Europ.J.clin.Pharmacol. 8, 327-336 (1975)

Evaluation of *in vivo* Parameters of Drug Metabolizing Enzyme Activity in Man after Administration of Clemastine, Phenobarbital or Placebo

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Received: August 16, 1974, accepted: Januar 7, 1975

Summary. The 24 h urinary excretion of 6β-hydroxycortisol and D-glucaric acid, the plasma half-lives and total clearances of aminopyrine, and serum gamma-glutamyl-transpeptidase activity have been measured in nineteen healthy male volunteers. The study was done double blind and was conducted as a test of induction of microsomal drug metabolizing enzymes during and after daily doses of 6 mg clemastine, 300 mg phenobarbital or a placebo. The urinary excretion of 6β-hydroxycortisol and D-glucaric acid was significantly increased in the phenobarbital group, the standard for induction. No changes were observed after treatment with clemastine or placebo. Phenobarbital also reduced the half life of aminopyrine, but it was not affected by clemastine or placebo. Gamma-glutamyl-transpeptidase activity increased only in the phenobarbital group. The elimination constant k_2 of aminopyrine and the excretion of glucaric acid in the pre-medication period were correlated ($p<0.05$). The results indicate that the tests were of diagnostic value in determination of microsomal enzyme induction by phenobarbital. Failure to observe similar changes after treatment with clemastine imply failure of induction of this activity under the experimental conditions.

Key words: Glucaric acid, aminopyrine half life, gamma-glutamyl-transpeptidase, 6β-hydroxycortisol, enzyme induction, drug metabolism in man.

Drug metabolism occurs mainly in the liver by enzymes of the endoplasmic reticulum. It can be stimulated by many compounds, a process known as enzyme induction (1, 2). Many drugs have been shown to be effective enzyme inducers in animals, but this has often involved doses well outside any possible clinical range, a fact which has cast doubt on the relevance of induction to the clinical situation.

In man apparent inducing agents seem to influence drug metabolism in various ways and to a different extent. Thus, halofenate, a hypolipidaemic agent, shortens the half-lives of antipyrine and dicoumarol, but lengthens that of warfarin (3). This can be explained by the existence of multiple drug metabolizing enzymes, which are differentially stimulated or inhibited by the various compounds. Therefore, induction must be examined by several tests, which might respond to a different extent to the effects of inducing agents on the various enzymes.

In the present study four different *in vivo* parameters have been employed to investigate the possible inducing effect on drug metabolism of clemastine (Tavegil®, HS 592, Tavist, 1-methyl-2-[2-(α-methyl-p-chlordiphenyl-methyloxy)ethyl] pyrrolidin-hydrogen fumarate). A double blind parallel group design with phenobarbital as a "standard" of induction, and a placebo as the control, has been used.

Methods and Materials

a) Volunteers

As partial compensation for modifying factors such as sex and age, only male volunteers, 20-32 years old (18 Caucasians, 1 African), were chosen. Careful supervision and stable environmental conditions (e.g. nutrition) were ensured by use of a hotel in Berlin (Raststätte Grunewald) as a metabolic ward. All volunteers were fully informed and had signed a written consent for the study.

b) Procedure

Each subject was studied for a maximum of seven weeks; one week for initial evaluation, two weeks

for double blind medication and four weeks for post-treatment observation. The stay in the metabolic ward lasted from the 5th day of week 1 to the 5th day of week 4.

The initial evaluation stage (study week 1) was conducted in an open design, in which no subject received any medication. During the evaluation stage (study weeks 2 and 3), 6 subjects received placebo, 6 received clemastine and 7 received phenobarbital.

A complete medical history was obtained from each subject prior to the study. A physical examination was performed before and at the end of the trial. Interim examinations were done at the end of weeks 1 and 3.

Laboratory tests were done prior to the study, at the end of the double blind evaluation stage and at the end of week 7. At the beginning of the study a narcotic screening test was also done.

c) Treatment

The medication consisted of two pairs of identical-appearing tablets of 1 mg clemastine (Cl) (Tavegil® Tavist) and matching placebo, or 50 mg phenobarbital (PB) and matching placebo, supplied by Sandoz Ltd. (Basle, Switzerland). The doses of both active drugs were in the upper therapeutic range to ensure maximal effects. The drugs were packed in separate bottles for each subject:

	Bottle I	Bottle II
A) Placebo group:	PB Placebo	Cl Placebo
B) Phenobarbital group:	PB active	Cl Placebo
C) Clemastine group:	PB Placebo	Cl active

Each subject received two tablets from each of these two bottles t.i.d., at 8 a.m., 2 p.m. and 8 p.m. daily, for 14 consecutive days.

d) Blood and Urine Samples

All blood for haematology and chemistry was taken in the morning in the fasting state. For determination of the half-life of aminopyrine about 10 ml of blood were collected. Serum samples were divided into two equal parts and frozen at -20°C.

Urinary excretion of 6β-hydroxycortisol (6β-OHF), 17-hydroxycorticosteroids (17-OHCS) and D-glucaric acid was measured in 24 h urine collections carried out on days 4, 5 and 6 of week 1 (control period), days 2, 4 and 6 of week 2, and days 3 and 7 of week 3 (treatment period). Samples were collected on days 1 and 3 of the washout period in week 4.

All urine voided between 8 a.m. on the collection day and 8 a.m. on the following day was collected, stored with toluol (3 ml) and refrigerated until the end of the period. Then the total 24 h volume was recorded and aliquots removed and frozen (-20°C).

e) Determination of 6β-Hydroxycortisol

I. Materials. All solvents were reagent grade. Thin layer chromatography silica gel GF 254 was precoated on glass plates 20 x 20 cm, 500 μ thick by Merck (Darmstadt, Germany).

