EFFECTS OF DIETARY LIPID AND PHENOBARBITONE ON THE DISTRIBUTION AND CONCENTRATION OF CYTOCHROME *P*-450 IN THE LIVER STUDIED BY QUANTITATIVE CYTOCHEMISTRY

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1. Introduction

Cytochrome *P*-450 is an important component of the NADPH-dependent electron-transport system located in the endoplasmic reticulum of liver parenchymal cells and is the terminal oxidase involved in the oxidative metabolism of drugs, anaesthetics, carcinogens, steroid hormones, insecticides, environmental pollutants and fatty acids. In the reduced form this haemoprotein binds carbon monoxide to produce a characteristic Soret absorption band at 450 nm and is therefore routinely assayed in the microsomal fraction by measuring the difference spectrum of reduced cytochrome *P*-450 in the presence and absence of carbon monoxide [1].

It is well established that phenobarbitone administration increases the concentration of cytochrome *P*-450 in liver microsomes [2,3], and that variations in the content and composition of the dietary lipid can also markedly affect the concentration of cytochrome *P*-450 in liver microsomes [4,5].

Quantitative cytochemical measurements of the concentration of cytochrome *P*-450 in small groups of cells located in unfixed tissue sections using a Zeiss Universal Microspectrophotometer (type I) have shown that cytochrome *P*-450 is not evenly distributed in rat liver and is induced selectively in the centrilobular hepatocytes by phenobarbitone [6,7]. The effects of changes in dietary lipid composition on the distribution of cytochrome *P*-450 within the rat liver lobule have not been investigated.

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Here, the microspectrophotometric assay procedure [6], for the determination of cytochrome *P*-450, has been adapted for use with the widely available Vickers M85/M85A/M86 series of microdensitometers. We have thus investigated the effects of phenobarbitone administration and variations in the dietary lipid on the distribution and concentration of cytochrome *P*-450 in rat liver. The validity of the microdensitometric assay has also been established by comparing the measurements with those obtained by a conventional biochemical assay using dilute liver homogenates.

2. Materials and methods

2.1. *Animals and diets*

Purified diets containing lipids of known composition were freshly prepared in a powdered form as in [8]. Refined herring oil was obtained from Marfleet Refining Co., Hull and corn oil from Mazola, Brown and Polson CPC, Esher. The fatty acid compositions of these dietary lipids have been described [8].

Three groups of 4 male albino Wistar rats (130–150 g body wt), were transferred from a stock diet (Spratts no. 1, Central House, Barking, Essex) to diets containing 10% corn oil, 10% herring oil or no lipid (fat-free diet) and fed for 10–24 days prior to killing by cervical dislocation. For comparison, one other group of 4 rats was maintained on the stock diet. All animals 180–270 g body wt at death. The fatty acid composition of the stock diet has been described [8]. One group of 4 rats was also maintained on the stock diet but were given a solution of sodium phenobarbitone (1 g/litre) as their sole source of drinking water for 5–7 days prior to killing.

2.2. *Experimental procedures*

Serial sections (10 and 40 μm thick) were prepared from chilled blocks of liver in a Bright's cryostat as in [6,9]. The 40 μm thick sections were taken directly from the cryostat and allowed to warm up to room temperature. They were then mounted in freshly prepared incubation medium, consisting of 20% polyvinyl alcohol (Wacker Chemicals, MO5/140 grade) and 150 mM KCl in 20 mM Tris–HCl (pH 7.4). Immediately before use, sodium dithionite (5 mg/ml) was added and the medium was saturated with carbon monoxide, then centrifuged at 1000 × *g* for 20 min to remove most of the gas bubbles and used immediately. The sections (40 μm) were incubated at room temperature (23°C) in the dark for 5 min to allow the reduced cytochrome *P*-450 to complex with carbon monoxide and were then transferred to the stage of a Vickers M85 microdensitometer. Clearly identifiable centrilobular and periportal areas, located within 5 cell layers of central and portal veins of <40 μm diam., were selected for measurement by comparing the 40 μm sections to serial Toluidine blue stained sections (10 μm) [9].

Absorption measurements were made in each of the selected areas at 20 different wavelengths between 420–480 nm, with a separate blank (for 100% transmission) at each wavelength. A × 20 objective, field size A4 (40 μm diam.), scan size 1 × 1 and spot size 2 (being 2 μm in the optical plane of the specimen) were used. The microdensitometer was fitted with a high sensitivity gating circuit (Vickers Instr., York) which enabled a monochromator bandwidth setting of zero to be used for all of the measurements. At 450 nm the bandwidth of the monochromator was <1.5 nm under these conditions. The concentrations of cytochrome *P*-450 in the selected areas were calculated by drawing in the continuous curve between 425–480 nm and using it as an artificial reference curve as in [6,7,10]. These calculations were facilitated by interfacing a Commodore PET microcomputer to the microdensitometer as in [11].

