

# Intracellular Antibiotics : what does it (really) mean ?

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[www.md.ucl.ac.be/facm](http://www.md.ucl.ac.be/facm)

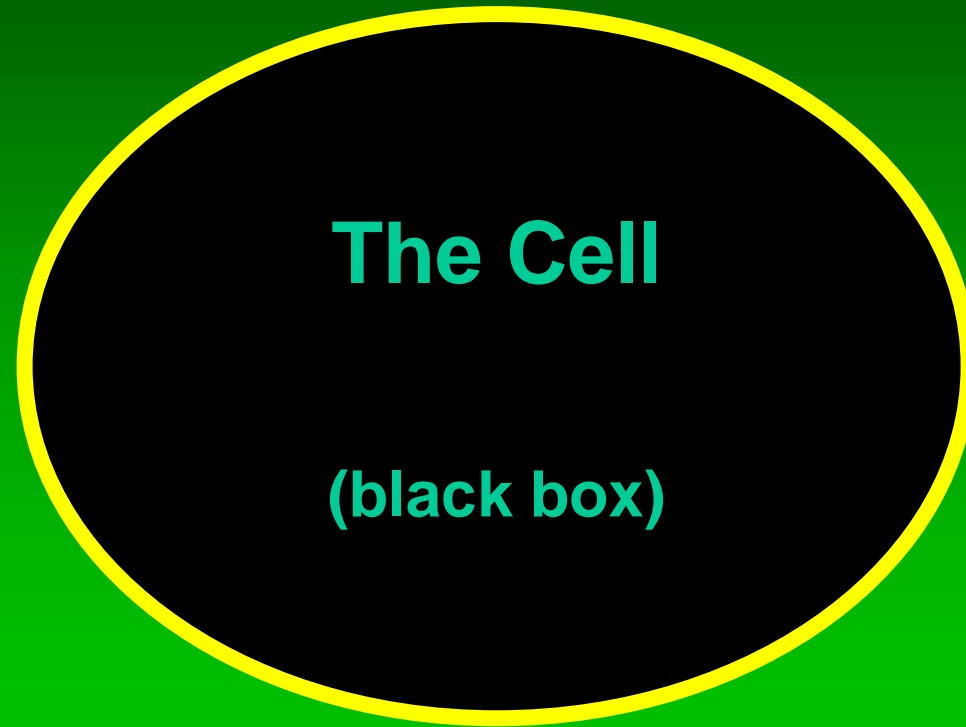
[www.isap.org](http://www.isap.org)

Melbourne, Victoria  
April 6th 2001

# What is Intracellular Infection ?

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**antibiotic**



**bacteria**

# Intracellular antibiotics: the issues

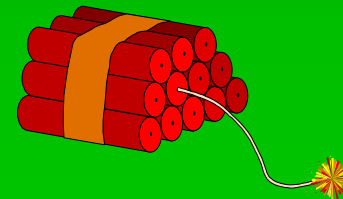
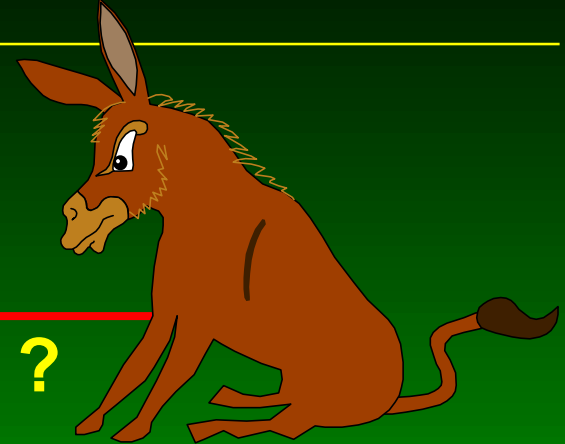
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**the bug**

1. which bacteria and where ?
  2. which antibiotics accumulate ?
  3. influx vs efflux ?
  3. where are antibiotics in cells ?
  4. intracellular expression of activity ?
  5. bacterial responsiveness ?
  6. cooperation with host defenses ?
  7. any toxicity ?
- 

**the pharmacologist**

**the toxicologist**



# Intracellular antibiotics: the issues

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**1. which bacteria and where ?**

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2. which antibiotics accumulate ?

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3. where are antibiotics in cells ?

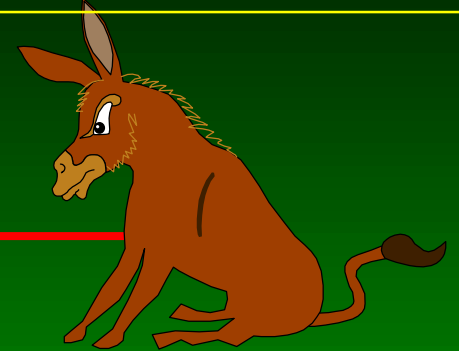
4. intracellular expression of activity ?

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7. any toxicity ?



# Which bacteria ... and which diseases ...

## ➔ Obligatory or mainly intracellular:

### ▶ respiratory infections (pneumopathies):

*Chlamydia pneumoniae*: 10% in children

*Legionella pneumophila*: frequent if immunosuppression

*Mycobacterium spp.*: frequent if immunosuppression

### ▶ sexually transmitted diseases

*Chlamydia trachomatis*: most common pathogen

### ▶ CNS infections + other sites:

*Listeria monocytogenes*: pregnant women; immunosuppression

## ➔ Facultative or mainly extracellular:

### ▶ digestive tract infections

*Salmonella spp.*, *Shigella spp.*

### ▶ respiratory, cutaneous, etc...tract infections

*Streptococcus spp.*, *Staphylococcus spp.*

etc...

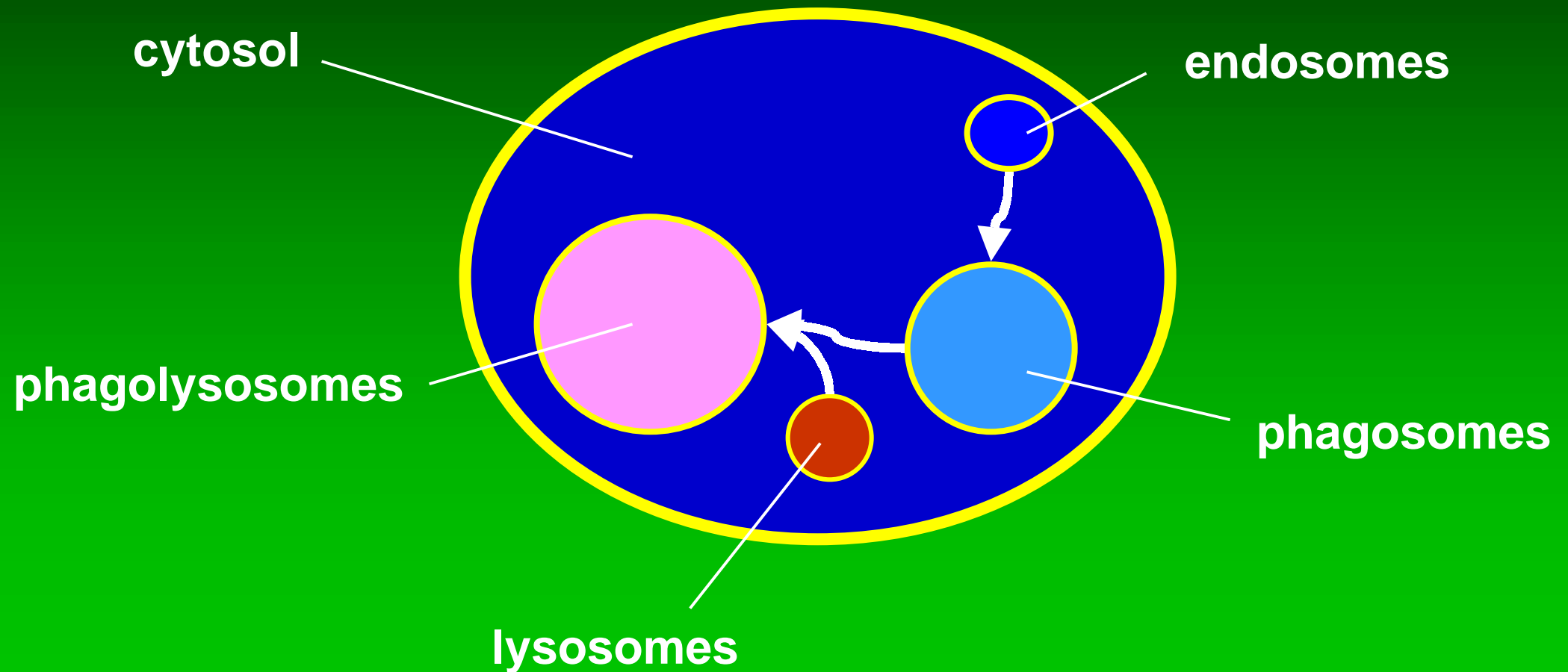
# **Question :** **where do intracellular bacteria sojourn and thrive ?**

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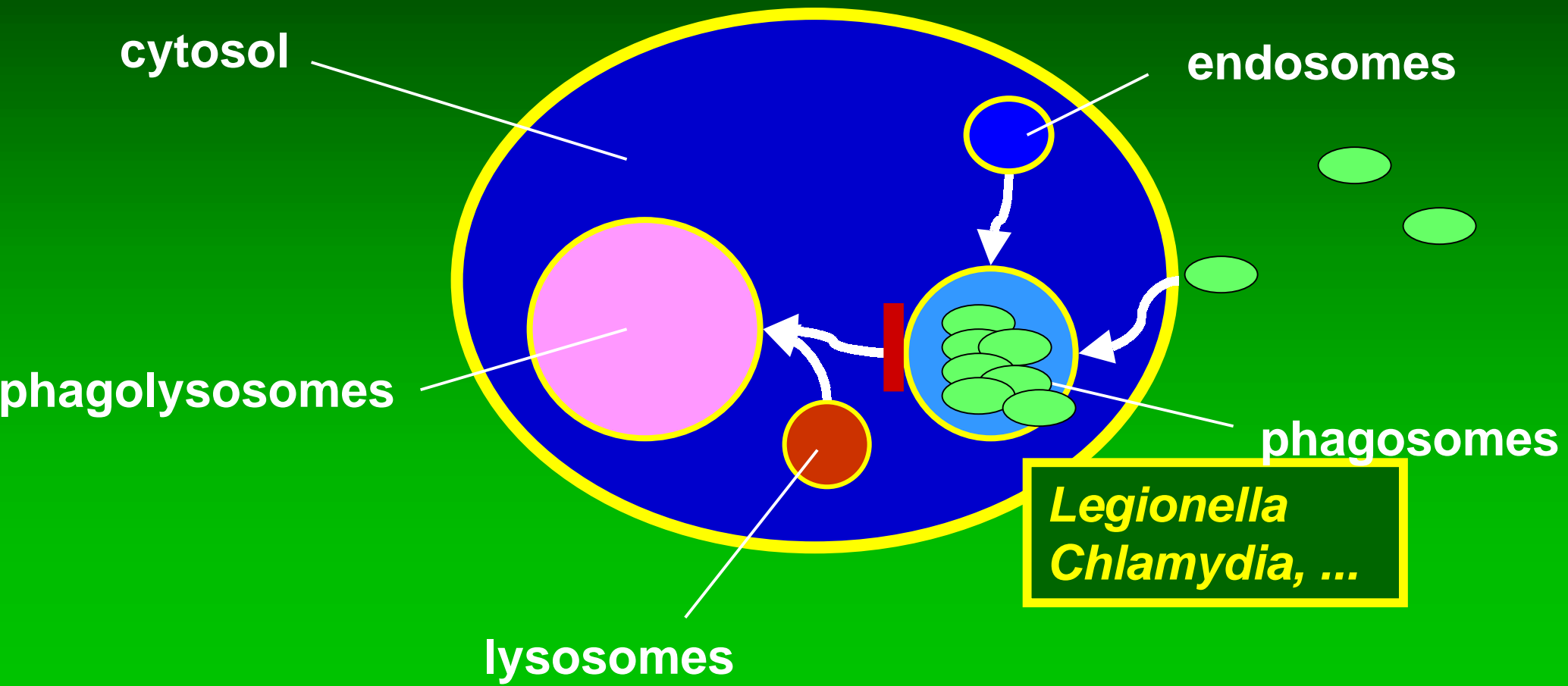
**somewhere in the vacuolar  
apparatus, but also outside ...**