Recovery was estimated by liquid scintillation counting of added ^{3}H-tetrahydrocortisol (THF) (NEN Chemicals) in a Packard Tricarb Spectrometer, Pure 6β- and 6α-OH-cortisol were kindly supplied by Dr. Seymour Bernstein, Lederle Laboratories, New York. ^{3}H-labelled 6β-OH-cortisol was prepared by NEN Chemicals and was purified by repeated thin layer chromatography.

II. Methods. Aliquots (4%) of 24 h urine specimen were washed for 15 seconds with twice their volume of chloroform, according to Frantz *et al.* (4). The urine phase was saturated with 20 per cent (w/v) sodium sulphate (anhydrous) and extracted once with four time its volume of ethyl acetate. The ethyl acetate extract was washed twice with 1/20 volume of 0.25 N sodium hydroxide, then once with 1/20 volume of 0.5 per cent acetic acid, and finally with 1/100 volume H_2O; all solvents were saturated with 20 per cent sodium sulphate. The ethyl acetate phase was evaporated to dryness in a rotary vacuum evaporator. For quantitative recovery, the residue was redissolved in 5 ml of ethanol, transferred to conical evaporation tubes and evaporated to dryness. The residue was redissolved in 0.6 ml of methanol (5). Fifty microliters were added to 10 ml scintillator cocktail.

The extract was applied to a thin layer silica gel plate with the aid of a Desaga (Germany) "Autoliner". Usually, two extracts were applied to one plate, each in a band exactly 5 cm wide, with a guide channel of 6β-hydroxycortisol standard in between. After primary chromatography in system I (chloroform:methanol:acetic acid, 115:15:3), the plates were airdried, and then rechromatographed in system II (104:26:3, chloroform:methanol:acetic acid).

Plates were viewed under UV-light (254 nm) to locate 6β-hydroxycortisol bands. Quantitative determination was performed by scanning the remission from 6β-OHF at 236 nm with the aid of a Camag-Z-Scanner (Muttenz, Switzerland), in conjunction with a Zeiss spectral photometer PMQ-2 and a Beckman recorder.

Experiments in which 6β-OH-cortisol and ^{3}H-6β-OH-cortisol were added to the urine samples, showed good recoveries after chromatography in systems I and II; the mean percentage ± standard deviation was 80% ± 4.5.

The concentration of 6β-OH-cortisol was estimated by planimetric integration of the area under the 6β-OH-cortisol peak, as recognized from the location of the reference material. Since ^{3}H-6β-OH-cortisol was not available in a sufficient quantity, 25,000 dpm tetrahydrocortisol was added to each sample after chloroform extraction to correct for possible losses during preparation. Simultaneous analysis of several samples by high pressure liquid chromatography confirmed this procedure (unpublished).

Additional elution of the 6β-OH-cortisol band and its subsequent rechromatography in system III (see below) was performed to confirm homogeneity

of this peak by its further separation from possible interference by other steroids or drugs, e.g. aminopyrine, clemastine, phenobarbital or their metabolites. Under these circumstances recovery was about 40% as determined by addition of 6β-OH-cortisol and ^{3}H-6β-OH-cortisol standards to urines which were treated as test samples. All the silica gel in the same place as the reference 6β-OH-cortisol was scraped off and transferred to dry, conical, glass-stoppered centrifuge tubes. 11 ml of ethyl acetate and 2 ml of water saturated with sodium sulphate were added. After mixing and centrifuging, 10 ml of the ethyl acetate phase was transferred to a 25 ml conical evaporation tube and dried. The residue was redissolved in 0.6 ml of methanol. 50 µl were withdrawn for liquid scintillation counting and 0.55 ml applied to a silica gel chromatographic plate. Usually, four extracts were put on one plate in 2 cm bands and chromatographed in system III (119:19:3, chloroform:methanol:acetic acid). After drying, the plates were scanned in an ascending direction, as described above, and compared to 6β-OH-cortisol standard applied to each plate.

f) Determination of 17-Hydroxycorticosteroids

17-Hydroxycorticosteroids (17,21-dihydroxy-20-ketosteroids) were determined after Silber and Porter (6) with slight modifications. 40 ml of urine was incubated for 15 hours at 38°C with 8 ml ketodase (5000 Fishman units/ml; Warner-Chilcott, USA), in the presence of 1.0 M sodium acetate-buffer 8.0 ml. The urine was then extracted into twice its volume of ethyl acetate, washed twice with 0.25 N NaOH and evaporated to dryness. The residue was redissolved in 5 ml ethanol, evaporated to dryness in a 25 ml evaporation tube, and the residue redissolved in 1 ml ethanol. 0.1 ml of the ethanol extract was placed in test tubes containing 1.5 ml absolute ethanol. 2.4 ml of Silber-Porter reagent were added and the sample allowed to stand overnight at room temperature. Optical density readings were performed at three wavelengths (370; 410 and 450 nm), and an Allen correction (7) applied. Recovery was determined with the aid of ^{3}H-cortisol.

