Guroff *et al.* (1967) have claimed that the N I H shift fully explains the reaction. However, if the N I H shift were typical the postulated positively charged intermediate formed by the action of OH^+ on the substrate would be stabilized by the presence of the hydroxyl group in *p*-hydroxyphenylpyruvate as an uncharged quinonoid structure. This would be expected to cause almost complete elimination of the side chain to yield *p*-quinol as the major product (Daly, Jerina & Witkop, 1968). Since homogentisate is the only detectable product an alternative mechanism was sought. The reaction can be explained if a negatively charged intermediate is formed in which the charge is localized at C-2 on the benzene ring. The presence of the hydroxyl group would not cause this charge to migrate to the *para* position, in contrast with the positive charge in the N I H shift. An intermediate of this type can be formed if C-1 of the benzene ring is attacked by molecular oxygen. During elimination of the carbonyl group the oxygen molecule could form a cyclic peroxide with the oxygen molecule attached to the carbonyl carbon and C-1. Migration of the charge would cause the side chain to migrate and the peroxide bridge to break with the formation of homogentisate. Unlike previously suggested mechanisms, the proposed one can explain the whole reaction sequence in terms of electronic configurations, and is not subject to the problems involved in the classical N I H shift.

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The State of the Iron Atoms in Hydroxylase Iron-Sulphur Proteins

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Adrenodoxin is an iron-sulphur protein associated with the steroid hydroxylase systems of adrenal glands. Like the plant ferredoxins it is a small protein, of molecular weight approx. 12000, containing two atoms each of iron and inorganic sulphide/molecule, and accepting one electron/molecule on reduction.

Mössbauer spectroscopy has been used to determine the state of the iron atoms in ferredoxins in their oxidized and reduced forms (Rao, Cammack, Hall & Johnson, 1971). The results were found to agree with the model proposed by Gibson, Hall, Thornley & Whatley (1966). According to this model, the two iron atoms are both tetrahedrally co-ordinated to sulphur ligands. In the oxidized ferredoxin they are both high-spin Fe^{3+} , antiferromagnetically coupled to give no net spin; in reduced ferredoxin one iron atom becomes high-spin Fe^{2+} , giving a net spin $S = \frac{1}{2}$ for the coupled system.

We have measured Mössbauer spectra of purified porcine adrenodoxin enriched with ^{57}Fe isotope by reconstitution. The spectra of oxidized adrenodoxin were similar to those of the ferredoxins, consisting of a doublet of narrow lines, with a small chemical shift and quadrupole splitting. This indicates that both iron atoms are Fe^{3+} in a non-magnetic state, and are in very similar environments. The Mössbauer spectra of reduced adrenodoxin showed magnetic hyperfine interaction at all temperatures up to 244°C , because of the long electron-spin relaxation time. The spectrum of adrenodoxin at 4.2°K was similar to those of the plant ferredoxins. In a small applied magnetic field the spectrum appeared to consist of two hyperfine splitting patterns due to Fe^{3+} and Fe^{2+} respectively; these were better separated than in the corresponding spectra for ferredoxin. The patterns were separated by application of a large magnetic field, indicating that the hyperfine fields at the two iron atoms were in opposite directions, and thus the atoms were antiferromagnetically coupled together. All of these observations are consistent with the model proposed by Gibson *et al.* (1966) and the interpretation of the electron-paramagnetic-resonance spectra of adrenodoxin and ferredoxin by Johnson, Cammack, Rao & Hall (1971).

The Mössbauer spectra of putidaredoxin, an iron-sulphur protein from the camphor hydroxylase system of *Pseudomonas putida* (Debrunner, 1970; Bearden & Dunham, 1970), appear to be similar to

the corresponding spectra of adrenodoxin. Thus according to our interpretation of the spectra both of these hydroxylase iron-sulphur proteins have a similar mode of action to the plant ferredoxins.