# where do intracellular bacteria sojourn and thrive ?

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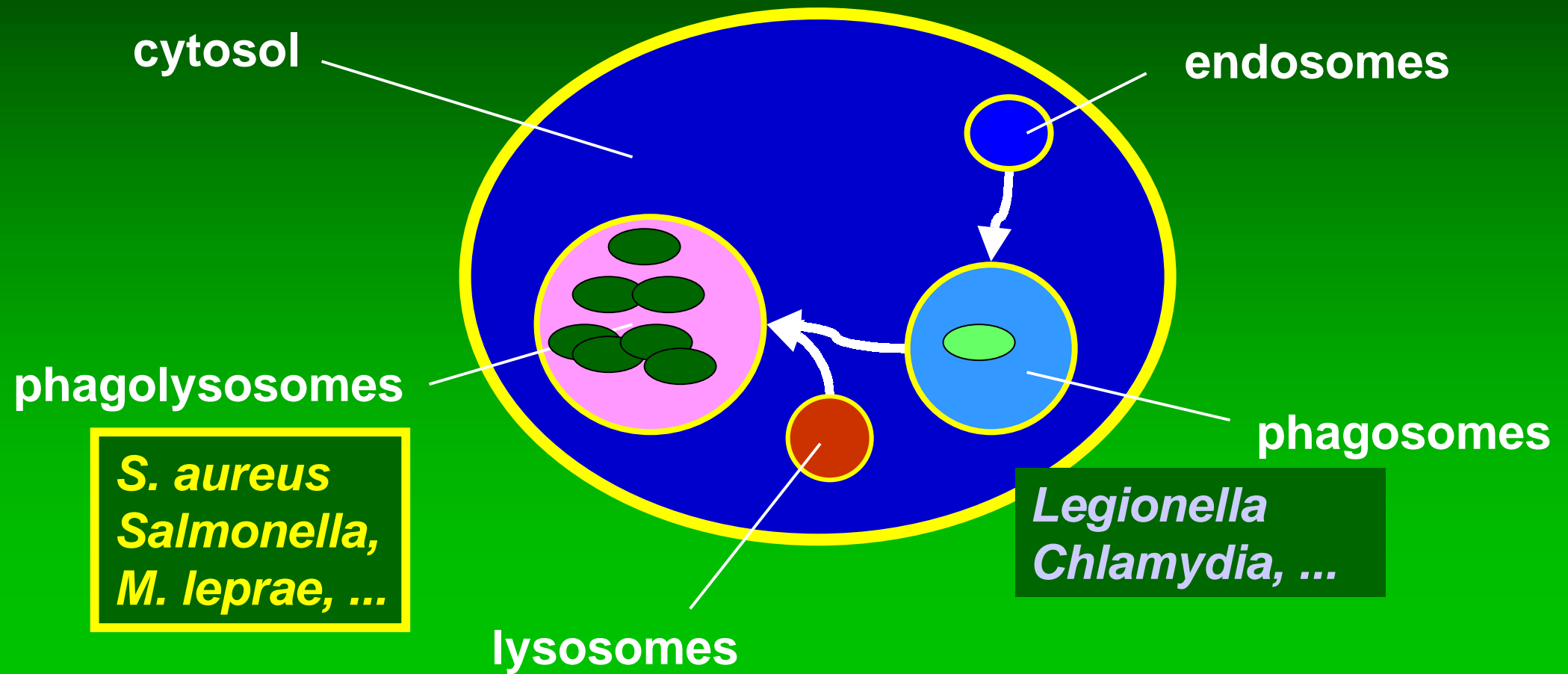


# where do intracellular bacteria sojourn and thrive ?

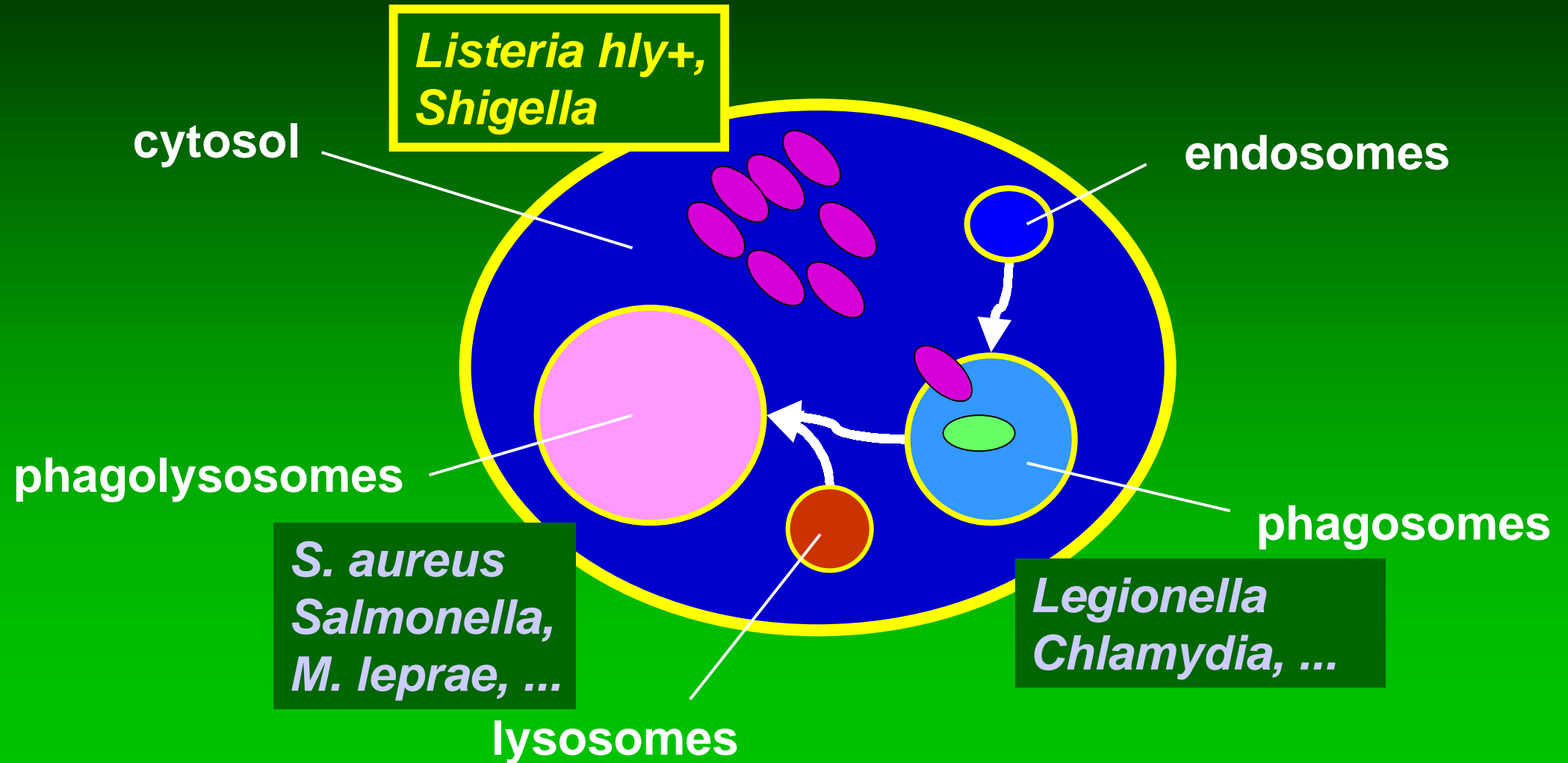




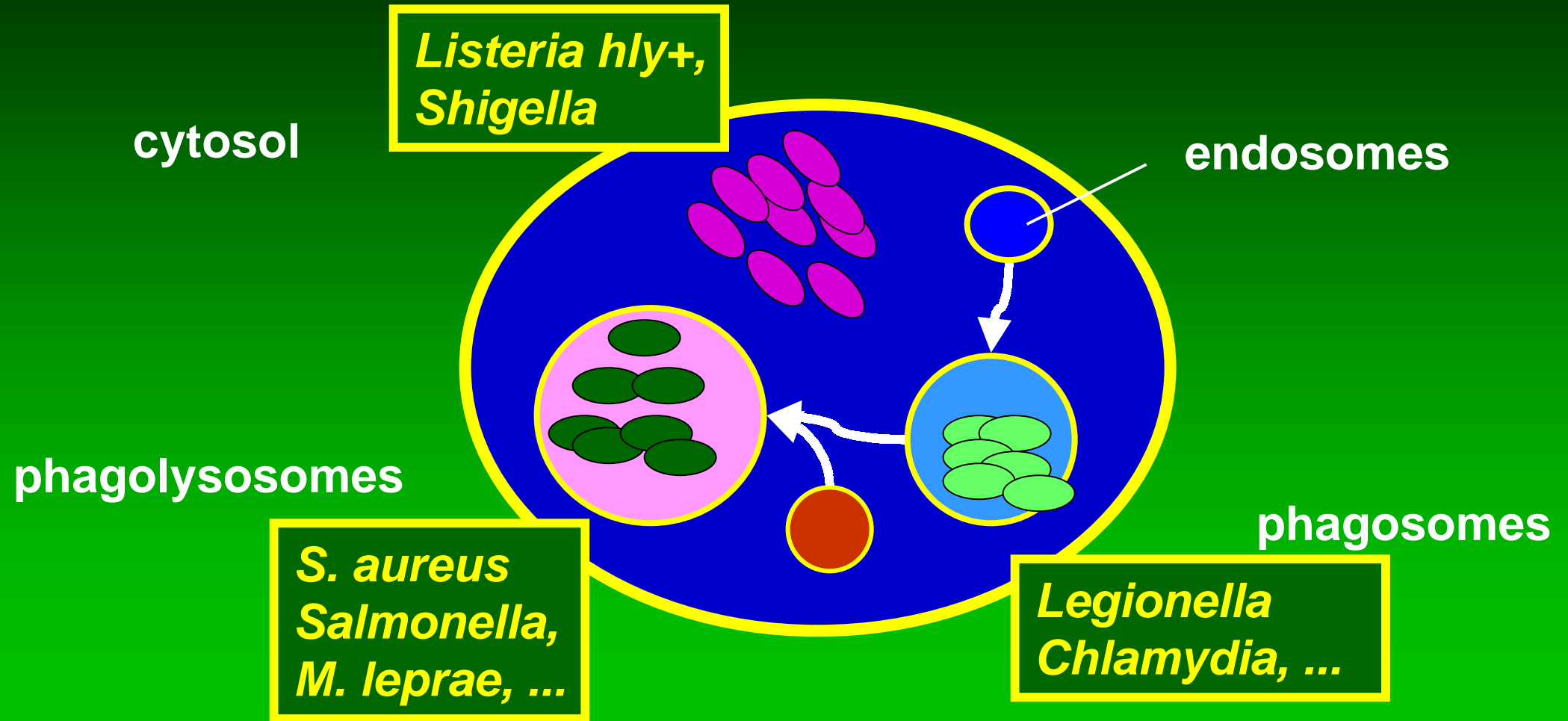
# where do intracellular bacteria sojourn and thrive ?



