glyoxylate considerably decreased $^{14}CO_2$ release from [1-^{14}C]glycollate and [2-^{14}C]glycollate.

These results indicate that glycollate is oxidized to glyoxylate before decarboxylation. Glyoxylate is further oxidized by H_2O_2, generated endogenously, to formate and CO_2 by a non-enzymic reaction. The formate, derived from C-2 of glyoxylate, can then be further oxidized (Leek & Butt, 1972). The release of CO_2 from both carbon atoms of glyoxylate and the low activities of the oxidizing system both argue against a significant participation of these oxidations in photorespiration.

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Kisaki, T., Yoshida, N. & Imai, A. (1971) *Plant Cell Physiol.* **12**, 275–288
Leek, A. E. & Butt, V. S. (1972) *Biochem. J.* **128**, 87 P
Tolbert, N. E. (1971) *Annu. Rev. Plant Physiol.* **22**, 45–74
Zelitch, I. (1966) *Plant Physiol.* **41**, 1623–1631

The Occurrence of an Induced Glyoxylate Cycle in Wheat Aleurone Tissue

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The glyoxylate cycle occurs in several oleagenous seeds during germination, whereas in carbohydrate-storing seeds the cycle has been reported only in the scutellum of maize (Oaks & Beevers, 1964). Little has been reported on the induction of glyoxylate-cycle activity during germination. Our previous studies in germinating wheat (*Triticum vulgare*) have indicated that in the bran (aleurone tissue) triglycerides probably constitute a major nutrient reserve whose metabolism is induced early during germination (Laidman & Tavener, 1971). We have therefore looked for a glyoxylate cycle during germation in this species and we have investigated the principle factors concerned with its induction.

During germination, isocitrate lyase and malate synthetase activities in the aleurone tissue rose from zero in the quiescent grain to a maximum at 6 days, after which both enzyme activities declined. No activity was found in other tissues. When de-embryoed grain (endosperm halves) were incubated alone for up to 8 days, isocitrate lyase failed to appear, and malate synthetase appeared at only low activities in the aleurone tissue. The addition of 100 nM-gibberellic acid to the incubating endosperm halves caused the development of enzyme activities up to the values found in the intact germinating grain. The hormone was effective at concentrations ranging from 0.01 nM to 1 mM with an optimum concentration at 100 nM. Of several other plant growth regulators tried, only 1 mM-glutamine and 1 mM-hydroxylamine induced any activity (to about 40% of that produced by gibberellic acid).

The present results show that the induction of glyoxylate-cycle activity in aleurone cells is dependent on the presence of the embryo, and that gibberellic acid is probably the principle inducing factor for the activity. This role of gibberellic acid is interesting in that it is the first recorded example of the complete hormonal induction of the glyoxylate cycle in plants, and is a rare example of a non-hydrolase non-secretory enzyme system induced by gibberellic acid in germinating cereals.

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Laidman, D. L. & Tavener, R. J. A. (1971) *Biochem. J.* **124**, 2 P
Oaks, A. & Beevers, H. (1964) *Plant Physiol.* **39**, 431

A Comparison of some Properties of the 1,2-Dihydroxynaphthalene Oxygenase and Catechol 2,3-Oxygenase Activities in Naphthalene-Grown *Pseudomonas* sp. N.C.I.B. 9816

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Evans *et al.* (1965) suggested that the ability of a single organism to open rings of 1,2-dihydroxyanthracene, 3,4-dihydroxyphenanthrene, 1,2-dihydroxynaphthalene and catechol by *meta* cleavage might be due to a single oxygenase of low specificity present under the various growth conditions. To test this hypothesis, the properties of the catechol 2,3-oxygenase and the 1,2-dihydroxynaphthalene oxygenase activities were compared in cell-free extracts of naphthalene-grown *Pseudomonas* sp. N.C.I.B. 9186.

Catechol 2,3-oxygenase activity was assayed at pH 7.5 by the usual spectrophotometric method (Nozaki *et al.*, 1963). 1,2-Dihydroxynaphthalene oxygenase activity was assayed in a Clark oxygen electrode by measuring the rate of O_2 uptake when 1,2-dihydroxynaphthalene (1 μmol) in anhydrous tetrahydrofuran was added to cell-free extract in 50 mM-phosphate buffer, pH 7.0; as long as the pH was not much above 7.0 and the amount of activity present was above a critical value, the auto-oxidation of substrate was negligible and the rate was directly proportional to the amount of extract added.

Both activities were unstable in air, each declining by 40% after 24 h at 4°C, and both were stabilized to

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the same extent by 10% acetone. As far as the 1,2-dihydroxynaphthalene oxygenase assay allowed, the two activities showed identical variation with pH.