g) Determination of D-Glucaric Acid Excretion

This was performed according to Marsh (8), with several modifications. Typical assays comprised 0.1 M acetate buffer pH 4.5, 0.005 M p-nitrophenylglucuronide (Serva, Heidelberg) and increasing amounts of urine, in a total volume of 1.6 ml. The urine was treated with alkali or acid according to Marsh (8). The reaction was started by addition of 0.6 µg(2.5 mU)1β-glucuronidase from Helix pomatia (EC 3 2.1.31) (Boehringer, Mannheim) in 0.1 M acetate buffer pH 4.5, and incubated at 30°C. The reaction was stopped after 20 min by adding 0.5 N NaOH 2.0 ml. The resulting extinction of p-nitrophenol was measured at 405 nm with a Zeiss spectralphotometer PMQ-2 and the blank subtracted. Straight lines were obtained by plotting the reciprocal of extinction versus concentration of urine in the incubate. From this Dixon-plot (9), I_{50}-values for acid- or alkali-treated samples were obtained to calculate the amount of "acid-potentiated inhibitor" (8) excreted in 24 h. Calibration curves were established by adding calcium-D-saccharate (Merck, Darmstadt) to urine.

h) Determination of Aminopyrine in Serum

To 2.0 ml serum in round-bottomed stoppered glass centrifuge tubes was added: 1.0 ml saturated NaCl, 1.0 ml tetraborate buffer pH 10.0 and 0.1 ml 4 N KOH. After addition of 10 ml chloroform the tubes were shaken mechanically for ten minutes, centrifuged and the chloroform phase removed by aspiration. The chloroform phase was re-extracted with 0.5 N HCl 5.0 ml, centrifuged, and the upper phase removed for further analysis. 1 ml NH_4Cl buffer pH 9.0 and 1 ml 4 N KOH were added to the HCl phase. A final extraction was performed in presence of 10 ml chloroform containing 40 µg of imipramine as an internal standard. After centrifugation, the chloroform phase was evaporated to dryness, the residue dissolved in 20 µl of ethanol and 1 µl injected into a gas chromatograph.

A Hewlett-Packard model 402 gas chromatograph was used with a 1.8 m, 2 mm i.d. glass "U" tube column, packed with 80/100 mesh chromosorb-G-HP coated with 3 per cent OV 17. The column, injection port and flame ionisation detector temperatures were 220, 240 and 260°C, respectively. The helium carrier gas flow rate was 30 ml/min, with hydrogen and air flow rates of 30 and 350 ml/min, respectively. The disappearance rate of aminopyrine from venous blood was estimated from samples collected at intervals after a single oral dose of 600 mg taken as two tablets of 300 mg (Drobena, Berlin), 30 min after breakfast. Logarithmic concentration decay graphs after dosing were calculated by the method of least squares, and were used to determine the plasma half-life. Back extrapolation of the line to zero was performed to estimate the theoretical serum concentration (C_o) and to calculate the apparent volume of distribution (V_d). Plasma clearance (Cl_{tot}) was calculated assuming complete bioavailability. For pharmacokinetic reasons, C_o, V_d and Cl_{tot} after oral and intravenous administration may differ slightly.

i) Serum γ-Glutamyl-Transpeptidase

The activity of this enzyme was determined using a commercial kit (Monotest γ-GT, Boehringer, Mannheim), which is based on the method of Szasz (10).

j) Statistical Evaluation

Values from each subject are averaged to obtain one mean value for each of the following groups (days have been counted from the beginning of the study in the "metabolic ward", Table 3):-

a) days 1-2-3 (premedication period)
b) days 6-8 (2nd and 4th days of medication period)
c) days 10-14-18 (6th, 10th and 14th days of medication)

d) days 19-21 (1st and 3rd days of washout period)
e) days 10-14-18-19-21

The significance of the differences between the mean values of b), c), d), and e) were tested against the mean value of a) by Student's t-test for paired samples.

Results

Determination of 6β-Hydroxycortisol Excretion

6β-Hydroxycortisol excretion (mean ± standard error of mean) after the various treatments is shown in Fig. 1. The mean 6β-OH-cortisol excretion for all subjects during the premedication period (see Methods, 'j'.) was 445 µg/day (S.D. = 79.9; n = 19). This is within the range of values reported previously (11, 12, 13, 14) and indicates normal excretion of 6β-hydroxycortisol; none of the subjects had a result outside the mean ± 2 standard deviations.

The data shown in Fig. 1 for placebo indicate the absence of an increase or a trend during the treatment. Determination of the values for particular individuals on several occasions indicates the occurrence of individual differences during the course of the experiment. This has usually been neglected in similar studies, in which only endpoint values have been quoted.

In the phenobarbital group, statistical comparison of mean values before treatment with the subsequent periods showed a significant increase (Table 1 and Fig. 1) in 6β-hydroxycortisol excretion.

The effect of treatment with clemastine has been shown in Fig. 1 by the dashed line. There was no significant effect on 6β-hydroxycortisol excretion (Table 1). Only a comparison between the pretreatment period and the mean of days 6 and 8 showed a decrease ($p<0.05$) in the urinary levels of 6β-OH-cortisol.

Determination of 17-Hydroxycorticosteroids and the Ratio 6β-OHF/17-OHCS

The general value of the ratio of 6β-OH-cortisol/17-OH-corticosteroids has been demonstrated in previous studies of cortisol metabolism (5,12,14). Conney (15) suggested its use to detect drugs that affect drug metabolizing enzyme activity, as it should compensate for changes due to increased adrenal 17-OH-corticosteroid excretion rather than enzyme induction.

