Catechol Oxygenase Induction in *Pseudomonas aeruginosa*

By D. R. FARR AND R. B. CAIN

Microbiology Group, Department of Botany, University of Newcastle upon Tyne

(Received 2 August 1967)

1. The transfer from benzenesulphonate to benzoate as a growth substrate for *Pseudomonas aeruginosa* strain A resulted in a change in the enzymic route by which catechol was degraded; at intermediate stages it was possible to obtain cells containing the enzymes of both the 'ortho' and 'meta' metabolic pathways. 2. A similar result was effected by the reverse transfer, benzoate to benzenesulphonate. 3. Catechol itself always elicited a catechol 2,3-oxygenase in uninduced cells, but the product of this reaction, 2-hydroxymuconic semialdehyde, and biochemically related compounds such as 4-hydroxy-2-oxovalerate, unexpectedly induced a catechol 1,2-oxygenase. 4. Both types of catechol oxygenase are strongly repressed by the metabolic end products of both the 'ortho' and 'meta' pathways, but there was no inhibition of enzymic activity by these end products.

The mechanisms for the regulation of enzymes induced by aromatic substrates in bacteria have recently received considerable attention (Mandelstam & Jacoby, 1965; Stevenson & Mandelstam, 1965; Cánovas, Agaard & Stanier, 1966; Hegeman, 1966; Ornstom, 1966). These studies were all concerned with the enzymes of the 'ortho' ring-cleavage route, i.e. those enzymes that convert benzoate through catechol and β -oxoadipate into succinate and acetate. The 'meta' ring-cleavage route (the enzymes degrading catechol through 2-hydroxymuconic semialdehyde and 4-hydroxy-2-oxovalerate to acetaldehyde and pyruvate) has not been examined, possibly because the individual enzymic steps, although identified (Dagley & Gibson, 1965; Bayly, Dagley & Gibson, 1966; Cain & Farr, 1968), have not been characterized in sufficient detail. During studies of the metabolic route of benzenesulphonate degradation by *Pseudomonas aeruginosa*, we examined some features of the induction of the catechol oxygenases in this organism by different aromatic and aliphatic compounds. The results substantiate several recent findings in other organisms and led to the observation that some intermediates of the 'meta' route will induce enzymes always associated with the 'ortho' route for catechol metabolism.

MATERIALS AND METHODS

Organisms. The organism *Pseudomonas aeruginosa* strain A was grown, and cell extracts prepared, as described in the preceding paper (Cain & Farr, 1968). *Moraxella calcoacetica* (*Vibrio* O1, N.C.I.B. 8250) was also used in some experiments.

Enzyme assays. The enzymes were assayed spectrophotometrically with a Unicam SP.800 recording spectrophotometer fitted with the SP.820 constant-wavelength attachment and a constant-temperature cuvette holder through which water at 30° was circulated by a Braun (Melsungen, W. Germany) Circotherm unit. Cuvettes contained: tris-HCl buffer, pH 7.2, 300 μ moles; substrate, 0.5 μ mole; extract equivalent to about 100 μ g. of protein; other additions and water to a total volume of 3 ml. Blank cuvettes contained no substrate. Catechol 1,2-oxygenase (EC 1.13.1.1) was determined by the method of Hegeman (1966) with the addition to the cuvettes of EDTA (1 mM). *cis-cis*-Muconate-lactonizing enzyme [(+)-4-carboxymethyl-4-hydroxyisocrotonolactone lyase (deacylizing), EC 5.5.1.1] was determined by following the disappearance of *cis-cis*-muconate by measuring E_{260} in cuvettes also containing MnCl₂ (1 mM). Catechol 2,3-oxygenase (EC 1.13.1.2) was determined by following the increase in E_{375} due to the formation of 2-hydroxymuconic semialdehyde. The rate of disappearance of this product in cuvettes containing crude extract was so slow compared with its formation from catechol (less than 2% at the protein concentration used) that it can be neglected. Protocatechuate 3,4-oxygenase (EC 1.13.1.3) was determined by the method of MacDonald, Stanier & Ingraham (1954). A linear response was obtained in all assays over the first 5 min. A unit of activity is defined as the change of 1 m μ mole of substrate or product/min. at 30° and pH 7.2. Benzoate oxidase activity could not be obtained in extracts, so it was measured manometrically in whole cells. Warburg flasks contained: 20% (w/v) KOH, 0.2 ml., in the centre well; potassium phosphate buffer, pH 7.0, 300 μ moles; sodium benzoate, 3 μ moles; a washed-cell suspension equivalent to about 5–10 mg. dry wt. of cells (individually measured for each experiment by drying a washed sample to constant weight); D-chloramphenicol, 100 μ g., to prevent further induction. Total volume was made to 3 ml. with water. Oxygen uptake was measured for 30 min. at 30° and corrected for endogenous respiration. Linear oxygen uptakes were always found.

