

of the non-sporulating clone was isolated by repeated subcultures on the antibiotic-supplemented medium. In a similar manner a penicillin-resistant strain (100 $\mu\text{g}/\text{ml}$.) of one of the sporulating clones was derived. Both these strains seem stable. They have been passed through a number of subcultures in the absence of their respective antibiotics without having lost their resistance.

Each of the resistant strains was tested for cross-resistance to the other antibiotic and showed no growth on antibiotic-supplemented media. They were also inoculated—separately or simultaneously—into media which contained both the antibiotics. No growth was found to have occurred in any case even after two months. However, when the two strains (designated as *S-Sm^rP^r* and *S⁺Sm^rPr*) were grown together in the basal medium containing no antibiotic and transferred five times in the same medium, and then inoculated in the mixture of the two antibiotics, growth occurred in two out of 47 culture tubes. This points to a case of genetic recombination due to the chance inclusion and rare distribution of recombinants in the two culture tubes where growth had occurred in the presence of both the antibiotics. The double-resistant recombinant also formed spores at low frequency. It has been passed five times in antibiotic-free medium without loss of resistance and spore character. It is highly probable that the recombinant strain (designated as *S⁺Sm^rPr*) is formed by the transfer of the genetic marker *Sm^r* of the non-sporulating clone to the sporulating clone showing penicillin resistance. These results seem to indicate the occurrence of sexual or parasexual phenomena in the blue-green alga, *Cylindrospermum majus* Kuetz.

The only other previously reported case of an apparently genetic recombination in a blue-green alga is that of *Anacystis nidulans* by Kumar², who found it impossible to grow clones of this alga.

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¹ Allen, M. B., and Arnon, D. I., *Plant Physiol.*, **30**, 366 (1955).
² Kumar, H. D., *Nature*, **196**, 1121 (1962).

Chloramphenicol Inhibition of the Growth of Green Algae

VAZQUEZ¹ has recently stated that D-threo-chloramphenicol does not inhibit the growth of protozoa (with the exception of *Tetrahymena pyriformis*) and plants.

Tamiya *et al.*² have given an account of the effect of chloramphenicol on the green alga *Chlorella ellipsoidea*, and shown that at the relatively high concentration of 323 $\mu\text{g}/\text{ml}$. the rate of growth was decreased after a lag period. At ten times this concentration, growth was virtually inhibited, but at 32.3 $\mu\text{g}/\text{ml}$. no effect was observed. The reduction in the growth rate was accompanied by a decrease in the size of the cells. *Chlorella* therefore appears to be a more resistant organism to chloramphenicol than the bacteria, but still shows similar effects.

Kumar³ has found that with the blue-green alga *Anacystis nidulans*, between 1–6 $\mu\text{g}/\text{ml}$. the growth rate is unaffected, but there is an increasing lag period with increase in concentration. At 8 and 10 $\mu\text{g}/\text{ml}$. no growth was observed after twelve days.

It may also be observed that the report concerning *Tetrahymena*⁴ is somewhat ambiguous. It is stated that "growth was considerably delayed or completely arrested at 25–150 $\mu\text{g}/\text{ml}$.". This may be taken to mean that there was a lag period before growth was commenced, but it does not specifically state that the growth rate itself was affected.

I have recently carried out some experiments on the effect of D-threo-chloramphenicol on the growth and metabolism of *Scenedesmus quadricauda*, a green alga. Here,

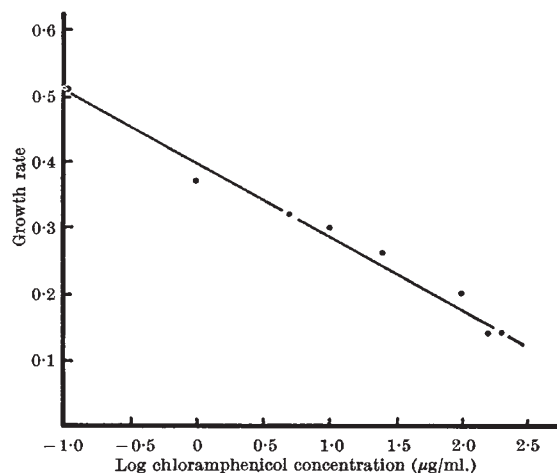


Fig. 1. Effect of D-threo-chloramphenicol concentration on the growth rate of *Scenedesmus quadricauda*. Control growth rate = 0.59. Correlation coefficient = 0.99; significant at $P = 0.001$. Growth rates are the coefficients b in the equation $\log N = a + bt$, where N = number of colony units (of four cells) per unit volume, and t is the time in days. The equation is calculated for the period of unrestricted growth only.

increasing the chloramphenicol concentration over the range 0.1–200 $\mu\text{g}/\text{ml}$. results in a progressive reduction in the growth rate (Fig. 1). This is accompanied by an increase in the lag period before unrestricted growth commences.

We have, therefore, two cases of the growth of green algae being affected by D-threo-chloramphenicol, though the levels which are effective differ considerably in the two organisms. *Scenedesmus* has more in common with the chloramphenicol-sensitive bacteria in this respect than with *Chlorella*.

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¹ Vazquez, D., *Nature*, **203**, 257 (1964).

² Tamiya, H., Morimura, Y., and Yokota, M., *Arch. Mikrobiol.*, **42**, 4 (1962).

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MICROBIOLOGY

Isoenzymes of Lactate-dehydrogenase in Micro-organisms

It is generally accepted that lactate-dehydrogenase (LDH) exists in more than one molecular species. LDH isoenzymes have been separated by (among other methods) using their eventual heat stability at 58° C (ref. 1). It has been shown that the LDH fraction prominent in heart muscle is more stable to heat than other fractions from, for example, liver tissue². There is good correlation between this heat-stable isoenzyme and the fastest moving, anodal LDH I (nomenclature of Wieme³) in electrophoresis.

In the present investigation, we examined suspensions of *Staphylococcus pyog.*, var. *aureus* (1 ml. containing approximately 10⁹ bacterial cells, as measured by turbidity) by the method of King⁴ for LDH activity. Aliquots of the suspension were simultaneously exposed to 58° ± 0.2° C for 60 min in a water-bath. Twenty-four samples were examined, all determinations being made in duplicate. After heating, the LDH activity of the samples decreased by 83 per cent ($s_x \pm 15.9$) with very good reproducibility.

In a second experiment, we examined strains of *Staphylococcus pyog.*, var. *aureus*, resistant to a variety of antibiotics (penicillin, streptomycin, chloromycetin, tetracycline). After exposure to 58° C, practically no LDH