# where do intracellular bacteria sojourn and thrive ?



# where do intracellular bacteria sojourn and thrive ?



# Finally the answer to the question... (where do intracellular bacteria sojourn and thrive ?)

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The subcellular localization of bacteria may be highly variable according to

- their type

- phagosomal
- phagolysosomal
- cytosolic

Very different environments...

pH  $\approx$  6

pH  $\approx$  5

pH  $\approx$  7

- the state of their journey in cell

Dynamics is essential ...

## 2d question (and answer): which antibiotics do or do not accumulate in cells ?

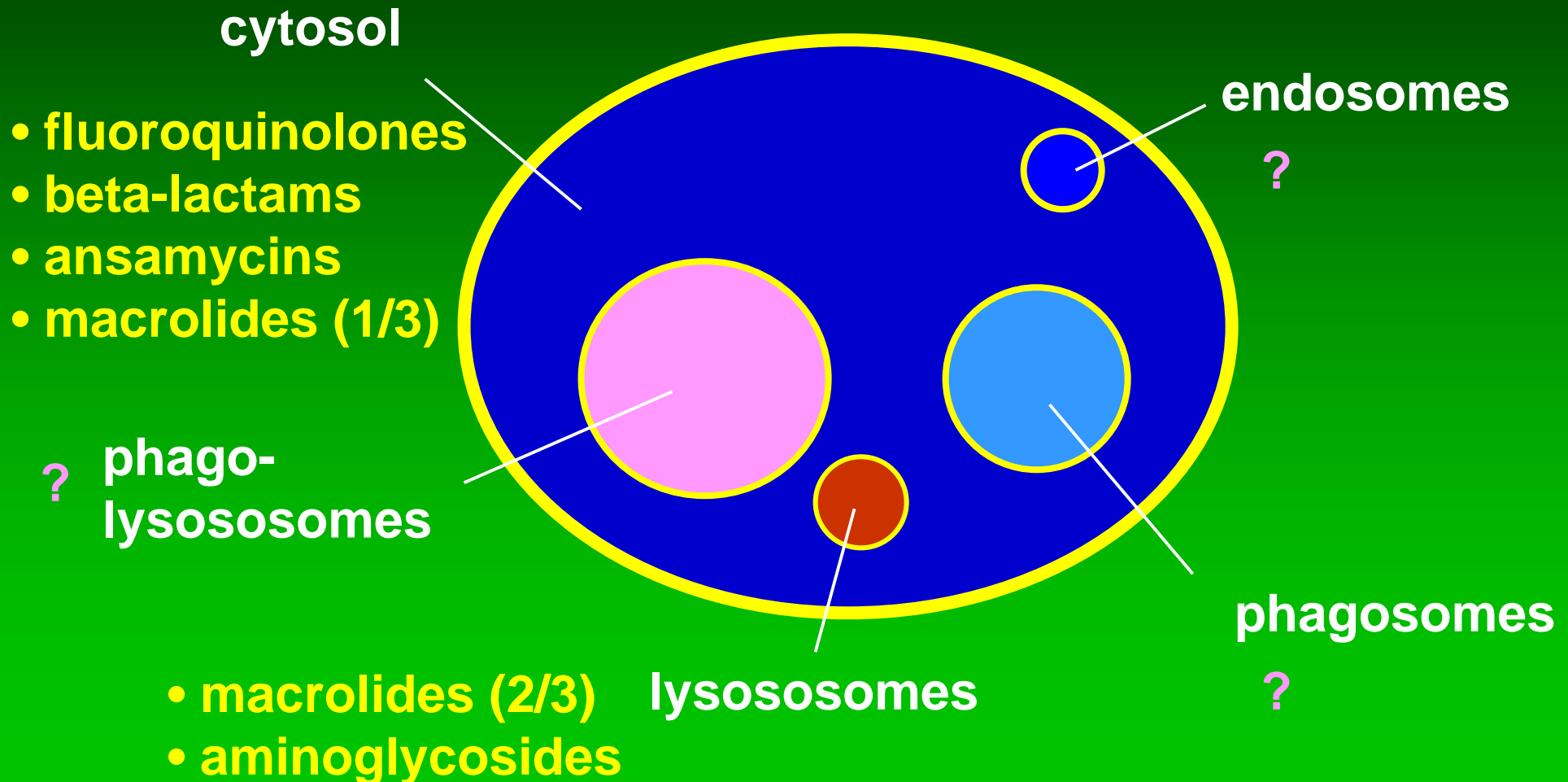
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- **beta-lactams:  $\leq 1x$**
- **aminoglycosides:  $<1$  to  $2x$**
- **ansamycins:  $2-3x$**
- **tetracyclines:  $2-4x$**
- **fluoroquinolones:  $5 - 20x$**
- **macrolides:  $4$  to  $> 100x$  \***
- **glycopeptides:  $1$  to  $400x$  !! \*\***

\* azithromycin, ketolides

\*\* LY 333328

# 3d question (and answer) : where are intracellular antibiotics located ?

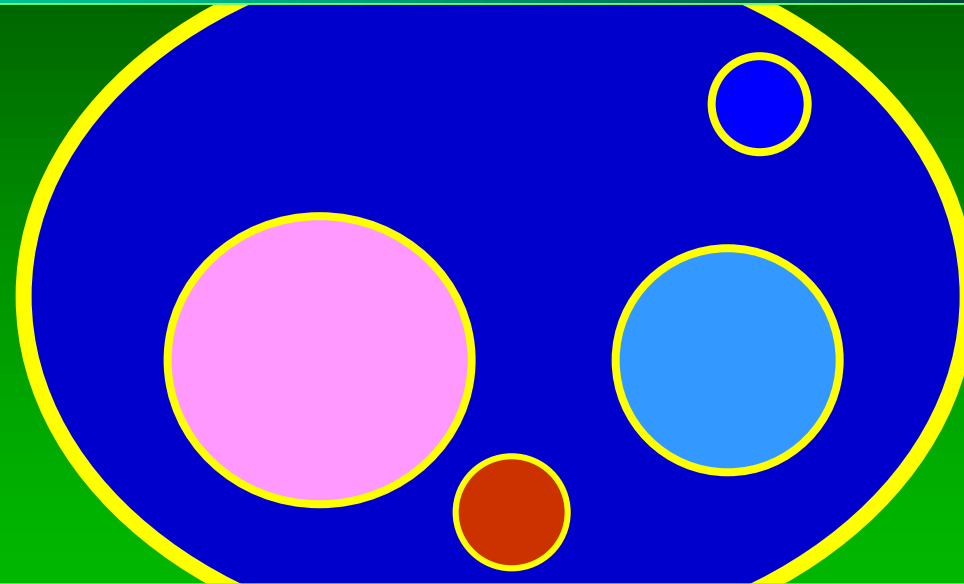


# 3d question bis (and answer) : what is the subcellular mobility of antibiotics ?

**High**

**Fair**

**Nil**



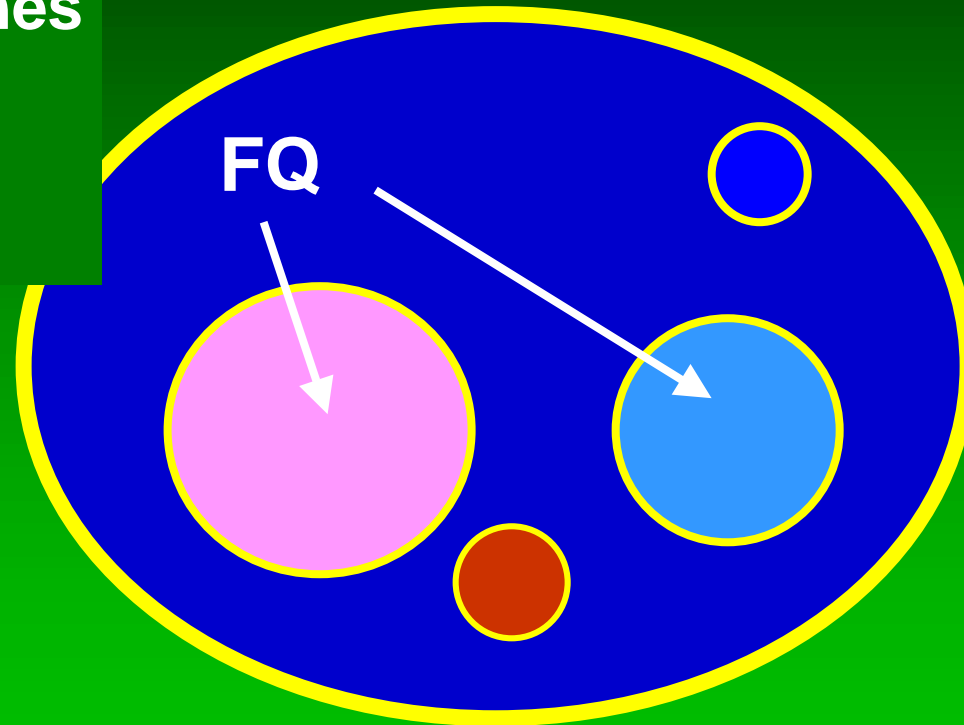
**FQ / Ansamyc. / cytosol. ML**

**lysosom. ML / AG**

# 3d question bis (and answer) : what is the subcellular mobility of antibiotics ?

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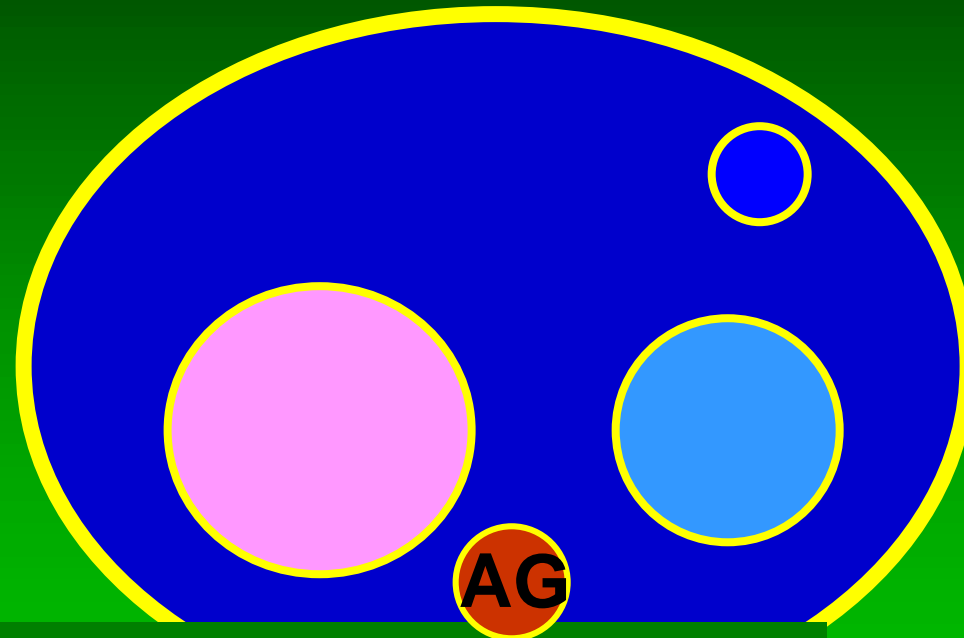
**Fluoroquinolones  
move easily  
across  
membranes**





