marked fall in the association constant for diazepam in the presence of valproic acid: $K_a = 8.9 \times 10^4 \text{ M}^{-1}$ (diazepam only); $K_a = 4.1 \times 10^4 \text{ M}^{-1}$ (diazepam and valproic acid).

The results suggest that diazepam, under the conditions used, has one primary binding site on human serum albumin. This is in agreement with the findings of previous authors (Müller & Wollert, 1973; Kober *et al.*, 1979). The inhibition of diazepam binding by valproic acid has been shown to be competitive since the number of binding sites for diazepam remained unchanged while the affinity of diazepam for these sites was reduced.

The disposition kinetics of many drugs can be influenced by changes in plasma protein binding. Dia-

zepam is a drug subject to restrictive elimination by hepatic metabolism and it has been shown that for such drugs the total clearance is directly proportional to the free fraction of the drug (Shand, Mitchell & Oates, 1975). Changes in diazepam free fraction may therefore cause an important alteration in disposition.

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EFFECT OF LOW-DOSE PHENOBARBITONE ON FIVE INDIRECT INDICES OF HEPATIC MICROSOMAL ENZYME INDUCTION AND PLASMA LIPOPROTEINS IN NORMAL SUBJECTS

Although the concept of dose-dependent enzyme induction with hypnotic doses of barbiturates is well established (Breckenridge *et al.*, 1973), little information is available on the minimum dosage of these drugs that is necessary to produce enzyme-induction

in man. We have therefore decided to evaluate the enzyme-inducing properties of low doses of phenobarbitone (15 and 30 mg daily respectively) following repeated administration in normal volunteers. Five indirect indices of hepatic microsomal enzyme-

Figure 1 Serum phenobarbitone (Pb) concentration profiles in the subjects included in the study.

induction have been measured: antipyrine clearance (Stevenson, 1977), oral availability of lignocaine (Perucca *et al.*, 1980), serum γ -glutamyl-transpeptidase activity (Whitfield *et al.*, 1973) and urinary excretion of 6- β -hydroxycortisol (Ohnhaus & Park, 1979) and D-glucaric acid (Hunter *et al.*, 1971). In view of the evidence that enzyme-induction may be responsible for biochemical disturbances in lipid metabolism (Perucca, 1978), plasma cholesterol, triglycerides and lipoprotein fractions have also been monitored.

Four healthy male volunteers, two cigarette smokers (average about 10 and 20 cigarettes/day) and two non-smokers (aged 26–40 years, weight 65–81 kg), participated in the study, which was approved by a local Ethics Committee. All had normal hepatic function as assessed by conventional laboratory tests and maintained dietary and smoking habits unchanged throughout the duration of the study. During the first week of the study, pretreatment measurements of plasma lipids and of the various parameters of enzyme induction were obtained. Each subject was then started on phenobarbitone using a dosing schedule especially designed to maintain stable serum phenobarbitone concentrations throughout each period of the study. Loading doses of phenobarbitone (60 mg nocte) were given on the first two treatment days, followed by 15 mg nocte for the next 26 days. After two further loading doses (90

mg) on days 29 and 30 respectively, phenobarbitone was continued at a dosage of 30 mg nocte until day 56 and then discontinued abruptly (Figure 1). Biochemical and pharmacological tests were repeated during the last week of each treatment period and again approximately 6 weeks after discontinuation of the drug. Antipyrine clearance was calculated from salivary concentration data determined by gas-liquid chromatography (Lindgren *et al.*, 1974) after intravenous dosing (940 mg). The apparent oral clearance of lignocaine was determined as dose:area under the blood concentration curve following administration of a single oral dose of lignocaine hydrochloride monohydrate (400 mg), according to Perucca *et al.* (1980). The concentration of 6- β -hydroxycortisol, 17-hydroxy-corticosteroids and D-glucaric acid were determined in 2×24 h urine collection aliquots by radioimmunoassay (Park, 1978) spectrophotometry (Sanghvi, Taddeini & Wight, 1973) and by an enzymatic method (modified from Marsh, 1963) respectively. Serum γ -glutamyl transpeptidase activity and phenobarbitone concentration were measured according to Szasz (1969) and Houghton & Richens (1974) respectively. Total cholesterol and triglycerides and individual lipoprotein levels were measured in plasma after a 14 h overnight fast. Cholesterol and triglycerides were measured by semi-automated fluorometric techniques (Technicon method N.77; Camp & Robertson, 1968). After

