The postantibiotic and sub-MIC effects in vitro and in vivo

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The postantibiotic effect in vitro

Postantibiotic effect; PAE in vitro

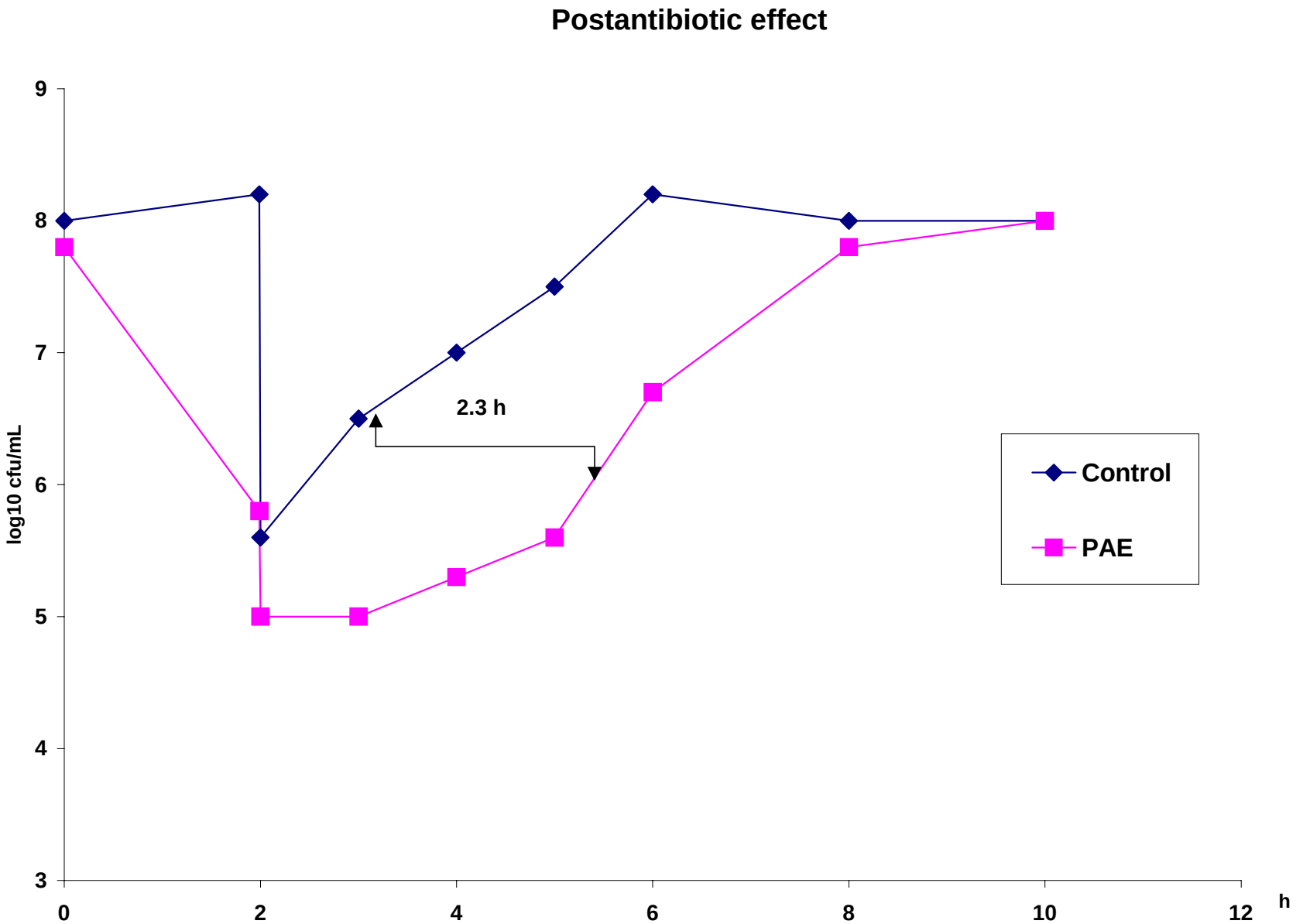
Definition:

- Suppression of bacterial growth after short exposure of organisms to antibiotics

  PAE=T-C

  T= The time required for the exposed culture to increase one $\log_{10}$ above the count observed immediately after drug removal

  C= The corresponding time for the unexposed control



Odenholt et al. SJID, 1988

Postantibiotic effect in vitro

The PAE is dependent on:

- **Type of antibiotic**
- **Type of bacterial species**
- **Concentration of the antibiotic**
- **Duration of exposure**
- **Size of the inoculum**
- **Growth phase of the organism**

PAE against Gram-positive bacteria

Antibiotics	*hours*
• Penicillins	1-2
• Cephalosporins	1-2
• Carbapenems	1-2
• Quinolones	1-3
• Proteinsythesis inhibitors	3-5