# 3d question bis (and answer) : what is the subcellular mobility of antibiotics ?

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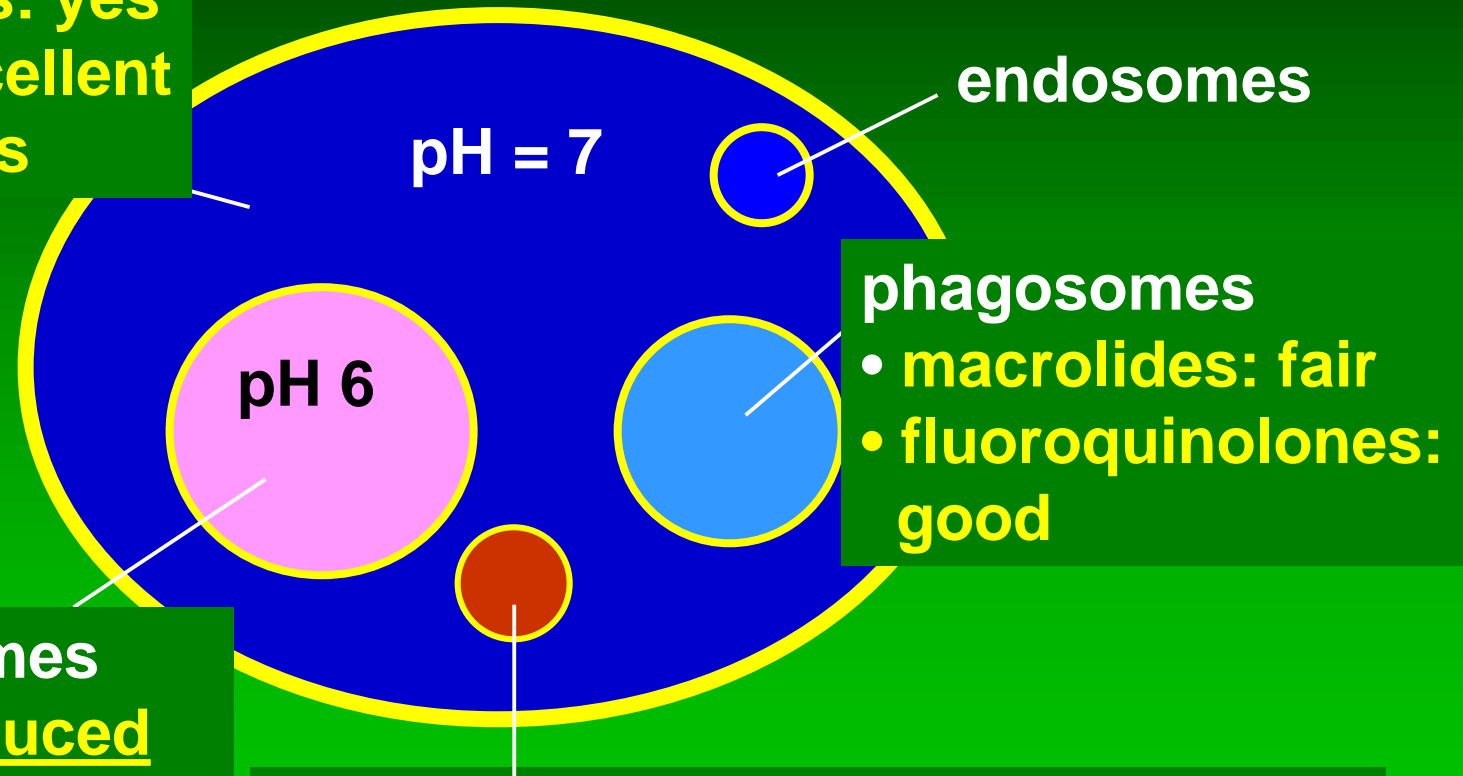