For this reason the total amount of 17-hydroxycorticosteroids was estimated (Fig.2) and the ratio calculated of 6β-OHF/17-OHCS (Fig.3). The excretion of 17-hydroxycorticosteroids (17-OHCS) remained unchanged during the period of treatment (Fig. 2). The increases observed towards the end may have been caused by administration of a test dose (600 mg) of aminopyrine; a similar effect has been reported for antipyrine (16). The mean value for all volunteers during the pretreatment period was 7912 µg (S.D. = 1559; S.E.M. = 367; n = 18). Throughout the study the mean of the phenobarbital group was lower than that of either the placebo or clemastine groups. However, statistical evaluation of the pretreatment period showed that all groups belonged to the same population. As shown in Fig. 1, 6β-hydroxycortisol pretreatment values tended to be higher in the phenobarbital than in placebo or clemastine groups and consequently, the ratio of 6β-OHF/17-OHCS was highest in the phenobarbital group (Fig. 3).

Again, there was no trend after treatment with placebo or clemastine, a result that differs from the significant increase observed after phenobarbital treatment (Table 1). No increase was found on days 19 and 21 in the washout period. The slight increase observed in 6β-OH-cortisol excretion (Fig. 1) in the clemastine and especially the phenobarbital groups has thus been compensated.

The population taken for calculation of the mean values shown in Figs. 1-3 consisted of a total of 18 volunteers (placebo n = 6, phenobarbital n = 7, clemastine n = 5). Although the latter group contained 6 volunteers, one (Sh) was excluded from this comparison for the following reason: the absolute values of 6β-OH-cortisol excretion during this pretreatment period fell within the range calculated for all 19 subjects during this period (445 µg/day; S.D. = 79.9; n = 19). However, his 17-OH-corticosteroid values were extremely low (Fig. 4), and values obtained for the ratio 6β-

Table 1. Statistical significance of differences between mean values obtained during premedication, medication and washout periods. For arrangements of groups see 'Methods, j'. [a] *indicates a decrease*

	mean of days 6 and 8	mean of days 10-14-18	mean of days 19 and 21	mean of days 10-14-18-19-21
Placebo (n=6)				
17-OHCS	n.s.	n.s.	n.s.	n.s.
6β-OHF	n.s.	n.s.	n.s.	n.s.
17-OHCS / 6β-OHF	n.s.	n.s.	n.s.	n.s.
Glucaric acid	n.s.	n.s.	n.s.	n.s.
Clemastine (n=5)				
17-OHCS	n.s.	n.s.	n.s.	n.s.
6β-OHF	$p<0.05$[a]	n.s.	n.s.	n.s.
17-OHCS / 6β-OHF	n.s.	n.s.	n.s.	n.s.
Glucaric acid	n.s.	$p<0.02$[a]	n.s.	$p<0.02$[a]
Phenobarbital (n=7)				
17-OHCS	n.s.	n.s.	n.s.	n.s.
6β-OHF	n.s.	$p<0.02$	$p<0.001$	$p<0.005$
17-OHCS / 6β-OHF	n.s.	$p<0.05$	$p<0.005$	$p<0.025$
Glucaric acid	$p<0.005$	$p<0.005$	$p<0.001$	$p<0.001$

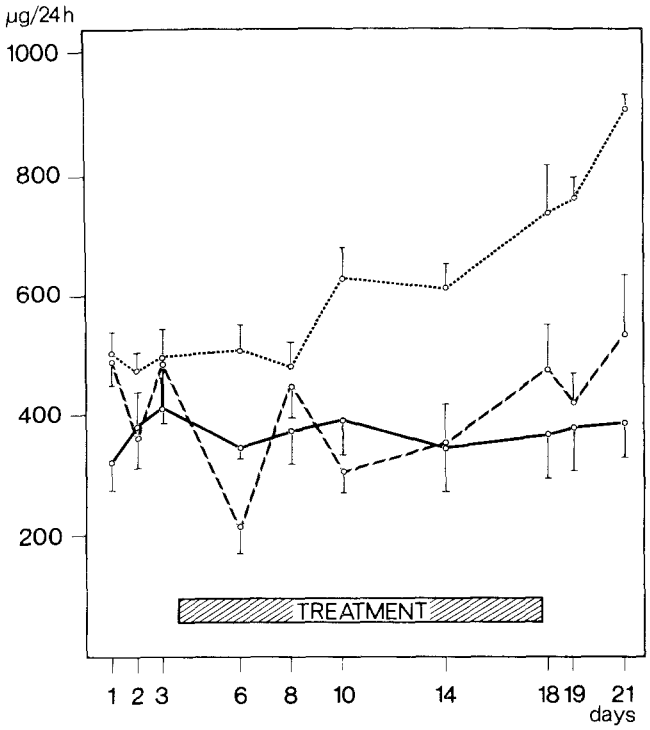

Fig. 1. Daily excretion of 6β-hydroxycortisol in urine of male volunteers. Days refer to sojourn in metabolic ward. ——— placeo (n = 6);....., 3x100 mg daily phenobarbital (n = 7); - - - - 3x2 mg daily clemastine (n = 5). Mean ± S.E.M.

