placenta could be due to the low content of cytochrome *P*-450 present or to a higher affinity and a high concentration of endogenous steroid substrates, which saturates the enzyme system and prevents access of drugs to the enzyme site.

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The Effect of Diet on Rat Plasma Corticosteroids and Liver Aromatic Hydroxylase Activity

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There is evidence that protein-deficient diets in children may result in adaptive changes of hepatic enzymes concerned with intermediary metabolism and that these are mediated by corticosteroid hormones (Alleyne & Young, 1967; Waterlow, 1968). Further, it has been shown that the microsomal drug-metabolizing enzymes of the liver of growing rats are dependent on the dietary intake of protein and total calories (Dickerson, Basu & Parke, 1971). These drug-metabolizing enzymes of the liver are also concerned with metabolic deactivation of the endogenous steroid hormones, and their activity may be increased by stress or administration of corticosteroids (Driever, Bousquet & Miva, 1966). It was therefore decided to ascertain whether the effect of diet on the activities of the hepatic drug-metabolizing enzymes observed previously were similarly mediated through corticosteroid hormones.

Male weanling rats (24 days old) were divided into three groups: group A, controls, were fed *ad lib.* on stock diet (Spiller's), 21% protein; group B, 'protein-deficient', were fed *ad lib.* on stock diet diluted with starch to contain 7% protein; group C, 'protein- and calorie-deficient', were fed on same amount of protein as group B but without starch. Liver biphenyl 4-hydroxylase activity was determined as a measure of hepatic drug metabolism (Creaven, Parke & Williams, 1965). Liver glycogen (Kemp & Kits van Heijningen, 1954), glucose

6-phosphatase (Cori & Cori, 1952) and plasma corticosteroids (Mattingly, 1962) were also determined.

The protein-deficient animals (group B) after 2 and 4 weeks on the diet showed increases in hepatic biphenyl 4-hydroxylase activity (70–100%) and plasma corticosterone (150–200%). The animals on the diet deficient in both calories and protein (group C) showed only moderate increases in both liver biphenyl 4-hydroxylase activity (25–50%) and plasma corticosterone. Studies of the kinetics of the hepatic biphenyl 4-hydroxylase activity showed that in both groups B and C the K_m value was the same as controls (0.23 nM) but the V_{max} was increased.

Since the hepatic cytochrome *P*-450 in similar protein-deficient rats was either diminished or showed no change (Dickerson *et al.* 1971) and the K_m for the biphenyl 4-hydroxylase was unchanged but the V_{max} increased, the increased hepatic biphenyl 4-hydroxylase activity is unlikely to be due to any change of the active sites or in the total amount or the terminal oxygenase. It is more likely to be due to an increase of some other component of the associated electron-transport chain, e.g. cytochrome *P*-450 reductase, or to some conformational change of the membrane of the endoplasmic reticulum or change of the membrane environment. The simultaneous increase in plasma corticosteroids and the known stimulating effect of these hormones might suggest that the increases observed in the hepatic drug-metabolizing enzyme were mediated through the endogenous steroid hormones.

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Studies on the Hydroxylation of Lysine Occurring in the Non-helical Region at the N-Terminal End of the Collagen Molecule

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In bone collagen from chick tibia (Miller, Lane & Piez, 1969; Lane & Miller, 1969), in contrast with all skin collagens so far examined (Bornstein & Piez, 1966; Kang, Bornstein & Piez, 1967; Kang, Piez &

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 period bridge is formed between the benzene ring and the side chain