**Aminoglycosides  
and lysosomal macrolides  
remain largely if not totally sequestered**

# 4th question (and answer): what is the antibiotic intracellular expression of activity

## In cytosol:

- fluoroquinolones: yes
- ansamycins: excellent
- beta-lactams: yes



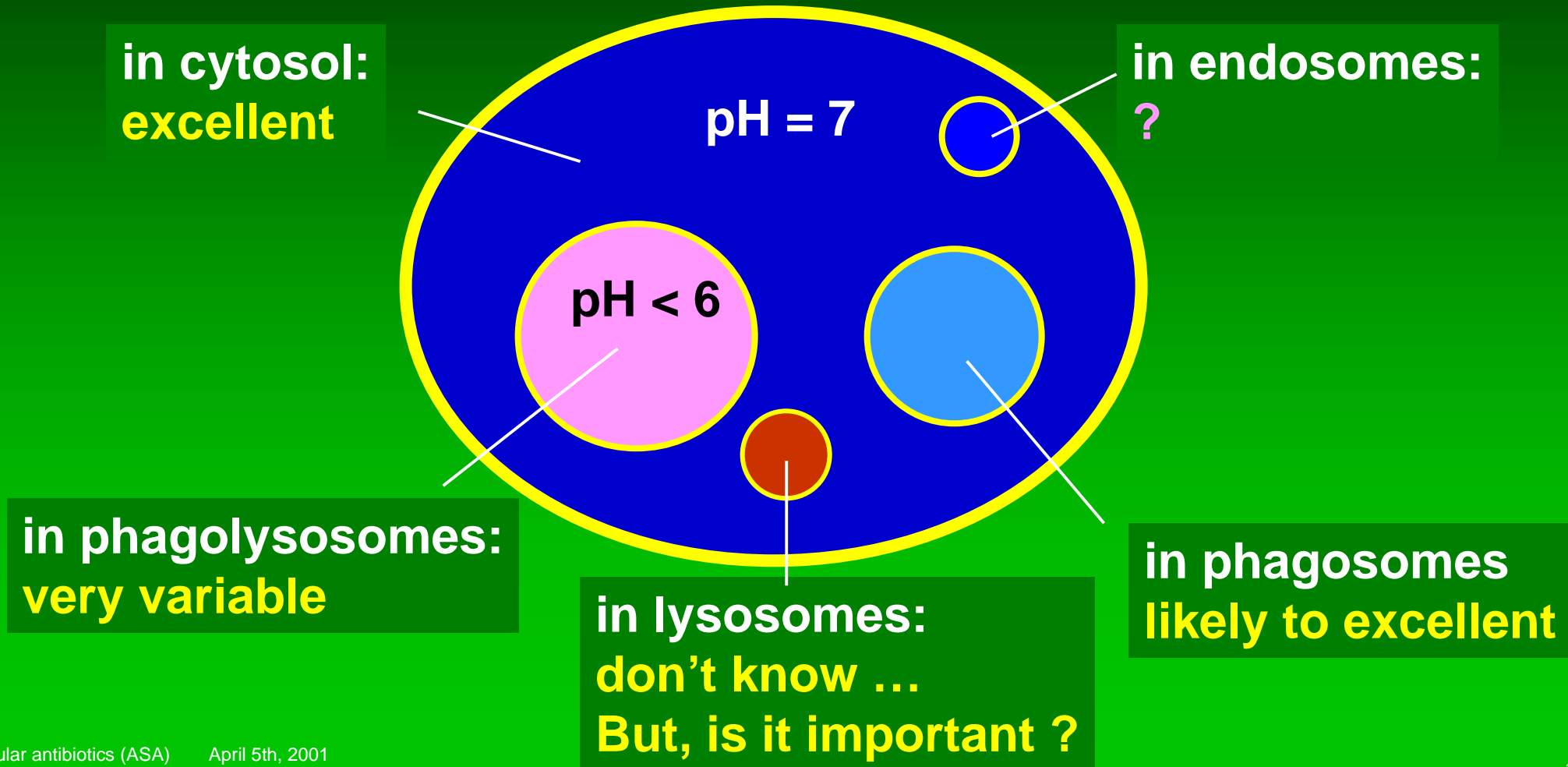
## in phagolysosomes

- macrolides: reduced
- fluoroquinolones: fair
- ansamycin: excellent

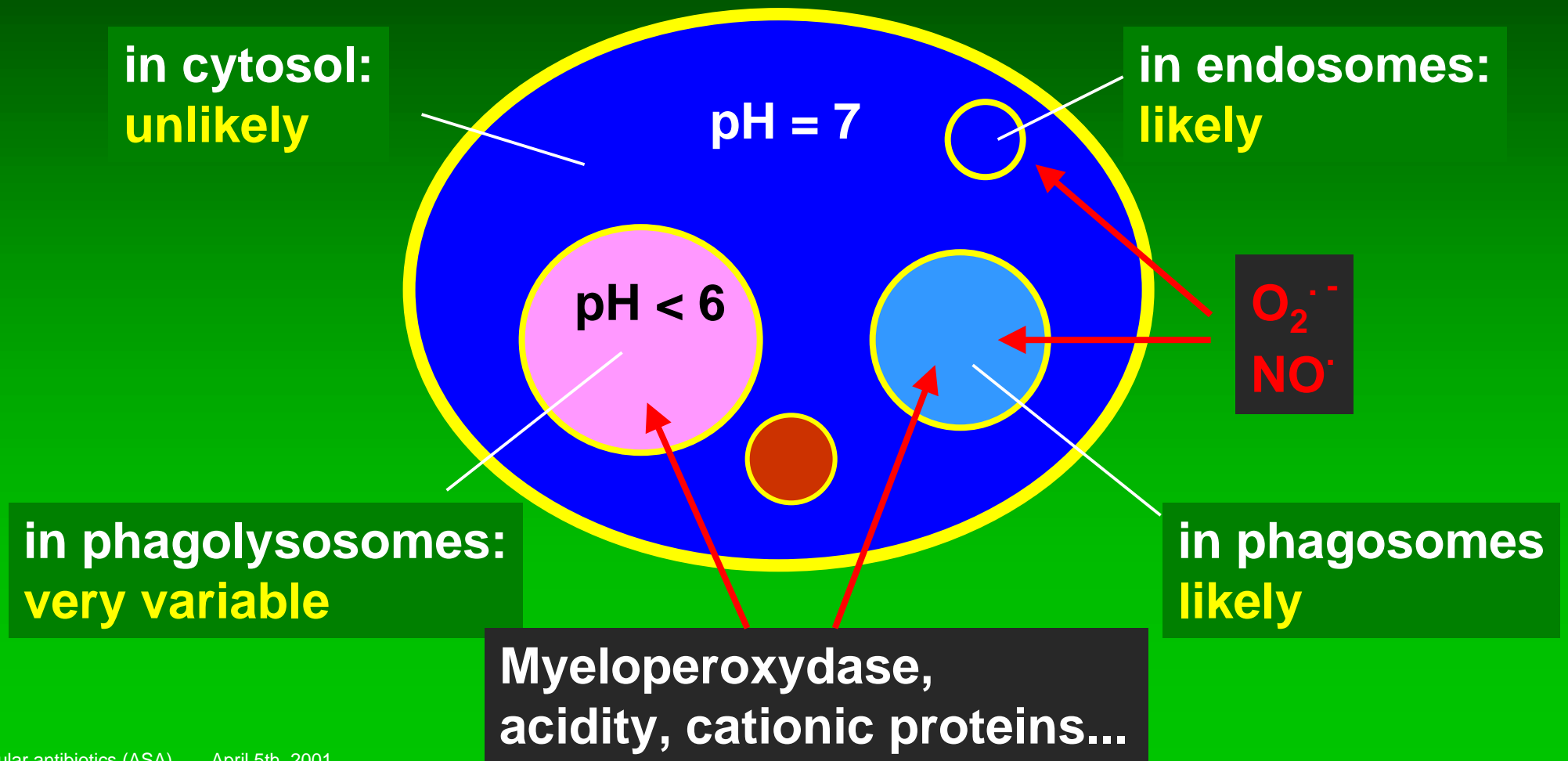
## in lysosomes (pH 5) :

- aminoglycosides: very reduced

# 5th question (and answer) : what is the bacterial responsiveness to the antibiotics ?



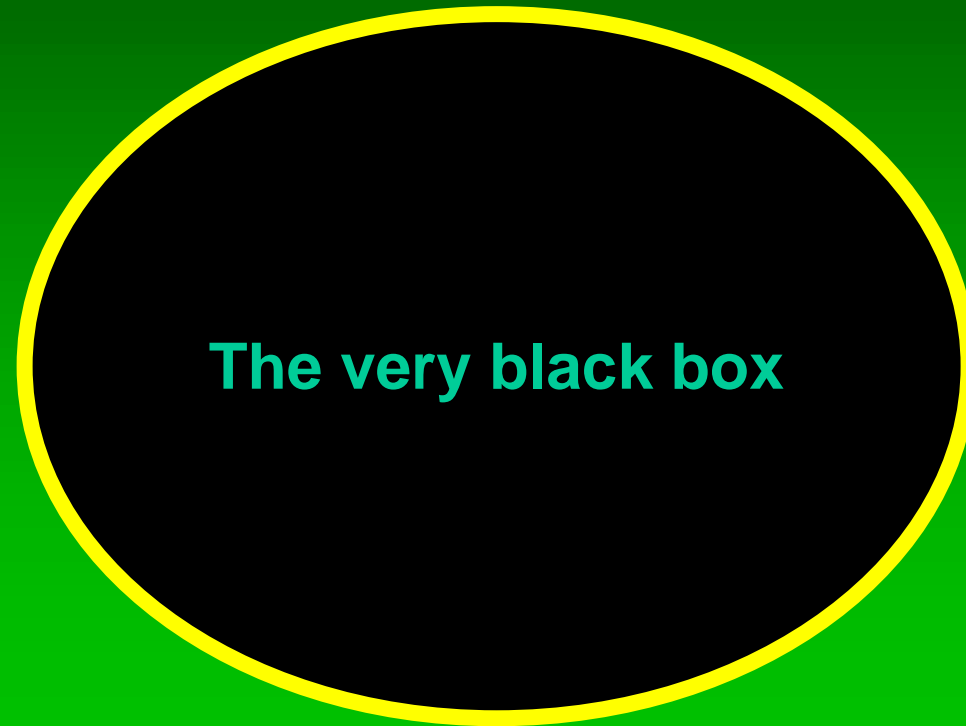
# 6th question [1 of 2] (and possible answer) : cooperation with the cell own defenses ?



# 6th question [2 of 2]: cooperation or antagonism with the actions of cytokines ?

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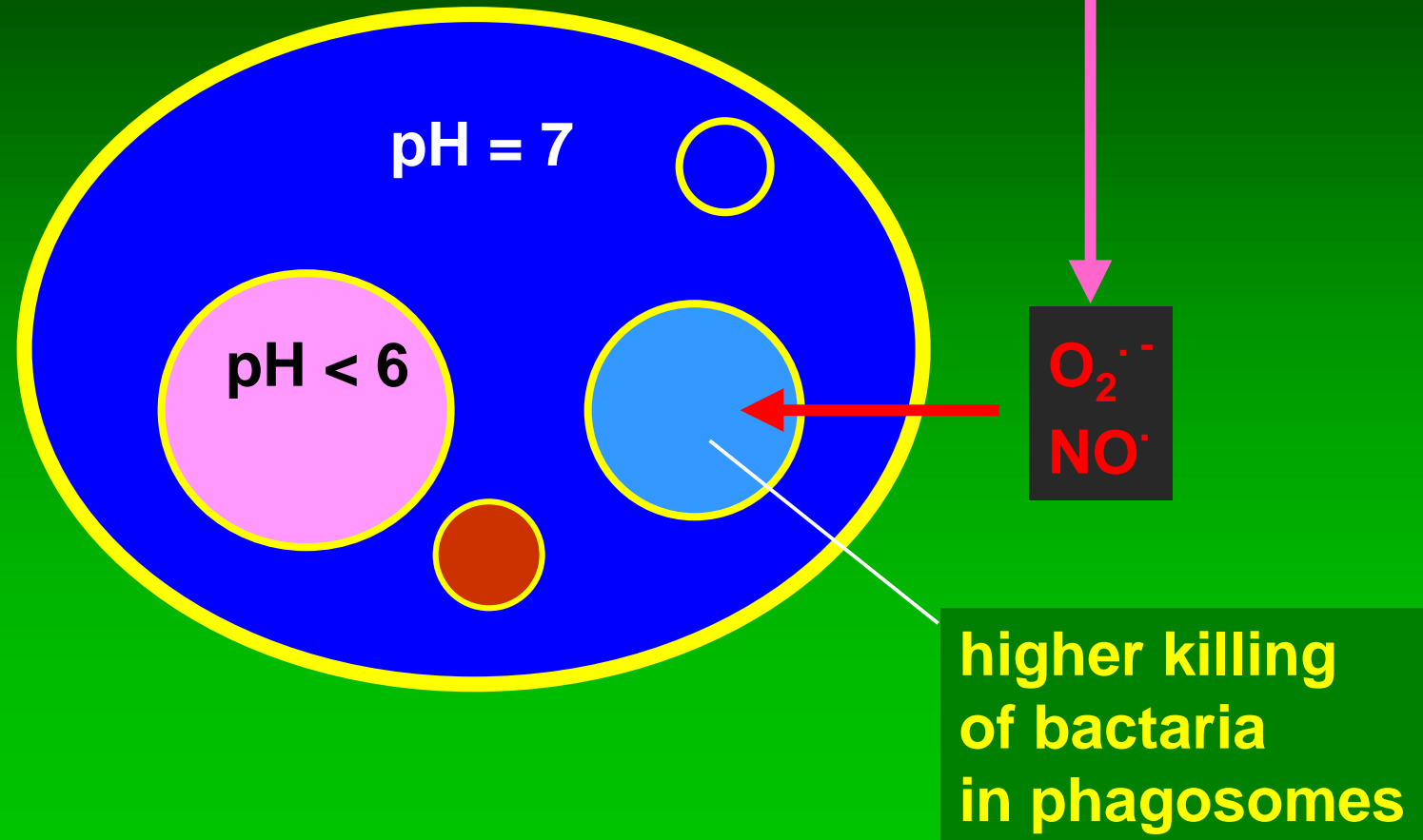
**antibiotic**



**interferons  
ILx  
growth fact.  
TNF  
...**

# cooperation with cytokines : one limited answer...

**Gamma-Interferon stimulates ORS and NRS production**



# Intracellular antibiotics: the issues

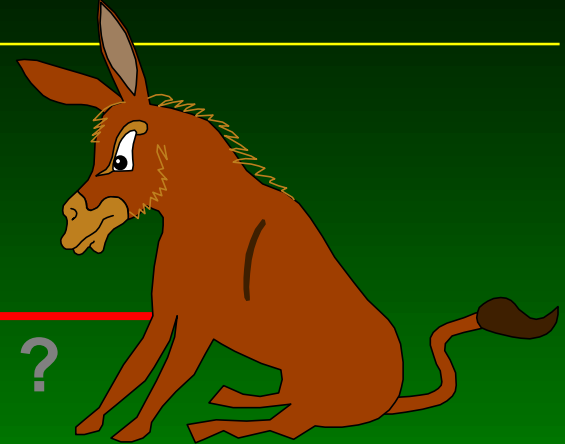
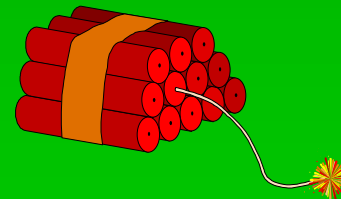
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**the toxicologist**