Nomenclature. The existence of two distinct pathways for

catechol metabolism results from the mechanism by which the aromatic ring of this substrate is cleaved. The trivial term '*ortho*' pathway refers to the route in which the catechol ring is broken by a catechol 1,2-oxygenase between the *o*-hydroxyl groups to form *cis-cis*-muconate, and this product degraded through (+)-muconolactone and β -oxoadipate enol lactone to β -oxoadipate. The '*meta*' pathway refers to the sequence initiated by cleavage of the catechol ring adjacent to one of the hydroxyl groups by a catechol 2,3-oxygenase to yield 2-hydroxymuconic semialdehyde; this intermediate is subsequently degraded to formate, acetaldehyde and pyruvate through 4-hydroxy-2-oxovalerate.

Chemicals. 2-Hydroxymuconic semialdehyde, after preparation from catechol on a large scale by using heat-treated extracts of *P. aeruginosa* strain A (Cain & Farr, 1968) and isolation by the method of Kojima, Itada & Hayaishi (1961), was recrystallized several times from ethyl acetate and then chromatographed as a band on several thin-layer plates (10 cm. $\times$ 20 cm.) of Kieselgel G (1 mm. thickness) in butan-1-ol-acetic acid-water (12:3:5, by vol.) to effect complete separation from any remaining catechol. The yellow band was scraped off the plates, the 2-hydroxymuconic semialdehyde was eluted from the Kieselgel G with ethanol and the ethanolic solutions were evaporated to dryness *in vacuo*. The residue was recrystallized again from ethyl acetate to give a product, m.p. 214° (decomp.), that was free from detectable catechol on paper chromatography and by infrared spectrophotometry. 5-Methyl-2-hydroxymuconic semialdehyde was prepared similarly from 4-methylcatechol. Synthetic 4-hydroxy-2-oxovalerate was kindly provided by Dr R. Bayly and 4-oxalocrotonate by Dr Y. Nishizuka. *cis-cis*-Muconic acid and β -oxoadipic acid were synthesized as described by Cain (1961). All other chemicals were obtained from commercial sources.

RESULTS AND DISCUSSION

Effect of growth substrate on the ring-fission mechanism employed

The nature of the growth substrate profoundly influenced the type of ring-fission mechanism adopted by *P. aeruginosa* strain A. Growth on fumarate yielded cells extracts of which had no catechol oxygenase activity. Benzoate induced a catechol 1,2-oxygenase, cleaving the catechol ring between the *o*-hydroxyl groups to form *cis-cis*-muconate, and the three enzymes that convert this product into β -oxoadipate (Ornston & Stanier, 1966). When the structurally similar benzenesulphonate or toluene-*p*-sulphonate was the carbon source (Table 1) a catechol 2,3-oxygenase was induced, converting catechol into 2-hydroxymuconic semialdehyde (Cain & Farr, 1968); this product was degraded to acetaldehyde and pyruvate by induced enzymes.

This difference of metabolic pathways suggested an examination of the enzymic changes brought about when cells growing on one aromatic source were transferred to another. In particular, we wished to learn whether benzenesulphonate-grown cells, after transfer into benzoate medium, formed only a benzoate oxidase (Ia) to produce catechol from benzoate but continued to use their existing '*meta*'-pathway enzymes (IIb-d) to degrade this catechol to acetaldehyde and pyruvate, or whether the whole distinct benzoate-to- β -oxoadipate '*ortho*'

Table 1. *Effect of growth substrate on the type of oxygenases induced in P. aeruginosa*

Cells were harvested from exponential-phase cultures grown in mineral salts medium with carbon source (25 mM). Assays were performed as described in the Materials and Methods section.

