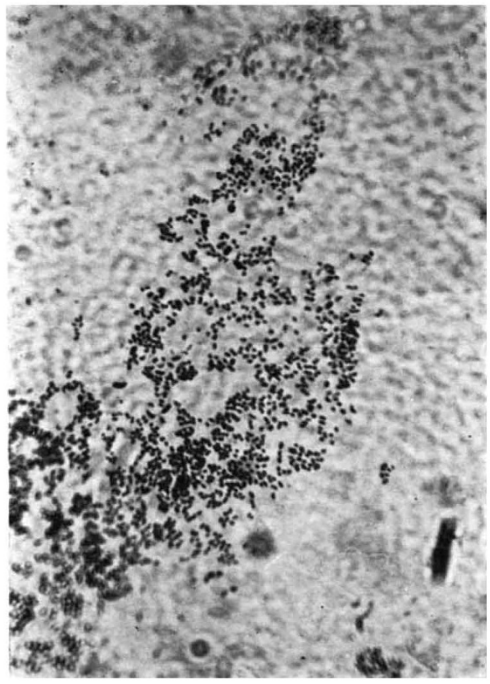

Fig. 4. *V. comma*, untreated

addition of certain classes of antibiotics but not by others. It appears that compounds which exert their antibacterial effects by interfering with protein synthesis suppress the induction of spheroplast and filament formation. Agents which act against organisms by interrupting the synthesis of the cell wall or by altering the cell membrane do not appear to interfere with the action of penicillin. These observations may cast some doubt on the efficacy of treatment of infected patients with combinations of tetracycline or chloramphenicol and penicillin—which are common forms of therapy. Furthermore, these findings may offer an explanation for the clinical failures that may result in cases where this type of antibiotic combination has been used to treat disease caused by penicillin-sensitive organisms. Removal of the "broad spectrum" agent has, in some cases, been found to result in prompt improvement and cure of the infection.

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[1] Jawetz, E., Gunnison, J. B., Speck, R. S., and Coleman, V. R., *Arch. Intern. Med.*, **87**, 349 (1951).
[2] Jawetz, E., and Gunnison, J. B., *Antibiotics and Chemotherapy*, **2**, 243 (1952).
[3] Garrod, L. P., and Waterworth, P. M., *J. Clin. Path.*, **15**, 328 (1962).
[4] Jawetz, E., and Speck, R. S., *Proc. Soc. Exp. Biol. and Med.*, **74**, 93 (1950).
[5] Lepper, M. H., and Dowling, H. F., *Arch. Intern. Med.*, **88**, 489 (1951).
[6] Jackson, G. G., Lepper, M. H., Seto, J., and Dowling, H. F., *Amer. J. Med. Sci.*, **225**, 525 (1953).
[7] Ahern, J. J., Burnell, J. M., and Kirby, W. M. M., *Proc. Soc. Exp. Biol. and Med.*, **79**, 568 (1952).
[8] Walker, S. H., *Antibiotics and Chemotherapy*, **3**, 677 (1953).
[9] Paredes, L., and DePablo, J., *Antimicrobial Agents and Chemotherapy*, 794 (1962).

Level of Vitamin B_{12} in Propionate-grown *Prototheca zopfii*

THE colourless alga *Prototheca zopfii* does not require vitamin B_{12} as a growth factor, whereas at least half the marine algal flagellates and diatoms which have been investigated in pure culture[1], the protozoan flagellate *Ochromonas malhamensis*[2], and *Euglena gracilis*[3], have all been shown to have a growth requirement for this compound.

It has previously been shown that *Prototheca* assimilates propionate by a route involving β-hydroxypropionate and malonic semialdehyde[4,5]. This pathway is presumably independent of vitamin B_{12}, whereas the succinyl-Co*A* pathway of propionate metabolism, present for example in *Ochromonas*[6], requires a coenzyme form of the vitamin which functions in the isomerization of methyl malonyl-Co*A* to succinyl-Co*A*. Data on the level of vitamin B_{12} in extracts of *Prototheca* grown in medium rich in propionate were obtained as further evidence that the proposed pathway of propionate assimilation is the only mechanism involved in growth on this carbon source.

The organism was grown on propionate–mineral salts–thiamine medium as previously described[4], and the cell concentration at the time of collecting was determined by counting in a haemocytometer cell. The cells were collected quantitatively and resuspended at a final population of 10^8 cells/ml. in acetate buffer (*p*H 4·6) containing one drop of 1 per cent (w/v) potassium cyanide solution per 5 ml. of buffer. After autoclaving at 10 lb./in.^{2} for 10 min, the suspension was passed twice through a French press; it was then frozen and thawed, and autoclaved once more. Microscopic examination revealed that almost all the cells had been disrupted. The presence of cyanide stabilizes any vitamin B_{12} present; the extracts were protected from strong light.

Microbiological assays were carried out by Dr. M. E. Gregory of the National Institute for Research in Dairying, Shinfield, Reading. The assay using *Ochromonas* as a test organism failed to detect any vitamin B_{12} activity. The more sensitive *Lactobacillus leichmannii* assay revealed that the extract contained 0·08 mμg of vitamin B_{12}/ml., but the possibility arises with this organism that it is deoxyriboside activity which is measured rather than true vitamin B_{12}. *Euglena gracilis* was finally used as the test organism. The extract was centrifuged and the clear supernatant was used undiluted and diluted ten-fold. The undiluted extract gave no growth, and from this it can be concluded that cyanide or some other substance was present at an inhibitory level. In the case of the diluted extract it was found that growth increased with increasing addition to the assay cultures and the results calculated at two different levels were similar. The value obtained, 0·40 mμg/ml., can be regarded as a measure of true vitamin B_{12} activity.

Droop[1] has calculated that in an alga which requires vitamin B_{12} for metabolic processes the level will correspond to about three molecules of vitamin/μ^3; Hutner *et al.*[3] have reached a similar conclusion.

Assuming a molecular weight of 1,357 for the vitamin it can be shown that the level of the compound in *Prototheca* corresponds to only 200 molecules/cell. However, if Droop's calculations are applicable to any algal species, *Prototheca* (mean volume 1,440 μ^3) would contain about 4,300 molecules/cell and it is evident that the level of vitamin B_{12} in propionate-grown *Prototheca* is some twenty times lower than would be expected if the cells required the vitamin for their metabolism.

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[1] Droop, M. J., *Nature*, **180**, 1041 (1957).
[2] Coates, M. E., and Ford, J. E., *Symp. Biochem. Soc.*, No. 13, 36 (1955).
[3] Hutner, S. H., Provasoli, L., Schatz, A., and Haskins, C. P., *Proc. Amer. Phil. Soc.*, **94**, 152 (1950).
[4] Callely, A. G., and Lloyd, D., *Biochem. J.*, **92**, 338 (1964).
[5] Lloyd, D., and Callely, A. G., *Biochem. J.*, **97**, 176 (1965).
[6] Arnstein, H. R. V., and White, A. M., *Biochem. J.*, **83**, 264 (1962).