Rega Institute :
my research contacts …
while in "Louvain" and in Brussels …

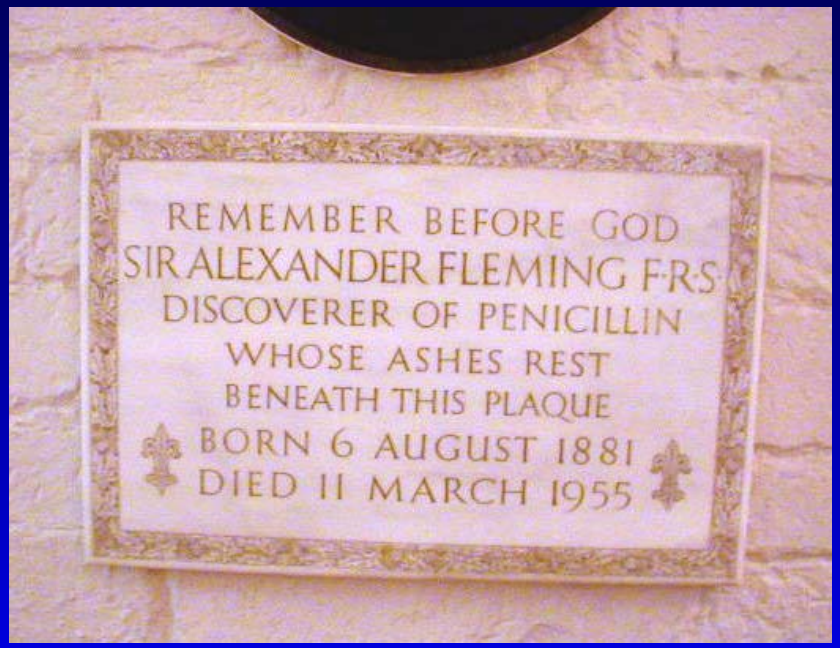
Paul M. Tulkens

Cellular and Molecular Pharmacology Unit
Université catholique de Louvain, Bruxelles

Human Biochemistry and Biochemical Pathology
Université de Mons-Hainaut, Mons

It all started with microbiology …

In the late 50's, I was offered a book by André Maurois about Fleming's life…



And the first chapter was: "*Au début était l'Ecosse* …"

… The Fleming children spent much of their of time ranging through the streams, valleys, and moors of the countryside. "We unconsciously learned a great deal from nature," said Fleming.

So, my first microbiological experience was in Scotland, and then in London …

Medicine & Veterinary Medicine at The University *of* Edinburgh

School of Biomedical and Clinical Laboratory Sciences - Division of Medical Microbiology

Welcome to the Division of MEDICAL MICROBIOLOGY

1963

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

A registered UK charity supporting biomedical and related research

1964

However, when arriving in Louvain in 1965, Cell Biology became more important …

I guess, it was because of the previous speaker…

But the green pastures of England were still attractive…

MRC | National Institute for Medical Research

Prof. H.G. Hers had the good idea to give me 3,000 Bfrs to spend 3 months at Mill Hill, where I learned Cell Culture …
and also to wave through a remarkable Library …

So, when I came back, we decided to launch Cell Culture (fibroblasts) at the "Dekenstraat" …

Nature. 1970 Dec 26;228(278):1282-5.

Immunological inhibition of lysosome function.

Tulkens P, Trouet A, Van Hoof F.

J Cell Biol. 1974 Nov;63(2 Pt 1):383-401.

FREE full text article at www.jcb.org

Analytical fractionation of homogenates from cultured rat embryo fibroblasts.

Tulkens P, Beaufay H, Trouet A.

