The Effects of Some Terpenoids and other Dietary Anutrients on Hepatic Drug-Metabolizing Enzymes

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At birth, many of the hepatic enzymes that metabolize foreign compounds are relatively inactive, but they increase rapidly to reach normal adult values a few weeks after birth. Further, these enzyme activities may be induced by treatment of the foetus or neonatal animal with phenobarbitone and other foreign chemicals (Fouts & Hart, 1965). This may suggest that these enzymes, which give protection against the toxic action of foreign chemicals, are normally induced by naturally-occurring foreign compounds present in the diet of the neonatal animal. A further induction of these enzymes may be obtained in the weanling or adult animal by pretreatment with various drugs, insecticides, polycyclic hydrocarbons and food additives (Conney, 1967), and it has been suggested that for a foreign compound to be an inducer of the drug-metabolizing enzymes it has to be lipid-soluble and not rapidly excreted (Gillette, 1963).

To elucidate these points, studies have been made of the effects of terpenoids, and other dietary anutrients, on the activities of hepatic drug-metabolizing enzymes. Rats were pretreated for 3 days with terpenoids administered by intraperitoneal injection or by admixture with their food, and biphenyl 4-hydroxylase (Creaven, Parke & Williams, 1965), glucuronyl transferase (Bollet, Goodwin & Bron, 1959), and 4-nitrobenzoate reductase (R. Gingell, personal communication) activities and cytochrome *P*-450 were determined in 10 000g supernatants of the liver homogenates.

Pretreatment with β -ionone resulted in increases in the activities of all three enzymes and cytochrome *P*-450 of about 50–75%, and hexobarbitone sleeping times were decreased to half the normal. Limonene, borneol, citral and terpineol produced smaller increases of the order of 25%. Linalool, nerolidol and squalene produced no increase in the activities of biphenyl 4-hydroxylase, glucuronyl transferase or cytochrome *P*-450, but increased the activity of 4-nitrobenzoate reductase by 25–50%. The induction produced by β -ionone was maximal after a single treatment and showed no further increase when treatment was continued daily for 28 days. Liver weights and liver protein did not increase significantly.

All of these terpenoids are very lipid-soluble, and it may be significant that β -ionone, which shows the maximum inductive effect, is the only one of those compounds studied that is known to be metabolized by hydroxylation. The other terpenoids are metabo-

lized by reduction and conjugation with glucuronic acid, and it would appear that, with this class of substances at least, a compound induces those enzymes that are involved in its own metabolism.

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The Effect of Pregnancy on the Hydroxylation and Reduction of Drugs and cytochrome *P*-450 content of Rat Liver Microsomes

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The hydroxylation of foreign compounds by microsomal preparations of rat liver is inhibited during pregnancy (Creaven & Parke, 1965), and by progesterone and progestogens (Neale & Parke, 1968), and it has been suggested that the inhibition during pregnancy might be due to the high blood concentrations of progestogens (Creaven & Parke, 1965).

We investigated the following parameters in liver microsomal preparations from full-term pregnant rats: hydroxylation of biphenyl, reduction of *p*-nitrobenzoic acid, microsomal protein and cytochrome *P*-450. When these are expressed as a function of liver weight, the reductase activity and the protein content of the pregnant livers are unchanged, but the 4-hydroxylation of biphenyl and cytochrome *P*-450 content are both significantly decreased by 22%. When the results are expressed as a function of total liver weight, all four parameters are significantly increased in the pregnant animals, as there is an increase in liver weight accompanying pregnancy. Thus the total capacity of the pregnant animal to metabolize foreign compounds is increased, but since the pregnant animal also increases in weight, comparable to the gain in liver weight, the hydroxylating activity and cytochrome *P*-450 content per unit body weight decrease. This is confirmed by the fact that the sleeping time in rats dosed with 100mg. of hexobarbital/kg. is increased by 32% in full-term pregnant rats.

Thus it is probable that the decreased biphenyl hydroxylase activity of liver microsomal prepara-

tions from pregnant rats is due to a decrease in the content of cytochrome *P*-450 and not to inhibition by progestogens.

