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Time Course of Phenobarbital and Cimetidine Mediated Changes in Hepatic Drug Metabolism

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Summary. Four healthy subjects were investigated weekly for 14 weeks by the antipyrine one sample saliva test, the 48-h urinary excretion of major antipyrine metabolites and the 2-h aminopyrine breath test before, during and after stimulation and inhibition of drug metabolism with phenobarbital and cimetidine, respectively. The phenobarbital-induced enhancement of antipyrine clearance (1.33–2.03 times) and of the aminopyrine breath test (0.94–1.19 times) occurred one week after beginning drug administration and persisted for 10 days after its cessation. The cimetidine-related inhibition of antipyrine clearance (0.62–0.85 times) and of the aminopyrine breath test (0.52–0.93 times) was observed 24 h after beginning cimetidine administration and subsided within two days after the last dose. During enhancement and inhibition the clearance of antipyrine to 3-hydroxymethyl-, 4-hydroxy- and norantipyrine varied as the total antipyrine clearance. The intraindividual variation in antipyrine clearance was 6–8%, and the corresponding variation in urinary excretion of antipyrine metabolites was 10–20%. It is concluded that the influence of phenobarbital and cimetidine on hepatic microsomal enzyme activity can be monitored simply by measurement of the blood concentration of the drug. Whether this simple relationship applies to other microsomally mediated drug interactions requires further evaluation.

Key words: microsomal drug metabolism, antipyrine; aminopyrine, antipyrine metabolism, phenobarbital, cimetidine, enzyme induction, enzyme inhibition

Many xenobiotics are able to alter microsomal enzyme activity in man to a clinically important extent [7, 27]. Enhancement of the activity is most frequently reported, but an increasing number of substances are recognized as inhibitors [37].

Microsomal enzyme activity has sometimes been assessed after discontinuation of drug administration [2, 5, 6, 8, 12, 13, 19, 24, 25, 28, 31, 38, 42], but the time course of drug-associated changes in microsomal enzyme activity in man has received limited attention.

Some of the drug-induced changes in microsomal enzyme function may cause serious drug interactions, so it is important to know the rate with which the changes may develop and subside. The information is necessary to explain the time course of microsomally mediated drug interactions, and in planning to prevent clinically important interactions.

The present study was done to examine the sequential changes in antipyrine and aminopyrine metabolism, as assessed by the antipyrine saliva test [39], the 48-h urinary excretion of the three major metabolites of antipyrine [9], and the 2-h aminopyrine breath test [16], before, during and following stimulation and inhibition of drug metabolism with phenobarbital [40] and cimetidine [35], respectively.

Material and Methods

Four healthy non-smoking volunteers, three men and one woman, aged 25–35 years, gave informed consent. None had taken any drugs for one month prior to the study. They all consumed alcohol socially, i.e. their average daily consumption was less than 10 g ethanol. No effort was made to control the dietary habits of the subjects.

The design of the study is illustrated in Fig. 1. A control period of two weeks was followed by two experimental periods, each lasting six weeks. During

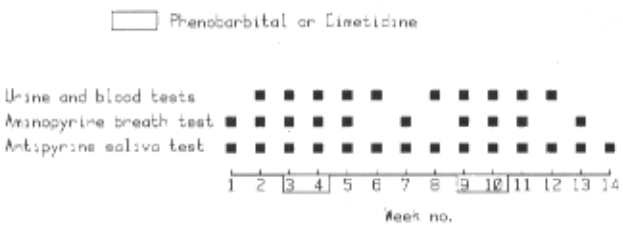

Fig. 1. Design of study. Four healthy subjects randomly received phenobarbital 100 mg daily, or cimetidine 1000 mg daily, for 13 days, separated by four weeks

Table 1. Coefficient of variation expressed as SD in % of the mean value $\bar{x}$ (SD rel.). The standard deviation of 450 duplicate gas chromatographic antipyrine analyses of was calculated from $\sqrt{\frac{\Sigma d^2}{2N}}$, where d is the difference between duplicates

