Letters to the Editor

(fig 1a). In one patient a large spiral bacterium resembling that recently observed by Dent et al was seen on carbol-fuchsin stained sections (fig 1b). This bacterium was not seen in tissue sections by PAP and haematoxylin and eosin staining. The histopathology of this patient's biopsy specimen showed active chronic gastritis. Culture and PAP method did not detect Campylobacter pylori.

Our findings show that carbol-fuchsin staining is simple, quick, cheap and very suitable for the routine demonstration of spiral bacteria in tissue sections of gastric mucosa.

Conversely, rapidly killed strains would show plate counts of < 10 cfu, thus presenting a situation in which too few or no colonies were present to show the paradoxical effect. If the authors had used inoculum preparations of higher density so that a 99-99% colony reduction breakpoint would have approximated 100 cfu/plate or more, the paradoxical effect would probably have been observed as often for rapidly killed strains as it was for slowly killed strains. In our own studies using an agar dilution plate-count method, we found that the paradoxical effect was demonstrable at some time in the killing sequence for all Haemophilus influenzae and Staphylococcus aureus strains that we studied and we would anticipate similar findings for viridans streptococci.

In the Discussion section of their paper Powley et al stated that: "the results illustrate the arbitrary nature of the 99-99% killing criterion usually applied to bactericidal activity", with which we agreed but were then disappointed with their conclusion that: "the present results suggest that this arbitrary cut off point is, perhaps fortuitously, a useful ...". As we and others have previously discussed, the measurement of bactericidal action cannot meaningfully be reduced to a single arbitrary and artificial index such as the MBC, the use of which has unfortunately promoted the "all-or-none" concept of antimicrobial tolerance. Our own studies with H influenzae and S aureus indicate that the bactericidal action of β lactam producing agents is strain dependent, with strains showing a spectrum ranging from some that are rapidly killed to others that are more slowly killed. This situation pre-empts absolute classification of a strain as tolerant or not. Much work needs to be done in developing useful methods for measuring bactericidal action and in defining species reference strains representing those that are slowly, moderately, and rapidly killed. The putative, relative nature of bactericidal action and the apparent universal presence and variation in form of the paradoxical effect must clearly be kept in mind for future studies designed to evaluate whether therapeutic success may in any way be related to bactericidal response.

Tolerance to penicillin in streptococci of viridans group

The paper by Powley et al that appeared in the Journal showed that there were some weak points in both methods and concepts.

Results obtained by the broth dilution plate-count method that was used to determine bacterial killing are now held to be unreliable because of their heavy dependence on minor variations in technical factors. Given that such a method was required, a more acceptable approach would have been the selection of the procedure described by Taylor and colleagues. Even this procedure, however, has many of the problems associated with previously used broth dilution plate-count methods. Another methodological problem in the study was the use of the agar droplet counting method in which the broth dilution samples were seeded into 45°C molten agar. In bacterial species injury to an inoculum may occur at temperatures above 40°C and may cause a rapid loss of viability for some strains, and slower or no loss for others thus resulting in counts of colony forming units that may actually represent thermal killing rather than antimicrobial agent activity.

The authors emphasised that there was a striking association between tolerance and the paradoxical effect, thus implying that isolates that are slowly killed more readily exhibit the paradoxical effect. This assumption stems from the use of insensitive methodology. In the method used, slowly killed or so-called tolerant isolates, by definition, had colony counts above the 99-99% colony reduction minimum bactericidal count (MBC) breakpoint (> 10 cfu/plate) and therefore would show a large and significant number of cfu per count plate, which would result in a discernible paradoxical effect.

Matters arising

Conversely, rapidly killed strains would show plate counts of < 10 cfu, thus presenting a situation in which too few or no colonies were present to show the paradoxical effect. If the authors had used inoculum preparations of higher density so that a 99-99% colony reduction breakpoint would have approximated 100 cfu/plate or more, the paradoxical effect would probably have been observed as often for rapidly killed strains as it was for slowly killed strains. In our own studies using an agar dilution plate-count method, we found that the paradoxical effect was demonstrable at some time in the killing sequence for all Haemophilus influenzae and Staphylococcus aureus strains that we studied and we would anticipate similar findings for viridans streptococci.

In the Discussion section of their paper Powley et al stated that: "the results illustrate the arbitrary nature of the 99-99% killing criterion usually applied to bactericidal activity", with which we agreed but were then disappointed with their conclusion that: "the present results suggest that this arbitrary cut off point is, perhaps fortuitously, a useful ...". As we and others have previously discussed, the measurement of bactericidal action cannot meaningfully be reduced to a single arbitrary and artificial index such as the MBC, the use of which has unfortunately promoted the "all-or-none" concept of antimicrobial tolerance. Our own studies with H influenzae and S aureus indicate that the bactericidal action of β lactam producing agents is strain dependent, with strains showing a spectrum ranging from some that are rapidly killed to others that are more slowly killed. This situation pre-empts absolute classification of a strain as tolerant or not. Much work needs to be done in developing useful methods for measuring bactericidal action and in defining species reference strains representing those that are slowly, moderately, and rapidly killed. The putative, relative nature of bactericidal action and the apparent universal presence and variation in form of the paradoxical effect must clearly be kept in mind for future studies designed to evaluate whether therapeutic success may in any way be related to bactericidal response.

References


Dr Greenwood comments:

I thank Drs Woolfrey and Lally for their interest in our paper. I am happy to be able to reassure them that we are very familiar with
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 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 streptococci 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. Four of the five strains of S. sanguis were 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 effect 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 outcomes, 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 peritumoral fibrosis and perivascular lymphocytic cuffing. They contrast their findings with ours, state