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Microsomal Dehydrogenation of *cis*- and *trans*-Acenaphthene-1,2-diol to Acenaphthenequinone

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1,8-Naphthalic acid has been isolated after the oxidation *in vivo* and the microsomal oxidation of *cis*- and *trans*-acenaphthene-1,2-diol (Hopkins & Young, 1966; Hopkins, 1968). It was suggested by Hopkins (1968) that the oxidation might involve an initial dehydrogenation of the diols to produce aldehydic or ketonic compounds. The presence of acenaphthenequinone in microsomal incubation mixtures of the diols has now been demonstrated by thin-layer- and gas-liquid-chromatographic methods, and the compound has also been isolated.

cis- or *trans*-Acenaphthene-1,2-diol (1.5 m-moles) in 0.1 M-phosphate buffer, pH 7.4, was incubated at 37° for 2 hr. in open flasks with NAD⁺ (0.15 m-mole), nicotinamide (4.5 m-moles), and a rat liver microsomal preparation. Acenaphthenequinone was isolated from the incubation mixture, and was characterized by comparison with the authentic compound and by conversion into the 2,4-dinitrophenylhydrazone.

The formation of carbonyl material was followed during microsomal incubations of each diol. Samples of the incubation mixture were treated at timed intervals with 2,4-dinitrophenylhydrazine and the increase in *E*₄₅₀ in the absorption curve of the resultant 2,4-dinitrophenylhydrazone was measured. No increase was observed in the absence of microsomes or in the presence of microsomes inactivated by heat. In the absence of NAD⁺, the rate of increase was much less. The appearance of the absorption curve resembled closely that of authentic acenaphthenequinone 2,4-dinitrophenylhydrazone.

The possibility arises that naphthalic acid, isolated by Hopkins (1968) from microsomal incubations of diols, was formed from acenaphthenequinone during the isolation procedure. In that procedure, the incubation mixture was left at room temperature overnight at pH 11, before adjustment

to pH 2. In the present work, acenaphthenequinone was kept overnight in the presence of a rat liver microsomal preparation at pH 11 and then the mixture was adjusted to pH 2. Naphthalic acid could readily be detected by gas-liquid chromatography, whether or not the microsomal preparation had been inactivated by heat. Only trace amounts of naphthalic acid could be detected, however, if the quinone had stood overnight in the microsomal preparation at pH 7.4, before adjustment to pH 2. Further aspects of this will be discussed.

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Modification of Methionine in the Haem Environment of Cytochrome *c*

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It has been suggested that the spectral changes when horse heart ferricytochrome *c* is modified by *N*-bromosuccinimide (NBS) are caused by tryptophan modification (Yonetani, 1968; Stellwagen, 1968). However, if methionine is a haem-coordinating ligand (Harbury *et al.* 1965), oxidation of methionine to sulfoxide (O'Brien, 1966) is more likely to explain the spectral changes. Formation of the NBS-modified product (I) resulted in a 4 nm. blue shift of the Soret band and disappearance of the 695 nm. band of the ferri form; the ferro form underwent a 2 nm. red shift of the Soret band and a decrease of the α - and β -bands. Tryptophan is probably not involved, as similar spectral changes occurred after reaction with NBS at pH 7 although less tryptophan was modified at this pH. At pH 3.0, NBS modified the cytochrome to a high-spin product (II) with a high-spin and mixed-spin-type spectrum up to pH 7.5. The high-spin type had a Soret band of 391 nm. whereas that of native cytochrome *c* was 394 nm. The spectrum of the ferro form was similar to reduced produce I.

A haem peptide isolated from a tryptic hydrolysate of cytochrome *c* formed low-spin haemochromes with imidazole, methionine and methionine sulfoxide. The α -bands for these haemochromes were 550 nm., 552 nm. and 547 nm. respectively. If these band positions correspond to the electronegativity of the ligand atom, the atoms involved may be nitrogen, sulphur and oxygen respectively. Methionine sulphone and the carboxymethylsulphonium salt of methionine did not form haemochromes.

Carboxymethylation of both methionine residues of cytochrome *c* (product III) resulted in a 2 nm.