Range of concentration [μg/ml]		SD [μg/ml]	SD rel. [%]
2.0–4.9	($\bar{x}$=3.5, n= 54)	0.18	5.1
5.0–9.9	($\bar{x}$=7.8, n=115)	0.23	3.0
10.0–14.9	($\bar{x}$=12.4, n=152)	0.38	3.1
15.0–19.9	($\bar{x}$=17.0, n=101)	0.62	3.7
20.0–25.0	($\bar{x}$=21.3, n= 28)	0.52	2.5

one period phenobarbital 100 mg was given at bedtime for 13 days, and during the other period cimetidine 200 mg three times daily plus 400 mg at bedtime was given for 13 days. No drugs were given for the rest of the periods. Two subjects received cimetidine first and then phenobarbital and two received the drugs in the opposite sequence. Venous blood samples for estimation of plasma phenobarbital and cimetidine during the appropriate periods were collected 12 h after the bedtime dose and before the morning dose, according to the time schedule in Fig. 1.

Plasma phenobarbital was determined by a spectrophotometric enzymatic EMIT® method [35]. Plasma cimetidine was determined by HPLC [21]. γ-Glutamyltranspeptidase (s-γ GT) was also determined in all blood samples.

Once weekly an oral dose of phenazone 1 g (antipyrine) was given simultaneously with ^{14}C-aminopyrine 2 μCi (98% radiochemically pure as judged by TLC), containing about 5 μg aminopyrine, with 20 ml of tap water. Aminopyrine was not given during Weeks 1, 7, 13, and 14 in order to avoid a cumulative radioactive dose exceeding 20 μCi. After aminopyrine and antipyrine administration the volunteers restricted their physical activity to a minimum in order to avoid changes in endogenous CO_2-production. Two h after drug administration breath samples were collected by exhaling through anhydrous calcium sulfate (for drying) and into a scintillating vial containing a trapping solution of 0.5 M hyamine hydrochloride-ethanol 4 ml and two drops of 10% thymolphtalein solution, until the indicator changed from blue to colourless, indicating trapping of CO_2 2 mmol. After addition of scintillation cocktail, samples were counted in a liquid scintillation spectrometer, with use of an external standard and dual channel correction for quench. Activity was expressed as % of the administered ^{14}C-label per mmol CO_2 multiplied by the body weight in kg $\left(\frac{\%\ \text{dose} \times \text{kg}}{\text{mmol}\ CO_2}\right)$.

Saliva 5 ml was collected about 24 h after administration of antipyrine and was kept frozen at −20 °C until analyzed by GLC [28]. The coefficient of variation of duplicate analyses ranged from 2.5 to 5.1% at antipyrine concentrations between 2 and 25 μg/ml (Table 1). The clearance of antipyrine was calculated from the dose (D), an assumed volume of distribution (V_D) and the salivary concentration of antipyrine at time t (c_t)

$$Cl_{AP} = \frac{\ln (D/V_D) - \ln c_t}{t} \times V_D \qquad [11]$$

Urine was collected for 48 h after antipyrine administration once before, twice during and twice after each drug regime (Fig. 1) [9], and was kept frozen at −20 °C until analysed for antipyrine (AP), 4-hydroxyantipyrine (4-OH), 3-hydroxymethylantipyrine (3-OH-M) and norantipyrine (NOR).

The urinary excretion of each of the three metabolites was expressed as % of the administered dose of antipyrine, assuming complete absorption. The clearance of each metabolite was calculated by multiplying its total urinary excretion as % of the dose of antipyrine by the total clearance of antipyrine, assuming first order elimination kinetics for each metabolite and complete metabolism of antipyrine within 48 h [9].

