Letters to the Editor

Clinical versus laboratory strains of Group B streptococci and human neutrophil chemiluminescence

Group B streptococci are implicated in serious infection, particularly during the neonatal period. Considerable attention has therefore been focused on the opsonisation and phagocytosis of these organisms by neutrophils in vitro since these are the principal means of host defence against group B streptococci. Neutrophil chemiluminescence, which provides an indirect measurement of membrane generated oxidative activity, is a sensitive technique for investigating opsonic requirements and phagocytosis of group B streptococci.¹ Furthermore, a positive correlation has been shown between chemiluminescence and phagocytosis for a limited number of strains of serotypes Ic, II, and III,² and in a separate report, serotype III.³

In a recent study we showed a significant, positive correlation between peak chemiluminescence values and the rate of phagocytosis of the five serotypes of group B streptococci, using the standard NCTC reference strains.⁴ Differences in opsonic requirements between strains of the same serological type have also recently been reported.⁵ The purpose of this study was to compare clinical isolates of group B streptococci with the NCTC reference strain, in terms of their ability to induce neutrophil luminol dependent chemiluminescence in an established assay system. The details of the experimental procedure have been described previously,⁶ and since type Ia (NCTC strain 090R) and type III (NCTC 11080) had consistently produced the lowest and highest chemiluminescence values respectively, eight clinical strains of type Ia and 10 of type III were tested.

The results are shown in the Table, with the peak chemiluminescence values, originally measured as millivoltage, expressed as a percentage of the maximum value (100%) produced within each serotype. All the clinical isolates of type Ia generated chemiluminescence far in excess of that for the NCTC strain, which always stimulated low chemiluminescence levels in our test system.^{4,6} Three of the strains were clearly very reactive, with the remaining five variably so. The range for the type III clinical strains was similar, but in this case the NCTC strain value was around the midpoint. To permit a comparison between the

Neutrophil chemiluminescence produced by stimulation with group B streptococci Ia and III strains

Group B streptococci strain	
Ia (n=8)	III (n=10)
100	100
100	90
100	83
95	70
79	67
76	63
69	60
51	53
	53
	49
7 NCTC 090R	74 NCTC 11080

two serotypes, the 100% value for the Ia was 950 mV and that for the III was 1110 mV.

In commenting on these results several points should be made. In all previous experiments we have shown a clear separation in the chemiluminescence induced by NCTC strains of group B streptococci serotypes Ia and III,⁶ which was subsequently correlated with their phagocytosis.⁴ For the clinical strains tested here this distinction was no longer apparent, and the overlap between values for the two serotypes was considerable. In terms of relating such results to the likely pathogenesis of group B streptococci infections, this clearly casts doubt on the relevance of using laboratory strains for such in vitro experiments. We have not attempted to correlate chemiluminescence with phagocytosis of clinical strains as yet, although in one study the relation has been shown to hold for four clinical isolates of type III.³ In our first report we suggested that a failure to induce neutrophil chemiluminescence, and therefore by inference oxygen dependent microbicidal mechanisms, may be indicative of a bacterial strain which is pathogenic relative to one which stimulates higher levels of chemiluminescence, and thus a more successful host response.⁶ This hypothesis remains to be tested by further investigation and comparison of the laboratory findings with clinical data.

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Inhibition of growth of *Staphylococcus aureus* on sensitivity testing agar by *Streptococcus faecalis* var *zymogenes*

In this laboratory the blotting paper strip method¹ is used to obtain a semiquantitative estimate of the number of bacteria in urine. The method is both economical and rapid but can provide problems in isolating individual colonies for antibiotic sensitivity testing.

A catheter specimen of urine received in this laboratory was cultured by the blotting paper strip method and yielded numbers of colonies indicative of appreciable bacteriuria on both blood agar and Clostridium medium (Oxoid). Growth on both media was semiconfluent but in each case appeared homogenous.

Antibiotic disc sensitivity tests were carried out on DST agar (Difco), and after incubation for 18 h at 37°C sensitivity to the antibiotics was determined by the comparative method.² The appearance of the sensitivity plate obtained is shown in the Fig.

