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The issue of the true postantibiotic effect

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Sir,

The postantibiotic effect (PAE) is often defined as the persistent inhibition of bacterial growth after a brief exposure to an antibiotic Viable counts have been the most widely used method for determining the duration of the PAE, but are subject to several problems. In particular, the results are distorted when tests are performed with antibiotics that cause filament formation. This point is relevant to some penicillins and cephalosporins. These agents preferentially bind to penicillin-binding protein 3 in *Escherichia coli*, causing the cells to grow as long filaments. When the antibiotic is removed from the medium the filaments undergo septation, and divide to give large numbers of individual bacteria. This causes an apparently faster increase in the viable counts in the antibiotic-exposed cultures than in the unexposed controls. The PAE thus appears negative when calculated by the widely used method of comparing the period for a 1-log increase in the viable counts of an antibiotic-exposed culture with the period required for the same increase in an antibiotic unexposed culture.

In an attempt to overcome this problem, Kroeker, Karlowsky & Zhanel (1995) described an alternative method for calculating the duration of the PAE from viable counts data. They suggested extrapolating the growth curve for the antibiotic-exposed culture "from the portion of the curve demonstrating conventional regrowth kinetics to a new point at time zero" and thereby, using the viable count at that point as the starting value for calculating the duration of the PAE. Whereas their calculation method attempted to overcome the problem of negative PAEs, as defined by the recovery of normal bacterial growth, it did not provide any clue as to what they call the "true" PAE.

The widely used definition of the PAE is a simplified view of what is happening in bacteria following exposure to antibiotics. A broader definition of the PAE was proposed by Bergeron (1992), who defined the PAE as "the persistence of an antibiotic effect for a variable period after cessation of exposure of microorganisms to an effective antibiotic". This definition acknowledges the fact that the activities of an antibiotic may be broader than simply inhibiting growth and that additional effects may be of clinical significance. This has been shown by several workers. For instance Hanberger *et al.* (1990) showed that when PAEs of ceftazidime were measured for *E. coli*, by a bioluminescent assay of ATP, which is not affected by cell morphology, PAEs with a positive duration were found. When measured by viable counts, negative PAEs were observed. Similarly, we showed that the total cellular metabolism, as measured by release of charged metabolites, remained inhibited after exposure to antibiotic, even though cells were multiplying normally on agar plates (Majcherczyk *et al.*, 1994). Ramadan *et al.* (1995) also showed that bacterial cell surface charge was altered during the PAE. This was unrelated to bacterial growth rates following exposure to antibiotic. Finally, *E. coli* and *Staphylococcus aureus* pre-treated with antibiotics were shown to be more susceptible to killing by leucocytes than untreated bacteria (Pruul & McDonald, 1990).

A further fundamental criticism of the currently used simple definition of the PAE stems from the fact that it assumes that the cell

population is homogeneous and that recovery of each individual cell follows an identical time-course. However, it has been shown that the cell size distribution of a bacterial population was significantly reduced, even though viable counts of the culture were increasing at the normal rate (Majcherczyk *et al.* 1994). Such physiologically altered populations are likely to differ in their susceptibility to phagocytosis, and in their ability to produce toxins and other pathogenic properties.

It is thus clear that the physiology (cell size, adherence, susceptibility to killing by host defences) of bacterial cells is greatly altered following exposure to antibiotics and that these parameters take a longer time to recover than the ability to form a visible biomass on an agar plate. Therefore, in attempting to establish the "true" PAE such parameters should be measured, rather than solely manipulating viable counts data.

DNA, RNA, protein and peptidoglycan synthesis can be measured in recovering cells by monitoring uptake of the radiolabelled precursors of these macromolecules. These results would give a measure of the recovery of the cellular metabolism. Moreover, these parameters are not artefactually affected by cellular morphology. Measuring release of charged metabolites by monitoring impedance changes of the culture medium could further give a measure to what extent cells had recovered from the effects of antibiotic treatment. Future studies of the PAE should certainly consider these factors. This would then give a clearer understanding of the "true" PAE. Ultimately though, significance of the "true" PAE would have to be correlated to the pharmacodynamics of antibiotics in animal models in order to determine the important parameters for antibiotic efficacy *in vivo*

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Reply

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Sir,

The postantibiotic effect (PAE) is defined as the period of bacterial growth suppression following complete removal of extracellular antibiotic. Using viable counts, the PAE is often calculated to be significantly less than zero (time units) for Gram-negative bacilli following exposure to β-lactam antibiotics. The aetiology underlying this phenomenon has been suggested to involve β-lactam induced bacterial filamentation. Following β-lactam removal, filaments separate into numerous cells and thus it appears that there has actually been a greater rate of regrowth in antibiotic exposed cultures than in growth control cultures. The intention of our study was to address and clarify the issue of filamentation and postantibiotic effect. More specifically, we wished to recommend a method for calculating the β-lactam induced PAE in Gram-negative bacilli using the viable count method. This method would ideally yield the actual or "true" numerical PAE and not an artificially negative PAE (Kroeker, Karlowsky & Zhanel, 1995).

Dr Majcherczyk suggests that a broader definition of the PAE should be considered and that it should account for "the persistence of an antibiotic effect for a variable period after cessation of exposure of microorganisms to an effective antibiotic". This would acknowledge that subinhibitory antibiotic concentrations have various physiological effects on bacteria besides simply affecting bacterial