The urine samples were assayed after hydrolysis with glucuronidase/arylsulfatase (Boehringer) for 3 h at 37 °C. Alkaline extraction with dichloromethane was performed for analysis of antipyrine and 3-OH-M, and acid extraction for analysis of 4-OH and NOR (dichlormethane/pentane 30/70 v/v). After evaporation to dryness and redissolving the residue in 100 μl mobile phase, 25 μl was injected into a high pressure liquid chromatographic (HPLC) system, consisting of a Waters pump and injection loop, a Waters μ Bondapack C18 column and a Waters UV detector (Model 440) with a fixed wavelength of 354 nm. The mobile phase was 0.01 M phosphate buffer/methanol (65/35, v/v), and the flow rate was 2 ml/min. Retention times for metabolites and internal standard (phenacetin) ranged from 2.6 to 7.1 min.

Table 2. Antipyrine clearance (Cl_{AP}), aminopyrine breath test (ABT) as % ^{14}C-label of ^{14}C-dose × kg body weight/mmol CO_2, serum γ glutamyltranspeptidase (s-γGT) plasma phenobarbital (P-Phen) and plasma cimetidine (P-Cim) in 4 subjects studied once a week for 14 weeks. Weeks of phenobarbital (Phen) or cimetidine (Cim) administration are indicated by boxes

Subject	Test	Week No.													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
				Phen						Cim					
HEP	Cl_{AP} [ml/min]	45.0	36.8	37.5	59.2	69.3	61.5	43.5	42.0	31.4	29.5	40.5	43.7	45.7	40.4
	ABT	0.37	0.37	0.35	0.35	0.36	–	0.32	–	0.27	0.30	0.35	–	0.33	–
	s-γGT [U/l]	–	27	37	32	35	39	–	36	33	39	35	38	–	–
	P-Phen [mg/l]		0	4	9	8	3	–	–	–	–	–	–	–	–
	P-Cim [mg/l]	–	–	–	–	–	–	–	0.0	1.00	0.23	0.0	0.0	–	–
				Phen						Cim					
BR	Cl_{AP} [ml/min]	52.4	57.7	56.8	71.9	73.5	63.0	61.5	63.1	57.1	46.6	61.4	60.5	56.9	50.4
	ABT	0.47	0.46	0.55	0.55	0.48	–	0.49	–	0.46	0.43	0.47	–	0.46	–
	s-γGT [U/l]	–	24	22	26	44	44	–	30	30	29	22	27	–	–
	P-Phen [mg/l]	–	0	3	9	8	2	–	–	–	–	–	–	–	–
	P-Cim [mg/l]	–	–	–	–	–	–	–	0.0	0.43	0.18	0.0	0.0	–	–
				Cim						Phen					
HP	Cl_{AP} [ml/min]	29.4	30.2	26.8	22.6	30.0	30.5	37.5	31.1	45.3	54.6	60.2	41.8	36.5	32.5
	ABT	0.31	0.28	0.22	0.18	0.26	–	0.21	–	0.20	0.23	0.28	–	0.22	–
	s-γGT [U/l]	–	17	16	16	18	16	–	10	18	15	18	21	–	–
	P-Phen [mg/l]	–	–	–	–	–	–	–	0	4	12	12	3	–	–
	P-Cim [mg/l]	–	0.0	1.08	1.04	0.0	0.0	–	–	–	–	–	–	–	–
				Cim						Phen					
MD	Cl_{AP} [ml/min]	59.3	54.2	36.1	38.9	47.3	55.6	59.0	57.7	60.5	81.3	85.8	65.3	60.3	58.5
	ABT	0.40	0.37	0.36	0.32	0.35	–	0.37	–	0.39	0.41	0.37	–	0.36	–
	s-γGT [U/l]	–	9	10	9	9	10	–	11	9	10	11	13	–	–
	P-Phen [mg/l]	–	–	–	–	–	–	–	0	3	12	11	4	–	–
	P-Cim [mg/l]	–	0.0	0.14	0.10	0.0	0.0	–	–	–	–	–	–	–	–

Reference metabolites were used for standard curves: NOR (EGA-Chemie), 4-OH (EGA-Chemie), and 3-OH-M, (kindly donated by Drs. Danhof, Eichelbaum, and Yoshimura). The coefficient of variation of duplicate analyses of the antipyrine metabolites in calculated as shown in Table 1, was about 3%, except for 3-OH-M, for which it was about 10%.