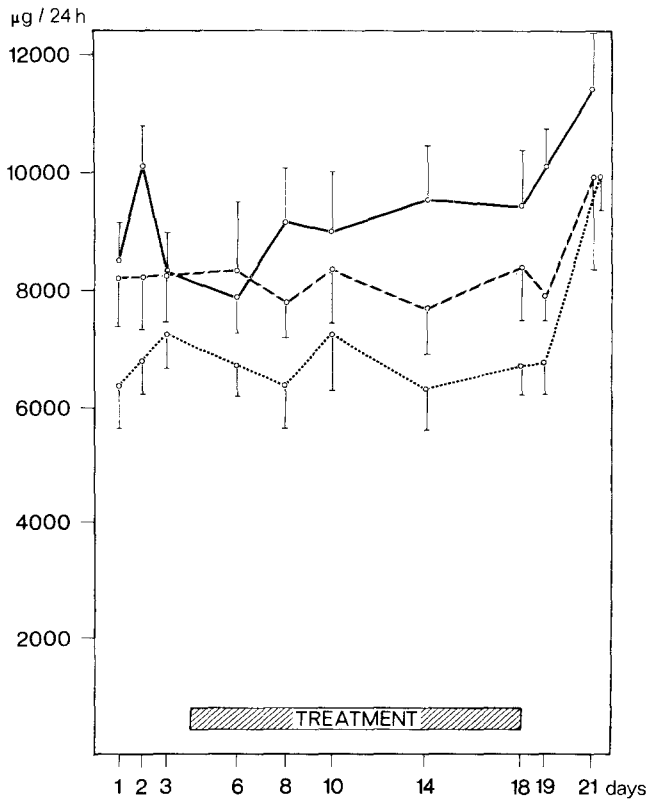

Fig. 2. Daily excretion of 17-hydroxycorticosteroids (Silber-Porter chromogens) in urine of male volunteers. Conditions as described in Fig. 1.

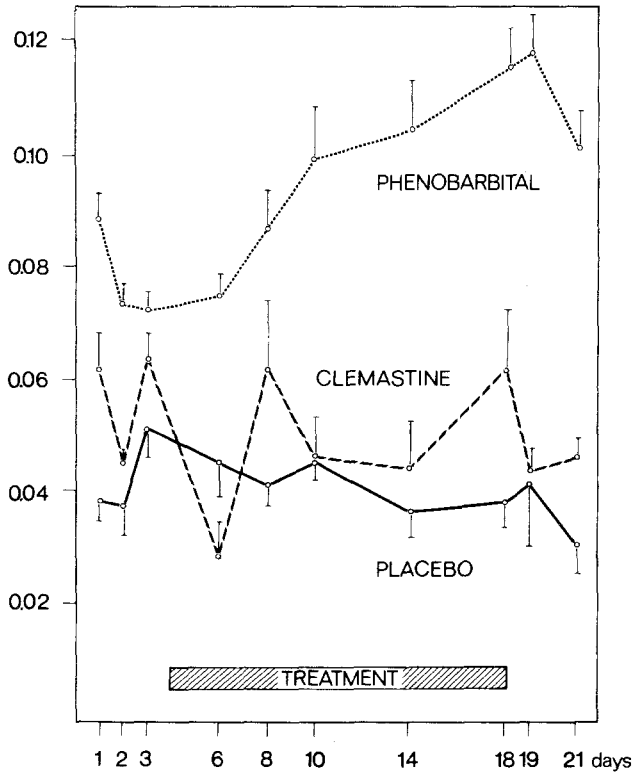

Fig. 3. Ratio of 6β-hydroxycortisol (6β-OHF) to 17-hydroxycorticosteroids (17-OHCS) calculated from data in Fig. 1 and 2 (mean ± S.E.M.)

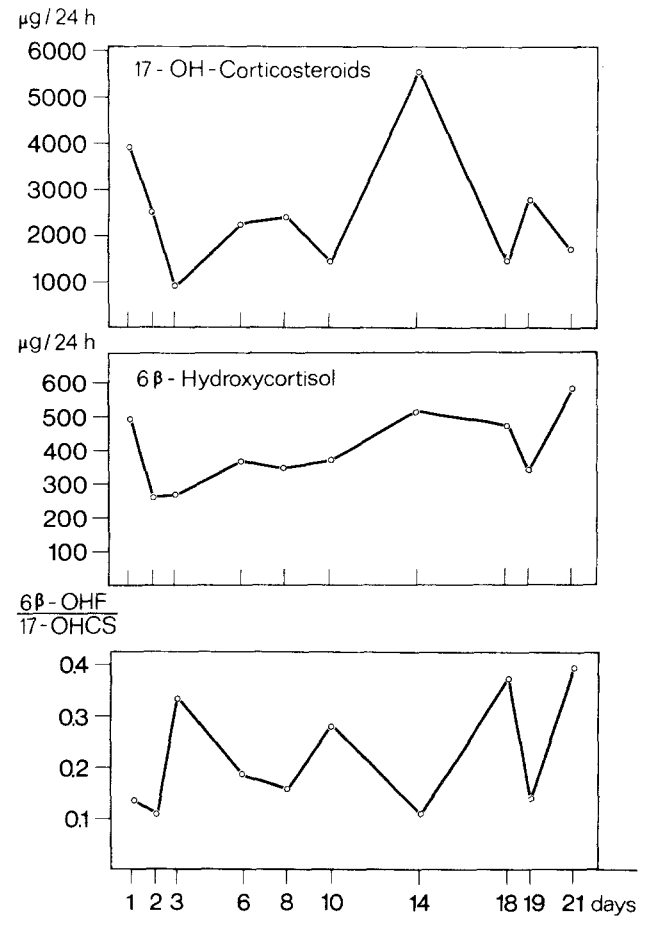

Fig. 4. Daily excretion of 17-hydroxycorticosteroids and 6β-hydroxycortisol, and their ratio in subject Sh.; 26 year old negro, given clemastine. Conditions as in Figs. 1-3

OHF/17-OHCS were much too high for comparison. It seemed justified, therefore, to treat these results separately. The very low 17-OH-corticosteroid values might be an accidental occurrence or due to racial differences (Sh was a negro). Other laboratory data and his physical examination were normal.

