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PHENOBARBITONE INTERACTION WITH ORAL CONTRACEPTIVE STEROIDS IN THE RABBIT AND RAT

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1 The effect of phenobarbitone on the single dose pharmacokinetics of the synthetic steroids, ethinyloestradiol (EE_2) and norethisterone, has been studied in the rabbit and rat.

2 EE_2 is subject to an extensive first pass effect (96%). The plasma clearance of EE_2 approaches total hepatic blood flow. It is suggested that a secondary peak in EE_2 plasma concentration time curves at 5 h is due to enterohepatic recycling. Phenobarbitone had no effect on plasma EE_2 concentrations following intravenous administration and produced a variable decrease after oral administration.

3 In phenobarbitone-treated rabbits, following intravenous administration of norethisterone there was no significant change in the area under the curve (AUC) compared to controls. In contrast, following oral administration of norethisterone to treated rabbits, the AUC was 20% and the peak plasma concentration 17% of that in controls.

4 The data in rabbits are consistent with drugs which are highly extracted by the liver.

5 In rats, phenobarbitone had no effect on plasma norethisterone concentrations following intravenous or hepatic portal (bolus) administration, but caused a decrease in systemic availability after both infusion into the portal vein (over a period of 5 min) and oral administration.

6 It is concluded that the rate of delivery of norethisterone to the liver is important in determining whether or not enzyme induction will cause an increased first pass effect.

7 Phenobarbitone caused an increase in conjugation of norethisterone in the gastrointestinal tract of rats.

Introduction

Breakthrough bleeding and failure of contraceptive therapy have been noted in patients taking antiepileptiform drugs (including phenobarbitone) concurrently with oral contraceptive steroids (Kenyon, 1972: Hempel, Bohm, Carol & Klinger, 1973; Janz & Schmidt, 1974). Chronic treatment with phenobarbitone results in induction of hepatic microsomal enzymes with the consequent acceleration of breakdown of many drugs. In man, phenobarbitone has been reported to decrease steady state plasma warfarin concentrations (Breckenridge & Orme, 1971); increase the metabolism of digitoxin (Jelliffe & Blankenhorn, 1966); and increase the rate of elimination of desmethylimipramine (Hammer & Sjoqvist, 1967). In animals, phenobarbitone induces microsomal enzymes responsible for the oxidative metabolism of many drugs (Conney, 1967) including steroids. Welch, Levin & Conney (1968) showed that chronic pretreatment of immature female rats with phenobarbitone caused accelerated disappearance of tritiated oestradiol-17β and oestrone from the body and formation of polar metabolites was increased. Levin, Welch & Conney (1968) studied the effect of pretreatment with phenobarbitone on the action of synthetic steroids (diethylstilboestrol, ethinyloestradiol (EE_2), mestranol, norethynodrel and norethisterone) on the immature rat uterus. The uterotrophic response to a 0.1 to 3.0 μg dose of synthetic oestrogen was markedly inhibited, although much less inhibition occurred when the dose of oestrogen was increased to 12 μg. The uterotrophic response to norethynodrel and norethisterone was also inhibited. In an *in vitro* study Kappus, Bolt & Remmer (1972) observed a three fold increase in the rate of demethylation of mestranol to EE_2 in phenobarbitone pretreated rats.

The aim of the work described in this paper was to study the pharmacokinetics of the synthetic progestogen, norethisterone and the synthetic oestrogen, EE_2, in rabbits and rats pretreated with phenobarbitone and compare findings to those in animals not so treated. Preliminary accounts of some of these findings have been published (Back, Breckenridge, Gay,

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Orme, Rowe & Smith, 1977a; Back, Breckenridge, Gay, Orme, Park, Rowe & Smith, 1977b).

Methods

Experimental procedure in rabbits

Thirty-five adult does (New Zealand White strain) weighing between 3.0 and 4.0 kg were used in 2 studies. They were housed individually in a well ventilated room at 24°C and were allowed unrestricted access to food (SG1 pellets, Nutrients Ltd., Liverpool) and water. Rabbits were deprived of food overnight prior to study.

