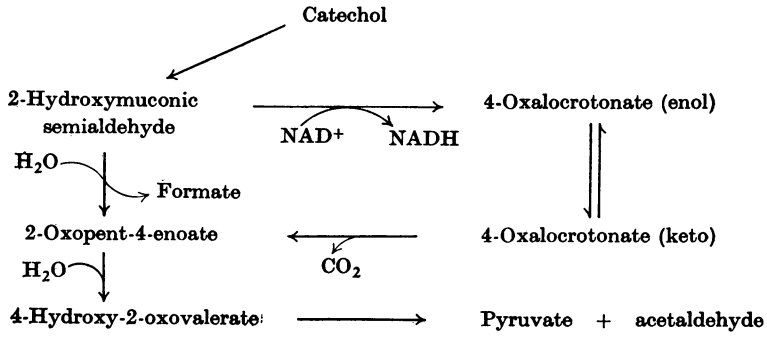

from catechol or 2-hydroxymuconic semialdehyde. These results implicate both NAD^+- and non-NAD^+-dependent activities in the metabolism of 2-hydroxymuconic semialdehyde; they also show that the rate of disappearance of 2-hydroxymuconic semialdehyde in the absence of added NAD^+ cannot be taken as a direct measure of the hydrolase activity, as has been done (Feist & Hegeman, 1969). We have confirmed in this strain all the metabolic steps of the scheme reported by Sala-Trepat & Evans (1971) in *Azotobacter* (see page 19).

Unlike the pattern observed in *Azotobacter* species, the 2-hydroxymuconic semialdehyde hydrolase activity was greatly increased by growth on phenol in this strain. The enzymes functional in this scheme were inducible. The high inducible activities of 4-oxalocrotonate tautomerase and 4-oxalocrotonate decarboxylase substantiate the physiological importance of the NAD^+-dependent dehydrogenase activity for the metabolism of 2-hydroxymuconic semialdehyde in this strain.

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The Physiological Significance of the Two Divergent Metabolic Steps in the *meta* Cleavage of Catechols by *Pseudomonas putida* N.C.I.B. 10105

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Cell-free extracts of *Pseudomonas putida* N.C.I.B. 10105 grown on phenol or any cresol (*o*-, *m*- or *p*-cresol) showed an identical pattern of enzyme activities, independent of growth substrate. These extracts contained high activities of a *meta*-cleaving oxygenase that attacked catechol, 4-methylcatechol and 3-methylcatechol at constant relative rates of 30:100:48 respectively. The low specificity of the oxygenase extended to the enzymes metabolizing the ring-fission products. The NAD^+-dependent aldehyde dehydrogenase present in all the extracts was able to metabolize 2-hydroxymuconic semialdehyde and the ring-cleavage product of 4-methylcatechol, namely 2-hydroxy-5-methylmuconic semialdehyde, at relative rates of 100:120 respectively, but did not attack the ring-cleavage product of 3-methylcatechol, namely 2-hydroxy-6-oxohepta-2,4-dienoate, in which the aldehyde group is absent. However, the non-NAD^+-dependent hydrolase activity was very active on 2-hydroxy-6-oxohepta-2,4-dienoate but much less active on 2-hydroxymuconic semialdehyde and 2-hydroxy-5-methylmuconic semialdehyde (relative rates 100:5:2.5 respectively). All these activities were inducible. All extracts also showed high induced activities of 4-oxalocrotonate tautomerase and 4-oxalocrotonate decarboxylase, and the 4-hydroxy-2-oxovalerate aldolase activity was increased fivefold over its activity in succinate-grown cells.

Feist & Hegeman (1969) have shown that all the enzymes of the metabolic scheme of Dagley & Gibson (1965) in this strain are induced from the top by the primary substrate (phenol and cresols). Our results agree with this view, and in addition show that the enzymes of the 4-oxalocrotonate branch of the pathway are induced with the oxygenase and hydrolase; the whole set of enzymes therefore appears to be induced from the top. Our results suggest that the two branches of the pathway are responsible for the metabolism of different catechols. The NAD^+-dependent aldehyde dehydrogenase and the other enzymes of the 4-oxalocrotonate branch are functional in the metabolism of catechol, 4-methylcatechol and their metabolic precursors, namely phenol and *p*-cresol,

whereas the physiological role of the hydrolase is mainly limited to the dissimilation of 3-methylcatechol and its metabolic precursors, namely *o*- and *m*-cresol. The coexistence of these two different branches in the metabolism of catechol and 4-methylcatechol, which at first sight may appear redundant, is a consequence of the low specificity of induction and action of the enzymes of the *meta*-cleavage pathway in this strain.