After dialysis for 24h at 4°C against 50mM-phosphate buffer, pH 7.0, containing 1mM-*o*-phenanthroline, 50% of both activities were lost; these could be restored by addition of Fe^{2+}, although in the case of 1,2-dihydroxynaphthalene oxygenase only to 70% of the original value. After dialysis for 24h at 4°C against phosphate buffer alone, catechol 2,3-oxygenase activity could be restored only by anaerobic incubation with Fe^{2+}, whereas 1,2-dihydroxynaphthalene oxygenase activity was restored by aerobic addition of Fe^{2+}.

The kinetics of thermal inactivation of the two activities at 55°C were very different. In the presence of 10% (v/v) acetone, both were fairly stable, declining by only 5–10% after 1h; in the absence of acetone, however, 1,2-dihydroxynaphthalene oxygenase activity was only marginally less stable than in the presence of acetone, whereas catechol 2,3-oxygenase activity was rapidly lost, decreasing by 60% after only 10min.

The results indicate the presence of two very similar but distinct enzymes involved in the *meta* cleavage of catechol and 1,2-dihydroxynaphthalene in this organism. It is also shown that the 1,2-dihydroxynaphthalene oxygenase has little or no activity against catechol, but it is not clear whether catechol 2,3-oxygenase has any activity against 1,2-dihydroxynaphthalene.

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Evans, W. C., Fernley, H. N. & Griffiths, E. (1965) *Biochem. J.* **95**, 819
Nozaki, M., Kaganuyama, H. & Hayaishi, O. (1963) *Biochem. Z.* **338**, 582

The Divergent *meta*-Cleavage Pathway for the Metabolism of Benzoate, 3-Methylbenzoate and 4-Methylbenzoate by *Pseudomonas arvilla* mt-2

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The coexistence of a branched pathway for metabolism of 2-hydroxymuconic semialdehyde, formed by *meta* cleavage of catechol, has been demonstrated in benzoate-grown *Azotobacter* sp. (Sala-Trepat & Evans, 1971*a,b*), a naphthalene-grown pseudomonad (Catterall *et al.*, 1971) and *Pseudomonas putida* N.C.I.B. 10015 grown on phenol or cresols (Williams *et al.*, 1971). In only *Ps. putida* are both branches induced to high activities, and consideration of the specificities of the enzymes involved suggests that the role of the 4-oxalocrotonate branch is the assimilation of catechol and 4-methylcatechol and their precursors, and that that of the hydrolytic branch is to assimilate 3-methylcatechol and its precursors (Sala-Trepat *et al.*, 1971).

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Pseudomonas arvilla mt-2, a representative of the species *Ps. putida*, biotype A, grows at the expense of benzoate, 3-methylbenzoate and 4-methylbenzoate. After incubation of cells under various conditions, typical *meta*-ring-fission products accumulated that indicated that benzoate was converted into catechol (as reported by Feist & Hegeman, 1969), 3-methylbenzoate into 3-methylcatechol and 4-methylbenzoate into 4-methylcatechol, and that the catechols were further metabolized by the *meta*-cleavage pathway.

High induced activities of six of the *meta*-cleavage suite of enzymes (catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde hydrolase, 2-hydroxymuconic semialdehyde dehydrogenase, 4-oxalocrotonate tautomerase, 4-oxalocrotonate decarboxylase and 4-hydroxy-2-oxovalerate aldolase) were found in extracts of cells grown on either substrate, showing the presence of both the hydrolytic branch (hydrolase) and the 4-oxalocrotonate branch (dehydrogenase, tautomerase and decarboxylase).

The substrate specificities of the activities early in the pathway were very similar in extracts of cells grown on either substrate, suggesting that the same enzymes are present, being non-specifically induced in the various media. Further, 2-hydroxymuconic semialdehyde dehydrogenase is active only on the ring-fission products of catechol and 4-methylcatechol (relative activities 83:100), whereas that of 2-hydroxymuconic semialdehyde hydrolase was much more active on the ring-fission product of 3-methylcatechol than on those of catechol or 4-methylcatechol (relative activities 100:4.2:3.6). It is therefore proposed that the physiological role of the two branches of the pathway in *Ps. arvilla* are the same as proposed for *Ps. putida* N.C.I.B. 10015 (Sala-Trepat *et al.*, 1971).

Comparison of enzyme activities in succinate-grown cells after incubation in liquid culture with either benzoate or catechol suggests that the *meta*-cleavage-pathway enzymes are coincidently induced from the top by the primary substrate.

Ps. arvilla cannot grow at the expense of 2-methylbenzoate, since it is neither a substrate for the benzoate oxidase system nor an inducer of the *meta*-cleavage-pathway enzymes.

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