Effect of phenobarbitone on plasma ethinyloestradiol concentrations after intravenous and oral administration Animals were allocated to 4 groups (4 or 5 animals per group). EE_2 (100 μg/kg; 200 μg/ml in saline:ethanol, 6:1 v/v) was administered either intravenously by the marginal ear vein or orally to 2 groups (controls). The remaining 2 groups were pretreated with phenobarbitone (1 g/l; w/v) in their drinking water for 5 days. Fluid intake was monitored and the average daily amount of phenobarbitone ingested was found to be 250 mg/day. On the 7th day following the start of treatment, EE_2 was administered either intravenously or orally. Blood samples (5 ml) were obtained from the marginal ear vein by venesection at 0, 5, 10, 20, 30 and 45 min and 1, 1.5, 2, 3, 4, 5 and 6 h. Blood samples were centrifuged (2000 *g* for 10 min) and the plasma removed and stored deep frozen.

At the termination of the study 2 rabbits from each group were killed for determination of the indices of enzyme induction (see below).

Effect of phenobarbitone on plasma norethisterone concentrations after intravenous and oral administration Animals were allocated to 4 groups (4 or 5 rabbits per group). Norethisterone (85 μg/kg: 85 μg/ml in saline: ethanol, 6:1 v/v) was administered either intravenously by the marginal ear vein or orally to two groups (controls). The remaining two groups were pretreated with phenobarbitone (as above) and on the 7th day norethisterone was administered either intravenously or orally to the respective groups. Blood samples (3 ml) were obtained from the marginal ear vein by venesection, at 0.15, 30 and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21 and 24 h. Blood samples were centrifuged (2000 *g* for 10 min) and the plasma removed and stored deep frozen.

At the termination of the study 2 rabbits from each group were killed for determination of the indices of enzyme induction (see below).

Experimental procedure in rats

Mature female rats (200 to 300 g) of the Wistar strain were housed in groups in cages in well ventilated rooms at a temperature of 24°C. All animals were fasted overnight prior to study.

Effect of phenobarbitone on plasma norethisterone concentrations after intravenous, hepatic portal or oral administration Rats were allocated to 8 groups (4 to 6 rats per group). They were anaesthetized with urethane (14% w/v in saline; 10 ml/kg intraperitoneally) and the carotid artery and femoral vein or hepatic portal vein cannulated as appropriate with polypropylene tubing (PP50). For hepatic protal vein catheterization a broken 23 gauge needle was attached to the catheter and the needle inserted into the vein for the duration of the experiment. Heparin (100 units) was given intravenously to prevent coagulation of the blood samples taken from the carotid artery.

Norethisterone (in saline:ethanol, 6:1 v/v) was administered by three different routes to 4 groups as follows: intravenously (50 μg/kg; 62.5 μg/ml), via the hepatic portal vein (either as a bolus, 50 μg/kg; 62.5 μg/ml; or infused over a period of 5 min, 500 μg/kg; 500 μg/ml) or orally (500 μg/kg; 500 μg/ml). Prior to steroid administration, rats were given physiological saline (0.1 mg/100 g body wt.: twice daily for 5 days). The remaining 4 groups were dosed orally, by gastric intubation, with phenobarbitone twice daily for 5 days (total daily dose, 80 mg/kg; 40 mg/ml, w/v in distilled water). On the 7th day, norethisterone was administered either intravenously, via the hepatic portal vein (bolus or infusion) or orally to the respective groups. Blood samples (0.3 ml) were obtained at 0, 2, 5, 10, 20, 30, 45, 60, 90, 120 and 180 min and centrifuged (2 min at 12,000 *g*) to obtain plasma which was removed and stored deep frozen.

At the termination of the study, 2 rats from each group were killed for determination of the indices of enzyme induction (see below).

Effect of phenobarbitone on the metabolism of norethisterone by the gastro-intestinal tract Rats were allocated to 2 groups (3 rats per group). One group (control) received physiological saline for 5 days, while the other group was treated with phenobarbitone (40 mg/kg twice daily for 5 days, orally). Rats were anaesthetized and the carotid artery and femoral vein cannulated; the former for collection of arterial blood samples and the latter for infusion of saline to maintain the blood volume during the study. A cannula inserted into the hepatic portal vein allowed withdrawal of portal venous blood. [^{3}H]-norethisterone (50 μCi/kg; 125 μg/kg) was administered into the duodenum. Arterial blood samples (0.3 ml) were collected

at 0, 2, 4, 6, 8 and 10 min and portal venous blood samples obtained by constant withdrawal at 0–2, 2–4, 4–6, 6–8 and 8–10 min. The rate of withdrawal was 0.2 ml/min. Since total liver blood flow in the female rat is approximately 5.0 ml min^{-1} 100 g^{-1} body wt. (Yates, Hiley, Roberts, Back & Crawford, 1978), only a small fraction of the blood flowing through the portal vein is removed. After centrifugation of the blood samples, total radioactivity was determined in an aliquot (20 µl) of plasma; the remaining plasma was subjected to ether extraction for determination of free steroid and conjugates. Metabolites were determined by thin layer chromatography (t.l.c.) as previously described (Back, Breckenridge, Crawford, Orme, Rowe & Smith, 1978a). In a solvent system of methylene chloride:diethyl ether (80:20 v/v) three distinct areas of radioactivity were designated, I (polar metabolites; $R_F = 0.09$); II (ring-A reduced metabolites; $R_F = 0.37$ to 0.46) and III (norethisterone; $R_F = 0.53$).