Growth substrate	Type of aromatic oxygenase	Sp. activity of oxygenase (units/mg. of protein)
Fumarate	None	0
Benzoate	Catechol 1,2-oxygenase	1410
Benzenesulphonate	Catechol 2,3-oxygenase	160
Toluene- <i>p</i> -sulphonate	Catechol 2,3-oxygenase	110
<i>p</i> -Hydroxybenzoate	Protocatechuate 3,4-oxygenase	1030

Scheme 1. Metabolic routes for benzoate and benzenesulphonate degradation by *P. aeruginosa* strain A.

Fig. 1. Enzyme changes effected by transferring benzenesulphonate-grown cells to 10mM-benzoate. ----, Growth (E_{540}); Δ , catechol 1,2-oxygenase (measured after inactivating the 2,3-oxygenase in air); $\circ$, benzoate oxidase (measured on intact cells); $\bullet$, catechol 2,3-oxygenase; $\blacktriangle$, *cis-cis*-muconate-lactonizing enzyme. Inset: enzyme activities plotted against growth.

pathway (enzymes Ia-e) was induced by this transfer (Scheme 1).

It is clear (Fig. 1) that initiation of growth on benzoate was coincident with the appearance of a benzoate oxidase (Ia) in the cells. There was, however, a considerable delay after the onset of growth (5hr. approx.) before significant activities of the catechol 1,2-oxygenase (Ib) and lactonizing enzyme (Ic) appeared (Fig. 1 inset), and during this time the existing catechol 2,3-oxygenase (IIa) and the other enzymes of the 'meta' route must have provided the cells with material for energy and growth.

After the appearance of the enzymes of the 'ortho' route, however, there was little change in the activity of the existing catechol 2,3-oxygenase. Addition of catechol to extracts prepared from cells taken at this time was followed by the rapid formation of 2-hydroxymuconic semialdehyde; some catechol was also being degraded by the 'ortho' route, however, because strong positive Rothera reactions for β -oxoadipate (Kilby, 1948) were obtained even in the presence of the intensely yellow 2-hydroxymuconic semialdehyde. Part of the 33hr. extract (Fig. 1) was vigorously aerated with oxygen for 30 min. at 30° to destroy the oxygen-labile catechol 2,3-oxygenase (Kojima *et al.* 1961; Hayaishi, Taniuchi & Kojima, 1962) and prevent the 'meta' route being used. The remainder was treated with sodium borohydride to restore maximum catechol 2,3-oxygenase

Table 2. Proportion of catechol metabolized to β -oxoadipate by *P. aeruginosa* extracts containing catechol oxygenases

Extract (4.2mg. of protein) from the 33hr. sample (Fig. 1), containing 92 units of catechol 1,2-oxygenase and 102 units of catechol 2,3-oxygenase/mg. of protein, was incubated for 1 hr. at 30° with 5 μ moles of catechol and 200 μ moles of tris-HCl buffer, pH 7.0, in Warburg flasks. Equivalent amounts of the same extract, treated to inactivate the 2,3-oxygenase, and a third extract containing 102 units of 2,3-oxygenase only, were similarly incubated. β -Oxoadipate was estimated by catalytic decarboxylation (Sistrom & Stanier, 1953). Results are corrected for controls containing no substrate.

Nature of extract	β -Oxoadipate formed (μ moles)
Extract with 2,3-oxygenase only	0.10
Extract with 1,2-oxygenase only	4.40
Extract with both oxygenases	2.52
Extract with 1,2-oxygenase only + extract with 2,3-oxygenase only	2.46

activity (Taniuchi, Kojima, Kanetsuna, Ochiai & Hayaishi, 1962). Equivalent amounts of these two preparations and a third specimen of 2,3-oxygenase free from 1,2-oxygenase activity (prepared directly from benzenesulphonate-grown cells) were then incubated with catechol (5 μ moles), and the β -oxoadipate was determined. Since both oxygenases have similar K_m values (0.5 μ M) for catechol

