Gross, 1969; Kang, Igarashi & Gross, 1969; Bornstein & Nesse, 1970; Chick & Bornstein, 1970; Fietzek, Munch, Breitzkreutz & Kuhn, 1970; Rauterberg & Kuhn, 1971), the lysyl residue occurring in the *N*-terminal non-helical telopeptide region of both α_1 - and α_2 -chains is partially hydroxylated, to the extent of approx. 50%, to hydroxylysine. In view of the involvement of the non-helical region of the collagen molecule in cross-linking (Bornstein, Kang & Piez, 1966; Bailey, Peach & Fowler, 1970a) and the occurrence in chick bone collagen (Bailey, Fowler & Peach, 1969) but absence from skin collagen (at least of the rat and calf; Bailey, Peach & Fowler, 1970b; A. J. Bailey, personal communication) of the hydroxylysine-derived intermolecular cross-link syndesine, this hydroxylation may be of particular significance. We have therefore examined the extent of hydroxylation of lysine in the *N*-terminal non-helical region of both α_1 - and α_2 -chains from several collagens. The following results, in which the extent of hydroxylation is expressed on a percentage basis, were obtained: collagen from rat skin, α_1 -chain, <1, α_2 -chain, <1; from rat tibia + femur, α_1 , 76, α_2 , 47; from rat tail tendon, α_1 , 3, α_2 , 9; from embryonic chick frontal bone, α_1 , 49, α_2 , 66; from embryonic chick leg tendon, α_1 , 31, α_2 , 43. The results suggest that extensive hydroxylation of the *N*-terminal telopeptide lysine is a common feature of bone collagen and also indicate some degree of hydroxylation in this region in tendon collagen, particularly so in the less-soluble leg tendon collagen from chick embryo known to contain syndesine (Bailey *et al.* 1970a).

In studies aimed at establishing which factors determine the extent of hydroxylation, we have so far demonstrated the inhibition of this hydroxylation by $\alpha\alpha'$ -bipyridyl and subsequently the hydroxylation *in vitro* of the *N*-terminal telopeptide lysine in leg tendon collagen from chick embryo to an extent similar to that occurring *in vivo*. Hydroxylation was achieved by incubation with an extract of embryonic chick tibiae in the presence of ferrous iron, α -oxoglutarate and ascorbate, known cofactors in the general hydroxylation of collagen proline and lysine (Prockop, 1970; Udenfriend, 1970). Conditions that might permit complete hydroxylation have yet to be ascertained. Attempts are also being made to hydroxylate the corresponding lysine in embryonic chick skin collagen.

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Hydroxylation of *p*-Hydroxyphenylpyruvate and the N I H Shift: a Hypothesis

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The conversion of *p*-hydroxyphenylpyruvate into homogentisate by *p*-hydroxyphenylpyruvate oxidase is virtually stoichiometric (La Du & Zannoni, 1955). The same enzyme oxidizes phenylpyruvate to *o*-hydroxyphenylacetate, but not quantitatively (Taniguchi & Armstrong, 1963). These reactions involve an oxidative decarboxylation and migration of the side chain during hydroxylation. Several mechanisms have been proposed that include the formation either of a semiquinone or of a peroxide bridge across the benzene ring. They are unsatisfactory because the hydroxyl group is obligatory (Goodwin & Witkop, 1957; Soloway, 1966; Davies, Hassall & Schofield, 1964). Crandall (1964) suggested an attack by an oxygen molecule on C-1 of the benzene ring, but did not develop a satisfactory reaction sequence. Lindblad, Lindstedt & Lindstedt (1970), who demonstrated that molecular oxygen is incorporated into the carboxyl and new hydroxyl groups of homogentisate, postulated a mechanism in which the hydroxyl group of the substrate is essential, whereby a peroxide bridge is formed between the benzene ring and the side chain

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