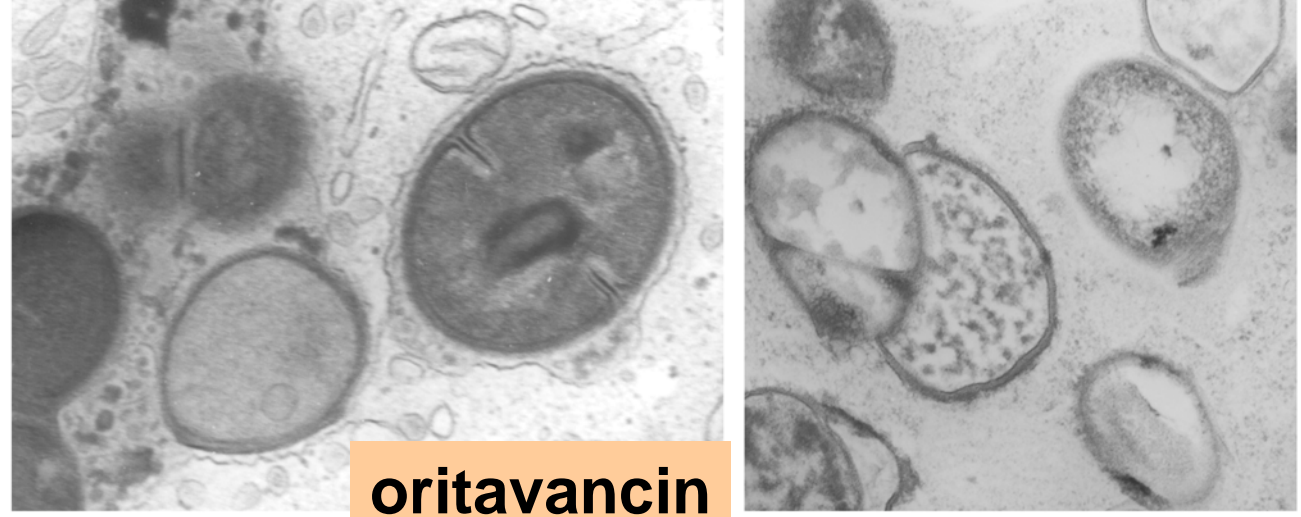
Intracellular killing is visible for antibiotics working on cell wall



control

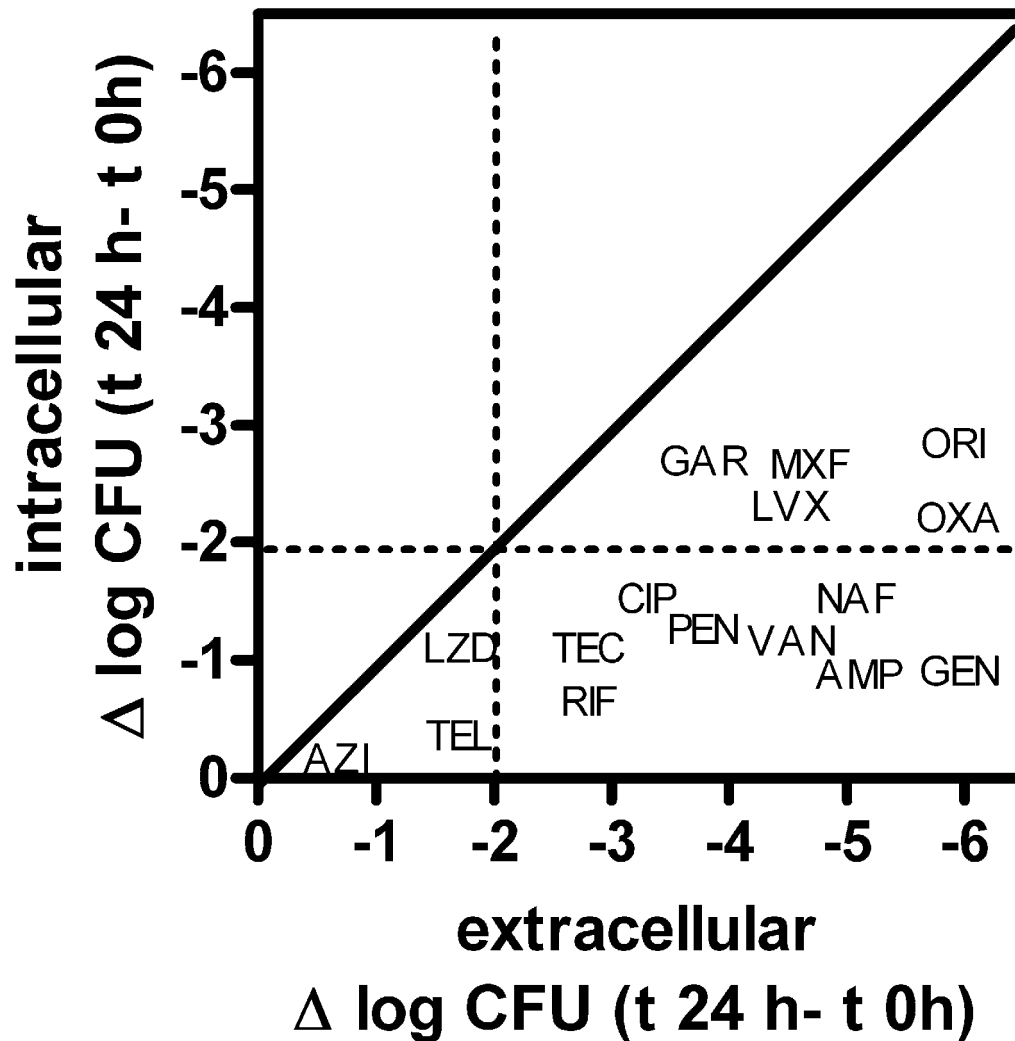


oxacillin



oritavancin

Smart choice of antibiotics based on balanced extra- / intra- activity



Intracellular activity : possible reasons for loss of activity



**Drug (lysosomal)
accumulation**

AB	Cc/Ce	Max. activity
OXA	< 4	-1.6
GEN	4	-2.5
MXF	8	-2.8
AZM	38	-0.2
ORI	148	-3.2