The concentration of cytochrome *P*-450 in dilute liver homogenates (0.5%, w/v) was also determined by an adaptation [10] of the method in [12]. Student's *t*-test was used to test the significance of the results.

3. Results and discussion

Concentrations of cytochrome *P*-450 in dilute liver homogenates prepared from the four rats in each experimental group are shown in table 1. Transferring rats from a stock diet to diets containing either 10% herring oil or 10% corn oil significantly increased the concentration of cytochrome *P*-450 in liver homogenate ($P < 0.002$), whereas transferring them to a fat-free diet significantly decreased the cytochrome *P*-450 concentration ($P < 0.001$). Phenobarbitone administration also significantly increased the concentration of cytochrome *P*-450 in dilute rat liver homogenate ($P < 0.001$) (table 1).

Although these biochemical measurements show that variations in the dietary lipid and phenobarbitone

Table 1

Effect of phenobarbitone and dietary lipid on the concentration of cytochrome *P*-450 in dilute rat liver homogenate and in the centrilobular and periportal regions of rat liver sections

Diet or treatment	Mean cytochrome *P*-450 concentration (±SEM)		
	Liver homogenate	Centrilobular region	Periportal region
Fat-free Diet	25.6 ± 0.4	34.2 ± 1.6	20.5 ± 0.4
Stock Diet	33.2 ± 0.3	42.2 ± 0.5	26.6 ± 1.3
10% Corn Oil Diet	38.6 ± 0.7	50.6 ± 2.1	30.5 ± 1.0
10% Herring Oil Diet	46.2 ± 1.7	59.5 ± 1.7	35.4 ± 1.1
Phenobarbitone + stock diet	74.0 ± 3.9	109.4 ± 2.7	30.4 ± 1.8

Diets were fed for 10–24 days before killing. Phenobarbitone was administered in the drinking water at 1 g/litre for 5–7 days prior to killing. Cytochrome *P*-450 concentrations are expressed in μmol/litre tissue and were measured in dilute liver homogenates by an adaptation of the method in [12] and in the centrilobular and periportal regions of rat liver sections (40 μm thick) by using a Vickers M85 microdensitometer. Each value shown is the mean (±SEM) of 4 rats in each experimental group

administration significantly alter the overall concentration of cytochrome *P*-450 in rat liver, these changes may not occur evenly throughout the rat liver lobule. The effects of phenobarbitone and dietary lipid on the distribution of cytochrome *P*-450 in the rat liver lobule were investigated by determining the concentration of cytochrome *P*-450 in the centrilobular and periportal regions of each individual rat's liver, from all 5 experimental groups, using the microdensitometric assay (table 1). The absorption spectra of 4 centrilobular and 4 periportal areas (40 μm diam.) were measured in 4 sections (40 μm), prepared from 2 chilled blocks of each rat's liver. Cytochrome *P*-450 concentrations were calculated from the absorption difference at 450–451 nm between the recorded spectrum and the constructed artificial reference curve [6].

From table 1 it can be seen that the microdensitometric assay gave values for cytochrome *P*-450 comparable with those obtained by the conventional spectrophotometric assay in dilute liver homogenates. Moreover, the results show that cytochrome *P*-450 is not evenly distributed in rat liver. The hepatocytes located in the centrilobular region of the liver contain ~1.6-times as much cytochrome *P*-450 as those in periportal region (table 1). This result is in agreement with early qualitative histochemical studies which suggested that the centrilobular hepatocytes were more active than the periportal hepatocytes in the metabolism of foreign compounds, such as benzo[a]-pyrene and bromobenzene [13–15]. The higher concentration of cytochrome *P*-450 in the centrilobular hepatocytes is also in agreement with the microspectrophotometric measurements [6,7] and the immunohistochemical findings [16]. It is therefore likely that the centrilobular hepatocytes are more important that the periportal hepatocytes in the oxidative metabolism of drugs and other foreign compounds

Phenobarbitone pre-treatment very significantly increased the concentration of cytochrome *P*-450 in the centrilobular regions ($P < 0.0001$), but had no significant effect ($P < 0.15$) on the concentration of cytochrome *P*-450 in the periportal region (table 1), in agreement with [6,7].

