LETTERS TO THE EDITORS

EFFECT OF LINCOMYCIN ON THE PRODUCTION OF HEAT-LABILE ENTEROTOXIN BY *ESCHERICHIA COLI*

In a recent paper by Burgess *et al.* (1979) describing the assay of heat-labile enterotoxin of *Escherichia coli* in infant rabbits, the authors discussed an attempt to increase yields of enterotoxin by the method of lincomycin induction, as originally reported from this laboratory (Levner, Wiener and Rubin, 1977). I feel obliged to take issue with certain conclusions arrived at by these authors which might unjustifiably discourage other workers from using this convenient technique.

Contrary to their statement, the authors did not demonstrate that "lincomycin . . . (was) found not to increase the yield of heat-labile enterotoxin from *E. coli* strain P307", since no data were presented, or referred to, regarding toxin production as a function of lincomycin concentration for strain P307. Indeed, the only data comparing toxin production for single strains grown with and without lincomycin (their table V) showed that, in five of five cases, increases of from 2-fold to more than 14-fold were obtained with lincomycin.

The basis for the above statement and for their statement that they were unable to confirm our report of lincomycin-induced increases in enterotoxin yield appears to be that spontaneously isolated lincomycin-resistant variants grown with lincomycin produced no more enterotoxin than did the sensitive parent strain P307 grown without it. In our 1977 paper we compared yields of enterotoxin from various enterotoxigenic strains of *E. coli*, and from *Vibrio cholerae* strain 569B, with yields from lincomycin-resistant isolates, grown with and without the drug. It is true that all the resistant isolates grown with lincomycin yielded much more toxin than did the sensitive parent strains grown without it. We have generally found this to be true, and the results of Burgess *et al.* (1979) with strain P307, which we have not examined, are certainly in contrast to our experience. However, since the resistant isolates were not characterised either by us or by Burgess *et al.* (1979) and have an undetermined genetic relationship to the parent strains, the significance of this latter comparison is unclear. Our point in presenting these data was that lincomycin resistance was not sufficient in itself to confer higher enterotoxigenicity; the presence of the drug during growth was required. Induction could be obtained with either "sensitive" or "resistant" strains as long as appropriate levels of lincomycin were used, although higher yields of enterotoxin were generally obtained in the latter case.

I suggest that anyone looking for a means of obtaining improved enterotoxin yield from any specific enterotoxigenic strain of *E. coli* or *V. cholerae* begin by looking at yield as a function of lincomycin concentration for that strain. Under our growth conditions (Levner *et al.*, 1977), optimal induction, in terms of total enterotoxin yield per ml of culture, was found to occur at lincomycin concentrations which resulted in optical densities (550 nm) at 24 h of about one-half that of cultures grown without the drug.

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Dr Levner is correct to point out that we compared toxin yields from lincomycin-resistant mutants of strain P307 grown in the presence of lincomycin, with the yield from strain P307 grown conventionally (Burgess *et al.* 1979).

We did this since it was apparent from the paper of Levner *et al.* (1977) that this approach led to the greatest increases in toxin yield for strain H197. The fact that we did not improve upon

yields of heat-labile toxin (LT) from strain P307 in conventional culture should not discourage others from investigating the effect of lincomycin on LT yields, although any useful toxin increase can be demonstrated only by comparison with the parent strain grown conventionally.

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REFERENCES

- BURGESS, M. N., COWLEY, C. M., MELLING, J., MULLAN, N. A. AND NEWSOME, P. M. 1979. Assay of the heat-labile enterotoxin of *Escherichia coli* in infant rabbits. *J. med. Microbiol.*, **12**, 291.
- LEVNER, M., WIENER, F. P. AND RUBIN, B. A. 1977. Induction of *Escherichia coli* and *Vibrio cholerae* enterotoxins by an inhibitor of protein synthesis. *Infect. Immun.*, **15**, 132.