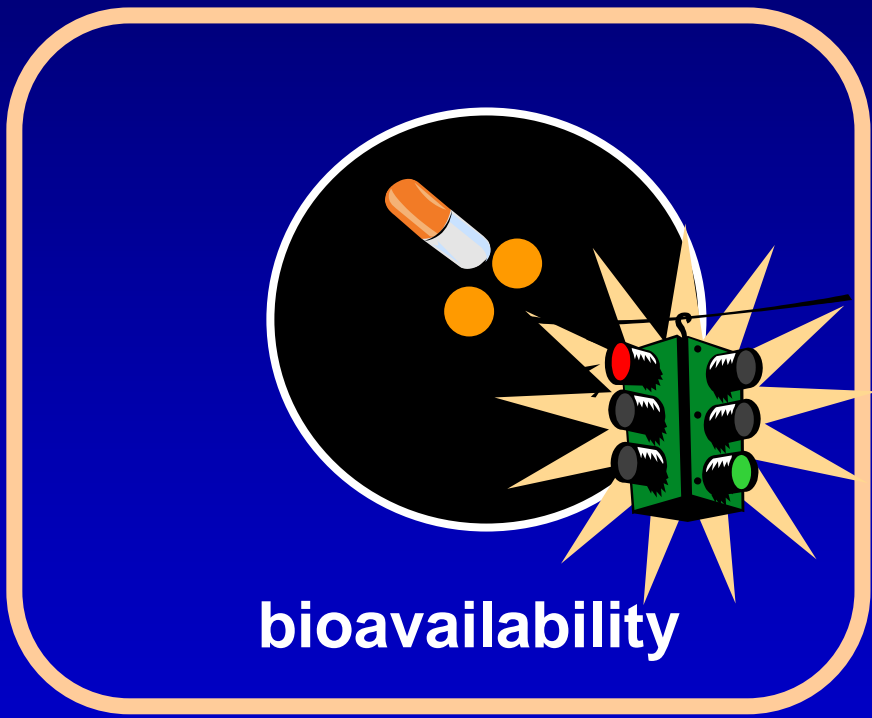
Intracellular activity : possible reasons for loss of activity



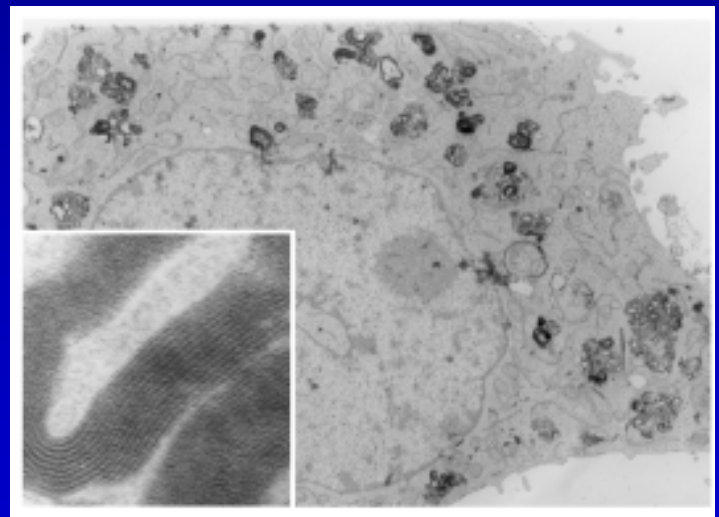
Lysosomal
acidic pH

AB	MIC	
	pH 7.4	pH 5.4
AZM	0.5	512
GEN	0.5	16
MXF	0.06	0.25
ORI	0.25	0.25
OXA	0.125	0.06

Intracellular activity : possible reasons for loss of activity



macrophages
exposed
to oritavancin



Intracellular activity : possible reasons for loss of activity



**Rapid equilibration
by diffusion**
quinolones
beta-lactams

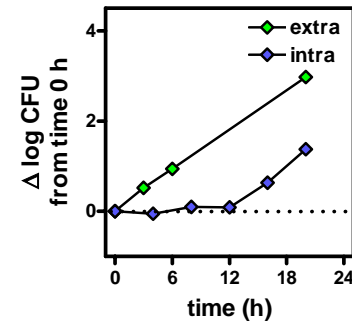
**slow accumulation
by endocytosis**
aminoglycosides
glycopeptides

Intracellular activity : possible reasons for loss of activity



Bacterial responsiveness

slow growth



metabolic changes
involved in
invasion and toxicity

Mol Microbiol. 2003 Aug;49(4):919-27.

Biphasic intracellular expression of *Staphylococcus aureus* virulence factors and evidence for Agr-mediated diffusion sensing.

Shompole S, Henon KT, Liou LE, Dziewanowska K, Bohach GA, Bayles KW.

Department of Microbiology, Molecular Biology and Biochemistry, Ag-Biotech Bldg. Laboratory 222, University of Idaho, Moscow, Idaho 83844 USA.

Take home message

- 1. identify your target**
- 2. choose the right weapon**



you may avoid serious problems ...

Thanks to ...



**Cristina
Seral**



**Maritza
Barcia-Macay**



**Sandrine
Lemaire**



**Marie-Paule
Mingeot-Leclercq**



**Paul M.
Tulkens**