(Hayaishi, Katagiri & Rothberg, 1957; Kojima *et al.* 1961; Nozaki, Kagamiyama & Hayaishi, 1963; R. B. Cain & D. R. Farr, unpublished work with this organism), it was expected that the affinity of the two enzymes for catechol would have little effect on the ultimate route chosen for catechol oxidation. Catechol was indeed metabolized about equally by each route in the extract containing enzymes of both pathways. When separate extracts, each containing the enzymes of only one route, were

mixed to give equivalent amounts of the two oxygenases (on a specific activity basis) a similar result was obtained (Table 2).

In the reverse experiment (Fig. 2) in which benzoate-grown cells, with an active catechol 1,2-oxygenase and lactonizing enzyme, were transferred to benzenesulphonate, there was a long growth lag during which the activities of both catechol-1,2-oxygenase and lactonizing enzyme fell, though at very different rates. All the lactonizing activity had disappeared at 15hr. whereas the existing 1,2-oxygenase persisted for some 72hr. Well within this period (36hr. approx.) catechol 2,3-oxygenase activity was induced and its appearance was coincident with growth recommencing on the new substrate. Hegeman (1966) has shown that, when an inducer substrate was supplied to *Pseudomonas putida*, there was an immediate synthesis of enzymes directly de-repressed by the inducer but a lag in the synthesis of enzymes behind the first sequential inductive step. The observation in cells transferred to benzoate of the delayed appearance of catechol 1,2-oxygenase and lactonizing enzyme (Fig. 1), both of which are behind the independently regulated benzoate oxidase (Stevenson & Mandelstam, 1965; Hegeman, 1966; Ornston, 1966), parallels this finding.

Induction of the catechol oxygenases in fumarate-grown cells. Although distinct aromatic substrates induce one or the other of the two catechol oxygenases in micro-organisms, there is no direct relation-

Fig. 2. Enzyme changes effected by transferring benzoate-grown cells to 10mM-benzenesulphonate. Symbols are as defined in Fig. 1.

Table 3. *Substrates for induction of catechol oxygenases in P. aeruginosa*

A washed suspension of fumarate-grown cells was distributed equally among several flasks containing mineral salts medium and a single carbon source (1 mM). The cultures were incubated for 12 hr. at 30° and the cells harvested and washed. A sample of the washed suspension was then taken for examination of the ability of the intact cells to oxidize the substrate (5 μmoles) in Warburg flasks containing: tris-HCl buffer, pH 7.0, 300 μmoles; KOH, 0.2 ml., in the centre well. Oxidation rates are corrected for endogenous respiration in control flasks containing no substrate. The remaining cells were used to prepare extracts that were assayed for the type and specific activity of oxygenase present. N.D., Not determined. Numbers in parentheses below column headings refer to separate experiments with different enzyme preparations. The lower limit of oxygenase activity that could be reliably detected was approx. 0.2 μmole/min./mg. of protein.

Inducer	Oxidation of inducer by intact cells (μl. of O ₂ /hr./mg. dry wt.)			Type of oxygenase induced	Sp. activity of oxygenase (units/mg. of protein)		
	(1)	(2)	(3)		(1)	(2)	(3)
None	—	—	—	None	0	0	0
Fumarate	20	16	19	None	0	0	0
<i>cis-cis</i> -Muconate	16	7	N.D.	1,2-Oxygenase	22	30	32
<i>trans-trans</i> -Muconate	0	N.D.	N.D.	1,2-Oxygenase	4	N.D.	N.D.
Catechol	24	21	N.D.	2,3-Oxygenase	69	85	80
2-Hydroxymuconic semialdehyde	46	51	N.D.	1,2-Oxygenase	41	47	73
2-Hydroxy-5-methylmuconic semialdehyde	N.D.	N.D.	N.D.	1,2-Oxygenase	N.D.	10	N.D.
4-Hydroxy-2-oxovalerate	N.D.	2	N.D.	1,2-Oxygenase	N.D.	22	20
4-Oxalocrotonate	N.D.	9	4	1,2-Oxygenase	N.D.	17	11
β-Oxadipate	N.D.	N.D.	11	[1,2-Oxygenase]	N.D.	N.D.	1.0
Pyruvate	N.D.	35	30	[1,2-Oxygenase]	N.D.	0	0.6

Table 4. *Substrates for induction of catechol oxygenase in M. calcoacetica*

Experimental conditions were as described for Table 3, but induction medium also contained 0.5 mg. of casein hydrolysate/ml. N.D., Not determined.