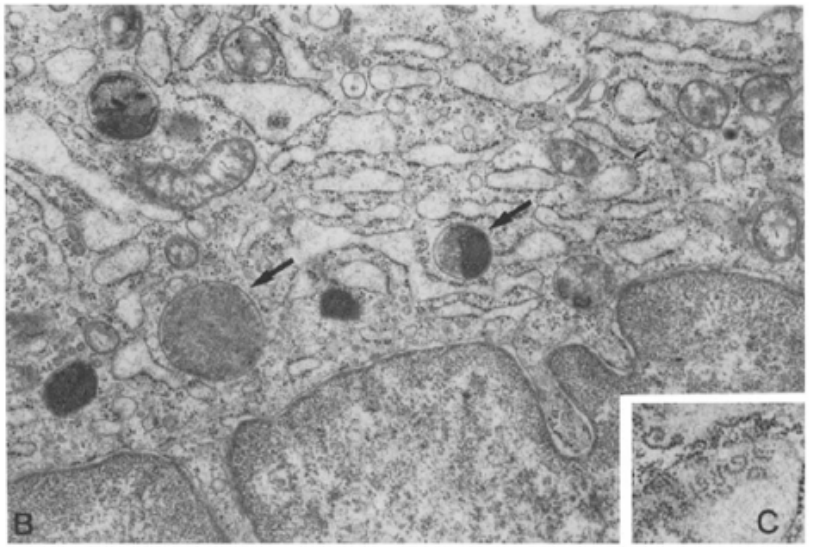


FIGURE 1 Ultrastructural aspec cultured rat embryo fibroblasts. Cells were collected and examined as previously described (56). A. General view of a peripheral part of the cytoplasm. Cisternae of the endoplasmic reticulum are dilated by an electron-dense material and are heavily coated with ribosomes. Many small smooth vesicles are observed, some of which are in continuity with the plasma membrane (arrows). Mitochondria, dense bodies, and lipid droplets are present (× 13,000). B. Alternating smooth and rough regions of the endoplasmic reticulum are clearly visible. Dense bodies (arrows) show a well-defined membrane surrounding a pleomorphic material. The convoluted aspect of the nucleus profile is illustrated (× 23,000). C. Polysomal arrangement of ribosomes, probably associated with a membrane of the endoplasmic reticulum seen in an oblique section (× 30,000). Courtesy of Dr. F. Van Hoof.

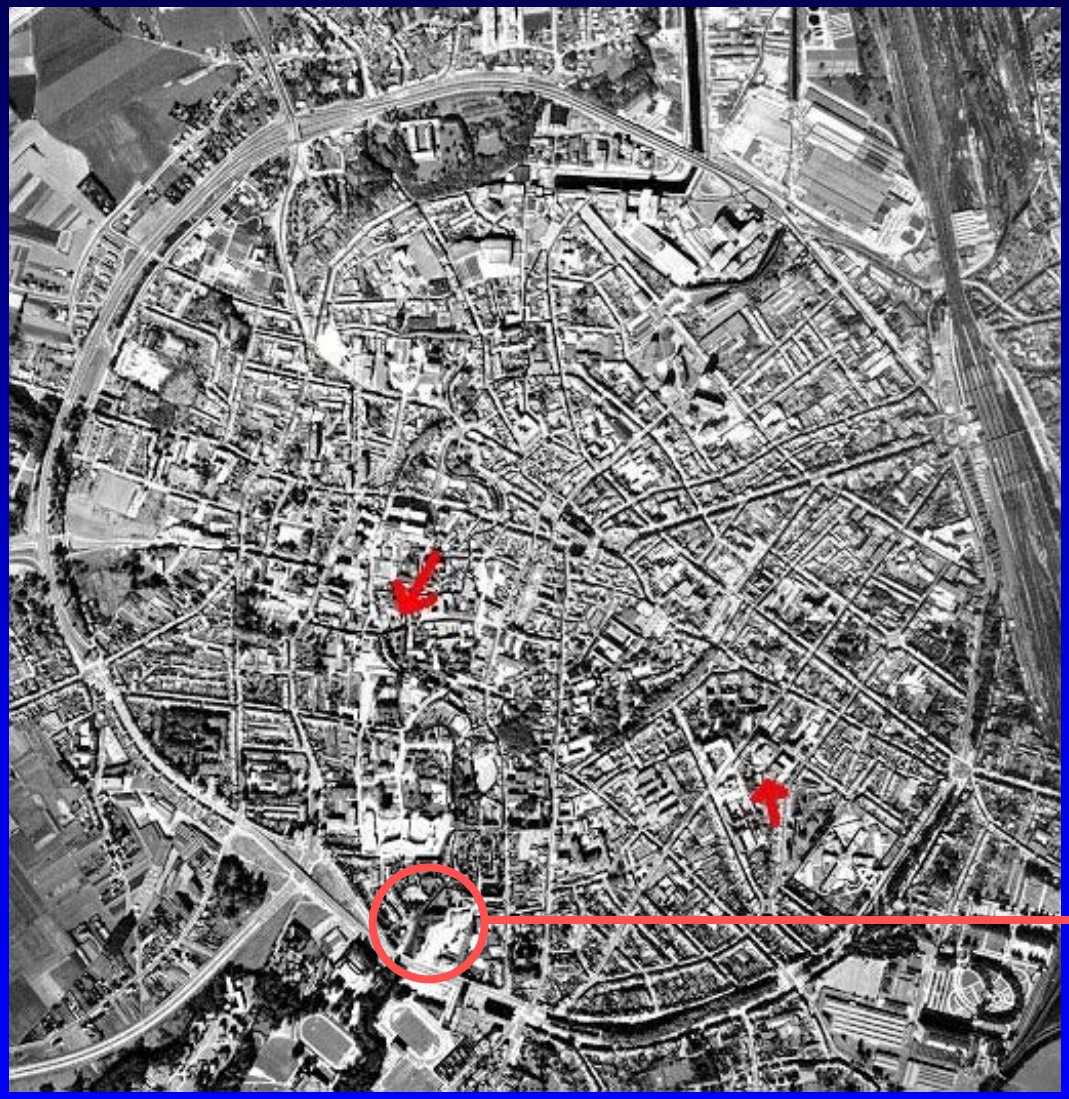
J Cell Biol. 1979 Aug;82(2):466-74. Related Articles, Link

FREE full text article at www.jcb.org

Fate of plasma membrane during endocytosis. II. Evidence for recycling (shuttle) of plasma membrane constituents.