The pattern was produced by the use of mixed culture on the sensitivity plates. The opaque pigmented colonies, present only as discrete bands around some antibiotic sensitivity discs, was confirmed to

Appearance of the sensitivity plate

Staphylococcus aureus. The staphylococcus could not be recovered from other areas of the sensitivity plates, which consisted of a pure semiconfluent growth of *Streptococcus faecalis* var *zymogenes*.

The appearance of the *Staphylococcus aureus* colonies only in discrete rings associated with particular antibiotic sensitivity discs is explained by an antagonism of the staphylococcus by the streptococcus. When a mixture of the two organisms is used as an inoculum for DST sensitivity plates, the staphylococcus forms colonies only where the streptococcus is preferentially inhibited by an antibiotic.

When antibiotic sensitivity determinations of the two organisms were carried out on DST agar alone, only with mezlocillin, trimethoprim, and nitrofurantoin did the staphylococcus yield a smaller zone than the streptococcus. On DST sensitivity test plates prepared from mixed inocula of the two organisms, staphylococci appeared as rings of growth around these antibiotics.

On ordinary blood agar staphylococci appeared similarly as rings of growth around mezlocillin and nitrofurantoin, but strangely appeared as a disc of growth growing around and right up to the trimethoprim disc. On their own, on blood agar both cultures grew almost right up to the trimethoprim disc. Haemolysis of the medium by the streptococcus was inhibited around the trimethoprim disc, however, and a ring of maximum haemolysis appeared as a ring around but some distance from the disc. This inhibition of haemolysis, but not growth, appeared to coincide with an inhibition of anti-staphylococcal activity by the streptococcus in the presence of certain concentrations of trimethoprim.

The inhibitory activity shown by the streptococci showed the properties previ-

ously ascribed to some streptococci. By the methods of Kekessy and Piguet³ the inhibitory range was shown to be restricted to related *Streptococcal* spp and to *Staphylococcus* spp, and a β -haemolytic activity associated with bacteriocin activity has been previously recorded with *S faecalis* var *zymogenes*.⁴ The properties of the bacteriocin like activity produced by *S faecalis* var *zymogenes* and reported here most clearly resembled those described by Brock⁵ as a type I bacteriocin.

These observations are reported for interest because they show one disadvantage that may ensue from using the primary urine culture method adopted here. If the inhibitory culture had been uniformly more antibiotic resistant than the other culture, the presence of the inhibited culture would have been overlooked and this might not have been corrected by reference to the primary isolation plates.

The effect which subinhibitory concentrations of trimethoprim may have on the production of metabolites by *S faecalis* var *zymogenes* and the inhibitory effect which that organism may exert on other Gram positive cocci is worthy of note because of the recent upsurge in interest in the effects of antibiotics at suboptimal concentrations.

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Measuring melanomas: the Vernier method

Breslow^{1,2} has clearly demonstrated that tumour thickness is the single most important histological criterion in determining the prognosis of stage 1 malignant

melanoma and in particular is a better measure than the level of invasion of the tumour. He measured the thickness from the granular layer of the epidermis to the deepest part of the tumour using an ocular micrometer and found a 100% 5 year tumour free survival rate for melanomas less than 0.76 mm thick, whilst those greater than 1.50 mm in thickness carried a bad prognosis.

This observation has now been confirmed by other workers,³⁻⁵ but thickness does not seem to be uniformly included in histopathological reports of melanomas. We think that this may be because pathologists think that they need an ocular micrometer or a computerised image analysis system⁶ to make the measurement and may not have the equipment readily to hand.

However, almost all modern laboratory microscopes have Vernier scales fitted on their stages, which are principally designed to give grid references for slides. In a brief survey of colleagues we found that many did not understand the principle or application of these scales, which actually provide an excellent way of measuring the thickness of melanomas.

The section of the melanoma should be orientated on the mechanical stage so that the tumour is perpendicular to one of the axes. The tumour is then aligned with a fixed point such as the edge of field of view. If a teaching pointer is fitted to the microscope this can be used instead. First of all the granular layer is aligned to the fixed point. The position on the Vernier scale is

Photographs of a Vernier scale on a microscope stage in two different positions, (a) showing a reading of 100.2 mm (arrowhead), and (b) showing a reading of 100.8 mm (arrowhead). The difference between the two readings is therefore 0.6 mm.