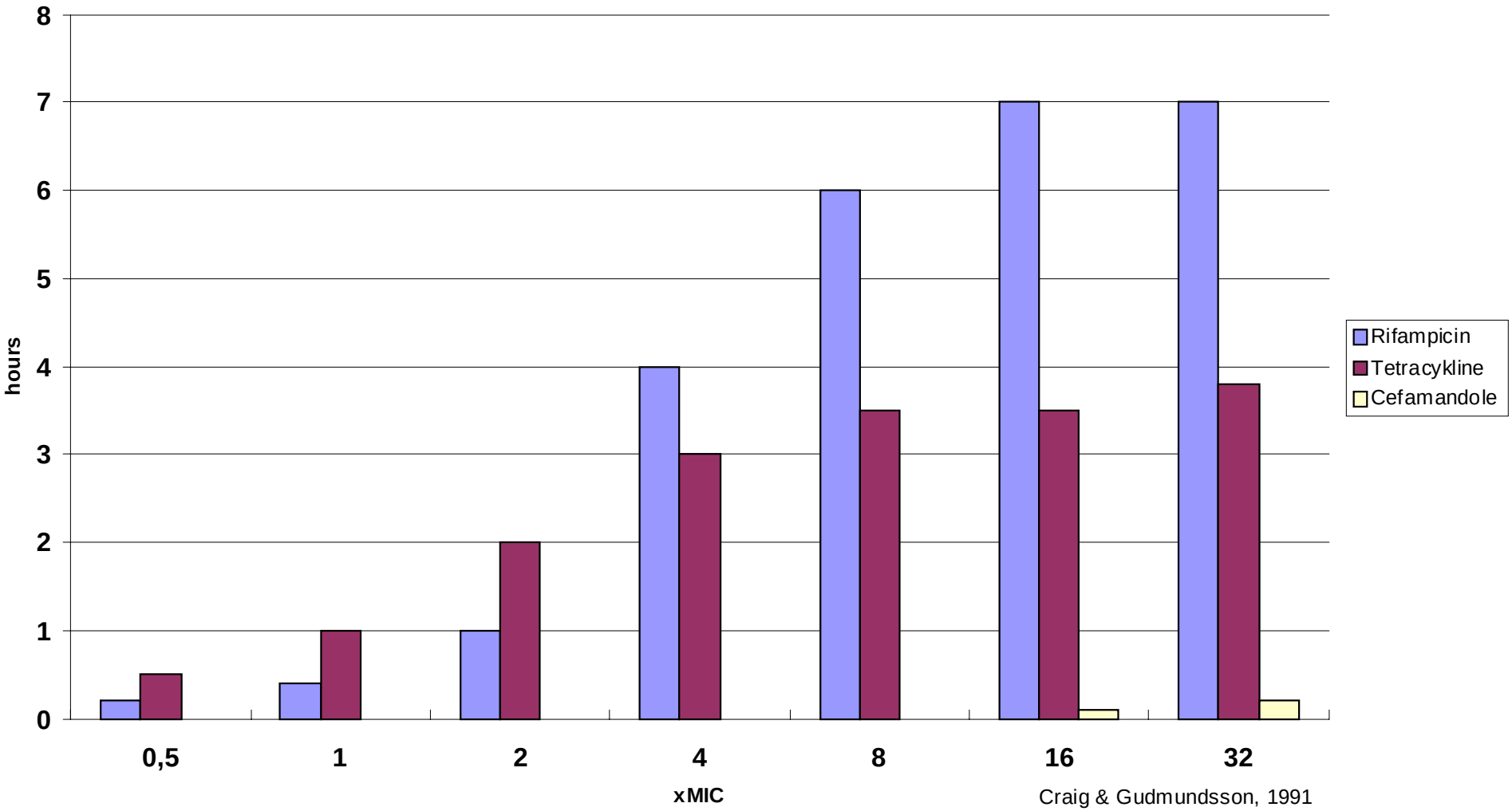
PAE against Gram-negative bacteria

Antibiotics	*hours*
• Penicillins	0
• Cephalosporins	0
• Carbapenems	(1)
• Quinolones	1-3
• Proteinsythesis inhibitors	3-8
• Aminoglycosides	2-4

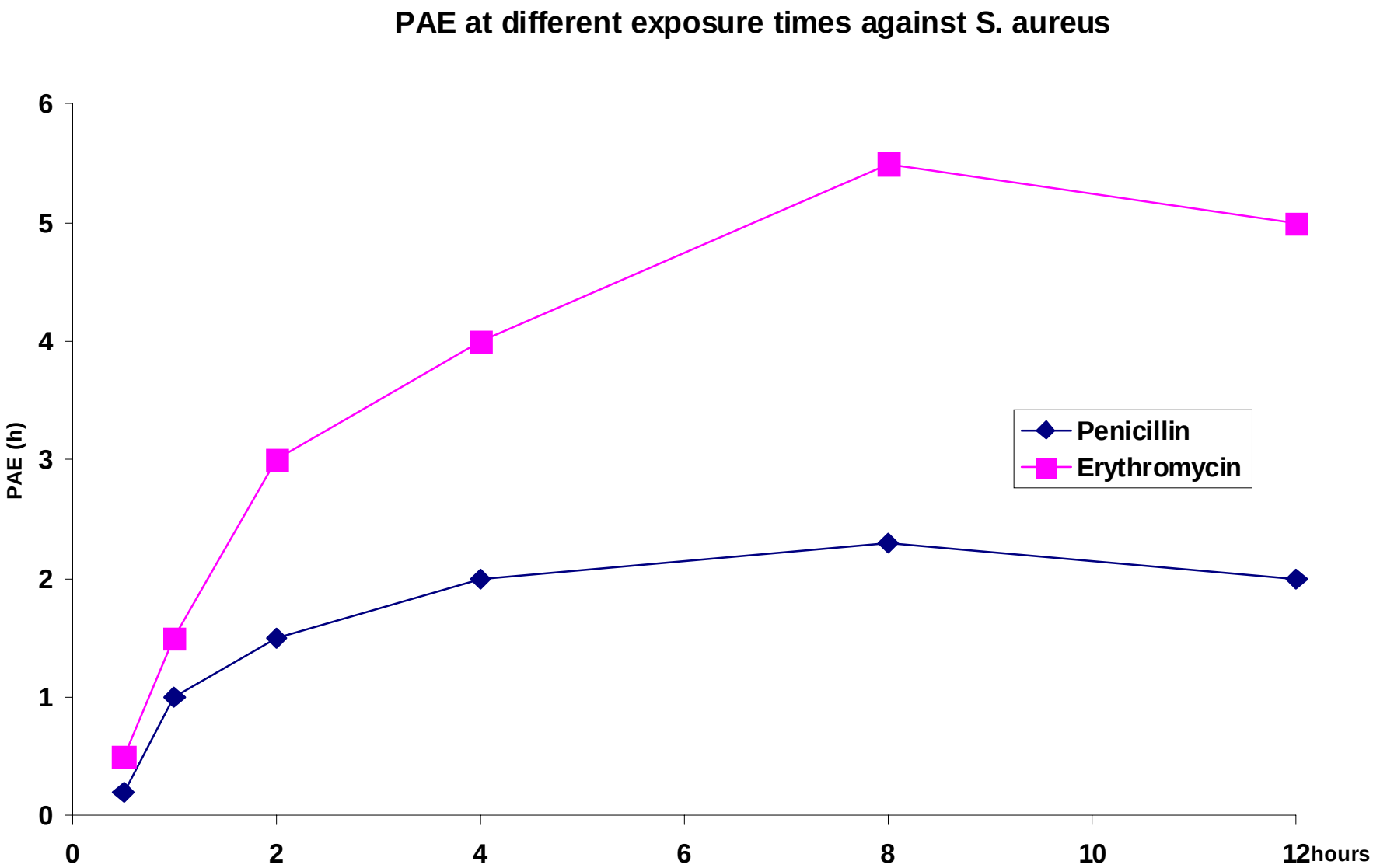
PAE against P. aeruginosa

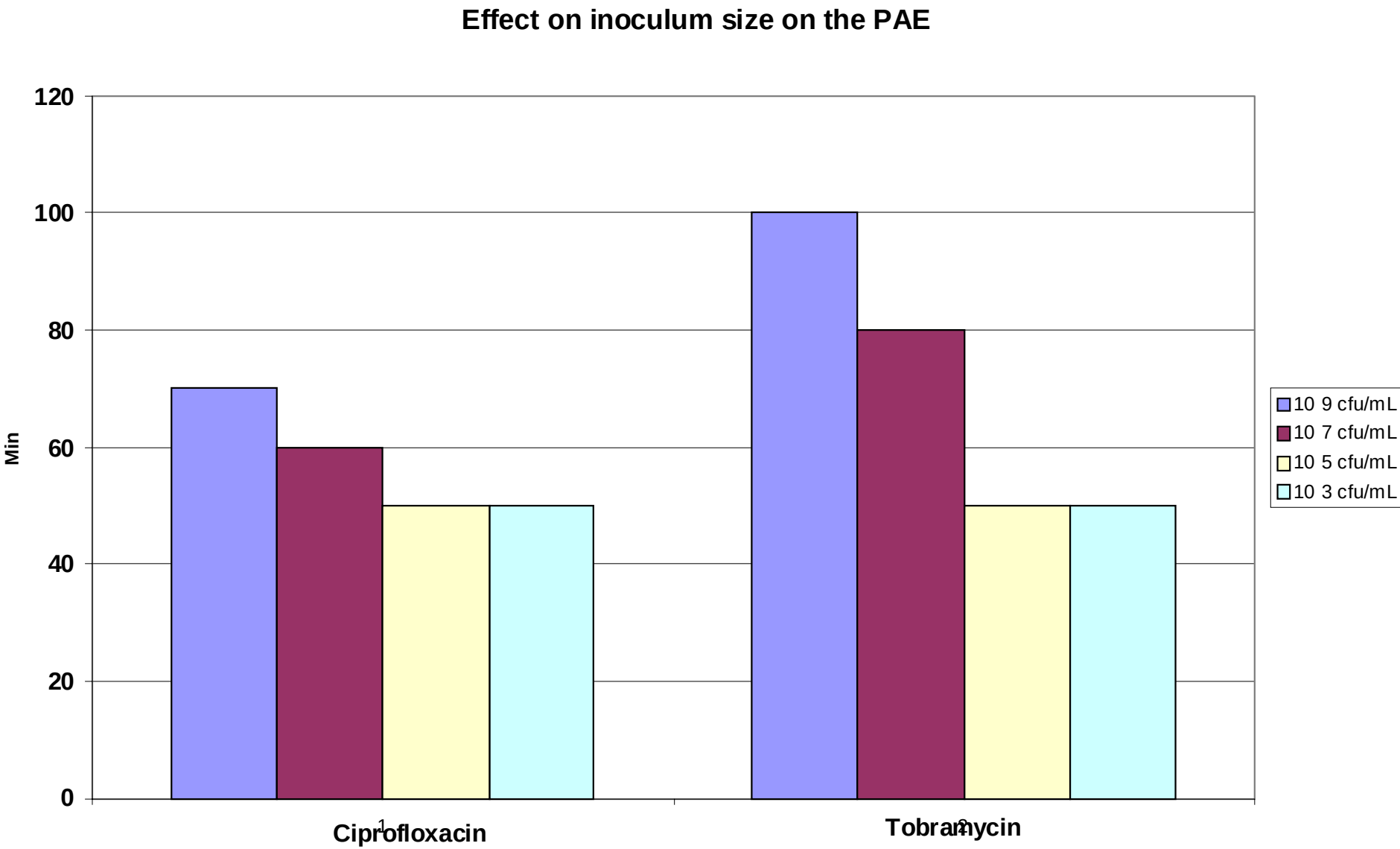
Antibiotics	*hours*
• Penicillins	0
• Cephalosporins	0
• Carbapenems	1-2
• Quinolones	1-2
• Aminoglycosides	2-3