Table 1 Indirect indices of hepatic microsomal enzyme-induction and plasma lipids (mean $\pm$ s.d.) before and after phenobarbitone (Pb) administration in the subjects included in the study.

	Serum Pb concentration ($\mu\text{mol/l}$)	Antipyrine clearance ($\text{ml h}^{-1} \text{kg}^{-1}$)	Lignocaine apparent oral clearance ($\text{l h}^{-1} \text{kg}^{-1}$)	Urinary 6- β -hydroxycortisol: 17-hydroxycorticosteroids ratio	Urinary D-glucuronic acid ($\mu\text{mol/24 h}$)	Serum gamma-glutamyl-transpeptidase (i.u.)	Total plasma cholesterol ^c (mmol/l)	Total plasma triglycerides ^c (mmol/l)	LDL:HDL ratio ^c
Pre-treatment period	—	38.4 ± 6.4	1.96 ± 0.55	4.6 ± 0.6	12.1 ± 5.3	16.4 ± 4.5	5.1 ± 0.9	1.2 ± 0.4	2.9 ± 0.8
Pb 15 mg/day for 4 weeks ^a	13.3 ± 3.6	48.5 ± 15.8	—	5.0 ± 0.9	10.6 ± 6.2	17.8 ± 5.1	5.2 ± 1.0	1.3 ± 0.6	2.7 ± 0.7
Pb 30 mg/day for 4 weeks	27.0 ± 7.2	57.7 ± 15.1^b	$3.06 \pm 0.83^*$	$7.3 \pm 1.3^*$	15.3 ± 5.3	17.9 ± 4.3	4.6 ± 1.0	1.2 ± 0.6	3.1 ± 1.0
Post-treatment period	—	40.2 ± 6.8	2.51 ± 1.68	—	12.4 ± 2.6	—	5.0 ± 0.8	1.0 ± 0.5	2.6 ± 1.0

^a loading doses of phenobarbitone given on the first 2 days (see text).^b value from one subject not available (pretreatment value = $34.3 \text{ ml h}^{-1} \text{kg}^{-1}$ in this subject).^c individual values used were the mean of two determinations in separate days.* $P < 0.05$ (Student's *t*-test for paired data).

precipitation, very low density lipoprotein (VLDL) cholesterol and VLDL-triglycerides were measured in the redissolved pellets according to Ononogbu & Lewis (1976). High-density lipoprotein (HDL)-cholesterol was determined in the supernatant according to Burstein, Scholnick & Morfin (1970). Low-density lipoprotein (LDL)-cholesterol was obtained by subtracting the sum of HDL and VLDL values from the total plasma cholesterol.