Schneider YJ, Tulkens P, de Duve C, Trouet A.

How did we do that ?



By rushing in bicycle between these two places …

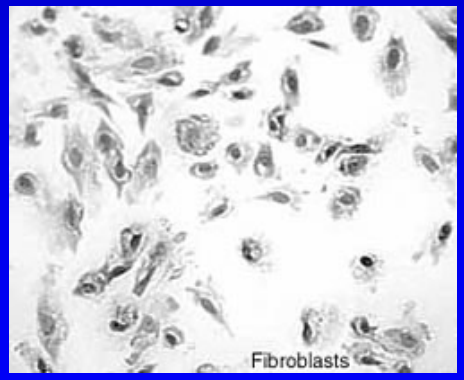
And getting some important ingredient from here …

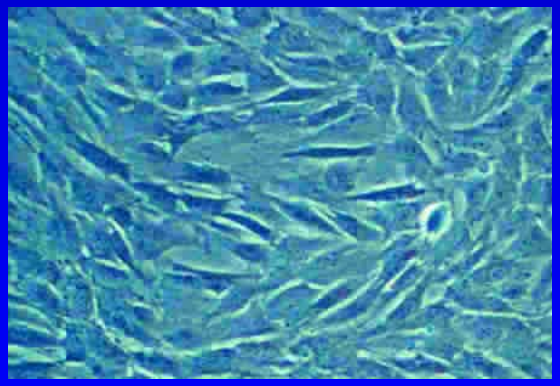
Thanks to …

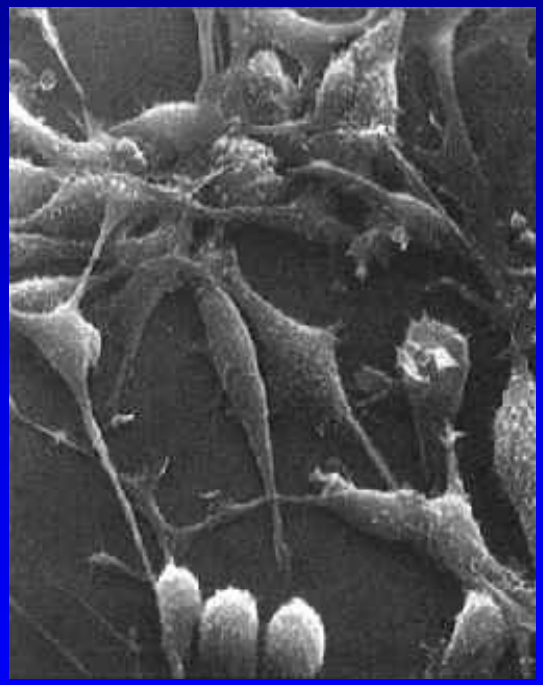
Mrs Lamy

Mr E. Schonne

Mr J. Desmyter







But fibroblasts eventually led me back to microbiology (or, at least antibiotics …)

Biochem Pharmacol. 1978 Feb 15;27(4):415-24. Related Articles, Lin

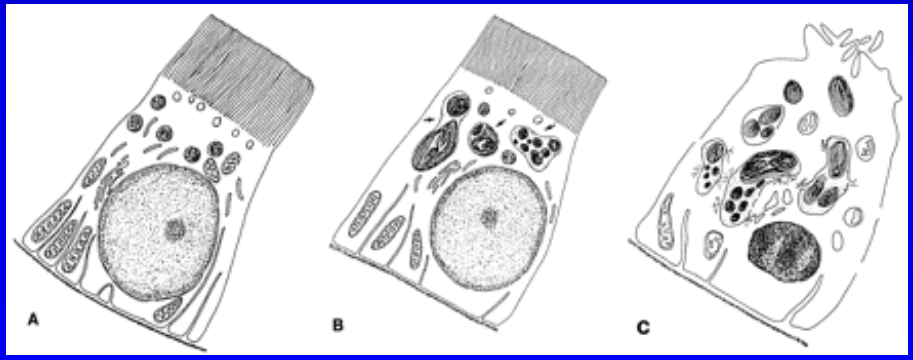
The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured rat fibroblasts.

Tulkens P, Trouet A.

Lab Invest. 1979 Apr;40(4):481-91. Related Articles, Links

Gentamicin-induced lysosomal phospholipidosis in cultured rat fibroblasts. Quantitative ultrastructural and biochemical study.

Aubert-Tulkens G, Van Hoof F, Tulkens P.



Antimicrob Agents Chemother. 1999 May;43(5):1003-12.

FREE full text article at aac.asm.org

Aminoglycosides: nephrotoxicity.

Mingeot-Leclercq MP, Tulkens PM.

