methodological problems of both bactericidal titrations and the expression of tolerance. The method we used was carefully standardised to take account of factors that influence the end point, including those highlighted in the paper by Taylor et al.1

The agar droplet method was used only in the construction of killing curves. The theoretical problems of thermal injury to bacteria diluted in molten agar at 45°C do not, in fact, seem to be borne out in practice.2 Even at 48°C, temperature effects seem to be small.3 Moreover, this technique, which is, in essence, a highly economical and simplified version of the pour-plate method, offers the important advantage that multiple counts (quintuplicate in the case of the work we describe) can easily be carried out at each data point—a statistical provision that is often overlooked in viable counting. A more serious pitfall than possible thermal injury in the estimation of the viability of streptococci is provided by the propensity of these bacteria to grow in chains of varying length; within these chains (colony forming units) most bacteria may be killed, but a single survivor has the potential to form a colony.

The question of the Eagle effect in non-tolerant strains is addressed in the paper: the Eagle effect was not displayed by any of 13 non-tolerant strains in which a sufficient number of survivors was detected. Furthermore, fig 2 (incorrectly described as fig 1 in the text) shows a strong correlation between the Eagle effect and the tolerance phenotype as expressed by logarithmic phase inocula.

I heartily agree with Woolfrey and Lally when they draw attention to the arbitrary nature of the 99-9% killing criterion of bactericidal activity, and the folly of using this endpoint uncritically. We approached our study in the expectation that we would find a continuous spectrum in the rate of killing of various strains of viridans streptococci, but in the event we did not: nearly all the “non-tolerant” strains yielded less than 0-02% of survivors after overnight incubation; with all the strains designated as “tolerant” >0-1% of the inoculum survived, by definition. Thus while the 99-9% criterion may be arbitrary, it seemed (in this admittedly somewhat circumscribed study) to be useful in separating the two halves of a bimodal distribution.

Much remains to be learned about penicillin tolerance and its expression and much nonsense has been written on the subject. Woolfrey and Lally are right to be sceptical (as I am) of findings in an area in which results can be manipulated almost at will by changes in the conditions of the test. There is little doubt, however, that tolerance to penicillin is a real phenomenon and that, when present, it has therapeutic importance in conditions in which bactericidal activity is crucial to therapeutic success. Indeed, in enterococci, in which tolerance to penicillin is the norm, this has been accepted for many years. The problem is to define the precise criteria by which a strain can be correctly and reproducibly defined as tolerant. Our study is a small contribution to this on-going debate.

References

Tolerance to penicillin in streptococi of viridans group

We read with great interest the paper by Powley, Meeson, and Greenwood regarding the phenomenon of tolerance in “viridans” type streptococci.1

We have just completed a similar survey of 16 of our strains isolated from patients with confirmed endocarditis. Of the five strains of S. sanguis found to be tolerant when tested in stationary phase as opposed to logarithmic phase. The fifth strain remained tolerant by both methods. All five strains of S. mitis tested were found to be fully sensitive in both phases, and the three strains of S. bovis were tolerant. A haemolytic Streptococcus Lancefield group G and a S. mutans would have been classified as sensitive if tested in the log phase, but exhibited tolerance in the stationary phase. A very slow growing strain of S. mutans was tolerant in both growth phases. Similar results were obtained when four hour “time kill” studies were carried out, where, in addition, a strong Eagle’s effect5 was shown at higher concentrations of antibiotics (MIC × 16 or MIC × 128) for the tolerant strains.

The concept of tolerance, although poorly understood, has important clinical implications, underlined by the treatment recommendations of the BSAC working party.1 This was further shown by our recent experience with three patients recently referred to this regional cardiothoracic centre. All three had received treatment for their endocarditis with a single agent, on the basis of MIC/MBC tests which did not seem to show tolerance. All three had been referred as “treatment failures”.

Stationary phase broth dilution MIC/MBC tests and kill curves were performed in our department, and the organisms reclassified as tolerant on the basis of MIC:MBC ratios of >32, or less than a 1000-fold reduction in viable counts over four hours. All three made good bacteriological and clinical recoveries following the addition of an aminoglycoside to the regimen, with predictably good results in their serum bactericidal tests.

In view of the paucity of information about the rate of in vivo bacterial multiplication in established vegetations, the assumption should not be made that all organisms are in log phase. In vitro tests carried out in stationary phase are probably a truer reflection of in vivo bacterial growth. This would permit a more predictable correlation between in vitro results and clinical outcome, and more patients would be treated in line with the recommendations of the BSAC working party.

References

Prognostic variables in large bowel cancer

Halvorsen and Seim claim to have identified two new independent prognostic variables in large bowel cancer. These are peritumoural fibrosis and perivascular lymphocytic cuffs.

They contrast their findings with ours, state