# Illustration: the *Listeria* story

## antibiotics:

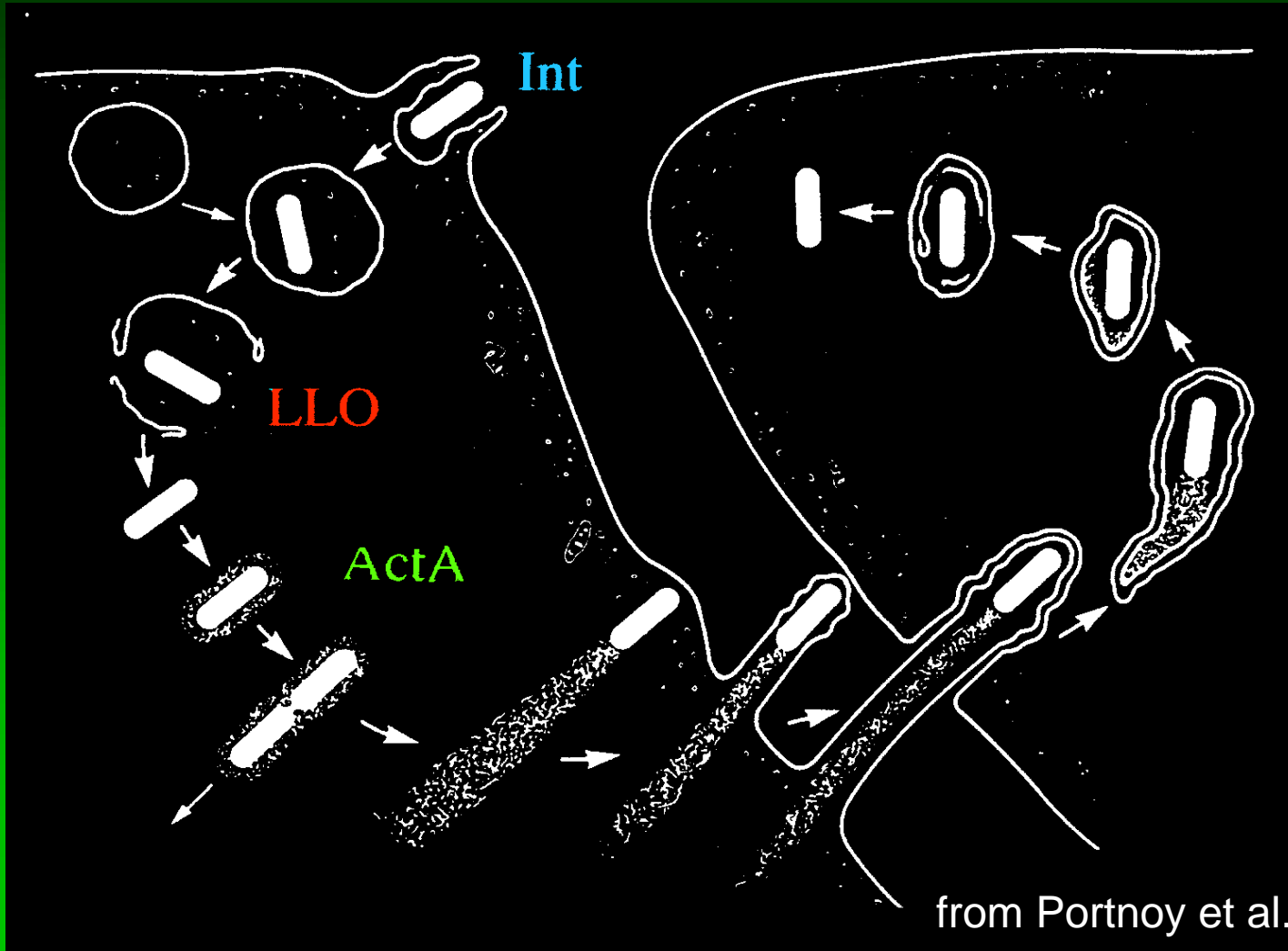
- ampicillin
- sparfloxacin
- moxifloxacin
- azithromycin

*Listeria monocytogenes*  
hly+ and hly-

gamma  
interferon



# Intracellular infection cycle of *Listeria monocytogenes* hly<sup>+</sup>

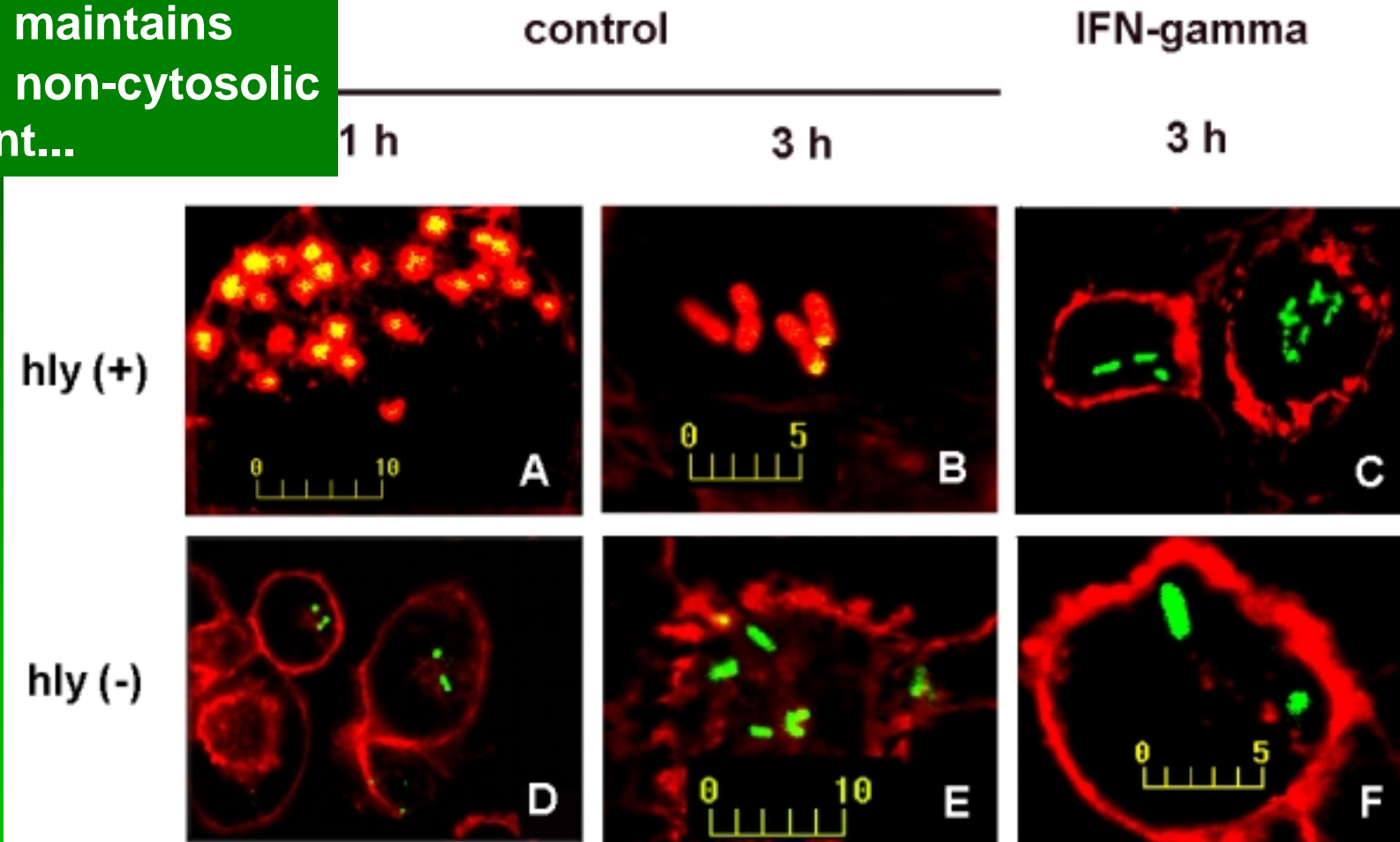


# *Listeria m.* and $\gamma$ -interferon : confocal microscopy

$\gamma$ -interferon maintains  
*L. m.* hly<sup>+</sup> in non-cytosolic  
compartment...

Bacteria  
stained with  
fluorescein  
(FITC) [green]  
and cell actin  
with phalloidin  
rhodamin  
[red];

hly<sup>+</sup>: virulent  
hly<sup>-</sup>: non virulent



# Following the intracellular fate of *Listeria m.* by EM

**A: phagocytosis**

**B: escape from vacuole**

**C: surrounded by actin**

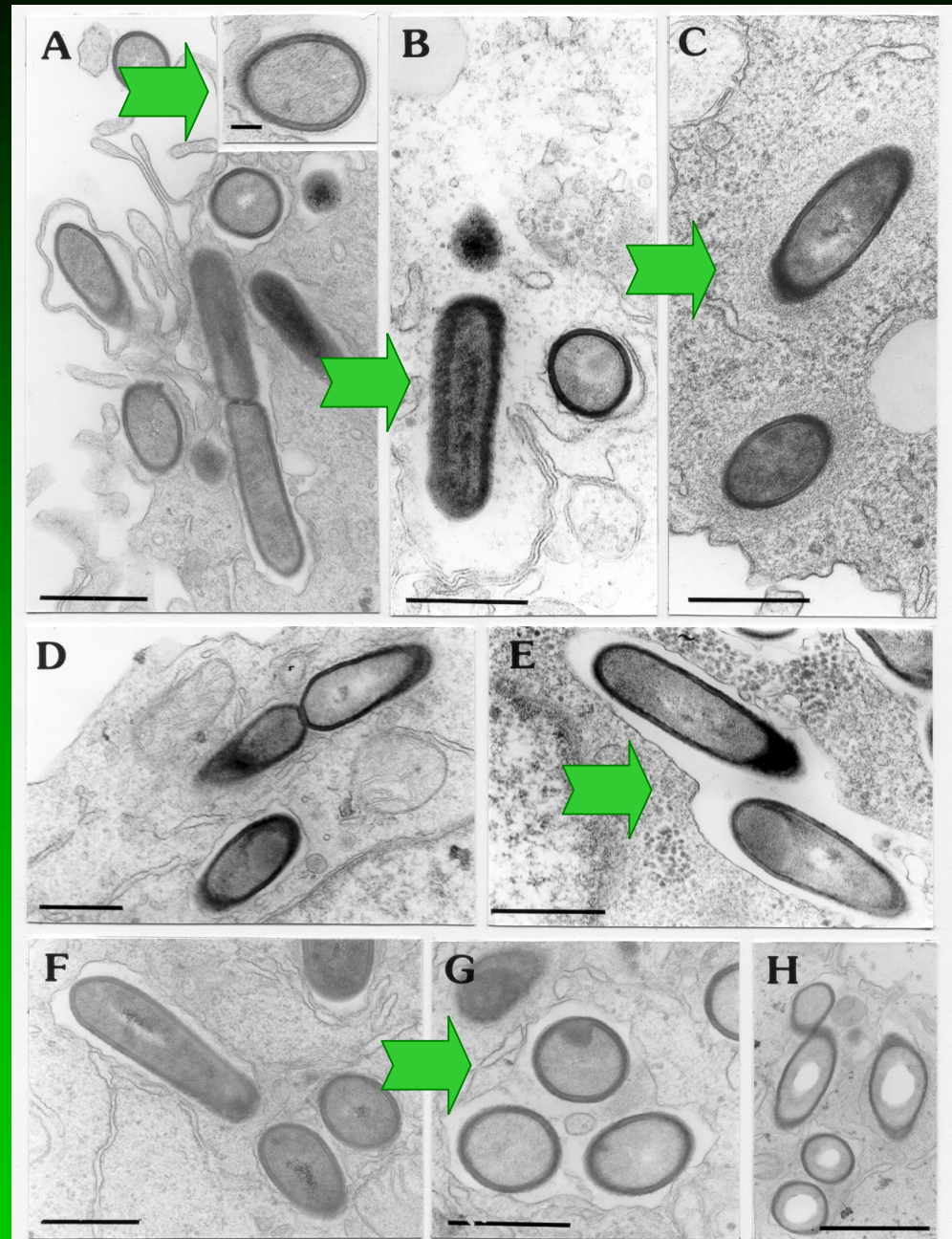
**D,E: cells pre-treated with interferon-gamma: *L. m.* hly+ remains in vacuoles.**