And this is where the Rega came back …

Trying to understand how the molecule interacts with phospholipids

But here are the real molecules

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1983, p. 440–449
0066-4804/83/030440-10$02.00/0

Vol. 23, No. 3

Inhibition of Lysosomal Phospholipases by Aminoglycoside Antibiotics: In Vitro Comparative Studies

MARIE B. CARLIER,[1] GUY LAURENT,[1] PAUL J. CLAES,[2] HUBERT J. VANDERHAEGHE,[2] AND PAUL M. TULKENS[1]*

Laboratoire de Chimie Physiologique, Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, B-1200 Brussels,[1] and Laboratorium voor Farmaceutische Chemie, Rega Instituut, Katholieke Universiteit Leuven, B-3000 Leuven,[2] Belgium

Received 8 October 1982/Accepted 23 December 1982

This has been the beginning of a long-lasting collaboration …

J Med Chem. 1991 Apr;34(4):1483-92. Related Articles, Links

New derivatives of kanamycin B obtained by combined modifications in positions 1 and 6". Synthesis, microbiological properties, and in vitro and computer-aided toxicological evaluation.

Van Schepdael A, Busson R, Vanderhaeghe HJ, Claes PJ, Verbist L, Mingeot-Leclercq MP, Brasseur R, Tulkens PM.

Laboratorium voor Farmaceutische Chemie, Rega Institut, Katholieke Universiteit Leuven, Belgium.

J Med Chem. 1991 Apr;34(4):1476-82. Related Articles, Links

New derivatives of kanamycin B obtained by modifications and substitutions in position 6". 2. In vitro and computer-aided toxicological evaluation with respect to interactions with phosphatidylinositol.

Mingeot-Leclercq MP, Van Schepdael A, Brasseur R, Busson R, Vanderhaeghe HJ, Claes PJ, Tulkens PM.

Laboratoire de Chimie Physiologique, Universite Catholique de Louvain, Belgium.

J Med Chem. 1991 Apr;34(4):1468-75. Related Articles, Links

New derivatives of kanamycin B obtained by modifications and substitutions in position 6". 1. Synthesis and microbiological evaluation.

Van Schepdael A, Delcourt J, Mulier M, Busson R, Verbist L, Vanderhaeghe HJ, Mingeot-Leclercq MP, Tulkens PM, Claes PJ.

Laboratorium voor Farmaceutische Chemie, Rega Instituut, Katholieke Universiteit Leuven, Belgium.

and several other papers on aminoglycosides…

This has been the beginning of a long-lasting collaboration …

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1987, p. 410–416
0066-4804/87/030410-07$02.00/0

Vol. 31, No. 3

Influence of Conversion of Penicillin G into a Basic Derivative on Its Accumulation and Subcellular Localization in Cultured Macrophages

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Laboratoire de Chimie Physiologique and International Institute of Cellular and Molecular Pathology, Université Catholique de Louvain, B-1200 Brussels,[1] and Laboratorium voor Farmaceutische Scheikunde, Rega Instituut, Katholieke Universiteit Leuven, B-3000 Louvain,[2] Belgium

Received 25 August 1986/Accepted 12 December 1986

β-Lactam antibiotics do not accumulate in phagocytes, probably because of their acidic character. We therefore synthesized a basic derivative of penicillin G, namely, ^{14}C-labeled *N*-(3-dimethylaminopropyl)benzylpenicillinamide (ABP), and studied its uptake and subcellular localization in J774 macrophages compared with that of ^{14}C-labeled penicillin G. Whereas the intracellular concentration (C_i) of penicillin G remained lower than its extracellular concentration (C_e), ABP reached a C_i/C_e ratio of 4 to 5. Moreover, approximately 50% of intracellular ABP was found associated with lysosomes after isopycnic centrifugation of cell homogenates in isoosmotic Percoll or hyperosmotic sucrose gradients. The behavior of ABP was thus partly consistent with the model of de Duve et al. (C. de Duve, T. de Barsy, B. Poole, A. Trovet, P. Tulkens, and A. Van Hoof, Biochem. Pharmacol. 23:2495–2531, 1974), in which they described the intralysosomal accumulation of weak organic bases in lysosomes. Although ABP is microbiologically inactive, our results show that β-lactam antibiotics can be driven into cells by appropriate modification. Further efforts therefore may be warranted in the design of active compounds or prodrugs that may prove useful in the chemotherapy of intracellular infections.

and also on β-lactams…

The fate of a basic derivative of penicillin ("**ABP**") synthesized at the Rega Institute

ANTIMICROB. AGENTS CHEMOTHER.

TABLE 2. Assignment of [^{14}C]ABP and [^{14}C]penicillin G to lysosomes and soluble fraction after fractionation of homogenates by isopycnic centrifugation

Gradient and drug	Percentage of drug assigned to[a]:		
	Lysosomes	Soluble fraction	Unassigned
Sucrose			
[^{14}C]ABP	48	46	6
[^{14}C]penicillin G	0	98	2
Percoll			
[^{14}C]ABP	60	38	2
[^{14}C]penicillin G	9	88	3

[a] Assignment of [^{14}C]ABP or [^{14}C]penicillin G present in homogenates of cells incubated with the drugs was made with respect to the lysosomes and the soluble fraction using the distribution of *N*-acetyl-β-hexosaminidase and α-galactosidase, on the one hand, and added [^{14}C]ABP or [^{14}C]benzylpenicillin, respectively, on the other hand. For [^{14}C]ABP the distribution patterns used are those obtained from the experiments shown in Fig. 3A and 4A. For [^{14}C]benzylpenicillin the distribution patterns are those obtained from the experiments shown in Fig. 3B and 4B (patterns of the lysosomal enzymes are not illustrated).