Inducer	Oxidation of inducer by intact cells (μ l. of O ₂ /hr./mg. dry wt.)	Type of oxygenase induced	Sp. activity of oxygenase (units/mg. of protein)
None	—	None	0
Fumarate	18	None	0
Catechol	65	1,2-Oxygenase	16
<i>cis-cis</i> -Muconate	11	1,2-Oxygenase	11
2-Hydroxymuconic semialdehyde	31	1,2-Oxygenase	13
2-Hydroxy-5-methylmuconic semialdehyde	N.D.	1,2-Oxygenase	2

ship between chemical structure of the inducer and the type of oxygenase induced. Nozaki *et al.* (1963), for instance, used benzoate to induce catechol 2,3-oxygenase in *Pseudomonas arvilla*; this substrate, in contrast, induced the 1,2-oxygenase in *P. putida* (Stevenson & Mandelstam, 1965; Ornston, 1966), *Moraxella calcoacetica* (Evans, 1947; Ornston & Stanier, 1966) and our *P. aeruginosa* strain A.

Exposure of fumarate-grown cells of *P. aeruginosa* strain A to catechol itself induced a 2,3-oxygenase (Table 3) and the other enzymes (IIc, d) catalysing further metabolism of the ring-fission product by the 'meta' route. When fumarate-grown *M. calcoacetica* was incubated with catechol and a nitrogen source, however, a catechol 1,2-oxygenase and the other enzymes (Ic-e) of the 'ortho' route were induced (Table 4). *M. calcoacetica* synthesized a catechol 2,3-oxygenase when grown with naphthalene as the carbon source (Griffiths, Rodrigues, Davies & Evans, 1964).

Transfer of benzenesulphonate-grown cells to benzoate (Fig. 1) and the appearance of benzoate oxidase (Ia) would supply the organism with increased internal concentrations of catechol, the bulk of which (at least initially) must be rapidly oxidized to 2-hydroxymuconic semialdehyde by the existing catechol 2,3-oxygenase. Under these circumstances, it is difficult to visualize the formation in the cells of appreciable amounts of *cis-cis*-muconate, which Ornston (1966) has shown to be the probable inducer of catechol 1,2-oxygenase in *P. putida*. Catechol was not completely ruled out as an inducer of this oxygenase, but Ornston (1966) believed that two compounds as widely disparate in chemical properties as *cis-cis*-muconate and catechol were unlikely to have equivalent inducing properties. Our observation that catechol and *cis-cis*-muconate induced distinct types of oxygenase in *P. aeruginosa* (Table 3) adds further support to this hypothesis.

Compounds structurally related to *cis-cis*-muconate were not examined by Ornston (1966), but we have found the structurally similar 2-

hydroxymuconic semialdehyde, and its 5-methyl homologue, to be inducers of the 1,2-oxygenase in fumarate-grown cells of both *P. aeruginosa* (Table 3) and *M. calcoacetica* (Table 4). These substrates (Scheme 2) did not induce the 2,3-oxygenase, which catalyses their formation from catechol and 4-methylcatechol respectively, but they were slowly degraded in the incubation flasks. Other products of 'meta' ring-cleavage pathway were also examined in *P. aeruginosa*. 4-Oxalocrotonate, structurally similar to *cis-cis*-muconate and 2-hydroxymuconic semialdehyde, was found to elicit appreciable activities of 1,2-oxygenase although the cells oxidized it rather slowly. A similar result was obtained with 4-hydroxy-2-oxovalerate but not with pyruvate, one end product of the 'meta' pathway. β -Oxoadipate, a fully saturated analogue of 4-oxalocrotonate, induced very little catechol 1,2-oxygenase in *P. aeruginosa*, although it is worth emphasizing that the level of activity found (1 μ mole/min./mg. of protein) was easily distinguished from the zero activity found in controls or fumarate incubations (Table 3). Ornston (1966) similarly found negligible catechol 1,2-oxygenase induction by β -oxoadipate in *P. putida*.