The PAE at different concentrations against E. coli



Craig & Gudmundsson, 1991


PAE at different exposure times against S. aureus
PAE (h)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
hours
Penicillin
Erythromycin

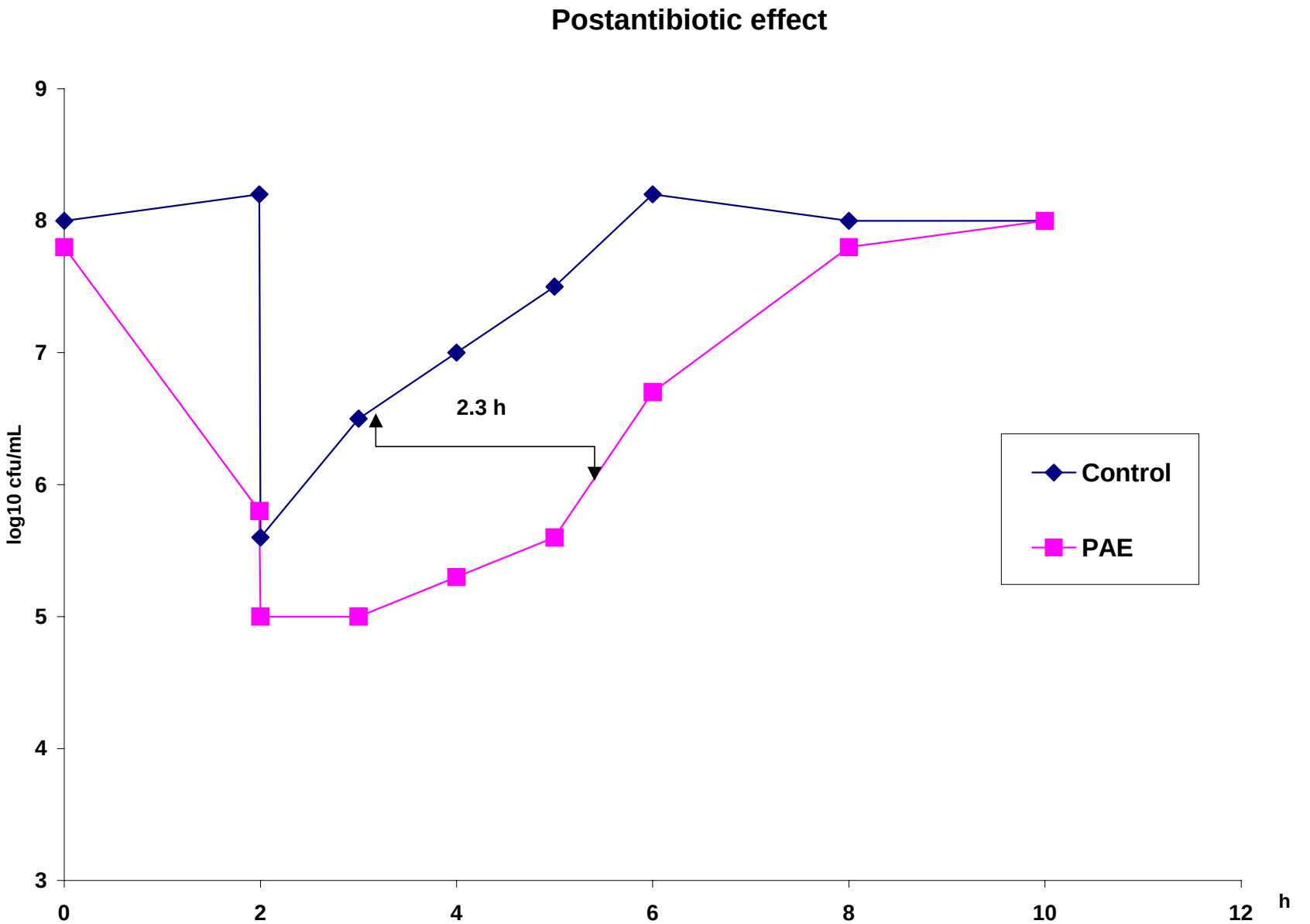

Effect on inoculum size on the PAE
120
100
80
60
40
20
0
Min
10 9 cfu/mL
10 7 cfu/mL
10 5 cfu/mL
10 3 cfu/mL
1
Ciprofloxacin
2
Tobramycin

PAE in vitro Methods

1. Viable counts

Methodological pitfalls

- may overestimate killing
- negative PAEs are common with ß-lactams and gram-negatives due to forming of filaments
- similar inocula of the control and the pre-exposed culture are desirable



Odenholt et al. SJID, 1988

PAE in vitro Methods

2. Optical density

Methodological pitfalls

- killing cannot be measured due to a detection limit of 10^6 cfu/ml

- control curves at different inocula and viable counts after drug removal are necessary to be performed to ensure that PAE culture and control are at the same inoculum