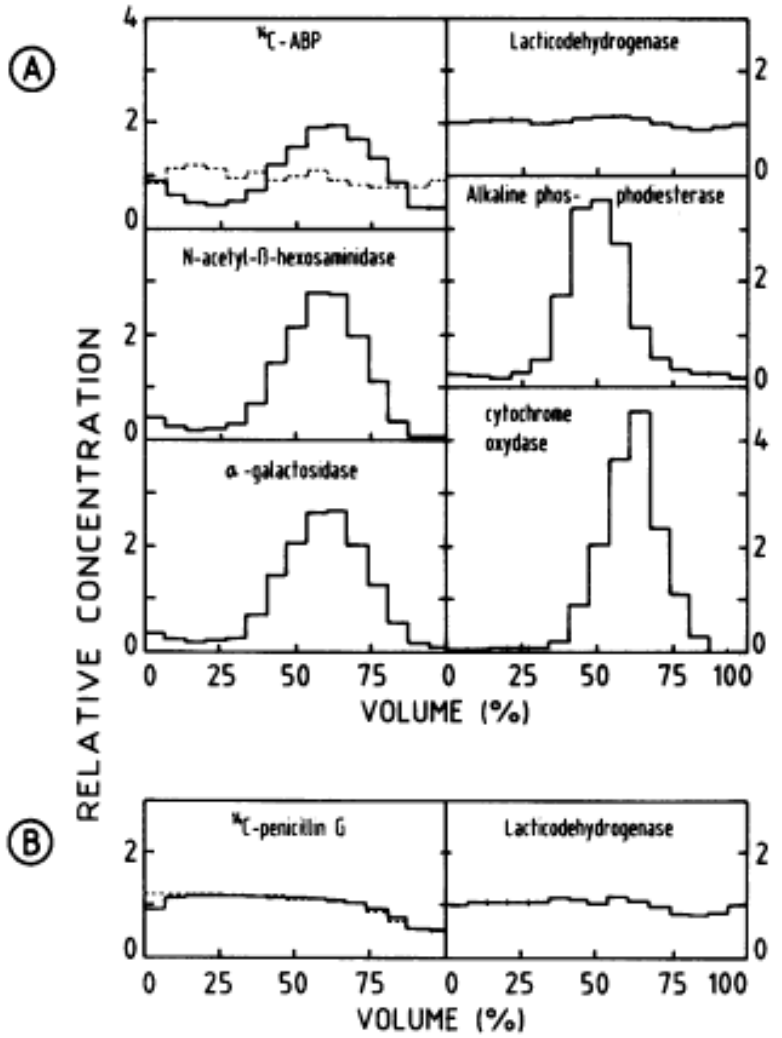


FIG. 4. Volume distribution patterns of [^{14}C]ABP (A), [^{14}C]penicillin G (B), and enzymes after fractionation of cytoplasmic extracts by isopycnic centrifugation in isoosmotic Percoll gradients. For [^{14}C]ABP (A) and [^{14}C]penicillin G (B), the solid line refers to the distribution of the drug found in homogenates of cells incubated with the corresponding drug (3 mM, 1 h, 37°C). The dotted line refers to free drug mixed throughout the tube content before centrifugation.

Where has that eventually led to with aminoglycosides…

TOXICOLOGICAL SCIENCES 56, 229–239 (2000)

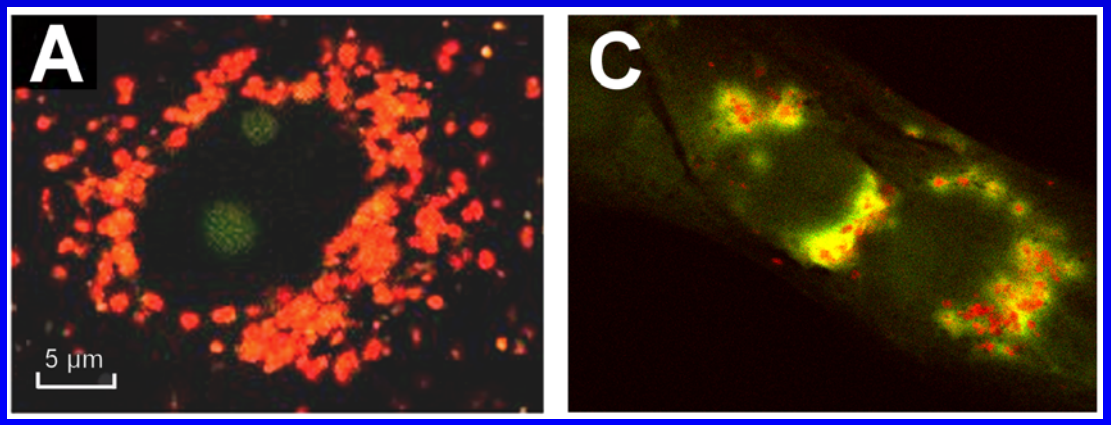
Gentamicin-Induced Apoptosis in Renal Cell Lines and Embryonic Rat Fibroblasts