Glucaric Acid Excretion

Glucaric acid was also estimated in all the urines examined for 6β-OH-cortisol excretion; the mean values and standard error of the mean are shown in Table 2, and the significances of the intrapair differences are listed in Tables 1 and 2. Placebo treatment did not influence glucaric acid excretion, nor did clemastine. There was only a slight fall in glucaric acid excretion for the mean of days 10, 14 and 18 and days 10 to 21. A parallel tendency, which was not statistically significant, was also observed in the placebo group (Table 2).

In contrast, treatment with phenobarbital increased the excretion of glucaric acid more than sixfold, a highly significant change.

Pharmacokinetics of Aminopyrine

The disappearance of aminopyrine from blood was linear when the logarithm of serum concentrations was plotted against time (Fig. 5). Neither the placebo or clemastine groups showed any significant differences before and after treatment (Table 3). However, some individuals may have had an increase in $t_{1/2}$ of aminopyrine after clemastine treatment, which might suggest an inhibitory effect (Table 4). This was ruled out by comparison of the total clearance of aminopyrine before and after clemastine, when changes in individual half-lives were found not to correspond to the appropriate alterations in total clearance.

In contrast, phenobarbital treatment resulted in a significant decrease in half-live time (Table 5), paralleled by a considerable rise in total clearance.

Determination of Serum Gamma-Glutamyl-Transpeptidase Activity

Individual activities of serum gamma-glutamyl-transpeptidase (γ-GT) are shown in Table 6 for each treatment group. No significant alterations were observed in volunteers treated with either placebo or clemastine. In contrast there was a significant and almost twofold increase in serum γ-GT after phenobarbital treatment. However, three volunteers (two in the clemastine and one in the phenobarbital group) had values definitely above normal levels, although all their other laboratory results appeared normal. Since γ-GT activity determination was not performed during the screening period, the subjects with elevated γ-GT levels had met the selection criteria.

Discussion

After chronic administration of certain commonly used drugs, drug metabolism by hepatic microsomal enzymes can be accelerated by a process known as induction (1,2,15). This process coincides with increased hepatic blood flow and activation of the metabolism of endogenous compounds, such as an increased hydroxylation of cortisol to 6β-hydroxycortisol (31). Stimulated metabolism of D-glucuronic acid to D-glucaric acid has been described (30, 17), as well as increase in the serum level of γ-glutamyl-transpeptidase activity (18).

Induction is associated with enhanced proliferation of endoplasmic reticulum and an increase in cytochrome P-450 content. Evidence from animal experiments (19, 20, 21, 22) has suggested that cytochrome P-450 has variable properties due to its existence in different forms, which may increase to a differing extent and have altered catalytic activity, according to the type of inducing agent used. Accordingly, various *in vivo* parameters of drug metabolism might be affected and respond differently to inducing agents. Thus, to test induction of drug metabolism in man, an important precondition would be to standardize the above-mentioned parameters by their ability to

Table 2. D-glucaric acid excretion (μmoles) in 24 h urines from male volunteers before and during treatment with placebo (n = 6), phenobarbital (300 mg daily; n = 7) or clemastine (6 mg daily; n = 5). Days refer to stay in the metabolic ward. p indicates significance of difference between each value and mean of control period. Mean and S.E.M. are given

	Control period			Treatment period					Washout period	
Day	1	2	3	6	8	10	14	18	19	21
Placebo	32.5	32.3	30.5	26.6	22.7	25.9	26.9	33.6	28.1	39.4
	4.921	3.392	4.853	2.521	4.264	2.223	4.540	6.475	3.297	6.416
Clemastine	30.1	30.6	33.7	26.5	21.4	26.2	22.8	23.5	24.8	35.8
	5.257	7.905	4.257	6.510	2.763	6.069	3.585	5.587	4.041	7.790
Phenobarbital	35.0	28.4	33.7	60.8	70.2	128	168	185	213	209
	9.729	8.890	9.628	15.18	17.96	34.34	28.99	31.66	36.55	32.97
				p<0.01	p<0.01	p<0.02	p<0.005	p<0.001	p<0.005	p<0.001

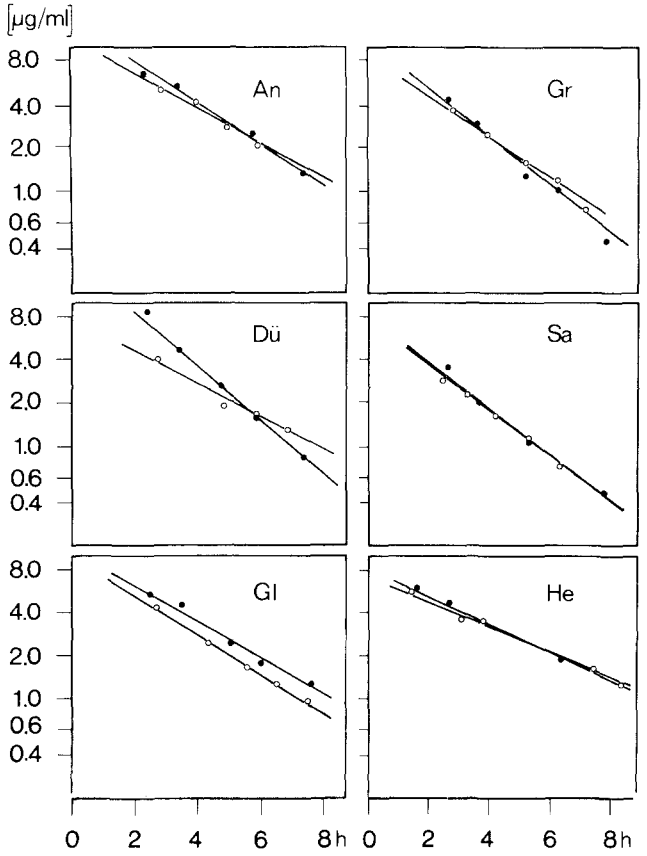

Fig. 5. Aminopyrine half-life in serum of male volunteers. A dose of 600 mg was given at zero time before (●) and after (○) 14 days treatment with placebo.