These observations permit an explanation of the induction of the enzymes of the 'ortho' ring-cleavage pathway in the experiment illustrated in Fig. 1. The 2-hydroxymuconic semialdehyde, produced by 'meta' cleavage of catechol by the existing 2,3-oxygenase, would induce a 1,2-oxygenase and lead to a significant increase in the internal concentration in the cells of *cis-cis*-muconate, the natural intermediate of the 'ortho' pathway. This metabolite is known to be the inducer of the three enzymes (lactonizing enzyme, muconolactone isomerase and β -oxoadipate enol lactone hydrolase) responsible for its conversion into β -oxoadipate in *P. putida* (Ornston, 1966) and *P. aeruginosa* (Cain & Farr, 1968).

The observation that catechol and 2-hydroxymuconic semialdehyde induce distinct catechol oxygenases also lends support to the suggestion

Inducers of 2,3-oxygenase

Inducers of 1,2-oxygenase

Compounds inducing no oxygenase

(≥ 10.0 units/mg. of protein)(≤ 1.0 unit/mg. of protein) Scheme 2. Structure of ring-fission metabolites inducing catechol oxygenases in *P. aeruginosa*.

(Stanier, 1966) that the induction of the 'meta' route is probably regulated 'from the top', i.e. by the primary substrate inducer, catechol, in contrast with the induction of the 'ortho' pathway in *P. putida*, which Ornston (1966) has shown convincingly to be regulated 'from the bottom', i.e. by the products.

Repression and inhibition of catechol 2,3-oxygenase by metabolic end products. Stevenson & Mandelstam (1965) observed that the benzoate oxidase and catechol 1,2-oxygenase of *P. putida* A3.12 were strongly repressed by succinate and acetate, the end products of the series of enzymes degrading the aromatic ring through β -oxoadipate to tricarboxylic acid-cycle intermediates. No studies have been made with catechol 2,3-oxygenase, although the enzyme is now well characterized. We have therefore examined the end products of both the 'ortho' route (acetate and succinate) and the 'meta' route (formate, acetaldehyde and pyruvate) for their inhibitory and repressive effects on both 1,2-oxygenase and 2,3-oxygenase in *P. aeruginosa*.

Catechol 2,3-oxygenase activity in extracts of this organism was not inhibited by any of the above five end products, alone or in combination, at concentrations up to 50-fold the substrate concentration. Induction of this enzyme in fumarate-grown cells by catechol, however, was strongly

Table 5. *Repression of P. aeruginosa catechol 2,3-oxygenase by aliphatic metabolites*

Cultures of *P. aeruginosa* strain A were grown in minimal medium with fumarate (1 g./l.) as carbon source, harvested and washed and resuspended in mineral medium containing catechol (3 mM) alone or with equimolar concentrations of the other components. Incubation was continued until turbidity measurements showed doubling of the cell mass. The individual cultures were harvested, the cells crushed and the extracts all adjusted to 2.6 mg. of protein/ml. Extracts were then assayed for catechol 2,3-oxygenase.

Inducer components	$\Delta E_{375}/5$ min.	Repression (%)
None	0	—
Catechol	0.42	0
Catechol + formate	0.30	29
Catechol + pyruvate	0.17	60
Catechol + acetaldehyde	0.25	40
Catechol + formate + pyruvate + acetaldehyde	0.08	81
Catechol + succinate + acetate	0.14	67

repressed by growth in the presence of equimolar concentrations of pyruvate or acetaldehyde and moderately so by formate (Table 5). The repression caused by pyruvate was still observable (11%) at

Table 6. *Repression of P. aeruginosa catechol 1,2-oxygenase by aliphatic metabolites*

Experimental methods were as described for Table 5, but benzoate (3mM) was used to induce the 'ortho' pathway. Protein concentrations were adjusted to 2mg./ml. before assay.