Mohammed El Mouedden, Guy Laurent,* Marie-Paule Mingeot-Leclercq, and Paul M. Tulkens[1]

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium; and
**Service d'Histologie et de Cytologie Expérimentale, Université de Mons-Hainaut, B-7000 Mons, Belgium*

Received December 24, 1999; accepted March 27, 2000

We know " tend to believe " that the link between lysosomal phospholipidosis induced by aminoglycosides and the ensuing cell toxicity could be apoptosis…

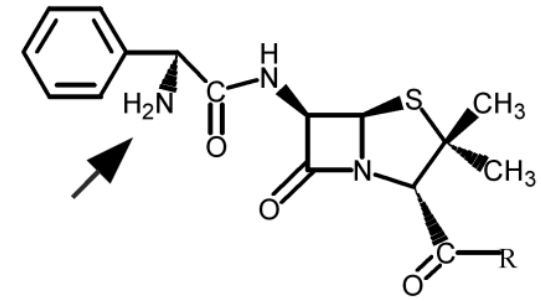


Perhaps caused by lysosomal rupture …

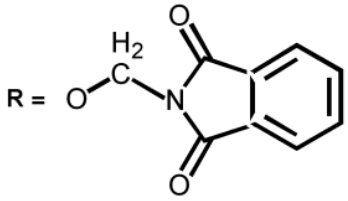
Servais *et al.* submitted

And with β-lactams …

New esters of ampicillin have been made and tested against intracellular *Listeria monocytogenes* …



ampicillin R = OH



PIMA R =

phthalimidomethyl

J Antimicrob Chemother. 2003 Oct;52(4):610-5. Epub 2003 Sep 12.

Related Articles, Link

Full text article at jac.oupjournals.org

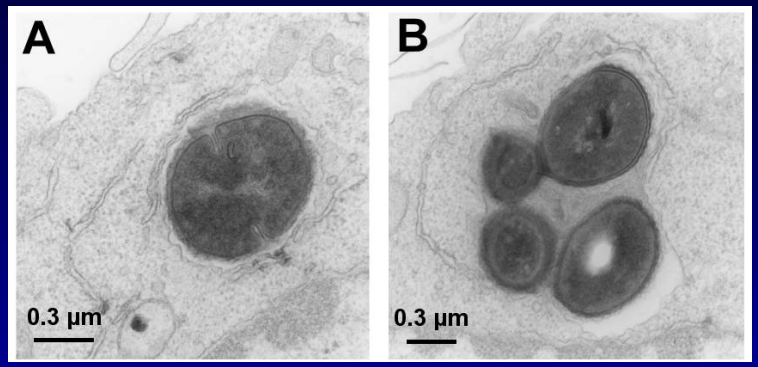
Intracellular accumulation and activity of ampicillin used as free drug and as its phthalimidomethyl or pivaloyloxymethyl ester (pivampicillin) against Listeria monocytogenes in J774 macrophages.

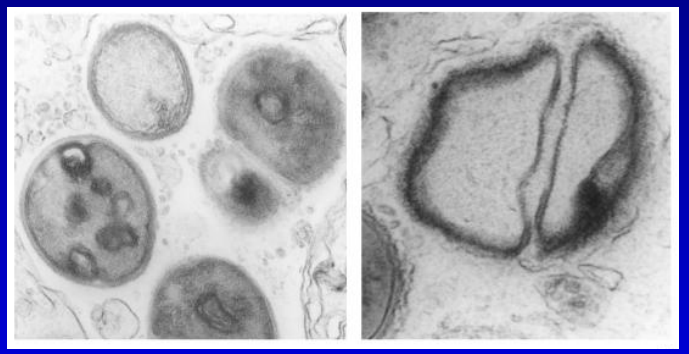
Chanteux H, Mingeot-Leclercq MP, Sonveaux E, Van Bambeke F, Tulkens PM.

