is also produced by cell-free extracts of *Pseudomonas solanacearum* (Phelps & Sequeira, 1967a,b) and by *A. tumefaciens*, which in addition produces indol-3-yl-lactic acid and tryptophol (Kaper & Veldstra, 1958). Plant epiphytic bacteria produce indol-3-ylacetic acid, indol-3-ylpyruvic acid, indol-3-ylcarboxaldehyde and indol-3-ylcarboxylic acid from tryptophan (Wichner & Libbert, 1968).

3-Methyleneoxindole formation during enzymic oxidation of indol-3-ylacetic acid has been reported by Hinman & Lang (1965), and during studies on the metabolism of indol-3-ylacetic acid by horseradish peroxidase Meudt (1965) isolated four indole derivatives one of which showed considerably greater auxin-like activity than that of indol-3-ylacetic acid. Indole and anthranilic acid were identified among products of indol-3-ylacetic acid metabolism by *C. fascians* (Dean, 1961).

The following experiments on the metabolism of indol-3-ylacetic acid by *C. fascians* suggest that tryptophol may be an important product formed by a reversal of the indol-3-ylacetic acid-synthetic pathway.

The organism was grown in nutrient salt medium containing indol-3-ylacetic acid (50 µg/ml). Changes in indol-3-ylacetic acid concentration were monitored by using the Salkowski reagent and reading the colours at 430nm (Marumo, Abe & Hattori, 1968). Breakdown products were recognized by paper chromatography.

Breakdown of indol-3-ylacetic acid by induced cells (previously grown on indol-3-ylacetic acid) in unbuffered medium (pH 6.9) lacking either a nitrogen or a carbon source was rapid (0.20 µmol of indol-3-ylacetic acid/min per mg of protein). In similar experiments with buffered media (pH 6.0 and 6.6) no indol-3-ylacetic acid breakdown products could be detected chromatographically.

In an unbuffered complete nutrient medium (pH 6.9) indol-3-ylacetic acid was metabolized less rapidly, and a number of breakdown products including indol-3-ylacetaldehyde, tryptophol, indole and anthranilic acid were detected. Low pH values between 5.0 and 6.0 favoured the production of tryptophol.

These results suggest that *C. fascians* may metabolize indol-3-ylacetic acid via a reversal of the normally accepted pathway for its synthesis, the major end products depending on the pH of the reaction medium, low pH values favouring tryptophol production.

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Metabolism of C₁ and C₂ Compounds by *Pseudomonas* AM1: a Glycerate Kinase Mutant and a Mutant Defective in Glyoxylate Formation from C₁ Compounds

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Glyoxylate is an intermediate in the serine pathway for the assimilation of C₁ compounds and oxalate by *Pseudomonas* AM1 (Blackmore & Quayle, 1970; Harder & Quayle, 1971).

There is considerable evidence that one of the enzymes required for glyoxylate formation from C₁ compounds is also involved in the pathway for assimilation of ethanol in *Pseudomonas* AM1 (Anthony, Dunstan & Drabble, 1971). It might be predicted that this common reaction is not involved in a direct condensation of C₁ units to give a C₂ compound, nor in the formation of glycolytic intermediates from serine. Experiments with the two mutants described here confirm this prediction, and support the suggestion (Anthony *et al.* 1971) that enzymes are present in *Pseudomonas* AM1 that convert acetate into glycollate and glyoxylate.

Mutant PCT-64 does not grow on any C₁ compound (even in the presence of glycollate or glyoxylate), nor on oxalate, but grows well on ethanol or succinate. No glycerate kinase could be detected in this mutant, but hydroxypyruvate reductase, serine-glyoxylate aminotransferase and enzymes for the oxidation of C₁ compounds are present. This result confirms that glycerate kinase is not essential for growth on ethanol, and that it is an essential enzyme of the serine pathway for the assimilation

of C₁ compounds, as proposed by Large & Quayle (1963).

Mutant PCT-57 grows well on ethanol, oxalate and succinate, but not on C₁ compounds, although serine-glyoxylate aminotransferase, hydroxypyruvate reductase, glycerate kinase and enzymes for the oxidation of C₁ compounds are present.

Methanol only supports growth when a C₂ compound (glyoxylate, glycollate or acetate) is present, thus indicating that mutant PCT-57 is unable to form glyoxylate from C₁ units. These results provide further evidence that enzymes catalysing the synthesis of glyoxylate and glycollate from acetate (or a derivative) are present in *Pseudomonas* AM1. Mutant PCT-57 has possibly lost an enzyme responsible for the condensation of C₁ units to one of these C₂ compounds, and hence the failure of C₁ compounds to support growth although good growth occurs with ethanol. Alternatively, if the cyclic scheme for glycine regeneration operates (Large, Peel & Quayle, 1962), mutant PCT-57 may have lost the enzyme that cleaves a C₄ compound to give two C₂ compounds.

We thank the Science Research Council for a grant to P. M. D.

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Poly(Adenosine Diphosphate Ribose) Polymerase in *Physarum polycephalum* Nuclei

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Poly(ADP-ribose) polymerase has been found in the nucleus of several cell types (Nishizuka *et al.* 1969), although rat liver nuclei have been used almost exclusively to characterize this enzyme (Fujimura, Haregawa, Shimizu & Sugimura, 1967; Nishizuka, Ueda, Nakazawa & Hayaishi, 1967). The substrate is NAD; the product is a polymer of ADP-ribose that may be covalently linked to protein. Nicotinamide inhibits polymer formation.

Poly(ADP-ribose) polymerase may participate in the regulation of DNA synthesis. Preincubation of liver nuclei with NAD inhibits subsequent incorporation of dTTP into DNA. This inhibition may be related to the synthesis of poly(ADP-ribose). Maximal inhibition, about 80%, occurs with 4mM-NAD (Burzio & Koide, 1970).

We have examined this enzyme in the nuclei of the slime mould *Physarum polycephalum*, which was grown on 1% Marmite-1% glucose, pH 4.6. Nuclei were isolated, purified (Mohberg & Rusch, 1964) and then broken in an Eaton press.

This preparation possesses a poly(ADP-ribose) polymerase activity. The assay method measures incorporation of [adenosine-³H]NAD into acid-insoluble products. The optimum temperature is 14°C; the organism is grown at 26°C. The optimum pH is 8.2, and Mg²⁺ is required. Incorporation of NAD continues for up to 2h. With optimum conditions the rate of incorporation is 23 pmol of ADP-ribose/min per mg of protein with 4mM-NAD. The reaction is almost completely inhibited by 10mM-nicotinamide.

We have confirmed that preincubation of rat liver nuclei with NAD inhibits subsequent incorporation of dTTP into DNA, and that this inhibition is greater at 25°C than at 37°C (Burzio & Koide, 1970). Isolated *Physarum* nuclei show increasing degrees of inhibition of subsequent dTTP incorporation into DNA after incubation in 0-4mM-NAD for 30min. We have also examined the variation of poly(ADP-ribose) polymerase activity during the cell generation.

The specific activity of NAD pyrophosphorylase in *Physarum* nuclei varies during each cell generation (Solao & Shall, 1971); it is high during DNA synthesis and low at other times. These two NAD-metabolizing enzymes may be closely involved in the regulation of DNA replication. This association would directly integrate NAD and energy metabolism with DNA biosynthesis both in progression through a single cell generation and in modulation of growth rate.

We thank the Cancer Research Campaign for their support of this work.

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