Estimation of indices of enzyme induction

Animals were killed by a blow on the head and the liver rapidly excised and placed in ice cold KCl (1.15% w/v; pH 8.0). The liver was weighed, homogenized with three strokes of a teflon pestle and used to produce a 30% homogenate in KCl (w/v). The homogenate was centrifuged at 10,000 *g* for 20 min at 4°C to yield the post mitochondrial supernatant (PMS). This was decanted off without disturbing the pellet of cell debris and further centrifuged at 105,000 *g* for 60 min at 4°C. The resulting pellet was resuspended in 0.2 M phosphate buffer (10 ml; pH 7.4) using two strokes by hand of a teflon coated homogenizer.

Microsomal protein, cytochrome P450 and cytochrome c-reductase were determined respectively by the methods of Lowry, Rosebrough, Farr & Randall (1951); Omura & Sato, (1964) and Williams & Kaiman (1962).

Pentobarbitone sleeping times

Groups of 4 rabbits or rats, were either pretreated with saline or phenobarbitone as described above. On the 7th day after start of the treatment, the duration of pentobarbitone (40 mg/kg i.p.)-induced sleep was determined. Sleeping time was defined as the time between loss and return of the righting reflex.

Radioimmunoassay

Ethinyloestradiol concentrations were measured by the radioimmunoassay procedure described by Back, Breckenridge, Crawford, MacIver, Orme, Rowe, Smith & Watts (1979). In this assay both standards and tests are extracted from plasma. The known major metabolites of EE_2 do not cross react significantly. The intra assay precision (expressed as 1 s.d. from the mean) assessed by measuring the same sample ten times within a single assay (mean 103 pg/ml) was 9.6%. The interassay precision assessed by measuring the same sample ten times in different assays (mean 179 pg/ml) was 15%.

Norethisterone concentrations were measured by the radioimmunoassay procedure previously described (Back, Breckenridge, Crawford, MacIver, Orme, Park, Rowe & Smith, 1978b). The known major metabolites of norethisterone do not cross react significantly. The mean percentage recovery of [^{3}H]-norethisterone from rabbit plasma (200 µl) was 86.5 ± 1.5 (s.e. mean, $n = 8$) and from rat plasma (50 µl) was 93.0 ± 1.1 (s.e. mean, $n = 8$). Assay precision (inter and intra) has been described elsewhere (Back *et al.*, 1978a).

Calculation of pharmacokinetic indices

(a) The plasma concentration time curves were best resolved in all experiments into 2 exponential components. The area under the plasma concentration-time curve (AUC_{0-T}) was calculated by the trapezoidal rule with a programmable pocket calculator (Texas SR 52). The fast disposition half life ($T_{\frac{1}{2}\alpha}$) and the slow disposition half life ($T_{\frac{1}{2}\beta}$) were determined from the bi-exponential curve by least squares regression analysis. In the plasma concentration-time profiles for EE_2 which included a secondary peak, half lives were derived from data obtained from $T = 0$ to the commencement of the secondary peak, although the value AUC_{0-T} includes the secondary peak.

(b) *Apparent volume of distribution* (Vd) The apparent volume of distribution (Vd) was calculated as:—

$$Vd = \frac{\text{Dose}}{\text{AUC i.v.} \times \beta} \quad \text{(Dost, 1968)}$$

where β is the rate constant of the terminal part of the concentration versus time curve

(c) *Plasma clearance* (Cl) Plasma clearance was calculated from the equation:—

$$Cl = \frac{F \times \text{dose}}{\text{AUC i.v.}} \quad \text{(Wilkinson \& Shand, 1975)}$$

where F is the fraction of dose absorbed into the systemic circulation. For intravenous administration F is unity.