Inducer components	$\Delta E_{260}/5 \text{ min.}$	Repression (%)
None	0	—
Benzoate	1.05	0
Benzoate + succinate	0.60	67
Benzoate + acetate	0.44	76
Benzoate + succinate + acetate	0.24	87
Benzoate + pyruvate	0.01*	99
Benzoate + pyruvate + formate + acetaldehyde	0.002*	100

* Assayed with a 20-fold increase in extract compared with others.

concentrations one-thirtieth of that of the inducer. Repression of nearly 70% in 2,3-oxygenase synthesis was also observed by growth of the organism in the presence of the products of the 'ortho' route, succinate and acetate.

Benzoate was used as a growth substrate to induce catechol 1,2-oxygenase in *P. aeruginosa*, but the presence of acetate or succinate, or both, in the growth medium led to strong repression (67–87%), similar to that found for this enzyme in *P. putida* (Stevenson & Mandelstam, 1965). The end products of the 'meta' route brought about virtually complete repression of catechol 1,2-oxygenase synthesis in *P. aeruginosa* (Table 6), confirming Ornston's (1966) findings that this enzyme is extremely sensitive to catabolite repression. In general, therefore, both catechol oxygenases are similar in their sensitivity to repression by catabolites of both 'ortho' and 'meta' routes. Catabolite repression of the two alternative oxygenases is therefore unlikely to play

a significant role in the selection by an organism of one of these two routes for aromatic ring cleavage.

This work was supported by a research grant from the Continental Oil Co. to R. B. C., which is gratefully acknowledged. A preliminary account was presented at the U.S.–Japan Symposium on Oxygenases at Kyoto in May 1966.

REFERENCES

- Bayly, R. C., Dagley, S. & Gibson, D. T. (1966). *Biochem. J.* **101**, 293.
- Cain, R. B. (1961). *Biochem. J.* **79**, 298.
- Cain, R. B. & Farr, D. R. (1968). *Biochem. J.* **106**, 859.
- Cánovas, J., Agaard, J. & Stanier, R. Y. (1966). In *Biological and Chemical Aspects of Oxygenases*, p. 113. Ed. by Bloch, K. & Hayaishi, O. Tokyo: Maruzen.
- Dagley, S. & Gibson, D. T. (1965). *Biochem. J.* **95**, 466.
- Evans, W. C. (1947). *Biochem. J.* **41**, 373.
- Griffiths, E., Rodrigues, D., Davies, J. I. & Evans, W. C. (1964). *Biochem. J.* **91**, 16r.
- Hayaishi, O., Katagiri, M. & Rothberg, S. (1957). *J. biol. Chem.* **229**, 905.
- Hayaishi, O., Taniuchi, H. & Kojima, Y. (1962). *Fed. Proc.* **21**, 52.
- Hegeman, G. D. (1966). *J. Bact.* **91**, 1140.
- Kilby, B. A. (1948). *Biochem. J.* **43**, v.
- Kojima, Y., Itada, N. & Hayaishi, O. (1961). *J. biol. Chem.* **236**, 223.
- MacDonald, D. L., Stanier, R. Y. & Ingraham, J. L. (1954). *J. biol. Chem.* **210**, 809.
- Mandelstam, J. & Jacoby, G. A. (1965). *Biochem. J.* **94**, 569.
- Nozaki, M., Kagamiyama, H. & Hayaishi, O. (1963). *Biochem. Z.* **338**, 582.
- Ornston, L. N. (1966). *J. biol. Chem.* **241**, 3800.
- Ornston, L. N. & Stanier, R. Y. (1966). *J. biol. Chem.* **241**, 3776.
- Sistrom, W. R. & Stanier, R. Y. (1953). *J. Bact.* **66**, 404.
- Stanier, R. Y. (1966). In *Biological & Chemical Aspects of Oxygenases*, p. 143. Ed. by Block, K. & Hayaishi, O. Tokyo: Maruzen.
- Stevenson, I. L. & Mandelstam, J. (1965). *Biochem. J.* **96**, 354.
- Taniuchi, J., Kojima, Y., Kanetsuna, F., Ochiai, H. & Hayaishi, O. (1962). *Biochem. biophys. Res. Commun.* **8**, 97.