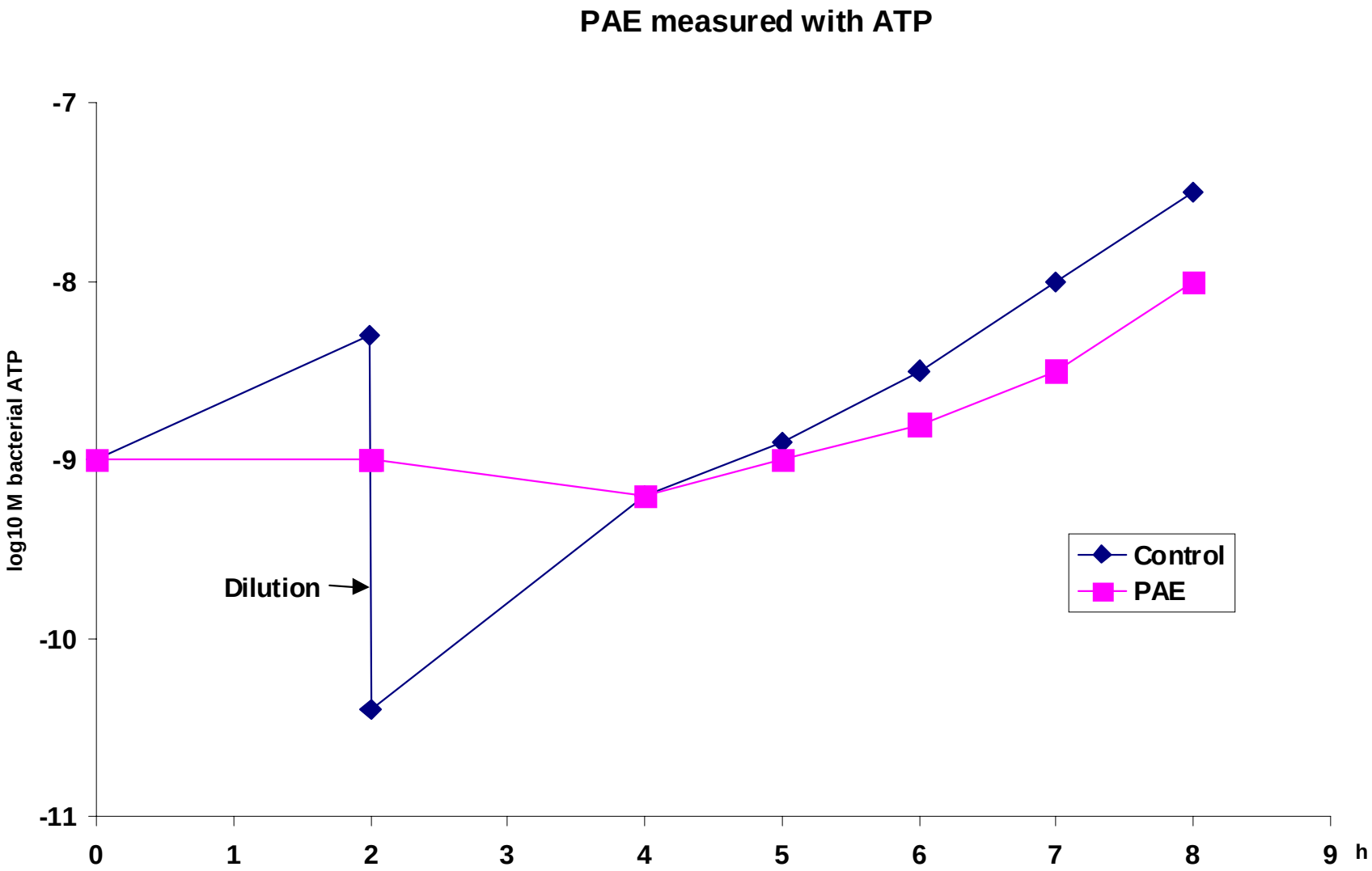
PAE in vitro
Methods

3. ATP measurement

Methodological pitfalls

- bactericidal activity is underestimated due to dead but intact (not lysed) bacteria still containing intracellular ATP

- PAE is overestimated due to falsely elevated ATP content


PAE measured with ATP
log10 M bacterial ATP
-7
-8
-9
-10
-11
0
1
2
3
4
5
6
7
8
9
h
Dilution
Control
PAE

PAE in vitro
Methods

4. Morphology

- Phase contrast microscopy
  - the time it takes for the bacteria to revert to 90% bacilli
- Ultrastructural changes
  - the changes in structure correlates well with the PAE measured with viable counting

5. ^{3}H-thymidine incorporation

-correlates well with the PAE measured with viable counting

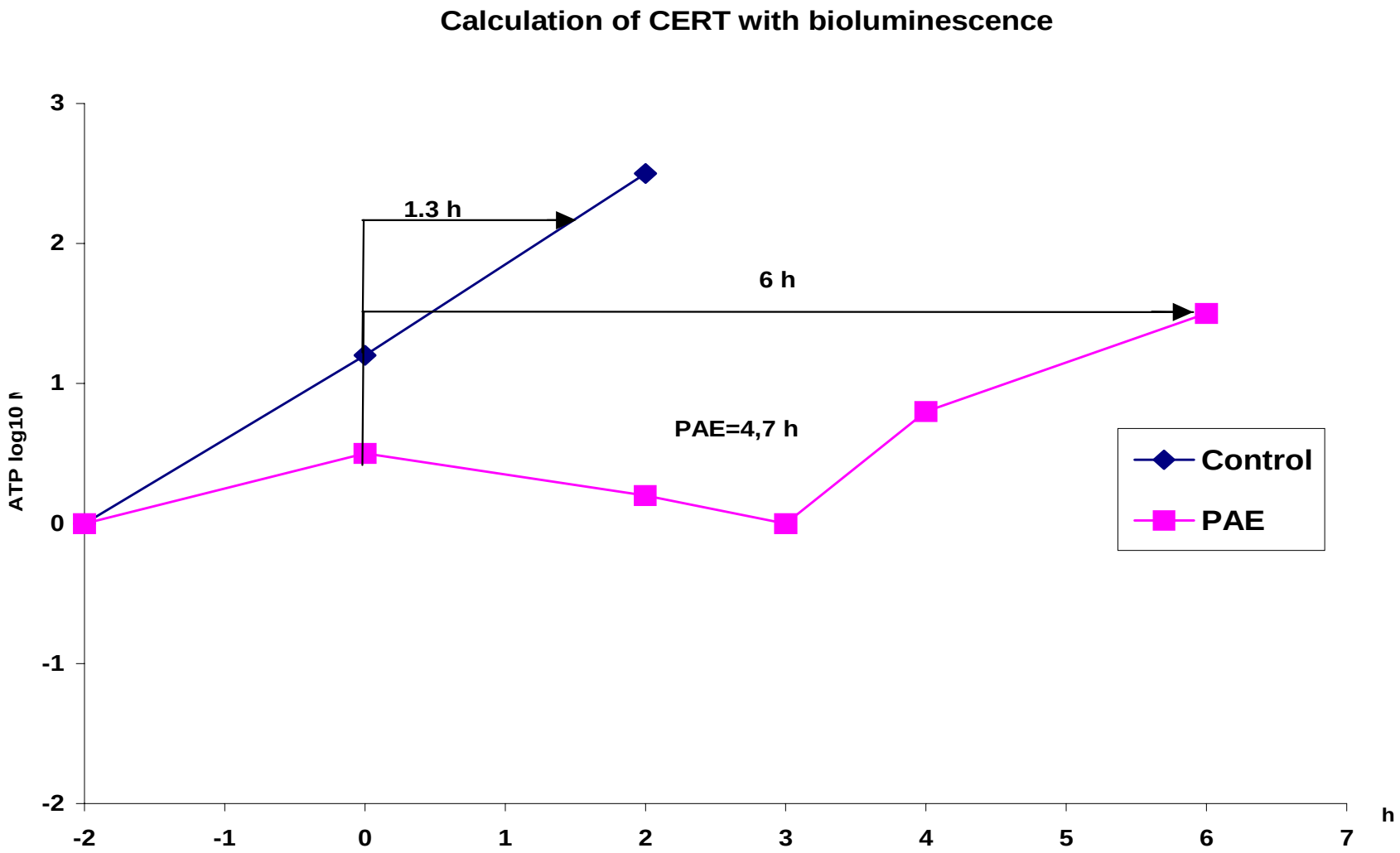
Control related effective regrowth time (CERT)

