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 24 h at 4°C against 50 mM-phosphate buffer, pH 7.0, containing 1 mM-*o*-phenanthroline, 50% of both activities were lost; these could be restored by addition of Fe²⁺, although in the case of 1,2-dihydroxynaphthalene oxygenase only to 70% of the original value. After dialysis for 24 h at 4°C against phosphate buffer alone, catechol 2,3-oxygenase activity could be restored only by anaerobic incubation with Fe²⁺, whereas 1,2-dihydroxynaphthalene oxygenase activity was restored by aerobic addition of Fe²⁺.

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 1 h; 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 10 min.

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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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, 1971a,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).

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 coincidentally 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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Fluorescence Studies on Ribonucleic Acid Polymerase

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Pyrenebutyric acid has been used as a label for estimating the rotational relaxation time of proteins (Weber, 1969a,b) by observing the depolarization of fluorescence. The chromophore is suitable for large protein molecules owing to the long lifetime of its excited state. We have applied this technique to a study of the shape and size of the *Escherichia coli* B RNA polymerase molecule.

Recrystallized pyrene-1-butyric acid was converted into its acid chloride (76.2 mg) and added in 100–150 molar excess to the enzyme (10–15 mg) in 0.1 M-phosphate buffer, pH 8.0, containing 0.25 M-KCl and 0.1 mM-EDTA. The 14S conjugate was purified from excess of reagent by passage through Whatman DE-23 DEAE-cellulose and Sephadex G-25 followed by centrifugation on a 10–30% (w/v) glycerol density gradient. The 14S enzyme bound 2–3 mol of reagent/mol of enzyme (estimated by E_{346} and the Lowry method) and retained its [8- 14 C]AMP-incorporating activity after this treatment. When more pyrenebutyryl groups were bound (>7 mol/mol of enzyme) the enzyme aggregated and moved faster on the density gradient.

The rotational relaxation time of RNA polymerase (ρ_h) was found from plots of polarization versus temperature/viscosity. In isothermal experiments at 25°C with glycerol being used to alter the viscosity a straight line was obtained. However, when the temperature was varied from 2° to 50°C a curved Perrin (1926) plot resulted, suggesting the possibility of an internal degree of freedom of rotation or a change in the lifetime of the ligand at higher temperatures. The ratio ρ_h/ρ'_0 (ρ'_0 being the rotational relaxation time of an anhydrous sphere of the same molecular weight) was found to be 2–3 over a range of temperature 2–40°C, suggesting no gross change in structure. However, at higher temperatures the

ratio increased to 8–11 and the enzymic activity decreased, presumably as a result of aggregation of the enzyme.

On the basis of these results an apparent volume of the hydrated enzyme was assigned as 810 litres/mol compared with an anhydrous volume of 340 litres/mol. This large discrepancy would be manifested by a 120%-solvated sphere or an anhydrous ellipsoid of revolution with an axial ratio of 5–5.5. These findings accord with those of King & Nicholson (1971) based on the frictional ratio f/f_0 determined from sedimentation coefficient and molecular weight.

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Thiol Groups of Ribonucleic Acid Polymerase

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It has frequently been observed that preparations of *Escherichia coli* B RNA polymerase (EC 2.7.7.6) maintain optimum activity only in the presence of added thiol compounds (see, e.g., Chamberlin & Berg, 1962), and that treatment with thiol-specific reagents inhibit its activity (Ishihama & Hurwitz, 1969). To further our understanding of the importance of the enzyme's thiol groups with regard to both structure and function we have titrated them with *p*-mercuribenzoate, 5,5'-dithiobis-(2-nitrobenzoic acid) and [14 C]*N*-ethylmaleimide.

Core RNA polymerase was isolated and assayed (without 2-mercaptoethanol) and its molar concentrations were estimated by the method of King & Nicholson (1971). Thiol groups were allowed to react with thiol-specific reagents at 20°C in a medium consisting of 50 mM-tris-HCl buffer, pH 8, 0.4 M-KCl, 5 mM-MgCl₂ and 0.1 mM-EDTA (except that 50 mM-triethanolamine-HCl buffer, pH 7, replaced the tris-HCl buffer when [14 C]*N*-ethylmaleimide was used).

Contrary to previous reports, it was found that 2-mercaptoethanol was unnecessary for maintenance of enzymic activity at 0°C for 24 h, during which time there was no decrease in the number of titratable thiol groups. Moreover, reaction of the first 5 mol of thiol groups/mol of enzyme with either [14 C]*N*-ethylmaleimide or 5,5'-dithiobis-(2-nitrobenzoic acid) resulted in no loss activity, and over 90% was