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Failure to detect Effects of Cycloheximide on Energy Metabolism in *Euglena gracilis*

Macdonald and Ellis report[1] that cycloheximide inhibits the uptake of chloride in storage tissue disks of beet, carrot and potato, and in roots of wheat and pea plants. It was also found that oxygen uptake was markedly stimulated by the antibiotic in beet and carrot disks, slightly stimulated in potato disks, and not affected in wheat or pea roots. These findings were considered to suggest that cycloheximide can disrupt cellular metabolism in other ways than the inhibition of protein synthesis.

Cycloheximide is an effective inhibitor of chlorophyll synthesis in the alga, *Euglena gracilis*[2,3]. This inhibition has been interpreted as a consequence of an inhibition of protein synthesis (on cytoplasmic 80S ribosomes). If in fact cycloheximide directly inhibits energy metabolism in *E. gracilis*, then this effect, rather than an interference with protein synthesis, might account for the inhibition of chlorophyll synthesis. The experiments reported here show that in *E. gracilis* cycloheximide has no effect on respiration or motility, suggesting that in this organism the drug does not inhibit energy metabolism.

The effect of cycloheximide at concentrations from 3·3 μg to 100 μg/ml. on the respiration of dark-grown cells of *E. gracilis* was studied manometrically. Up to 2 h after addition of the drug there was no significant effect, stimulatory or inhibitory, on the rate of oxygen uptake (Fig. 1 shows the time course of respiration at 33 μg cycloheximide/ml.). In the case of dark-grown cells of *E. gracilis* greening in the light, cycloheximide causes 75 per cent inhibition of the chlorophyll synthesis that takes place during the 2 h after addition of the drug[4] (suggesting that the inhibition of chlorophyll synthesis comes into effect at much less than 2 h). In long-term experiments there was a slight diminution in the respiration rate in the presence of antibiotic (compared with the control), but it amounted only to about 10 per cent even after incubation for 5 h with cycloheximide: this effect can plausibly be attributed to a slow loss of enzymes of carbohydrate metabolism resulting from turnover of protein without resynthesis.

Cells of *E. gracilis* possess a flagellum and are motile: their motility requires energy. To test whether cycloheximide interfered with the supply of this energy, cells were incubated in simple defined growth medium[5] in the presence or absence of cycloheximide (10 μg/ml.) in test tubes at room temperature in the dark. The suspensions were inspected visually at intervals up to 4·5 h. In the presence or absence of the drug the cells remained approximately evenly distributed throughout the medium, indicating unimpaired motility in both cases (cells deprived of their motility by freezing and thawing, or by adding the respiratory inhibitor, sodium azide, sediment to the bottom of the flask within minutes on standing). This result confirms the findings of Rosenbaum[6], who also showed that high concentrations of cycloheximide had no effect on motility of this alga.

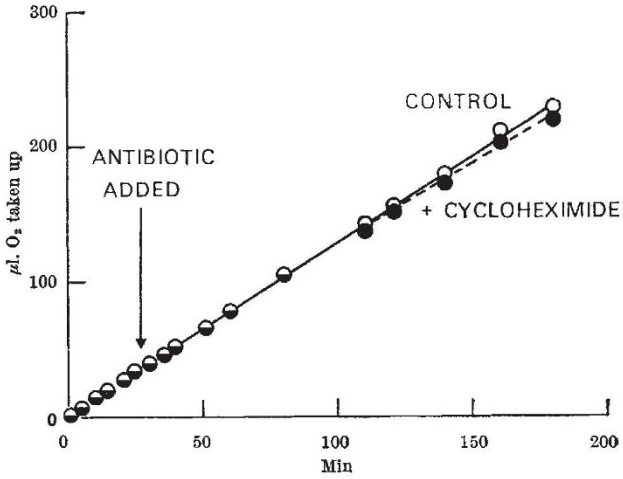

Fig. 1. Washed suspensions of dark-grown cells of *E. gracilis* strain Z were prepared as described previously[3]. 2·9 ml. aliquots of suspension containing $2·0 \times 10^6$ cells/ml. in 0·04 M KH_2PO_4–Na_2HPO_4 buffer (*p*H 7·0), with 1·0 mM $MgSO_4$, were incubated in Warburg manometer flasks at 26·4° C. After 25 min 0·1 ml. of water or 0·1 ml. 0·1 per cent cycloheximide (final concentration 33 μg/ml.) was added from a sidearm. Oxygen uptake was measured at intervals both before and after the antibiotic was added.

The lack of effect of cycloheximide on respiration or motility of *E. gracilis* suggests that the antibiotic does not inhibit energy metabolism in this organism. Further, in *E. gracilis* cycloheximide has relatively little effect on carotenoid synthesis up to 4 h (refs. 2 and 4), does not affect the operation (although it inhibits the formation) of an energy-requiring amino-acid accumulation system[7], and does not inhibit ^{14}C-adenine incorporation (in periods up to 4 h—unpublished results of R. L. Allen and myself). These findings for *Euglena* are in accord with the original report by Kerridge[8] that cycloheximide does not affect respiration or fermentation in yeast, and with the finding that the drug has no effect on respiration in the mould, *Blakeslea trispora*[9].

In short, it seems that there is no good reason yet to suppose that, in *Euglena gracilis*, cycloheximide directly inhibits any cellular process other than protein synthesis. Accordingly, the inhibitory effects of this antibiotic on chlorophyll synthesis can still most plausibly be explained in terms of a primary inhibition of protein synthesis. In this connexion, Ellis and Macdonald have made the interesting observation (unpublished) that cycloheximide, even at 100 μg/ml., has no effect on respiration or chloride uptake, in light or darkness, in plants of *Lemna gibba*, and in leaf disks of lettuce, leek and cabbage; that is, it seems as if in green tissue these cellular processes are immune to cycloheximide, and so this antibiotic can still be used as a selective inhibitor of protein synthesis on 80S ribosomes, in green cells at least.

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