**F,G, H: *L.m.* hly- remains constantly in vacuoles**

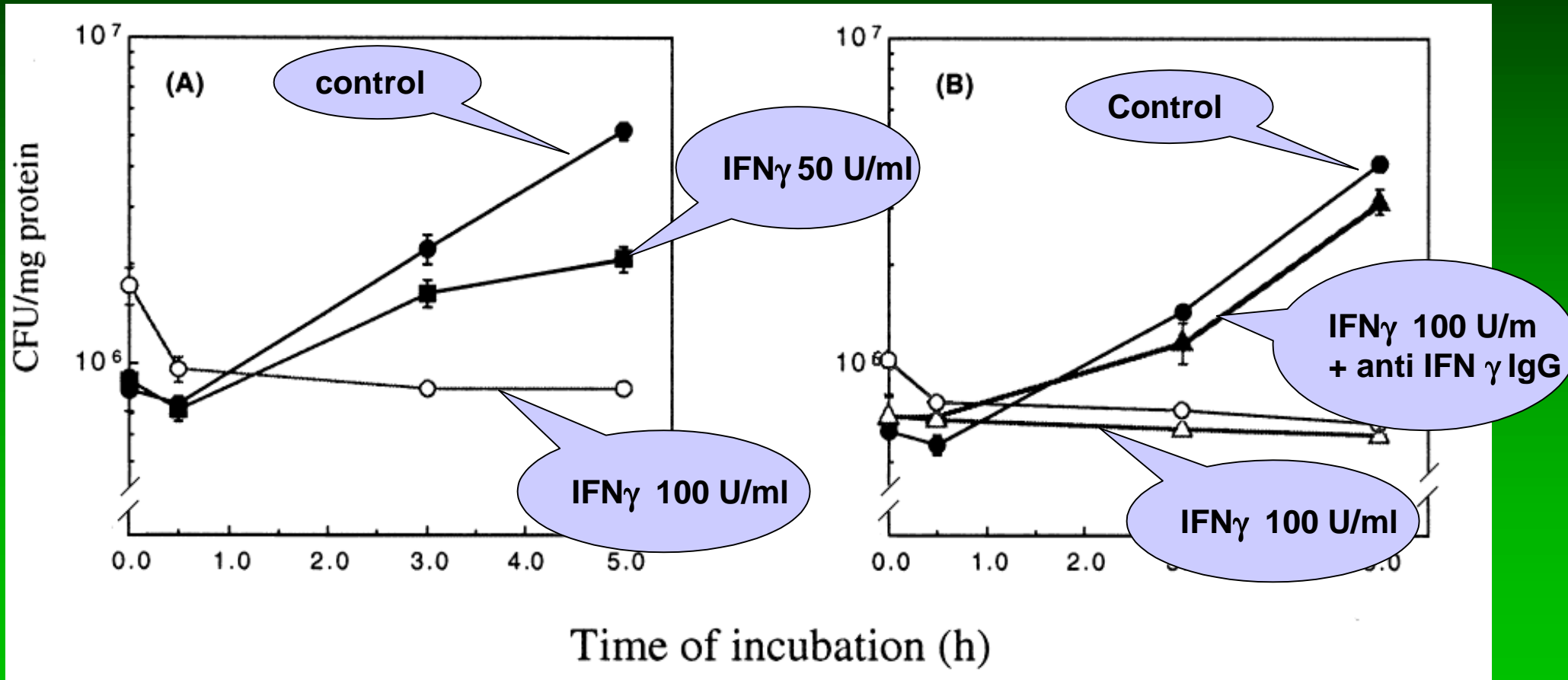
A = 1 h post infection;

B, C, D, F, G = 3 h post infection

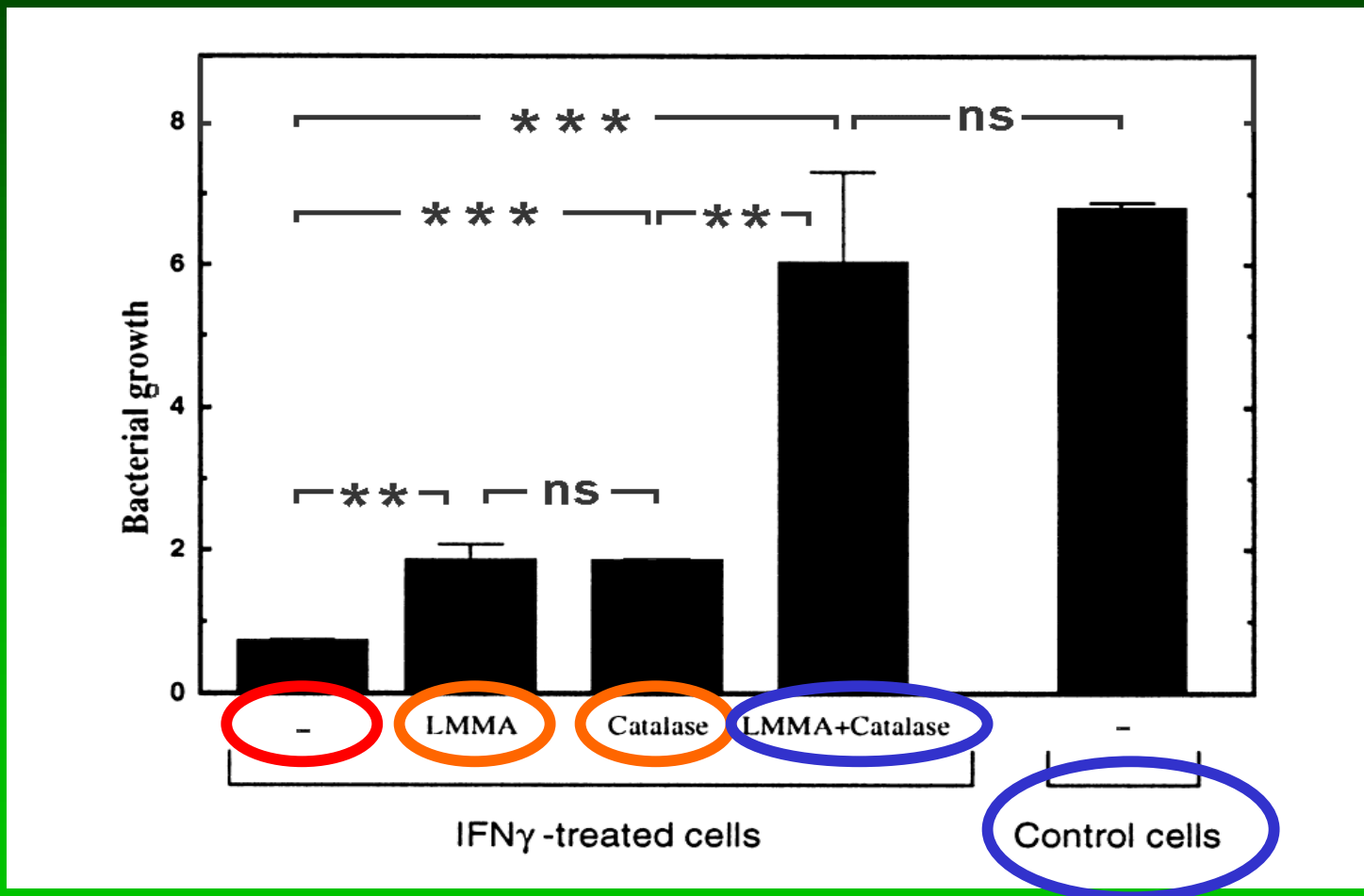
E, H = 5 h post infection



# $\gamma$ - IFN blocks the apparent growth of *L. m.* A: dose-dependency; B: specificity



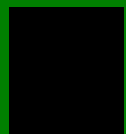
# The lack of apparent growth of *L. m.* with $\gamma$ -IFN is due to NO and H<sub>2</sub>O<sub>2</sub> production...



# Cooperation between $\gamma$ -IFN and antibiotics...

Intracellular activity:

$$\log_{10} \frac{\text{CFU @ 5 h}}{\text{CFU @ 0 h}}$$



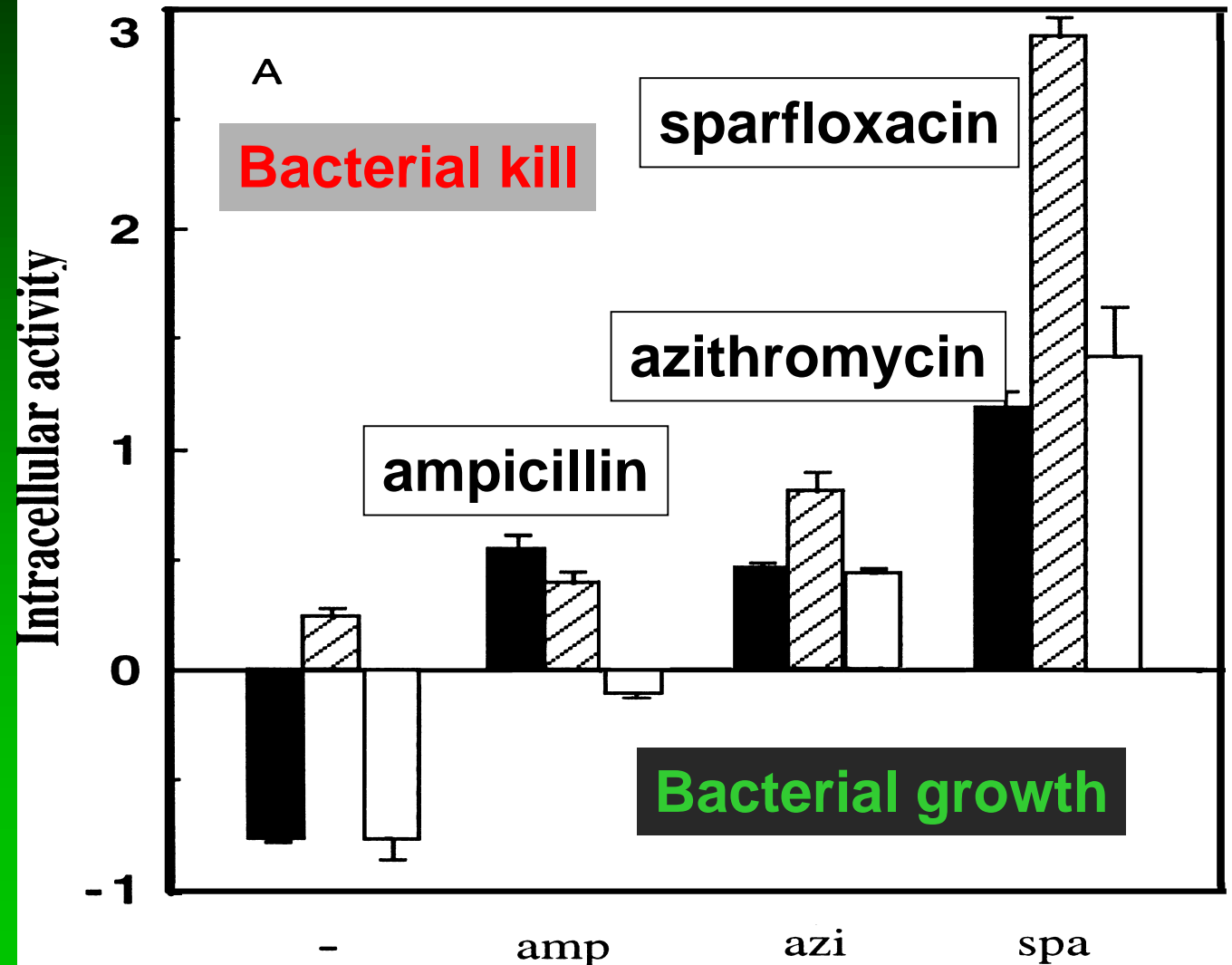
control



+  $\gamma$ -IFN



+  $\gamma$ -IFN  $\gamma$  and  
LMMA +  
catalase



# *Listeria m.*, ampicillin and $\gamma$ -interferon

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1. ampicillin is poorly active against intracellular *Listeria m.* ;
2. ampicillin does NOT cooperate and is actually antagonized by  $\gamma$ -IFN



Why do you keep ampicillin ?