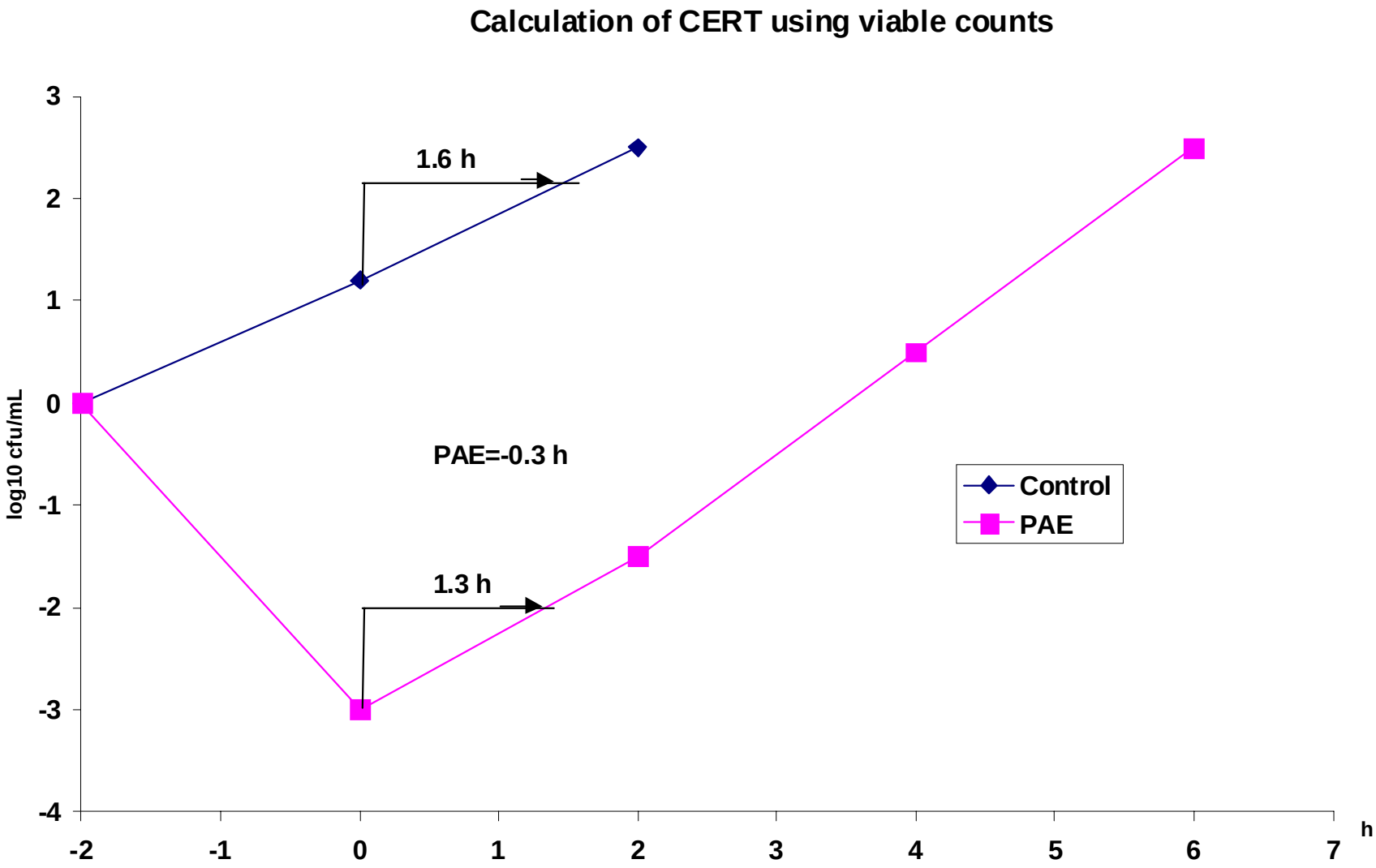
CERT

- Definition

  CERT=T-C

  T=the time required for resumption of logarithmic growth and increase of one $\log_{10}$ to occur over the preexposed inoculum of the test tube


Calculation of CERT with bioluminescence
3
2
1
0
-1
-2
ATP log10 r
1.3 h
6 h
PAE=4,7 h
Control
PAE
-2
-1
0
1
2
3
4
5
6
7
h


Calculation of CERT using viable counts
log10 cfu/mL
h
1.6 h
1.3 h
PAE=-0.3 h
Control
PAE
3
2
1
0
-1
-2
-3
-4
-2
-1
0
1
2
3
4
5
6
7