Aminopyrine, cimetidine and phenobarbital did not interfere with the HPLC analysis of metabolites.

In a separate study the intraindividual variation in antipyrine clearance was studied in five healthy volunteers (two men and three women) two of whom were smokers. The three non-smokers (HEP, HP and MD) also participated in the study with cimetidine and phenobarbital. Antipyrine administration, collection of saliva and urine, and analyses were performed by the methods described above. The measurements were made once a week, 14 times in each subject. Urine was only collected 10 times by the three non-smokers, with an interval of at least one week during the 14 weeks [9].

In each subject the amount of creatinine in each sample of urine was almost constant from week to week, indicating completeness of the 48-h collection.

Permission to use radiolabelled aminopyrine was granted by the Danish Health Authorities, and ethical approval according to the Declaration of Helsinki was given by the Committee of Ethics of Copenhagen.

The data for antipyrine clearance and aminopyrine were tested by two-way analysis of variance. The period effect was considered statistically significant if P were less than 0.05.

Results

Antipyrine clearances and the aminopyrine breath test results during the cimetidine and phenobarbital experiments are given in Table 2.

The mean plasma concentration of phenobarbital was 3.5 (0.3), 10.5 (0.9), 9.8 (1.0), and 2.5 (0.9) mg/l (± SD) during the two weeks of dosing and the

Table 3. Antipyrine clearance [ml/min] measured in 5 subjects once a week for 14 weeks (control experiment). BK and LN were smokers

Subject	Week No														$\bar{x} \pm SD$
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
HEP	49.1	50.7	40.6	47.5	42.5	49.5	38.7	41.8	47.7	45.7	45.8	45.2	41.9	38.6	44.7 ± 4.0
HP	31.5	30.8	27.9	33.7	29.3	27.7	28.3	33.7	34.6	32.7	33.5	32.0	28.8	31.1	31.1 ± 2.5
MD	52.1	44.8	52.4	47.3	55.1	55.3	53.4	54.0	59.4	55.5	48.8	56.5	50.0	44.2	52.0 ± 4.5
BK	66.6	59.7	69.2	72.5	71.7	64.5	66.5	73.7	64.8	68.5	59.0	62.1	66.3	65.6	66.5 ± 4.4
LN	51.1	53.2	50.0	52.5	55.7	57.0	52.9	63.5	54.8	59.3	58.0	54.8	56.5	52.4	55.1 ± 3.6

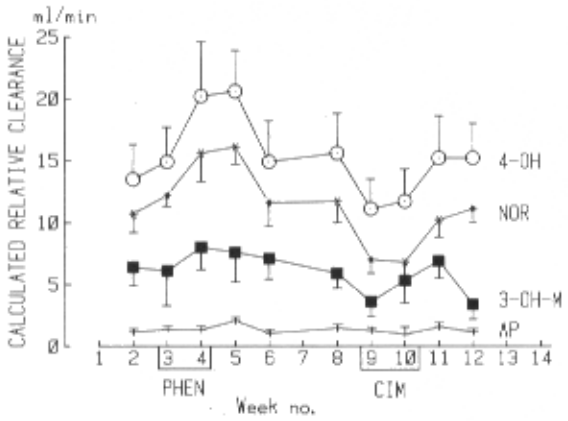

Fig. 2. Time course of the calculated relative clearance (metabolite % of antipyrine dose recovered in 48-h urine multiplied by total salivary antipyrine clearance) of 4-hydroxyantipyrine (4-OH), norantipyrine (NOR), 3-hydroxymethylantipyrine (3-OM-M) and unchanged antipyrine (AP) in 4 subjects before, during, and after phenobarbital (PHEN) or cimetidine (CIM); mean ± SEM

two weeks after withdrawal. The second sample was taken four days before cessation of phenobarbital administration. Plasma phenobarbital continued to increase (verified in two subjects) during the last four days of dosing. This explains why the plasma phenobarbital level was almost as high three days after the cessation of treatment as on Day 9 of treatment. Phenobarbital was not completely eliminated until 15 days after cessation. Plasma cimetidine was 0.7 (0.2) and 0.4 (0.2) mg/l during treatment and was only present in plasma during the period of administration.

