original diagnosis of carcinoid tumour were also retrieved. Many of these diagnoses had been made before the concept of atypical carcinoid had been defined, and so each case was examined to permit reclassification, using established criteria. Four of the cases originally designated carcinoid tumour were found to be typical; five were atypical. Four of the cases also diagnosed as small cell carcinoma seemed to be examples of atypical carcinoid. New sections were cut and stained with a modification of Ploton's technique developed in this laboratory. AgNORs are seen as well defined, dark dots in each nucleus. This modification uses higher temperatures for shorter times than the usual histological methods, but requires glycine preincubation to suppress background staining.

The number of AgNORs in 200 nuclei was counted in each section, and a mean AgNOR count calculated for each case. The counts for the various diagnoses show a very large overlap (figure): that for the typical carcinoids was 5-10 (range 1-26-7-12), that for atypical carcinoids was 4-60 (1-72-8-98), and that for small cell carcinoma was 5-67 (2-67-7-25). This last value compares closely with the value of 5-7 (4-2-7-3) obtained for small cell carcinomas by Crocker et al. This similarity is important because doubt has been expressed about the reproducibility of AgNOR methods; in particular, it shows that reproducibility survived our technical modifications. Unfortunately, the overlapping ranges indicate that such methods are quite useless as an aid to differential diagnosis of neuroendocrine bronchial tumours. Furthermore, because these values are not absolute counts, it is impossible to come to any conclusions about the biology of these tumours. Advances on that front will only be made when cell imprints or smears can be studied.

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References

Simple carbol-fuchsin staining for showing C pylori and other spiral bacteria in gastric mucosa

After the discovery of Campylobacter pylori on gastric mucosa of patients with gastritis and peptic ulcer, several simple and good methods have been used to show the presence of this bacterium in gastric tissue. We propose here another simple method which we have been using very successfully for over a year, and which clearly shows the characteristic morphology of the microorganism. This method uses just a dilute carbol-fuchsin stain.

To check on the accuracy of the carbol-fuchsin stain in identifying C pylori we compared its results with culture and peroxidase anti-peroxidase (PAP) method in 30 patients with symptoms associated with the upper gastrointestinal tract. Two endoscopic antral biopsy specimens were obtained from each patient. The first was used for C pylori culture and the second was fixed in 4% neutral formaldehyde for histology, PAP, and carbol-fuchsin staining. Dewaxed tissue sections were taken to water and stained for five minutes in carbol-fuchsin solution prepared as follows: 0.4 g basic fuchsin; 2 g phenol crystals; 4 ml absolute alcohol and 100 ml distilled water. After rinsing in tap water the sections were briefly decolourized with acetone. The slides did not need to be mounted and they were examined under an oil immersion lens.

Histopathological study of the biopsy specimen sections showed chronic gastritis in 29 of 30 (97%) patients. Biopsy specimens from 25 (83%) patients were shown by culture to harbour C pylori, of which 23 (77%) were positive by the carbol-fuchsin and 24 (80%) by the PAP method. All those positive by culture showed chronic gastritis. The biopsy specimens which were C pylori negative by culture were also negative by PAP and carbol-fuchsin staining. The sensitivity, specificity, and predictive value of carbol-fuchsin compared with culture were 92%, 100% and 100%, respectively.

The carbol-fuchsin positive sections showed spiral bacteria as dark red bodies in gastric mucus or adjacent to the surface of epithelial cells against a reddish background.

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Fig 1 (a) Campylobacter-like bacteria (arrow) in a gas-
tral biopsy specimen. (b) Large spiral bacterium (arrow) in lumen

of antral gland.

Figure Mean numbers of AgNORs per nucleus in typical carcinoids, atypical carcinoids, and small cell carcinomas.
Matters arising

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.1 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.3 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 others4 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 method.

In the method used, slowly killed or so-called tolerant isolates, by definition, had colony counts above the 99.9% 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.

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 99.9% 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 studied5 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 99.9% 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 we6 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 Lilly for their interest in our paper. I am happy to be able to reassure them that we are very familiar with