Table 3. $t_{1/2}$ (hours) of Aminopyrine in male volunteers before and after 14 days treatment with placebo (n = 6), clemastine (n = 6), and phenobarbital (n = 6). A posttreatment test was not be done on one volunteer (Le)

	Placebo		Clemastine 6 mg/d		Phenobarbital	
	before	after	before	after	before	after
mean	2.05	2.37	1.97	2.57	2.05[a]	1.32[a]
S.D.	0.516	0.491	0.653	1.041	0.388	0.300
S.E.M.	0.210	0.200	0.266	0.425	0.158	0.122

[a]Significance of difference p 0.001

Table 4. Individual values of half-life and total clearance of aminopyrine in male volunteers (n = 6) before and after clemastine treatment. Total clearance has been calculated from the equation:

$$Cl_{tot} = \frac{dose \cdot 0.69}{t_{1/2} \cdot C_o}$$

C_o represents theoretical serum concentration at zero-time

	$t_{1/2}$ (h)		Total clearance (ml/min)	
Name	Control	Clemastine	Control	Clemastine
Dü	1.58	1.30	392	309
Ku	1.94	1.90	357	308
Zü	1.08	1.82	281	416
Zu	2.35	3.85	204	225
Sh	1.89	3.00	215	210
Eh	2.99	3.58	151	199
MEAN	1.97	2.57	267	278
S.D.	0.654	1.05	94.0	83.1
S.E.M.	0.267	0.427	38.4	33.9

Table 5. Individual values of half-life and total clearance of aminopyrine in male volunteers (n = 6) before and after phenobarbital treatment

[a]Significance of difference p<0.02,
[b]p<0.001

	$t_{1/2}$ (h)		Total clearance (ml/min)	
Name	Control	Phenobarbital	Control	Phenobarbital
Wa	1.82	1.18	267	449
Gu	2.20	1.28	231	442
Ha	2.11	1.50	239	701
Ze	1.72	0.90	228	263
Fe	1.75	1.28	274	554
Ri	2.74	1.79	271	896
MEAN	2.05[b]	1.32[b]	251[a]	551[a]
S.D.	0.388	0.300	21.21	222.0
S.E.M.	0.158	0.122	8.660	90.66

Table 6. Activity of serum γ-glutamyltranspeptidase (mU/ml) before and after treatment with placebo, clemastine, or phenobarbital

Placebo			*Clemastine*			*Phenobarbital*	
name	before treatment	after treatment	name	before treatment	after treatment	name	before treatment	after treatment
An	14	11	Dü	21	23	Wa	16	44
Gr	19	20	Ku	38	40	Gu	16	20
Dü	14	10	Zü	16	17	Le	45	78
Sa	26	23	Zu	32	35	Ha	17	31
Gl	11	15	Sh	75	93	Ze	17	35
He	11	12	Eh	31	11	Fe	20	27
						Ri	10	16
MEAN	15.80	15.20	MEAN	35.50	36.50	MEAN	20.10[a]	35.90[a]
S.D.	5.78	5.27	S.D.	20.90	29.70	S.D.	11.40	20.80
S.E.M.	2.36	2.15	S.E.M.	8.54	12.10	S.E.M.	4.30	7.85

[a]Significance of difference: $p<0.02$

indicate quantitatively the effects of phenobarbital treatment on drug metabolism.

In order to show whether clemastine was an inducing agent it would have been of value to determine whether there was increased biotransformation of it upon chronic administration. However, as has been pointed out, due to the existence of multiple rate-limiting steps or enzymes in drug metabolism, lack of self-induction by this compound would not deny its possible inducing activity upon other paths of drug metabolism. For that reason, it was necessary to conduct induction studies with a broad spectrum of tests.

Some evidence has been reported (23) on the coincidence in man of the relative rates of metabolism of a variety of drugs. This has suggested use of the "prototype drug" for demonstration of correlations between the metabolism of the latter (half-life or clearance) and similar kinetic data for other drugs. Antipyrine has often been used as an index of drug metabolism in man and its kinetics have been compared with other compounds. The results obtained so far have not been encouraging, mainly because of the lack of correlation between it and other compounds. In accordance with this, a recent study by Smith and Rawlins (24) in untreated volunteers demonstrated lack of correlation between the plasma half-lives of antipyrine, phenylbutazone and warfarin, excretion of D-glucaric acid and 6β-hydroxycortisol in urine, and the activity of γ-glutamyltranspeptidase in serum. The present studies (Table 7) have revealed a significant correlation between glucaric acid and the elimination constant k_2 of aminopyrine before treatment. However, such a correlation in untreated volunteers can primarily not be expected, due to the various enzymes and other factors involved in drug metabolism. It would have been of interest to investigate how treatment with a known inducing agent, such as phenobarbital would have affected the correlation coefficients of the various parameters studied, but the limited number of subjects in each group did not allow such an evaluation.