The antipyrine clearance and aminopyrine breath test were significantly influenced by the drug regimens ($p < 0.01$). Phenobarbital increased antipyrine clearance to 1.60-times the control value (range 1.33–2.03), whereas the increase in the aminopyrine breath test result was only 1.07-times the control value (range 0.94–1.19). The antipyrine clearance was not significantly changed on the second day of phenobarbital administration, but the increase was apparent on Day 9 of treatment and was still at its maximum three days after cessation, when the plasma phenobarbital level was still high. The antipyrine clearance had decreased to its control level about 15 days after phenobarbital withdrawal, corresponding to the disappearance of phenobarbital from plasma.

During cimetidine administration the antipyrine clearance was reduced to 0.74-times the control value (range 0.62–0.85; $p < 0.01$), and the aminopyrine breath test result was reduced to 0.72 (range 0.52–0.93; $p < 0.01$). As indicated by the antipyrine and aminopyrine measurements on Days 2 and 9 of cimetidine dosing, the decrease in microsomal enzyme activity was confined to the period of administration of cimetidine (Table 2).

No difference was found between antipyrine clearance measured at the same time as the aminopyrine breath test during the third week and antipyrine clearance measured alone in the fourth week after cimetidine withdrawal (49.3 ± 10.3 vs. 44.9 ± 10.6 ml/min (mean ± SD; Table 2).

The intraindividual variation in total antipyrine clearance, expressed as the coefficient of variation, was 6.5 and 6.7% in the smokers (LN and BK), and 7.9, 8.7, and 8.9% in the non-smokers (HP, MD, and HEP) during the 14 week control period without cimetidine or phenobarbital administration (Table 3).

The relative clearance of each of the three major antipyrine metabolites before, during and after each drug regimen is shown in Fig. 2. During cimetidine administration the clearances of all three metabolites were significantly depressed and to almost the same extent to 0.55-times (range 0.43–0.91) the control values. The corresponding increase in the metabolites during phenobarbital was 1.50-fold (1.13–1.86).

During the periods of cimetidine and phenobarbital administration the clearance of each metabolite varied significantly in all four subjects ($p < 0.01$), whereas no significant variation in clearance was observed in the ten control measurements taken over 14 weeks in three subjects ($p > 0.1$; Fig. 3). The intraindividual variation in the clearance of the antipyrine metabolites ranged from 10.6 to 20.0% (Fig. 3).

No significant change in s-γGT was observed during either of the drug treatments (Table 2).

Discussion

Few studies have been made of the time course of drug-related changes in microsomal enzyme activity, but several authors have measured enzyme activity once after withdrawal of the causative agent [2, 5, 6, 8, 12, 13, 24, 25, 28, 31, 34, 39, 43].

The time course of enhanced and depressed antipyrine metabolism in the present study was closely related to the plasma concentrations of phenobarbital and cimetidine, respectively. Accordingly, the elimination or synthesis of increased or decreased amounts of microsomal enzyme appears to be only of minor importance. The half-life of microsomal enzyme turnover has been calculated as ranging from one to six days [20]. Since phenobarbital is eliminated at about the same rate, its elimination is probably the time limiting factor in reversal of phenobarbital -induced enhancement of microsomal enzyme activity. However, this may not apply to drugs that are eliminated more rapidly than the excess microsomal enzymes, in which case enzyme turnover may be the limiting factor [39]. The cimetidine-related inhibition of microsomal enzyme activity found in this study, and in another study with sulfaphenazole [34], occurred and subsided within hours. It is unlikely that the depressed activity have been associated with a corresponding change in the quantity of the enzyme involved, but may be explained by competitive inhibition, the degree of which was probably determined by the amount of modulating substance at the active site of the enzyme.