(d) *Apparent oral total drug clearance* (Cl_o) The apparent oral total drug clearance was calculated from the equation:—

$$Cl_0 = \frac{Dose_0}{AUC_0}$$ (Wilkinson & Shand, 1975)

(e) *Extraction ratio* (E) The extraction was calculated from the equation:—

$$E = 1 - \frac{AUC\ portal}{AUC\ i.v.}$$

(f) *Hepatic clearance* (Cl_H) The hepatic clearance was calculated from the equation:—

$$Cl_H = QE$$ (Wilkinson & Shand, 1975)

where Q is the total liver blood flow.

(g) *Intrinsic clearance* (Cl_{Int}) The intrinsic clearance of drug was calculated from the equation:

$$Cl_{Int} = \frac{QE}{1 - E}$$ (Wilkinson & Shand, 1975).

All the data were tested for statistical significance by Student's t test. Results are expressed as mean ± s.e. mean.

Results

Rabbits

The effect of phenobarbitone on liver weight and hepatic enzyme activity is shown in Table 1. In phenobarbitone-treated rabbits there was a significant increase in liver weight (41.3%; $P < 0.05$), cytochrome P-450 content (116%; $P < 0.05$), cytochrome c-reductase activity (76%; $P < 0.01$) and a significant decrease in pentobarbitone sleeping time (69%; $P < 0.001$) compared to control animals.

The effect of phenobarbitone on the plasma concentration time curves of EE_2 following intravenous and oral administration is shown in Figure 1. Pharmacokinetic parameters are summarised in Table 2. Phenobarbitone had no effect on plasma EE_2 concentrations following intravenous administration; plasma clearance (ml min^{-1} kg^{-1}) was identical in control (52.5 ± 11.4; mean ± s.e. mean) and treated (52.4 ± 3.2) rabbits. After oral administration there was a decrease in the mean values for AUC (by 43%) and peak concentration (by 40%) in phenobarbitone treated rabbits, but due to inter-animal variability this failed to reach significance. There was no change in the elimination (β) phase half life.

Figure 2 shows a comparison of the plasma concentration-time curves for norethisterone in control and phenobarbitone-treated rabbits following either intravenous or oral administration of the steroid. Pharmacokinetic parameters are summarized in Table 3. Following intravenous administration there was a significant reduction in peak concentration in treated animals. However, neither the AUC or plasma clearance or elimination phase half life was significantly changed. In contrast, following oral administration of norethisterone, phenobarbitone treatment caused a significant reduction in the peak plasma concentration and AUC and a significant increase in apparent oral clearance. In treated rabbits the AUC was only 20% and the peak plasma concentration 17% ($P < 0.01$) of that in control rabbits. There was no significant change in the elimination phase half-life.

Rats

In phenobarbitone-treated rats (Table 4) there was a significant increase in liver weight (25%; $P < 0.01$), cytochrome (P-450 content (144%; $P < 0.01$) and

Table 1. Effects of phenobarbitone (1 g/l in drinking water for 5 days) on liver weight and liver enzyme activity in female rabbits

	Control $n = 6$	*Phenobarbitone* $n = 6$	*% change*	*P*
Body wt. (kg)	4.27 ± 0.28	4.63 ± 0.31	+8	NS
Liver wt. (g)	96.5 ± 9.1	136.4 ± 16.8	+41	<0.05
Liver wt. (g/100 g body wt.)	2.30 ± 0.29	2.92 ± 0.16	+27	<0.05
Microsomal protein (mg/g liver)	14.4 ± 2.5	14.7 ± 0.7	+2	NS
Cytochrome P-450 (nmol/mg protein)	0.90 ± 0.19	2.12 ± 0.32	+116	<0.05
Cytochrome c reductase (nmol mg^{-1} protein min^{-1})	23.74 ± 0.62	41.74 ± 3.38	+76	<0.01
Pentobarbitone sleeping time (h)	3.60 ± 0.60	1.09 ± 0.17	−69	<0.001

Values are given as mean ± s.e. mean.
Student's t test was used to determine statistical significance.