The pharmacokinetics of aminopyrine have also been used to characterize drug metabolism (e.g. 25, 26, 27). This compound has found widespread use as an analgesic, antirheumatic and antipyretic agent, administered alone or in various combinations; although rare, its possible toxicological effects should not be neglected. Aminopyrine is rapidly absorbed after oral intake (25, 26, 28). As shown in Fig. 5, its half-life was very reproducible, and the rapidity of elimination permits a convenient sample collection period of 8 h. In addition, the decline in serum concentration primarily reflects its metabolism, as shown by the fast rate of excretion in urine of 4-amino-antipyrine and its acetylated derivative (26). Furthermore aminopyrine has been used for "*in vitro*" studies with microsomes from animals and from human liver biopsy samples to determine activities of drug metabolizing enzymes under various conditions. All these factors favour employment of amino-pyrine for studies of drug metabolism.

6β-Hydroxycortisol is a major unconjugated urinary metabolite of cortisol in man. Its excretion increases under various physiological and pathological circumstances (1, 4, 11, 12, 13, 14, 31), and further increases have been observed following administration of certain drugs, as described by Conney (15). As shown in Figs. 1 and 3, the rise in excretion of 6β-hydroxycortisol after treatment with phenobarbital confirms the

Table 7. Correlation coefficients (r) between ratio of 6β-OHF/17-OHCS, single-dose elimination constant (k_2) of aminopyrine, 24 hour excretion of D-glucaric acid, and serum γ-glutamyl-transpeptidase activity in 18 healthy subjects

		r-values			
		I	II	III	IV
I.	6-OHF/17-OHCS	-	0.151	-0.0619	0.158
II.	k_2 Aminopyrine	-	-	0.586[a]	0.179
III.	Glucaric acid	-	-	-	0.307
IV.	γ-GT	-	-	-	-

[a] $p<0.05$

previous reports. The failure of clemastine to produce a similar increase suggests that it had not produced significant induction.

The levels of 6β-hydroxycortisol were in the range for this age group reported by Yamaji (12), and others (4, 5, 14, 29), and so were the data on excretion of 17-hydroxycorticosteroids and the ratio 6β-OHF/17-OHCS.

The method for determination of urinary 6β-OH-cortisol was derived from reported techniques (4, 5, 13, 14, 31), which were modified to allow multiple analyses within a reasonable time. Due to the different TLC separation steps, it was possible to discriminate between 6β-OHF and 6α-OHF, although significant amounts of 6α-OHF were not detected, as has been reported previously (12). The excretion of 6β-OH-cortisol, as well as the ratio of 6β-OHF/17-OHCS, indicate changes in drug metabolizing enzyme activity, as established previously and as confirmed here, but its utility suffers from the occurrence of large intra- and interindividual differences, the lack of correlation with other parameters (24 and Table 7), the relatively complicated determination, the different levels of urinary 6β-OH-cortisol excretion reported from various laboratories (24, 29, 31), as well as variations due to sex, age and nutritional state of the subjects. The selection of male volunteers with small age differences, and their sojourn in a metabolic ward permitted partial control of these factors, as demonstrated by relatively small values of the S.E.M.

Marsh (17) and Aarts (30) found that treatment of people with phenobarbital stimulated excretion of D-glucaric acid in urine. They suggested that the output of D-glucaric acid might be a useful index of drug-induced alterations in drug metabolism. The present results, expressed in molar concentrations of D-glucaric acid excreted (not D-glucaro-1,4-lactone), agree with their findings in showing a highly significant increase in D-glucaric acid excretion in phenobarbital-treated volunteers. The lack of stimulation by clemastine was striking confirmation of the absence of induction after treatment with it.

The early rise in glucaric acid excretion, at a time when no significant increase in 6β-OHF excretion was observed, shows the sensitivity of the test.

Whether rises in γ-glutamyltranspeptidase activity represent a specific increase in synthesis of this enzyme, an effect of stimulated blood flow in liver, or enhanced cellular breakdown, is not known. The specificity of this enzyme as an indicator of alterations in drug metabolism has still to be proven and it requires comparison with other *in vivo* parameters of induction.

Interaction of drugs with drug metabolism in man is not limited to induction, since examples are known in which one drug can inhibit the metabolism of another. As pointed out in 'Results', the data obtained with clemastine do not necessarily reveal a trend towards inhibition. The partial prolongation of $t_{1/2}$ of aminopyrine, although not significant, was not correlated with a similar change in aminopyrine clearance, as observed after phenobarbital treatment. The remarkable increase in clearance values after the latter treatment might be explained by an increased V_d, resulting perhaps from enhanced hepatic storage.

With respect to the methodological approach employed here, experience was gained in the evaluation of new drugs in man and how to avoid severe interference by the inhomogeneity of subjects, as well as the environmental conditions. Continuous surveillance of volunteers was necessary since phenobarbital was given in high therapeutic amounts, as well as for proper collection of urine and blood. The sojourn in a hotel, which was less expensive than in a university hospital, offered healthy volunteers more comfortable surroundings than the isolated atmosphere of a hospital ward, with all the attendant problems that can arise during a test period of three weeks.

Acknowledgements. The authors thank Dr.W.H. Aellig, Sandoz Ltd., Basle, for his constant interest and advice. The excellent technical aid of Mrs. E. Berg, Mrs. B. Schwaneberg, and Mrs. I. Tinnes is acknowledged. The investigation was supported in part by Deutsche Forschungsgemeinschaft (Schwerpunktprogramm: Biologische Grundlagen der Arzneimittel- und Fremdstoffwirkungen (Hi 156/2)), and by Sandoz Pharmaceuticals, Hanover, N.J.

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