The increased enzyme activity attributed to DDT exposure lasted for three months [18], the effect of phenobarbital was reversed in 2–6 weeks (depending on the dose and duration of treatment [13, 19, 24]), the effect of rifampicin was reversed in 2–3 weeks [24, 39], that of glutethimide within two weeks [12], and the effect of diets rich in brussel sprouts and cabbage or charcoal broiled beef had returned to its control value 1 week after withdrawal of the diets [8, 25].

The inhibitory effect of disulfiram on antipyrine clearance lasted for at least 10 days after discontinuation of treatment [43], whereas the inhibition of tolbutamide metabolism was reversed within hours after withdrawing sulfaphenazole [34].

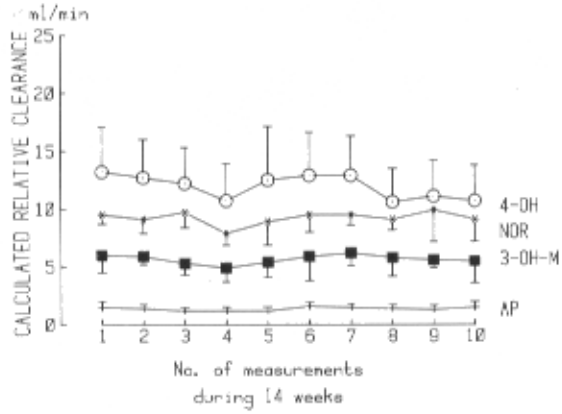

Fig. 3 Time course of the calculated relative clearance (metabolite % of antipyrine dose recovered in 48-hour urine multiplied by total salivary clearance) of 4-hydroxyantipyrine (4-OH), norantipyrine (NOR), 3-hydroxymethylantipyrine (3-OH-M), and unchanged antipyrine (AP) in a control experiment in 3 of the 4 subjects who participated in the phenobarbital-cimetidine study; mean ± SEM

Table 4. Previous studies on the effect of phenobarbital and cimetidine on antipyrine half-life ($t_{½}$) and clearance (Cl) expressed as a multiple of the control value

Number of persons	Dose/day	Number of day given	Effect on $t_{½}$, Cl	Design of study	Author	
			Phenobarbital			
16	2 mg/kg 150 mg	14	$t_{½}$ 0.63	before/during	Vesell and Page 1969	[40]
3	3.6 mg/kg 100–250 mg	21	$t_{½}$ 0.60	before/during/ after	Kampffmeyer 1971	[19]
10	180 mg	21	$t_{½}$ 0.84 Cl 1.82	before/during	Roberts et al. 1976	[30]
7	100 mg	14	$t_{½}$ 0.64 Cl 1.56			
22	100 mg	7	$t_{½}$ 0.65 Cl 1.60	before/during	Danhof et al. 1982	[10]
			Cimetidine			
6	400 mg	14	$t_{½}$ 1.38 Cl 0.80	before/during	Serlin et al. 1979	[35]
6	400 mg	21	$t_{½}$ 1.21 Cl 0.90	before/during	Puurunen et al. 1980	[29]
8	1000 mg	7	$t_{½}$ 1.32 Cl 0.73	before/during	Henry et al. 1980	[14]
9	1000 mg	21	$t_{½}$ 1.36 Cl 0.46	before/during	Neuvonen et al. 1981	[23]
6	1000 mg	7	$t_{½}$ 1.21 Cl 0.74	before/during	Staiger et al. 1981	[36]
7	1000 mg	7	$t_{½}$ 1.31 Cl 0.77	before/during	Breen et al. 1982	[4]

Table 5. Reported studies of the effect of phenobarbital and cimetidine on aminopyrine metabolism (2-h breath value, half-life ($t_{½}$) and clearance (Cl)) expressed as a multiple of the control values