Transferring rats from a stock diet to a fat-free diet significantly decreased the concentration of cytochrome *P*-450 in both the centrilobular ($P < 0.006$) and periportal ($P < 0.008$) regions of the rat liver lobule, but when rats were transferred from a stock diet to a diet containing 10% herring oil, which contains a high proportion of polyunsaturated fatty acids, the concentration of cytochrome *P*-450 was significantly increased in both the centrilobular ($P < 0.004$) and periportal ($P < 0.005$) regions of rat liver. Feeding a fat-free diet therefore significantly decreased the concentration of cytochrome *P*-450 throughout the rat liver lobule, whereas feeding a 10% herring oil diet significantly increased it.

When rats were transferred from a stock diet to a diet containing 10% corn oil the concentration of cytochrome *P*-450 was increased in both regions of the rat liver lobule, but only the increase in the centrilobular region was significant ($P < 0.02$). However, the concentration of cytochrome *P*-450 in corn-oil fed rats was significantly higher in both regions of the liver lobule than that of the rats fed a fat-free diet ($P < 0.002$).

It can therefore be concluded that alterations in the dietary lipid cause uniform changes in the concentration of cytochrome *P*-450 throughout the rat liver lobule, whereas the administration of phenobarbitone increases the concentration of cytochrome *P*-450 only in the centrilobular hepatocytes.

The high cytochrome *P*-450 concentrations and high rates of oxidative demethylation observed in liver microsomes after feeding diets containing 10% corn oil or herring oil are believed to be related to the larger proportions of ω6 or ω3 polyunsaturated fatty acids incorporated into the membranes of the endoplasmic reticulum [17]. The finding that variations in the dietary lipid alter the concentration of cytochrome *P*-450 to an approximately equal extent throughout the liver lobule therefore suggests that a relatively even distribution of fatty acids within the liver is produced by feeding these diets. The membrane matrix, when of suitable composition and configuration, may therefore, either stimulate the synthesis of more cytochrome *P*-450 in the endoplasmic reticulum irrespective of the cellular location or provide a suitable lipid environment for the accommodation of more cytochrome. It is significant that phenobarbitone induction causes the synthesis of membranes containing a relatively high proportion of linoleic acid in livers of animals fed on stock diets [18]. Phenobarbitone induction, without enhanced supplies of linoleic acid, is apparently limited to centrilobular cells, either on account of the specific accumulation and metabolism of phenobarbitone in these cells or because these cells possess components required in the induction which are not present in the periportal cells.

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References

[1] Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2370–2384.
[2] Orrenius, S. and Ernster, L. (1964) Biochem. Biophys. Res. Commun. 16, 60–65.
[3] Conney, A. H. (1967) Pharmacol. Rev. 19, 317–366.
[4] Rowe, L. and Wills, E. D. (1976) Biochem. Pharmacol. 25, 175–179.
[5] Wade, A. E. and Norred, W. P. (1976) Fed. Proc. FASEB 35, 2475–2479.
[6] Gooding, P. E., Chayen, J., Sawyer, B. and Slater, T. F. (1978) Chem.–Biol. Interact. 20, 299–310.
[7] Chayen, J., Bitensky, L., Johnstone, J. J., Gooding, P. E. and Slater, T. F. (1979) in: Quantitative Cytochemistry and its Applications (Pattison, J. R. et al. eds) pp. 129–137, Academic Press, London, New York.
[8] Hammer, C. T. and Wills, E. D. (1978) Biochem. J. 174, 585–593.
[9] Chayen, J., Bitensky, L. and Butcher, R. G. (1973) Practical Histochemistry, Wiley, London.
[10] Smith, M. T. (1980) PhD Thesis, University of London.
[11] Smith, M. T., Wills, E. D., Drew, K., Maxwell, C., Daly, J. R., Reader, S. C. J. and Robertson, W. R. (1980) Histochemistry 68, 321–323.
[12] McLean, A. E. M. and Day, P. A. (1974) Biochem. Pharmacol. 23, 1173–1180.
[13] Wattenberg, L. W. and Leong, J. T. (1962) J. Histochem. Cytochem. 10, 412–420.
[14] Koudstaal, J. and Hardonk, M. J. (1969) Histochemie 20, 68–77.
[15] Brodie, B. B., Reid, W. D., Cho, A. K., Sipes, G., Krishna, G. and Gillette, J. R. (1971) Proc. Natl. Acad. Sci. USA 68, 160–164.
[16] Baron, J., Taira, Y., Redick, J. A., Greenspan, P., Kapke, G. F. and Guengerich, F. P. (1980) in: Microsomes, Drug Oxidations and Chemical Carcinogenesis (Coon, M. J. et al. eds) vol. 1, pp. 501–504, Academic Press, London, New York.
[17] Hammer, C. T. and Wills, E. D. (1979) Brit. J. Nutr. 41, 465–475.
[18] Davison, S. C. and Wills, E. D. (1974) Biochem. J. 140, 461–468.