→ extracellular bacteria

→ get activity with very large doses

# *Listeria m.*, azithromycin and $\gamma$ -interferon

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1. azithromycin accumulates much more than ampicillin but is not much more active ...

2. azithromycin is indifferent to host defenses\* ...

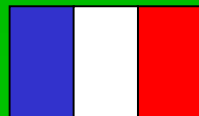


“Intracellular” antibiotics are NOT the panacea



Oh, yeah, but, did you test the ketolides ?

Sorry ...



Avez-vous évalué les **kétolides** ?



# *Listeria m.*, fluoroquinolones and $\gamma$ -interferon

1. Sparfloxacin IS **not** the active agent against intracellular *Listeria m.* (FQ move throughout the cell and their activity is not defeated by pH locally !! )
2. Sparfloxacin activity is **not** inhibited by the presence of  $\gamma$ -IFN ( $\gamma$ -IFN and FQ cooperate !)

FQ move throughout the cell and their activity is not defeated by pH

We don't know the reasons... Any suggestion ?? ( a big prize in Euros...)

Why don't you use fluoroquinolones ?

→ too low intrinsic activity ...

**This is where we are ...**

# Where was this stuff published ?

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**Scoreneaux et al.,  
*Antimicrob. Agents Chemother.* 40:1225-30, 1996**

**Ouadrhiri et al.,  
*Antimicrob. Agents Chemother.* 43:1242-51, 1999**

**Ouadrhiri et al.,  
*J. Infect. Dis.* 180:1195-204, 1999**

## **see also:**

Renard et al., *AAC* (1987) 31:410-416

Carlier et al., *JAC* (1990) 26(B):27-39.

Tulkens, *Eur. J. Clin. Microbiol. Infect. Dis.* (1991) 10:100-106.

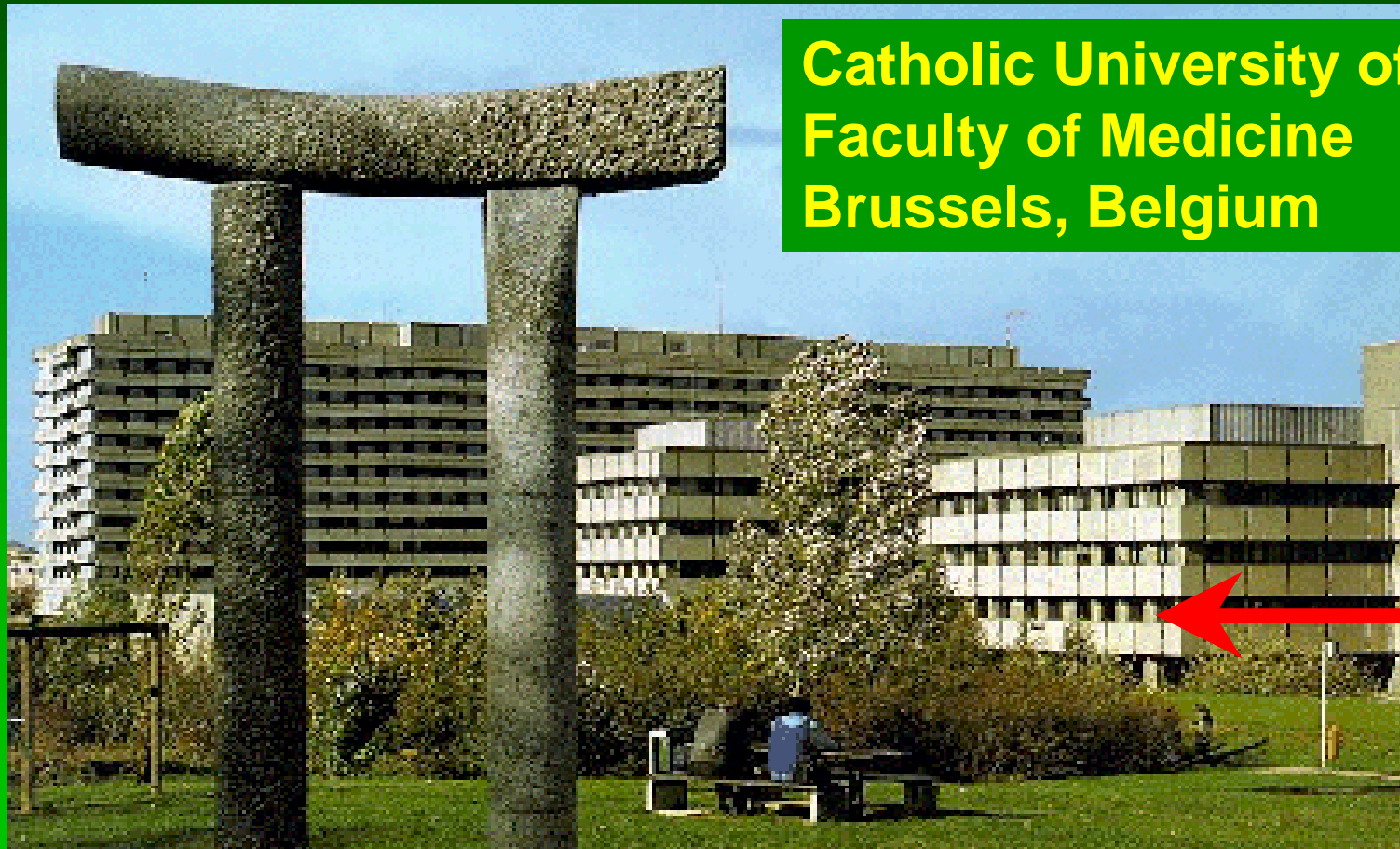
# Thanks to the crew ...

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- 😊 Zubida Bumkhalla (beta-lactams [chemistry])
- 😊 Marie-Béatrice Carlier (macrolides, fluoroquinolones)
- 😊 Séphane Carryn (méropenème, moxifloxacine, ...)
- 😊 Huajuan Fan (betalactams [chemistry] )
- 😊 Christine Renard (beta-lactams)
- 😊 Yousef Ouadrhiri (*Listeria*, interferon, IL6, fluoroquinolones)
- 😊 Isabelle Paternotte (beta-lactams [chemistry], efflux mech.)
- 😊 Bernard Scorneaux (*Listeria*, interferon, fluoroquinolones)
- 😊 Etienne Sonveaux (beta-lactams [chemistry] )
- 😊 Andrée Zenebergh (lincosaminides, macrolides)

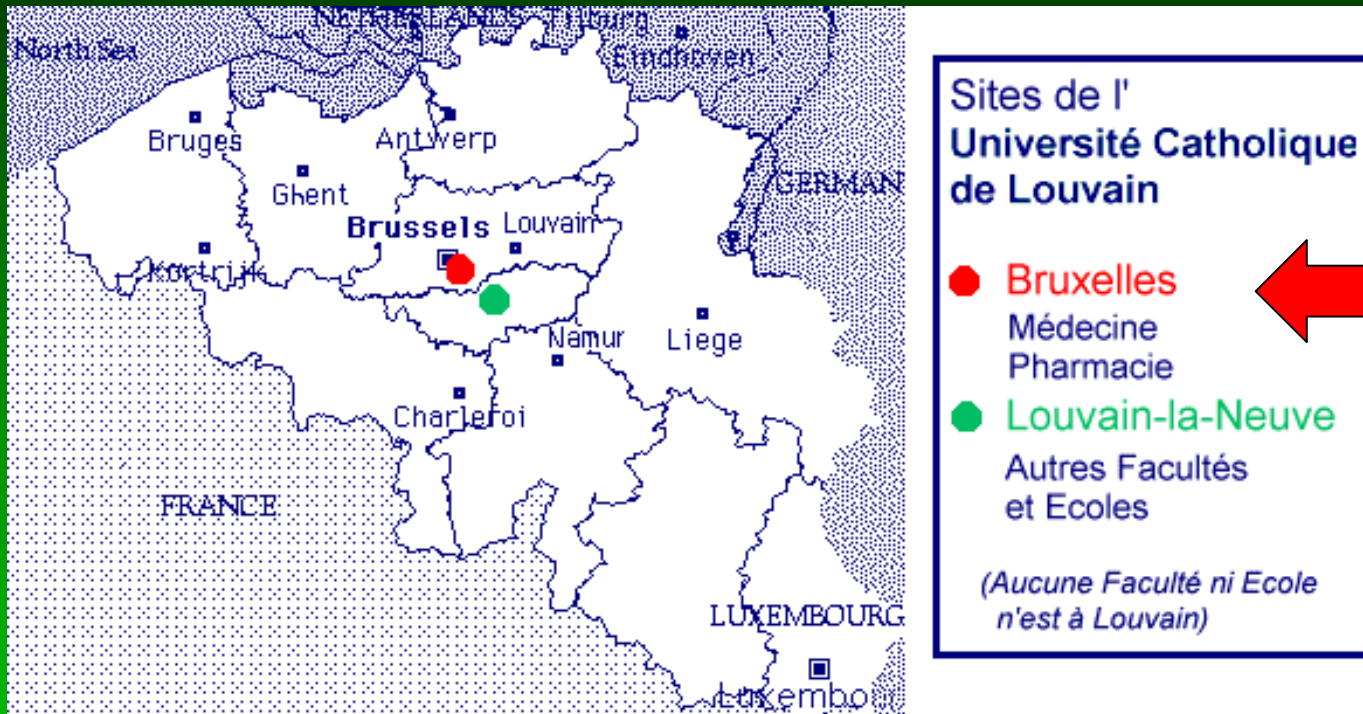
# But where do they work ?

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**Catholic University of Louvain  
Faculty of Medicine  
Brussels, Belgium**

# But, where the hell is that University?



**Cellular and Molecular Pharmacology Unit  
Catholic University of Louvain, Brussels, Belgium  
<http://www.md.ucl.ac.be/facm>**

# But don't forget ISAP...

population  
pharmacokinetics

tissue  
concentrations

efficacy/toxicity ratios

postantibiotic  
effect...

AUIC

intracellular  
pharmacodynamics



**ISAP**

**International Society of Anti-Infective Pharmacology**

Founded in 1991

<http://www.isap.org>

# Let's hope it helps you in Australia