Number of persons	Dose/day	Number of days given	Effect on $t_{½}$ Cl or 2-h breath test	Design of study	Author	
			Phenobarbital			
6	300 mg	14	Cl 1.19	before/during	Roots 1972	[32]
9	not reported	not reported	2-h breath higher during treatment	before/during	Hepner et al. 1974	[16]
11 treated 14 controls	not reported	not reported	2-h breath 1.77	treated/controls	Lewis et al. 1977	[22]
8	150 mg	7	2-h breath 1.46	before/during	Piken and Hepner 1979	[26]
			Cimetidine			
8	1000 mg	7	$t_{½}$ of breath 1.33 Cl (plasma) 1.32	before/during	Henry 1980	[14]

In all these studies the microsomal enzyme activity seems to have been roughly correlated with the half-life of the causative agent, but since no data on the blood concentrations of the modulating compounds have been given the exact relationship cannot be described.

The degree of phenobarbital-related enhancement, and cimetidine-associated depression of total antipyrine clearance observed here is in agreement with previous studies, where total antipyrine clearance was measured before and during drug administration (Table 4). The change in antipyrine clearance was considerably greater than the observed intraindividual coefficient of variation, which amounted to 6–8%, as calculated from 14 measurements made once weekly in five healthy subjects. This intraindividual variation is also consistent with a recent study in which, as in the present study, no effort was made to control the life style of the persons investigated [1].

Cimetidine was found to decrease both antipyrine and aminopyrine elimination to the same extent, in agreement with a recent study [14]. However, the induction of antipyrine elimination after phenobarbital was more pronounced than that of aminopyrine elimination. This indicates that whereas oxidation of antipyrine and N-demethylation of aminopyrine are equally depressed by cimetidine, the enzyme activities are differentially affected by phenobarbital. A similar discrepancy was previously reported by Hepner et al. [16], and has also been observed after administration of glutethimide [15]. In all the previous studies in which phenobarbital has been found to enhance aminopyrine metabolism, larger doses of phenobarbital were administered than in the present experiment (Table 5). Several studies have shown faster antipyrine elimination during treatment with doses of phenobarbital of about 100 mg daily as were used here (Table 4). Thus, it appears that antipyrine metabolism is more sensitive than that of aminopyrine to changes induced by small doses of phenobarbital.

The change in the total clearance of antipyrine during cimetidine and phenobarbital administration was accompanied by similar changes in the calculated clearance of its three major metabolites, viz. i.e. 3-hydroxymethyl-, 4-hydroxy-, and norantipyrine. Contrary to the selective inhibition of the urinary excretion of 3-hydroxymethylantipyrine by propranolol [3], the clearance of antipyrine to each of the three metabolites was depressed in parallel by cimetidine. The recently described dissociation by phenobarbital of the degree of enhancement of antipyrine metabolite excretion [10] was not found here. However, the small number of subjects investigated and the large intraindividual variation in the calculated clearance of antipyrine metabolites found in the present study do not permit exclusion of minor differences in the effects of cimetidine or phenobarbital on the metabolic pathways of antipyrine.

A one-sample method was used for determination of total antipyrine clearance. It was previously shown to give a result identical to the clearance estimated in the conventional way if the volume of distribution is unchanged [11]. The body weight, and thereby the total body water (equal to the distribution volume of antipyrine), was constant during the study. Moreover, it is well established that neither phenobarbital nor cimetidine change the volume of distribution of antipyrine [10, 35, 37, 41]. Therefore, the recorded changes in antipyrine clearance were probably caused by phenobarbital and cimetidine.

It has previously been shown that a large dose of aminopyrine depresses antipyrine clearance [42]. In this study aminopyrine 5 μg did not inhibit antipy-

rine metabolism, and the one-sample antipyrine saliva test and the aminopyrine breath test could be used simultaneously. With these two simple noninvasive tests for assessment of hepatic microsomal enzyme activity, it would be easy to investigate whether the simple relationship between the plasma concentration of a modulating compound and the microsomal enzyme activity found here could be extended to other drugs.

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