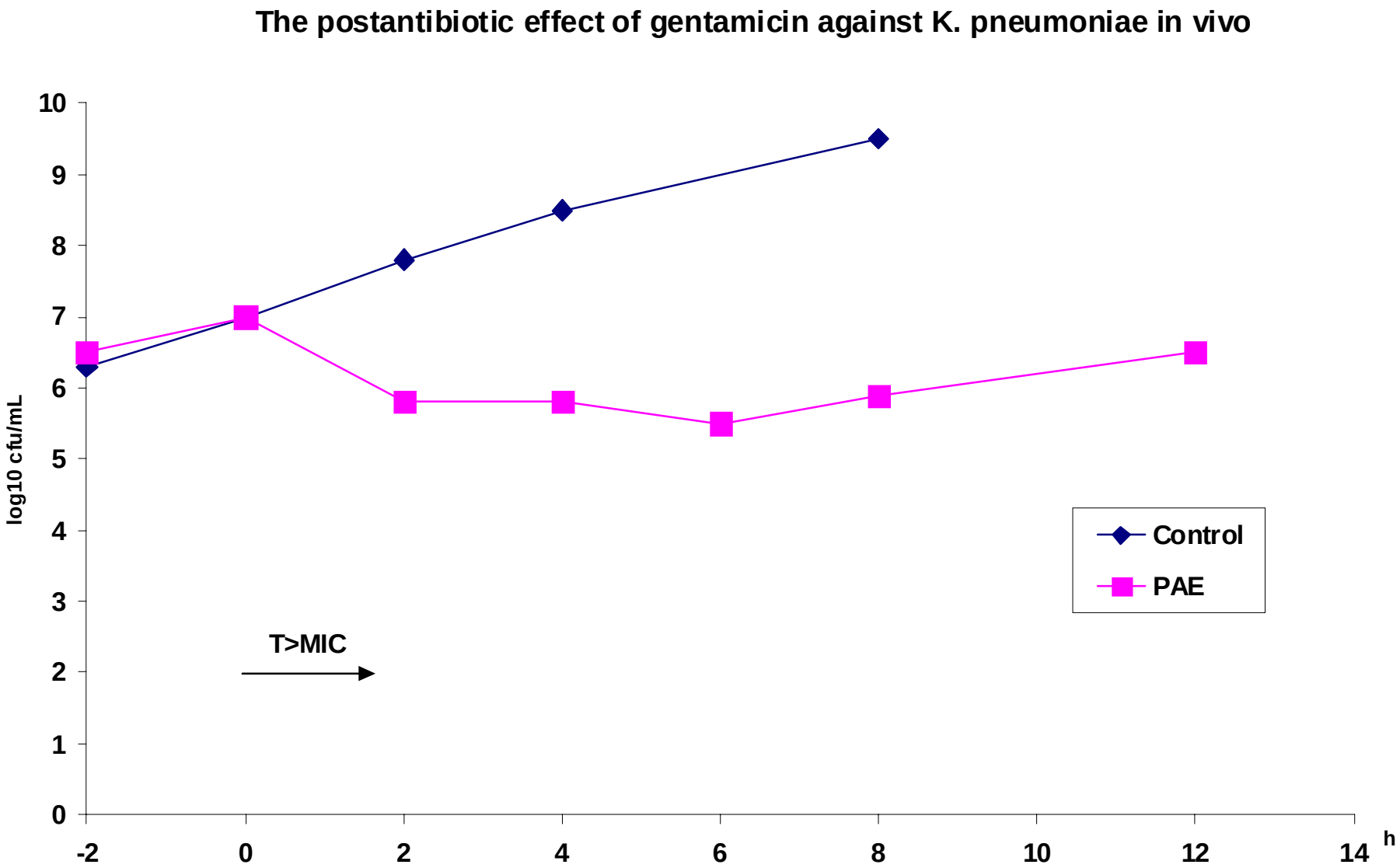
The postantibiotic effect in vivo

Postantibiotic effect in vivo

Definition

PAE= T-C-M

- T= the time required for the counts of cfu in thighs of treated mice to increase one $\log_{10}$ above the count closest to but not less than the time M
- C= the time required for the counts of cfu in thighs of untreated mice to increase one $\log_{10}$ above the count at time zero
- M= the time serum concentration exceeds the MIC



Fantin et al. JAC, 1990

PAE in vivo

- Observed in several animal models
- In vitro data are predictive of in vivo results except that in vivo PAE are usually longer due to the effect of sub-MICs and/or the effect of neutrophils
- The major unexplained discordant results are for ß-lactams and streptococci

PAE in vivo
Animal models

- Thigh infections in mice
- Pneumonia model in mice
- Infected treads in mice
- Infected tissue cages in rabbits
- Meningitis model in rabbits
- Endocarditis model in rats

Mechanisms of PAE

- *β-lactam antibiotics.*

  At least for *S. pyogenes* and penicillin it has been shown that PAE stands for the time it takes for the bacteria to resynthesize new PBPs

Mechanisms of PAE

- *Erythromycin and clarithromycin*:

  50S ribosomal subunits were reduced during 90 min and protein synthesis during 4 h (PAE) due to prolonged binding of the antibiotics to 50S.

Mechanisms of PAE

- *Aminoglycosides:*

  Binding of sublethal amounts of drug enough to disrupt DNA, RNA and protein synthesis. The time it takes to resynthesize these proteins.

  With a half-life of >2.5 h, the PAE disappears, reflecting a sufficient time for the repair mechanism to be restored.

The postantibiotic sub-MIC effect in vitro

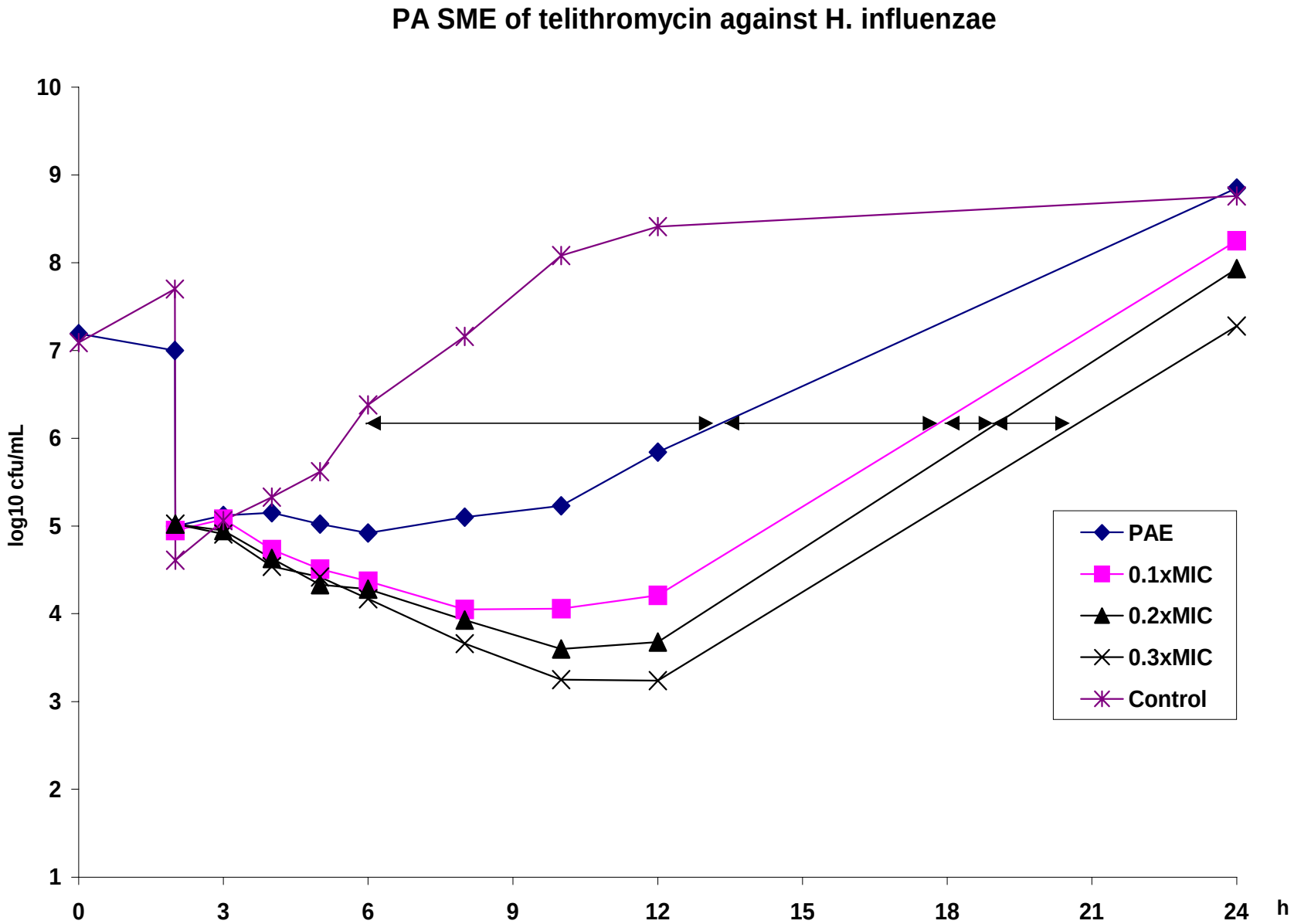
Postantibiotic sub-MIC effect; PA SME

Definition

- The effect of subinhibitory antibiotic concentrations on bacteria previously exposed to suprainhibitory concentrations

PA SME= T_{PA}-C

- T_{PA}=the time it takes for the cultures previously exposed to antibiotics and thereafter to sub-MICs to increase by one $\log_{10}$ above the counts observed immediately after washing.
- C=corresponding time for the unexposed control

