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.