Unite de Pharmacologie Cellulaire et Moleculaire, UCL 73-70, avenue E Mounier 73, B-1200 Brussels, Belgium.
hugues.chanteux@facm.ucl.ac.be

And with intracellular infection

We may be on the track of very active compounds against intracellular *S. aureus* …





Lemaire et al., submitted

Antimicrob Agents Chemother. 2003 Jul;47(7):2283-92. Related Articles, Links

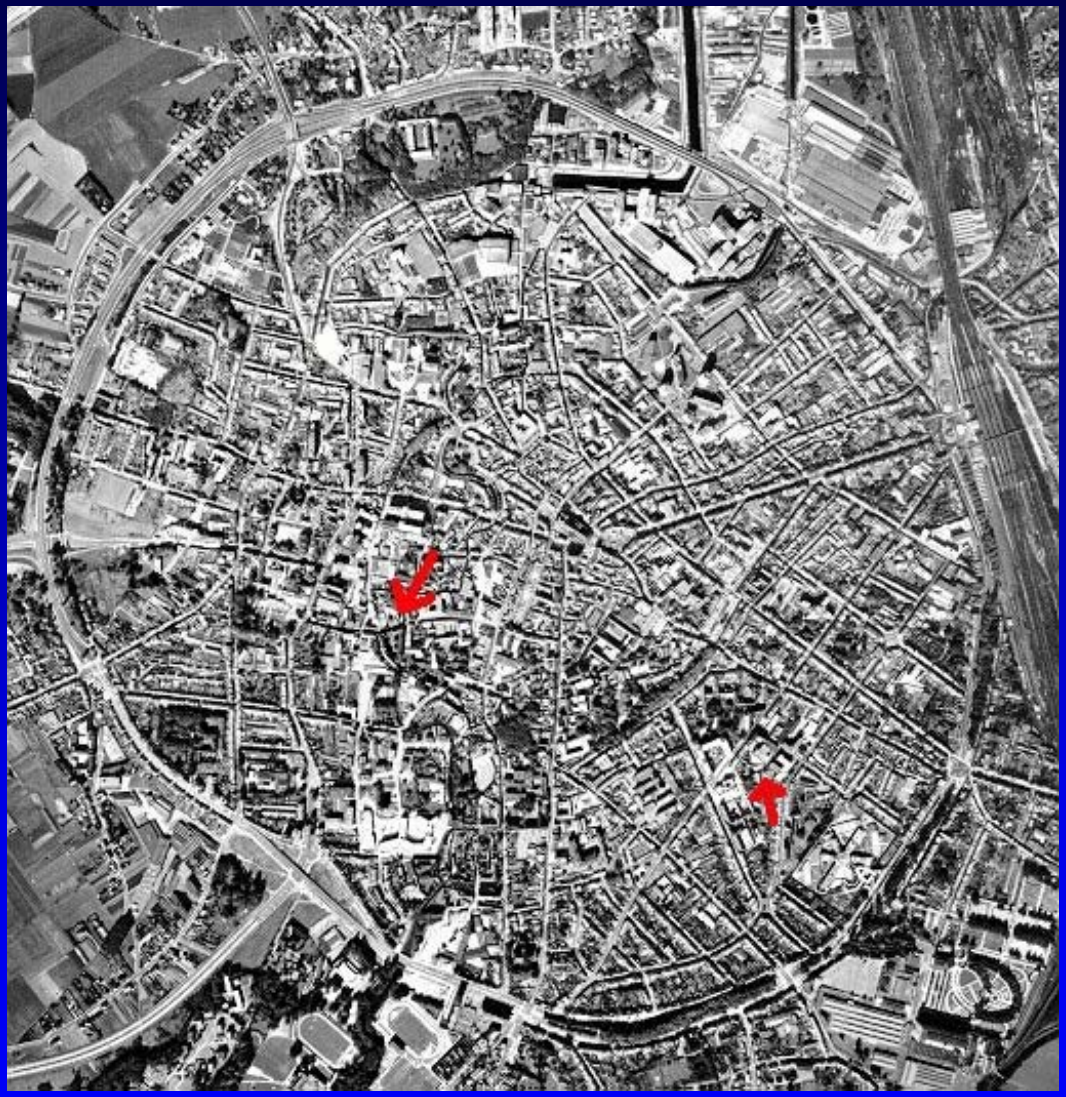
Full text article at aac.asm.org

Quantitative analysis of gentamicin, azithromycin, telithromycin, ciprofloxacin, moxifloxacin, and oritavancin (LY333328) activities against intracellular Staphylococcus aureus in mouse J774 macrophages.

Seral C, Van Bambeke F, Tulkens PM.

