Fig. 2. *Growth and cercosporin production by C. beticola on various carbon substrates*

For explanation of symbols, see Fig. 1.

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**Ethylene Glycol and Polyethylene Glycol Catabolism by a Sewage Bacterium**

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Polyethylene glycols are an important group of polymers used in the manufacture of explosives, cosmetics and pharmaceuticals. In addition, the polyoxyethylene group is the hydrophilic head group of non-ionic surfactants such as the alcohol ethoxylates used in detergent formulations. Ethylene glycol and diethylene glycol are also used as anti-freeze and deicing agents. Use of these man-made chemicals results in their discharge to the environment, where they are attacked by the micro-organisms of sewage, river waters and soil. Although biodegradation of polyethylene glycols has been studied by several investigators (Patterson *et al.*, 1967; Sturm, 1973; Pitter, 1973; Evans & David,
little is known about the mechanisms involved in enzymic cleavage of the ethoxylate chain.

Several strains of bacteria, able to use polyethylene glycols as sole carbon source for growth, have been isolated from sewage by elective culture, and the catabolism of ethylene glycol and polyethylene glycol by one of the isolates, possibly an *Acinetobacter*, has been studied in some detail. The bacterium would grow on ethylene glycol, diethylene glycol, triethylene glycol, polyethylene glycol 200 (a mixture of telomers with average mol wt. of 200) and polyethylene glycol 400 at 1g/litre in a mineral-salts medium containing 50mM-KH$_2$PO$_4$/Na$_2$HPO$_4$ buffer, pH 7.5. Higher-mol wt. polyethylene glycols supported poor growth and polyethylene glycol 1000 was not attacked to any appreciable extent. Cell-free extracts of the *Acinetobacter* grown on ethylene glycol contain high activity (0.175 μmol/min per mg of protein) of an ethylene glycol dehydrogenase, although ethanol and butan-1-ol were also oxidized. Both NAD$^+$ and NADP$^+$ were utilized and the pH optimum was 9.8. The ethylene glycol dehydrogenase activity of extracts of cells grown on succinate was low (3 nmol/min per mg). Growth on ethylene glycol also resulted in a 40-fold induction of a NAD$^+$-dependent glycolaldehyde dehydrogenase (specific activity 0.65 μmol/min per mg) that had a pH optimum of 9.5 and would also oxidize acetaldehyde and glyceraldehyde. We were unable to detect metabolism of glycollate by extracts, but glyoxylate was identified as a product of ethylene glycol and glycolaldehyde metabolism. The enzymes responsible for metabolism of glyoxyxlate to pyruvate by the glyceral pathway (Kornberg & Gotto, 1961) were also induced by growth on ethylene glycol and glycollate, but not detected in extracts of cells grown on succinate or glycollate. Tartronic semialdehyde, the product of glyoxylate carboligase in *Pseudomonas*, was not found after metabolism of glyoxylate. However, activity of a hydroxypyruvate reductase was found, suggesting that hydroxypyruvate rather than tartronic semialdehyde might be the first C$_3$ intermediate in the glyceral pathway in our organism.

Growth of the *Acinetobacter* on polyethylene glycol 200 also induced ethylene glycol dehydrogenase, glycolaldehyde dehydrogenase and the glyceral-pathway enzymes. However, ethylene glycol dehydrogenase had no detectable activity with diethylene glycol or polyethylene glycol, suggesting that the initial attack of polyethylene glycol was by a different enzyme. The ability of washed-cell suspensions to oxidize polyethylene glycol was rapidly lost when the cells were frozen or incubated at 30°C, and we have had great difficulty in demonstrating metabolism of polyethylene glycol by cell-free extracts, even when extracts were prepared immediately after harvesting the cells. In some extracts the oxygen uptake, determined in a Clarke-type oxygen electrode, was increased by the addition of polyethylene glycol and metabolism was confirmed by t.l.c. analysis. In dilute or aged extracts no additional O$_2$ uptake was found in the presence of polyethylene glycol, and this failure to metabolize the polyethylene glycol was confirmed by t.l.c. analysis. The immediate product of this enzyme has not yet been identified, but glycolaldehyde, identified as the 2,4-dinitrophenylhydrazone, which forms a purple colour in alkaline solution (Banks et al., 1955), has been tentatively identified in incubation mixtures after metabolism of polyethylene glycol.

These results suggest that the mechanism of polyethylene glycol catabolism might be different from the extracellular enzyme responsible for conversion of polyethylene glycol 20000 into diethylene glycol and ethylene glycol in *Pseudomonas aeruginosa* (Haines & Alexander, 1975) or the flavin-dependent dehydrogenase involved in tetraethylene glycol metabolism by a soil bacterium (Payne & Todd, 1966).