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 pen-

icillin 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.

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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 effect² 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.³ 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.

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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 cuffing. They contrast their findings with ours, stat-

Matters arising

ing that we could not show any prognostic influence for tumour fibrosis.^{2,3} In fact, like them, we showed tumour fibrosis to be an adverse factor, but in our particular model this was not an independent variable.² The important point is that it is not correct to compare their model with ours. Firstly, we were looking only at rectal cancer. Secondly, we broke up Dukes' stage into discrete variables. Considerably more prognostic information is provided by entering the extent of direct spread and number of affected lymph nodes as discrete variables. Although peritumoral fibrosis does not feature in our prognostic model, the observation may be of considerable biological importance. There is growing interest in stromal-epithelial interactions, and further study of the mechanisms of tumour fibrosis may provide new insight into the basis of tumour aggressiveness.

It is gratifying to note that a lack of prominent inflammatory cell reaction (assessed in the same way as our lymphocytic infiltration) confers independent prognostic information when others have reported difficulties with the reproducibility of this variable.⁴ Interestingly, we were unable to show that lymphocytic infiltration confers independent prognostic information for colonic cancer.⁵ In fact, different prognostic models may be required for colonic and rectal cancer. Clearly, a good deal more research is required and there is a need for a comprehensive, prospective, multicentre study of prognostic variables. It should be emphasised, however, that a prognostic classification is unlikely to become widely used unless it is based on a small number of important features.

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Drs Halvorsen and Seim comment:

We unfortunately referred inaccurately to their findings regarding the prognostic influence of tumour fibrosis in rectal cancer.^{1,2} This we regret. As highlighted by Dr Jass, the selection of different patient groups will make any comparison between clinicopathological series difficult. Some additional problems, however, pertain to comparing results from different proportional hazards regression analyses (Cox models) or to multiple regression models in general. First, the choice of variables with possible independent prognostic influence may have substantial impact on the results. Secondly, the variables are often categorised differently by different investigators, as emphasised by Dr Jass. Thirdly, some users of the Cox model prefer to treat the categories of a single variable by assuming "equidistance" between them, whereas others, as we do, prefer to recode the variable categories into dichotomous variables. Fourthly, few authors say anything about the proportionality assumption of the Cox model and the question of whether interaction terms should be included in the model. In a Cox analysis with no interaction terms it is implicitly assumed that each of the independent variables represents an unchanged risk factor, regardless of the values of the other variables, and also that a particular level of a risk factor implies an increase (or decrease) in the hazard rate that is constant throughout the follow up time.

Based on plots of the log minus log survival function,³ we have chosen a Cox model in which the grade and stage related risk factors influence the prognosis equally in the colon and rectum. We admit, however, that significant deviations from such assumptions may be found in data sets with considerably more observations than in our study. So, we agree with Dr Jass that such analysis should take into consideration the question of whether different models are required for colonic and rectal cancer.

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EVA SEIM

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Fine needle aspiration of thyroid nodules

Kendall¹ did not describe adequately the technique he used, merely mentioning that a standard technique was used.^{2,3} Fine needle aspiration is developing rapidly and a standard technique is probably not yet consolidated. To allow other workers to share his experience it would be important to state the size of the needle used, and whether a syringe was used and of what volume.

The author also did not describe the details of the cytospin preparation—for example, what solution was used to suspend the cells, what model of equipment was used, and what was the speed used. There was also no explanation for why direct smears were considered to be unsatisfactory.

The technical aspects are very important as they strongly influence the accuracy of interpretation of the morphology in fine needle aspiration. The standard technique varies from one laboratory to another. Most workers use 22 or 23 gauge needles,^{2,3} but others prefer the 21 gauge needle.⁴ Similarly, some are in favour of the haematoxylin and eosin stain while others prefer the Giemsa stain. I am certain other workers would like to hear his experience in this very important field in diagnostic pathology.

In our laboratory we perform over 2000 fine needle aspirations a year; 634 were on the thyroid in 1988. We use the 21 gauge needles with 10 ml syringes, loaded on a Cameco syringe holder⁵; we use the rehydration technique and haematoxylin and eosin stain for direct smears,⁶ and we prepare cell blocks from direct fixation of the aspirate in 7.5% buffered formalin as a routine.⁶ The rehydration technique gives us excellent results. Among other advantages it also lyses red blood cells without resorting to the use of acetic acid.¹

Kendall was of the opinion that specific diagnosis could not be achieved in most cases in fine needle aspiration of the thyroid. He had difficulty in occasionally distinguishing cellular colloid goitre from neoplasms. We addressed this point specifically in a previous study.⁴ Our experience is that a specific diagnosis can be made in most cases, even in cellular aspirates. For fine needle aspiration to be useful, more attention should be put on the technical aspect of this developing field, so that a specific diagnosis can be offered