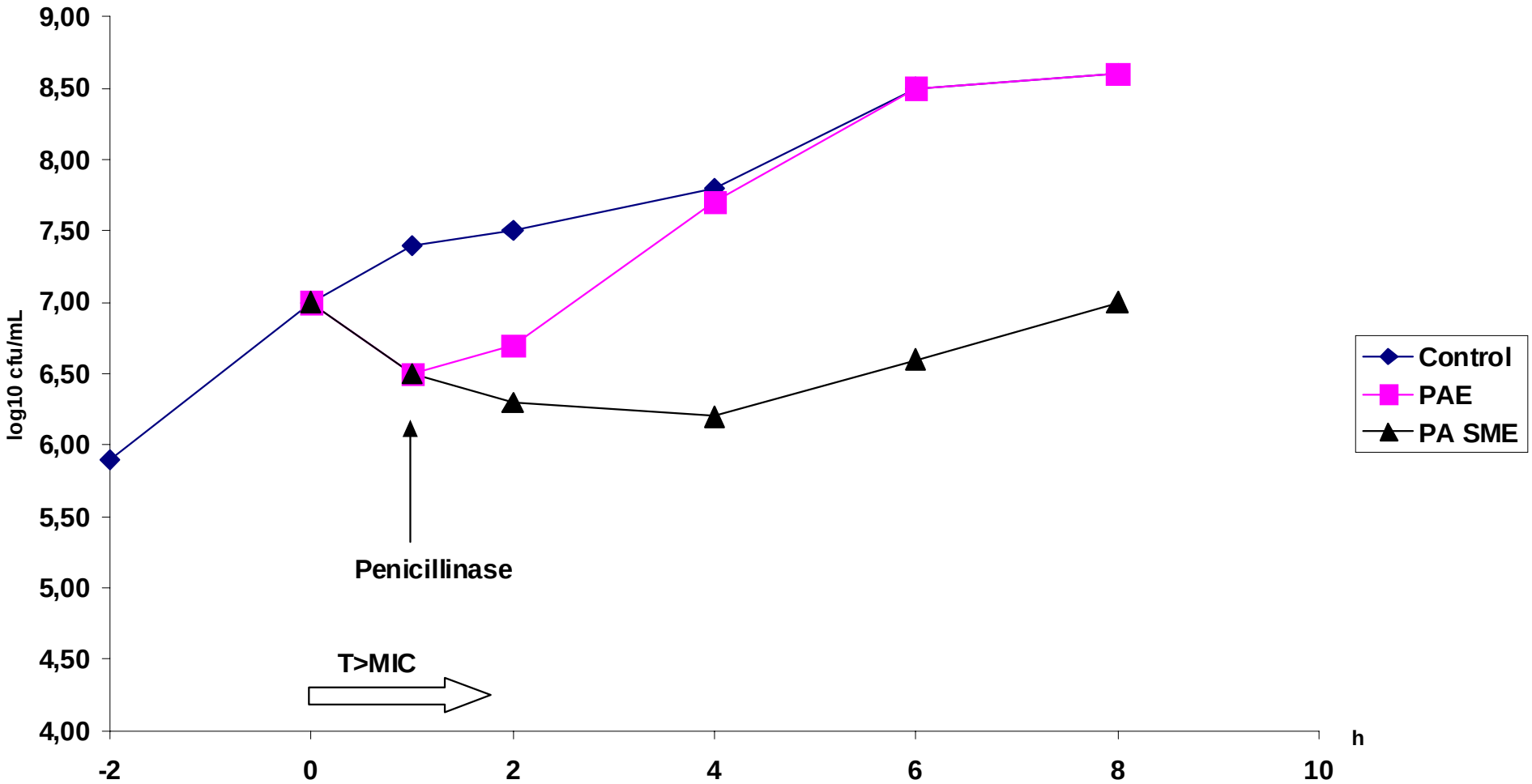

PA SME of telithromycin against H. influenzae
log10 cfu/mL
10
9
8
7
6
5
4
3
2
1
0
3
6
9
12
15
18
21
24
h
PAE
0.1xMIC
0.2xMIC
0.3xMIC
Control

The postantibiotic sub-MIC effect in vivo

PAE (PASME) in vivo of amikacin against K. pneumoniae in a thigh-infection model in mice

	PAE
• Normal mice (half-life 19 min)	5.5 h
• Uremic mice (half-life 98 min)	14.6 h

The PAE and PA SME of piperacillin against S. aureus in vivo



Oshida et al. JAC, 1990

Post-MIC effect (PME)

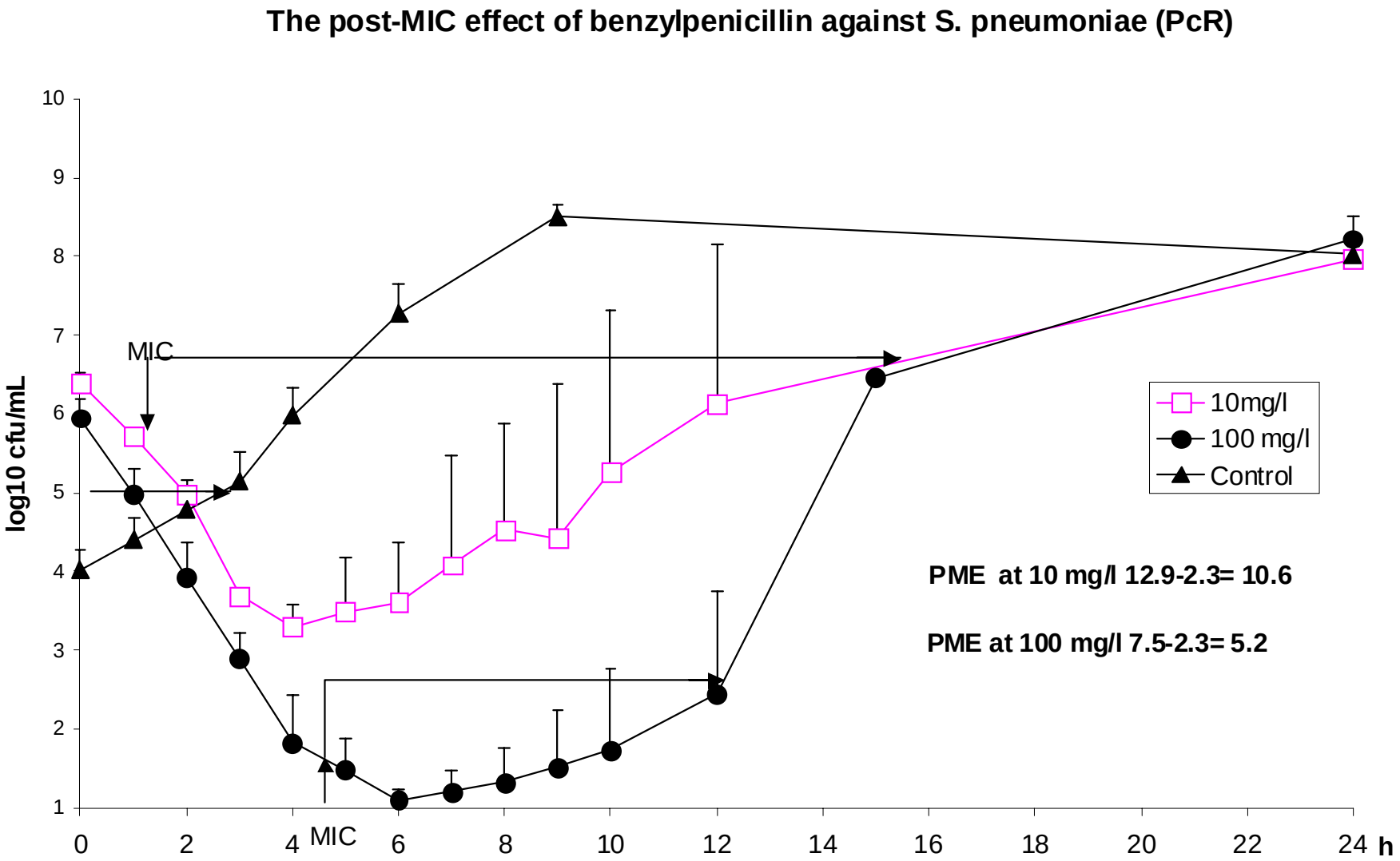
Post-MIC effect; PME

Definition

- The effect of sub-MICs on bacteria previously exposed to a constant decreasing antibiotic concentration

