(17 α ,21-dihydroxypregn-4-ene-3,11,20-trione, 17 β -hydroxy-1 α -methyl-5 α -androstan-3-one and 5 α -androstan-3,16-dione) by using an improved assay procedure. Replicate rate determinations allowed regression lines to be calculated for the reciprocal reaction rate on the reciprocal substrate concentration, by using weighting (Wilkinson, 1961) and non-weighting procedures. In this way, estimates of apparent K_m , apparent V_{max} , and the standard errors of these were obtained. The rates found for equimolar mixtures of pairs of the substrates were lower than the values calculated for wholly independent reaction, and the difference was not within the limits of experimental uncertainty. They were also a poor fit for values calculated for the simplest type of substrate competition for one binding site, although the standard errors were large enough to make the significance of this discrepancy doubtful. As a working hypothesis for further studies, it is suggested that the activities towards 3 α - and 20 β -hydroxy steroids may depend on one active centre with complex steroid-binding characteristics.

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Biological Oxygenation of Drugs and Steroids in the Placenta

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Cytochrome *P*-450 is the terminal component of an enzyme system of the hepatic endoplasmic reticulum that oxygenates drugs and endogenous steroid hormones (Ernster & Orrenius, 1965). Drugs may be metabolized by the other tissues of the body besides liver (Mason, 1957), and recent investigations have suggested that such activity is to be found in placental tissues (Mirkin, 1970), thus possibly compensating for the low drug-metabolizing activity of the foetal liver (Fouts & Adamson, 1959). The placental drug-metabolizing activities reported are modest and the concentration of cytochrome *P*-450 present is correspondingly low (Meigs & Ryan, 1968). This contrasts with the high

activities of enzymes present in placenta that aromatize steroids. This steroid-aromatizing system involves a series of oxygenations at C-19 and C-1, which are dependent on NADPH and molecular oxygen for activity (Ryan, 1959; Akhtar & Skinner, 1968). We have investigated this apparent anachronism to ascertain whether and to what extent the steroid-aromatizing enzyme system is mediated through cytochrome *P*-450 and to seek the reason for the low drug-metabolizing activity found in the placenta.

Our investigations of the human placental cytochrome *P*-450 agree with the findings of Meigs & Ryan (1968). In the rat placental cytochrome *P*-450 was not detectable in the mitochondria, whereas in the microsomal fraction it was 25% of that found in the human placenta at full term (0.042 nmol/mg of protein). In contrast, in the rabbit cytochrome *P*-450 was present both in the mitochondrial and microsomal fractions, being 50% (0.06 nmol/mg of protein) and 150% (0.063 nmol/mg of protein) respectively of that found in the corresponding human placental fractions. The cytochrome *P*-450 contents of rat and rabbit placenta did not significantly change during the period of gestation or after pretreatment with phenobarbital (50 mg/kg body wt.) or 3-methylcholanthrene (20 mg/kg body wt.). Inhibition studies with carbon monoxide showed that the placental steroid-aromatizing enzyme system was unlikely to be mediated via cytochrome *P*-450.

The metabolism *in vitro* of a number of drugs and xenobiotics has been studied with various cellular fractions of rat, rabbit and human placental tissue. The rat microsomal fraction was shown to contain slight aromatic hydroxylase activity and catalysed the 4-hydroxylation of biphenyl (0.05 μ mol of product/h per g of tissue) (Creaven, Parke & Williams, 1965), but this activity was not detected in the rabbit and human placenta (<0.01 μ mol/h per g of tissue). Human placental microsomal preparations, which effectively aromatized androst-4-ene-3,17-dione to oestrone (1.9–3.7 nmol produced/h per mg of protein), did not hydroxylate zoxazolamine, *N*-demethylate ethylmorphine or *O*-demethylate *p*-nitroanisole, nor form the glucuronide of 4-methylumbelliferone. Rat and rabbit 10000g supernatants reduced *p*-nitrobenzoate to a small extent (0.05 μ mol of *p*-aminobenzoate formed/h per g of tissue) and this activity did not respond to inducers. Human placenta readily hydrolysed phenolphthalein glucuronide, but this activity seemed not to be associated with any one particular subcellular fraction.

These results indicate that the biological oxygenation of steroids and drugs in different organs may involve different enzyme systems. The apparent lack of drug-metabolizing activity in the

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 &