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Some Studies on the Purified 2-Furoyl-Coenzyme A Hydroxylase from *Pseudomonas putida* F2

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The direct conversion of the carbon skeleton of furan-2-carboxylate (2-furoate) into 2-oxoglutarate by *Pseudomonas putida* F2 has been demonstrated by Jones & Trudgill (1967). The sequence of intermediates furan-2-carboxylate $\rightarrow$ 2-furoyl-CoA $\rightarrow$ 5-hydroxy-2-furoyl-CoA $\rightarrow$ 5-oxo- Δ^2 -dihydro-furoyl-CoA $\rightarrow$ 2-oxoglutaryl-CoA $\rightarrow$ 2-oxoglutarate has been proposed, and the 2-furoyl-CoA hydroxylase has been separated from 2-furoyl-CoA synthetase and the other enzymes of the sequence (Trudgill, 1969; Kitcher & Trudgill, 1970).

The 2-furoyl-CoA hydroxylase draws its oxygen from water and, like other enzymes that catalyse analogous hydroxylations of nicotinate (Hunt, Hughes & Lowenstein, 1958) and picolinate (Dagley & Johnson, 1963), has an obligatory requirement for an electron acceptor. This requirement may be satisfied by Methylene Blue, Nitro Blue Tetrazolium or the membrane fraction from furan-2-carboxylate-grown *Ps. putida* F2. Measurement of the reduction of the artificial electron acceptor under suitable conditions or coupling of Methylene Blue reduction to oxygen consumption provide acceptable methods for detection and assay of the enzyme.

The 2-furoyl-CoA hydroxylase has been purified about 100-fold by ammonium sulphate fractionation (0–30% saturation fraction), chromatography on Agarose A5-m and sucrose-density-gradient centrifugation. Sufficient purified enzyme has not yet been obtained for an assessment to be made of its degree of purity. Its high molecular weight, about 900 000, estimated by comparison with marker

proteins on the agarose column and sucrose density gradients, precludes the use of such micro techniques as isoelectric focusing and polyacrylamide-gel electrophoresis on the native protein.

On spectral analysis the enzyme displays a single absorption peak with $\lambda_{\max}$, 273 nm. This observation, coupled with the lack of significant inhibition by metal-ion-chelating agents, implies that this hydroxylase, in contrast with those that utilize molecular oxygen directly, contains neither metal ion nor flavin cofactors (Hayaishi, 1969).

The purified enzyme displays K_m 2×10^{-5} M for 2-furoyl-CoA. In the presence of an electron acceptor the purified enzyme catalyses the formation of a product that displays an absorption maximum at 373 nm. Addition of crude extract, which contains the enzymes required to convert the hydroxylation product into 2-oxoglutarate (Kitcher & Trudgill, 1970), catalyses a time-dependent decrease in the 373 nm extinction.

On the basis of a comparison with the spectral properties of 2-carbethoxy-5-hydroxythiophen it is proposed that the reaction product showing $\lambda_{\max}$, 373 nm is 5-oxo- Δ^7 -dihydro-2-furoyl-CoA.

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Hydroxylation and Toxicity of Aflatoxins

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There are at least four routes for the biological hydroxylation of this group of potent hepatotoxins: (1) a hydroxyl group may be inserted at the junction of the two fused furan rings, e.g. aflatoxin B₁ is converted into aflatoxin M₁; (2) the single methoxyl group in the aromatic portion of the molecule may be hydrolysed to form a phenolic derivative; (3) the vinyl ether double bond of aflatoxins B₁ and G₁ may be hydrated, yielding aflatoxin hemicetals B_{2a} and G_{2a}; (4) the cyclopentenone ring in aflatoxins B₁ and B₂ may be reduced to form the secondary alcohols, aflatoxicol and dihydroaflatoxicol.

Liver preparations of most species hydroxylate aflatoxins by pathway (1) to some extent (Portman, Plowman & Campbell, 1968; Schabert & Steyn,