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Desulphation of L-Fucose Monosulphates by an Enzyme from *Patella vulgata*

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It was demonstrated earlier that sulphatase-containing preparations from the viscera of *Patella vulgata* were active against mono- and oligosaccharide sulphate mixtures derived from fucoidin (from *Fucus vesiculosus*) by graded acid hydrolysis (Lloyd & Lloyd, 1961). The enzyme, a sulphohydrolase (Lloyd, Lloyd & Owen, 1962), also showed activity towards synthetic substrates: unresolved L-fucose mono-, di- and tri-sulphates suffered partial de-esterification. Analysis of the products of these enzymic hydrolyses suggested that the sulphatase might catalyse the hydrolysis of a sulphate ester group located at one position only of the fucose molecule. To enable further studies to be made the three isomeric monosulphate esters of L-fucopyranose have been synthesized.

Direct sulphation of L-fucose with pyridine–sulphur trioxide (Baumgarten, 1926; Duff, 1960) gave a mixture of mono-, di- and tri-sulphates. The monoesters were separated from the polyesters by column chromatography on DEAE-cellulose with 1M-ammonia as eluent; this method is of general application and monosulphates of L-rhamnose, D-glucose and D-galactose have also been isolated in this way (Lloyd, Stuart & Fielder, 1968). Separation of the isomeric fucose monosulphates was effected chromatographically by using a DEAE-cellulose column with 0.1M-borate buffer, pH 10, as eluent. The fractions were desalted on columns containing Sephadex G-10. The separated L-fucose 2-, 3- and 4-sulphates (barium or sodium salts) were identified by electrophoretic, periodate oxidation and other studies.

L-Fucose 2-sulphate was also synthesized definitively by successive sulphation and de-*O*-acetylation of 1,3,4-tri-*O*-acetyl-L-fucopyranose, but yields were low. A more effective synthetic route to the 2-sulphate furnished, in the penultimate stage, methyl-α-L-fucopyranoside 2-sulphate, which underwent hydrolysis in dilute acetic acid to give methyl-α-L-fucopyranoside and L-fucose 2-sulphate. The extreme sensitivity to acids of 2-sulphate esters of glycopyranosides has been shown to extend to other sugars (Lloyd & Forrester, 1971).

The sulphohydrolase from *Patella vulgata* de-esterified the purified L-fucose 2-, 3- and 4-sulphates at closely similar rates.

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Localization of Monomethyl- and Dimethyltocols on Cytoplasmic Particles in Plant Tissues

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Preliminary studies on the intracellular distribution of the tocopherols showed α-tocopherol to occur in the chloroplast, whereas β-, γ- and δ-tocopherols were found in the extrachloroplastidic part of the cell (Newton & Pennock, 1971). It was further shown that in *Fucus spiralis* the β- and γ-tocopherols appeared in a fraction that sedimented after chloroplasts and part of the δ-tocopherol could be precipitated by further centrifugation. This paper reports further studies on the localization of non-α-tocopherol in *F. spiralis*, *Coleus hybridus*, *Phaseolus vulgaris* and *Rumex sanguineus*.

After removal of chloroplasts by low-speed centrifugation of homogenates of the plants mentioned above, γ-tocopherol was found in a fraction sedimenting at 25000*g* for 45min. This heterogeneous fraction contains some fragments of nuclei, chloroplasts and cell wall (cell walls are mostly removed from the homogenate by passage through muslin) as well as mitochodria and some cytoplasmic organelles such as Golgi bodies, lysosomes, spherosomes, lomasomes and microtubules (Clowes & Juniper, 1968). Little or no γ-tocopherol was found in preparations of nuclei, mitochondria, cell walls or chloroplasts.

In earlier work δ-tocopherol was not found in