

LETTERS TO THE EDITOR

The Effect of pH on the Stability of Penicillin-induced Spheroplasts

SIR,—Edebo (1961) has made a detailed study of the effect of pH on the osmotic stability of lysozyme-induced protoplasts and spheroplasts. Protoplasts of *Bacillus megaterium*, prepared as described by Weibull (1953), were found to be more stable on the acid than on the alkaline side of neutrality, and did not burst below pH 5 when the osmotic pressure of the medium was suddenly reduced. Spheroplasts of *Escherichia coli*, obtained by the lysozyme-versene technique of Repaske (1958), and dialysed to pH values less than pH 5.5, showed greater resistance to disintegration than spheroplasts at higher pH; in addition, the optical density was higher in samples of lower pH.

In our experiments, spheroplasts of *E. coli*, formerly NCTC 5934, were induced by treatment of the organism with penicillin in 10 ml. nutrient broth containing 0.33M sucrose and 0.25 per cent w/v $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, at pH 7.2. After incubation for 5 hr. at 37°, tubes were centrifuged at a low speed, to avoid disintegration of the spheroplasts, and the spheroplast residue suspended in 10 ml. sucrose-Mg⁺⁺-broth, containing penicillin, at a pH value within the pH range 5-8. Tubes were re-incubated at 37°, and samples from each examined by interference microscopy at frequent intervals. It was found that the spheroplasts were less susceptible to disintegration at acid pH values. These results confirm those obtained by Edebo (1961) with lysozyme-induced spheroplasts and are also in agreement with the finding that penicillin-induced spheroplasts of *Aerobacter aerogenes* are more stable at acid pH values than at alkaline pH (Gebicki and James 1960).

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REFERENCES

- Edebo, L. (1961). *Acta Path. Microbiol. scand.*, **53**, 121-128.
Gebicki, J. M. and James, A. M. (1960). *J. gen. Microbiol.*, **23**, 9-18.
Repaske, R. (1958). *Biochim. Biophys. Acta*, **30**, 225-232.
Weibull, C. (1953). *J. Bacteriol.*, **66**, 688-695.