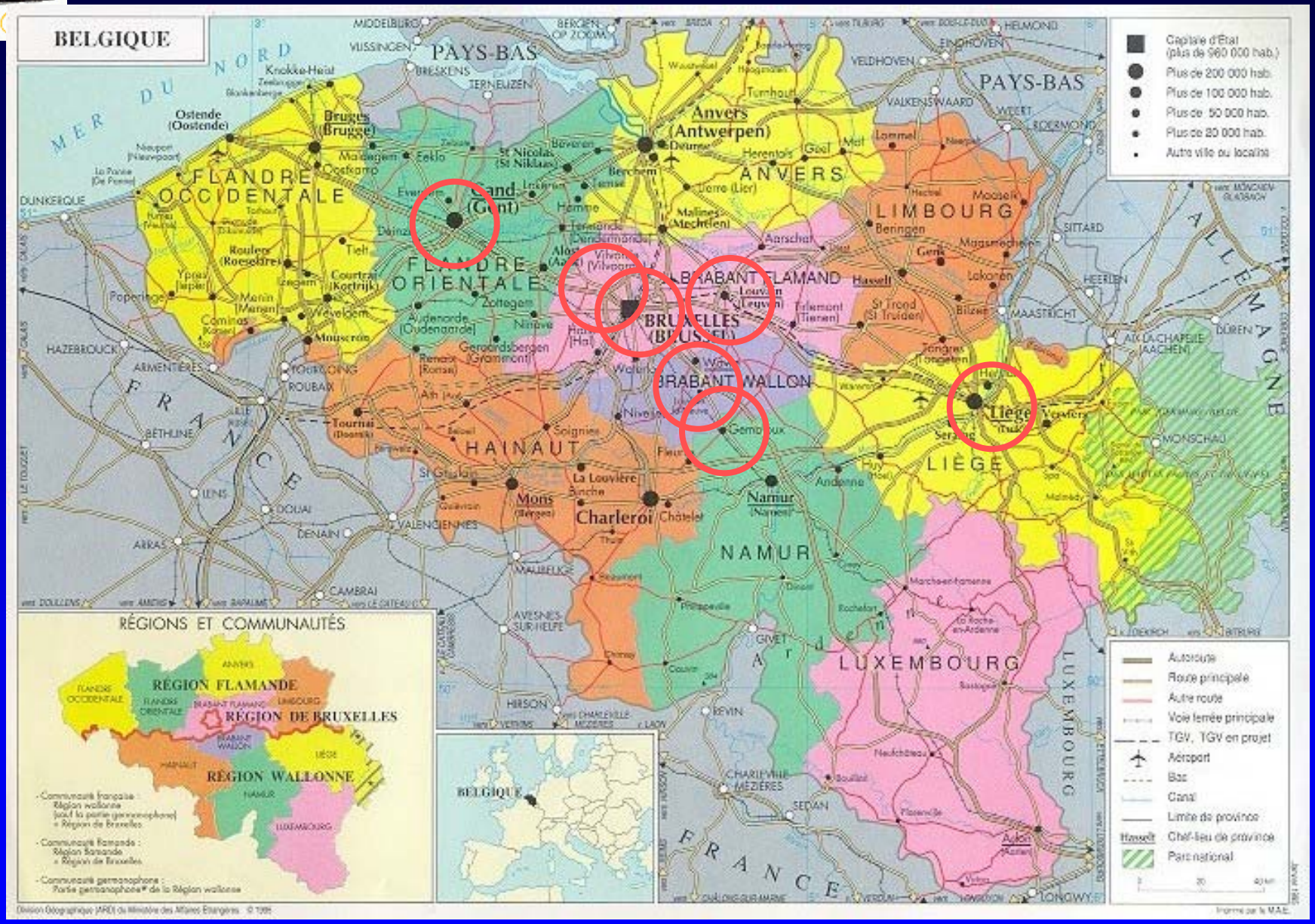
Unite de Pharmacologie Cellulaire et Moleculaire, Universite Catholique de Louvain, Brussels, Belgium.

Beyond dreams, reality started somewehere in beween…



Perhaps here … or there …

But continues on larger scale …



Indeed …

Personen :

- Prof. dr. FRERE J.-M. Université de Liège (ULG)
  Coördinator van het project
  Betoelaagde Belgische partner
  Duur: 1/1/2002-31/12/2006
- Prof. dr. BRASSEUR R. Faculté des Sciences Agronomiques de Gembloux (FSAGX)
  Betoelaagde Belgische partner
  Duur: 1/1/2002-31/12/2006
- Prof. dr. HERDEWIJN P. Katholieke Universiteit Leuven (KUL)
  Betoelaagde Belgische partner
  Duur: 1/1/2002-31/12/2006
- Prof. dr. TULKENS P. Université Catholique de Louvain (UCL)
  Betoelaagde Belgische partner
  Duur: 1/1/2002-31/12/2006
- Prof. dr. FASTREZ J. Université Catholique de Louvain (UCL)
  Betoelaagde Belgische partner
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- Prof. dr. VAN BEEUMEN J. Universiteit Gent (RUG)
  Betoelaagde Belgische partner
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- Prof. dr. WYNS L. Vrije Universiteit Brussel (VUB)
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- Prof. dr. ENGELBORGHS Y. Katholieke Universiteit Leuven (KUL)
  Niet-betoelaagde Belgische partner
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- Prof. dr. VAN DER EYCKEN J. Universiteit Gent (RUG)
  Niet-betoelaagde Belgische partner
  Duur: 1/1/2002-31/12/2006
- Prof. dr. DOBSON C. University of Cambridge (CAM)
  Betoelaagde buitenlandse partner
  Duur: 1/1/2002-31/12/2006

Ad multos annos …

En hartelijke dank aan
- Ann Van Schepdael
- R. Busson
- J. Desmyter,
- J. Balzarini,
- E. De Clercq,

… voor voodrachten, seminaries, …