PME=Tpme-C

- Tpme= The time for the counts in cfu of the exposed culture to increase one $\log_{10}$ above the count observed at the MIC level
- C= the time for an unexposed control to increase one $\log_{10}$


The post-MIC effect of benzylpenicillin against S. pneumoniae (PcR)
log10 cfu/mL
10
9
8
7
6
5
4
3
2
1
MIC
MIC
0
2
4
6
8
10
12
14
16
18
20
22
24
h
10mg/l
100 mg/l
Control
PME at 10 mg/l 12.9-2.3= 10.6
PME at 100 mg/l 7.5-2.3= 5.2

Mechanism of PA SME?

- The PAE of ß-lactam antibiotics seems to represent the time necessary to synthesize new PBPs. When bacteria in the PA-phase are exposed to sub-MICs, most PBPs are still inactivated and only a small amount of the drug is needed to prolong the inhibition of cell multiplication until a critical number of free PBPs are once more available

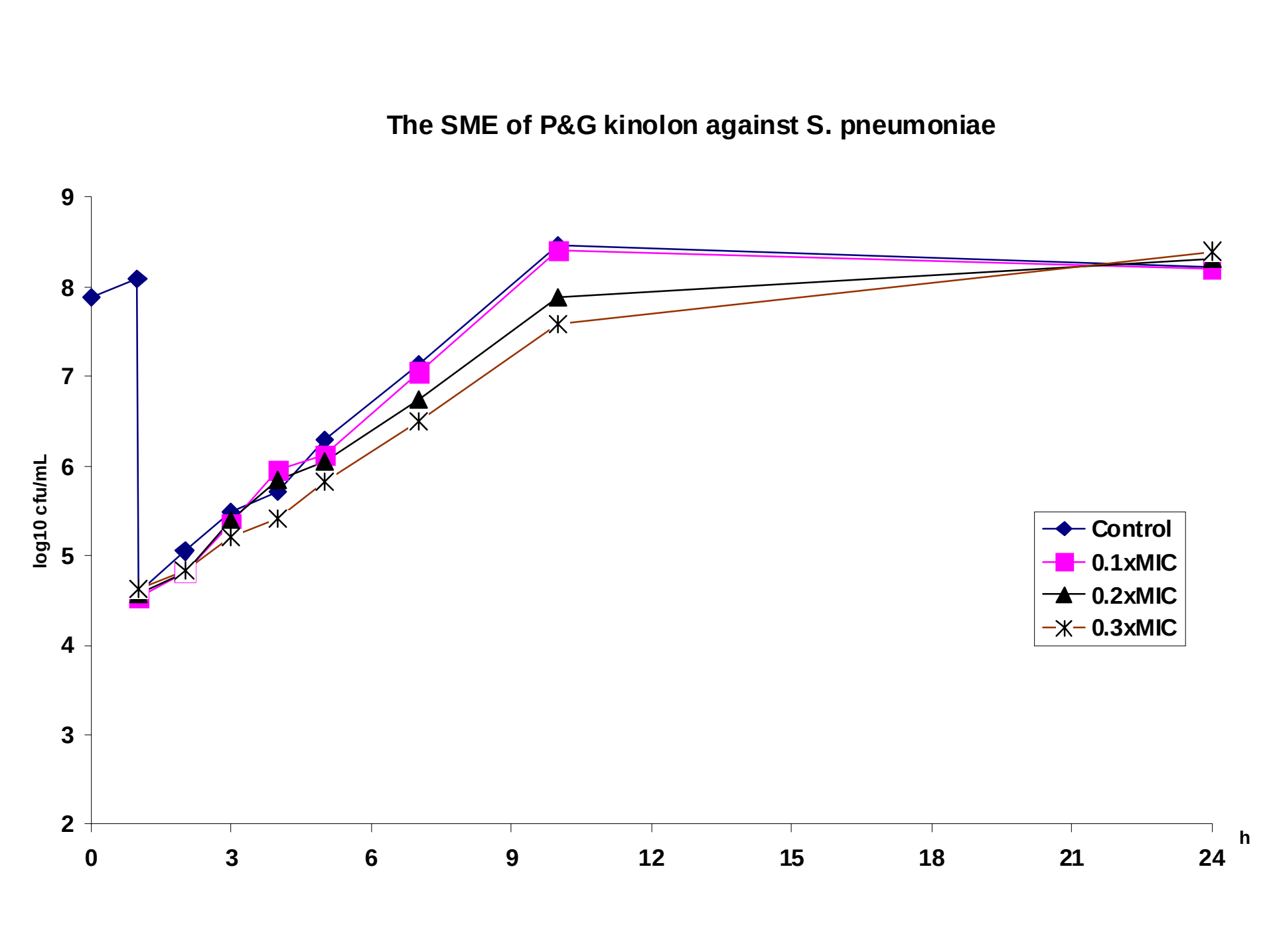
Postantibiotic leucocyte enhancement

Postantibiotic leucocyte enhancement; PALE

- Bacteria pretreated with antibiotics for a brief period of time show increased susceptibility to intracellular killing and phagocytosis

- In general, antibiotics that produce the longest PAEs exhibit maximal PALEs

Sub-MIC effects


The SME of P&G kinolon against S. pneumoniae
log10 cfu/mL
9
8
7
6
5
4
3
2
0
3
6
9
12
15
18
21
24
h
Control
0.1xMIC
0.2xMIC
0.3xMIC

Sub-MIC effects; SME

Definition

- The effect of subinhibitory antibiotic concentrations on bacteria not previously exposed to suprainhibitory concentrations

SME= T_s-C

- T_s=the time it takes for the cultures exposed to sub-MICs to increase by one $\log_{10}$ above the counts observed immediately after washing
- C=corresponding time for the unexposed control

Sub-MIC effects

- The minimum antibiotic concentrations that produces a structural change in bacteria seen by light or electron microscopy
- The minimum antibiotic concentration that produces a one $\log_{10}$ decrease in the bacterial population compared to the control
- Loss or change of bacterial toxins

Sub-MIC effects

- Loss of surface antigens resulting in decreased adhesion
- Increased rates of phagocytic ingestion and killing
- Increased chemotaxsis and opsonization

Mechanism of sub-MIC effects

- SME probably tests the distribution of antibiotic susceptibility in the bacterial population, in which there are subpopulations that are inhibited by concentrations less than the MIC. The SME would therefore represent the time it takes for the population with the higher MIC to become dominant

Implications

- The combined effects of supra- and subinhibitory concentrations seem to be more important for dosing regimens then PAE itself.

- A long PA SME/PME indicate that longer dosing intervals may be used even for antibiotics, which are dependent on the T>MIC for efficacy