No major adverse effects were observed during the study. All subjects, however, complained of reduced ability to concentrate and a general slowing in intellectual performance, especially during the last month. The effect of phenobarbitone treatment on the various indices of enzyme induction examined in the study are illustrated in Table 1. After phenobarbitone, 15 mg daily, a possible increase in both antipyrine clearance and urinary 6- β -hydroxycortisol:17-hydroxycorticosteroids ratio was found, but neither effect was statistically significant. Measurements of lignocaine kinetics at this dose level were not obtained. Administration of phenobarbitone 30 mg daily for 4 more weeks produced a significant reduction in lignocaine bioavailability, as assessed by comparison of the areas under the serum concentration curves before and after treatment (762 ± 237 vs $478 \pm 122 \mu\text{mol l}^{-1} \text{min}$ respectively, $P < 0.05$). As a result, the apparent oral clearance of lignocaine was significantly increased. The urinary 6- β -hydroxycortisol:17-hydroxycorticosteroids ratio was also significantly increased during phenobarbitone treatment. Although a comparatively similar elevation in antipyrine clearance was observed in three subjects, meaningful statistical analysis on this data was prevented by the accidental loss of the samples collected from the fourth subject. Both the antipyrine clearance and the apparent oral clearance of lignocaine reverted to near-baseline values after discontinuation of treatment. No consistent changes in urinary excretion of D-glucaric acid, serum γ -glutamyltranspeptidase activity and fasting plasma concentration of total cholesterol, total triglycerides, HDL, LDL and VLDL-lipoprotein fractions were observed during the study (cholesterol, triglycerides and LDL/HDL ratios are reported in Table 1).

The serum phenobarbitone concentration profiles in the four subjects are illustrated in Figure 1. With the loading doses used, stable serum phenobarbitone levels were maintained throughout each period of the study. In each of the four subjects, doubling the dose of phenobarbitone resulted in doubling of the steady state serum concentration. A significant correlation was found between the steady state serum phenobarbitone concentration and percent increase in urinary 6- β -hydroxycortisol:17-hydroxycorticosteroids ratio on the 30 mg dose ($r = -0.96$, $P < 0.01$).

Although the small number of subjects included in

the present study does not allow a precise evaluation of the data in statistical terms, the reduction in oral availability of lignocaine, a putative measure of induction of first-pass metabolism (Perucca & Richens, 1979; Perucca *et al.*, 1980), the increase in antipyrine clearance, and the elevation in the urinary 6- β -hydroxycortisol:17-hydroxycorticosteroids ratio provide evidence that as little as 30 mg daily of phenobarbitone was sufficient to stimulate the activity of the hepatic drug metabolizing enzymes. The effect of phenobarbitone, 15 mg daily, was less clear cut, even though the small increase in both antipyrine clearance and 6- β -hydroxycortisol excretion suggests that enzyme induction might in fact have occurred in some volunteers. No significant changes, on the other hand, could be demonstrated in plasma lipids after phenobarbitone administration. This is in line with the results obtained in a recent study in which considerably larger doses of phenobarbitone were used, even though for a shorter time (Ohnhaus, Kirchof & Peheim, 1979). The observation that the serum γ -glutamyl-transpeptidase activity and the urinary excretion of D-glucaric acid, unlike the other parameters of enzyme induction, did not change consistently during phenobarbitone administration is difficult to evaluate in view of the limited number of subjects. In any case, a lack of correlation between D-glucaric excretion, serum γ -glutamyl-transpeptidase activity and the enhanced rate of elimination of marker drugs has been commented upon previously (Freundt, 1979; Makki, Beetham & Richens, 1980; Ohnhaus & Park, 1979; Smith & Rawlins, 1974) and probably underlines the poor value of the former two parameters as indices of hepatic drug metabolizing activity. Unlike 6- β -hydroxycortisol, D-glucaric acid is synthesized by a non-microsomal enzyme system, which is independent of the mixed function oxidase (Breckenridge, 1975). Serum γ -glutamyltranspeptidase is also non-microsomal in origin (Huseby, 1979; Ratanasavanh *et al.*, 1979) and has been shown to increase in the circulation in a number of clinical conditions that are clearly unrelated to enzyme-induction (Rosalki, 1975).

The possibility of obtaining hepatic microsomal enzyme-induction with low doses of phenobarbitone is of interest, especially in relation to the much larger doses of the drug which are generally used to produce enzyme-induction in animal models. Furthermore, it may have therapeutic implications since small doses of barbiturates are used, often irrationally, in combination products (antihypertensive and antianginal preparations, gastrointestinal 'sedatives', analgesics, antiasthmatics, etc.) whose barbiturate content is often not appreciated by the prescriber. We wish to thank the Medical Research Council for financial support.

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