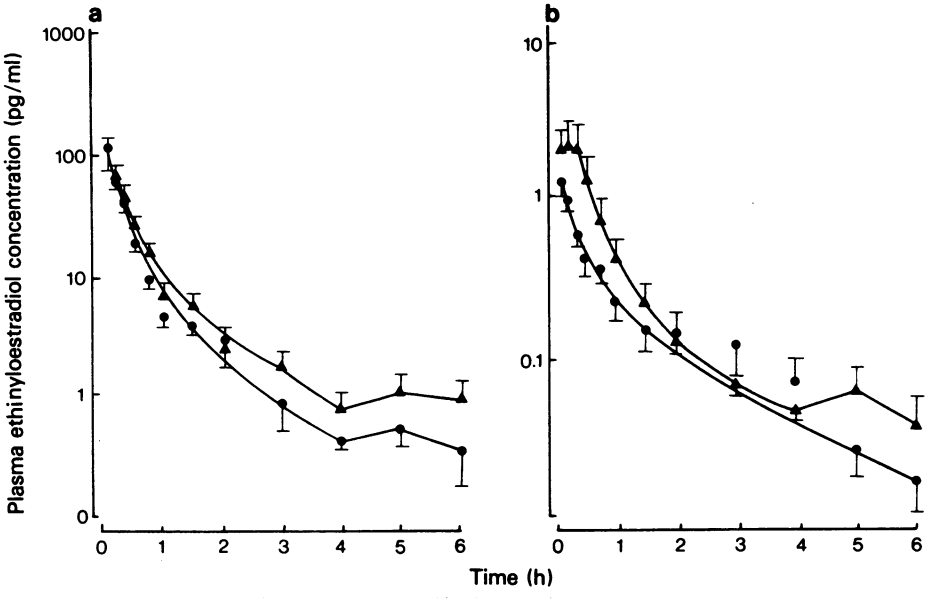

Figure 1 Plasma ethinyloestradiol concentrations (pg/ml) in control (▲) and phenobarbitone pretreated (●) rabbits after (a) intravenous and (b) oral administration of ethinyloestradiol (100 μg/kg). Each point is the mean of at least 4 experiments; vertical lines show s.e. mean.

cytochrome c-reductase activity (70%; $P < 0.01$). Pentobarbitone sleeping time was reduced to 23% ($P < 0.001$) of the control values.

Figure 3 and Table 5 show the results of phenobarbitone treatment on the kinetics of norethisterone given either intravenously or by the hepatic portal vein (bolus). In both cases, phenobarbitone pretreatment had little effect on the handling of norethisterone. There was no significant change in any of the pharmacokinetic indices (Table 5).

In contrast, following oral administration of norethisterone, phenobarbitone pretreatment caused a significant reduction in the AUC (43% of control; $P < 0.01$) and peak height (37% of control; $P < 0.05$) and a significant increase in oral clearance (149%; $P < 0.01$). These data are shown in Figure 4 and Table 6.

Constant infusion of norethisterone into the hepatic portal vein over 5 min reduces the rate of delivery of the drug to the liver in comparison to a bolus injection. Figure 4 and Table 6 show that in phenobarbitone-treated rats the AUC is reduced by 39% ($P < 0.05$) indicating a reduction in overall systemic availability.

Values for hepatic drug clearance (Cl_H) and intrinsic clearance (Cl_{Int}) in control and phenobarbitone-treated rats are given in Table 7. Following infusion of norethisterone into the hepatic portal vein of phenobarbitone-treated rats, there was an increase in Cl_{Int} of 92.5% compared to controls. In contrast, an increase in Cl_{Int} of only 10.2% was calculated in the comparable groups of rats in which norethisterone was given as a bolus injection into the portal vein. The values for liver blood flow in control and pheno-

Table 2 Pharmacokinetic parameters of ethinyloestradiol in control and phenobarbitone-treated rabbits

Intravenous	*Control* $n = 5$	*Phenobarbitone* $n = 4$
$T_{\frac{1}{2}\alpha}$ (h)	0.17 ± 0.03	0.15 ± 0.02
$T_{\frac{1}{2}\beta}$ (h)	1.41 ± 0.45	1.06 ± 0.14
AUC (ng ml^{-1} h)	41.4 ± 9.8	32.4 ± 2.2
Vd(1)	26.1 ± 10.4	20.2 ± 2.1
Peak height (ng/ml)	101 ± 24.4	112 ± 24.3
Plasma clearance (ml min^{-1} kg^{-1})	52.5 ± 11.4	52.4 ± 3.2
Oral	$n = 4$	$n = 4$
$T_{\frac{1}{2}\alpha}$ (h)	0.28 ± 0.01	0.17 ± 0.03
$T_{\frac{1}{2}\beta}$ (h)	1.05 ± 0.13	1.21 ± 0.18
AUC (ng ml^{-1} h)	1.71 ± 0.64	0.99 ± 0.29
Peak height (ng/ml)	2.12 ± 0.91	1.28 ± 0.29
Oral clearance (ml min^{-1} kg^{-1})	1307 ± 273	2310 ± 606

Values are given as mean ± s.e. mean.
Student's *t* test was used to determine statistical significance. There was no significant difference in any parameter, $P > 0.05$.

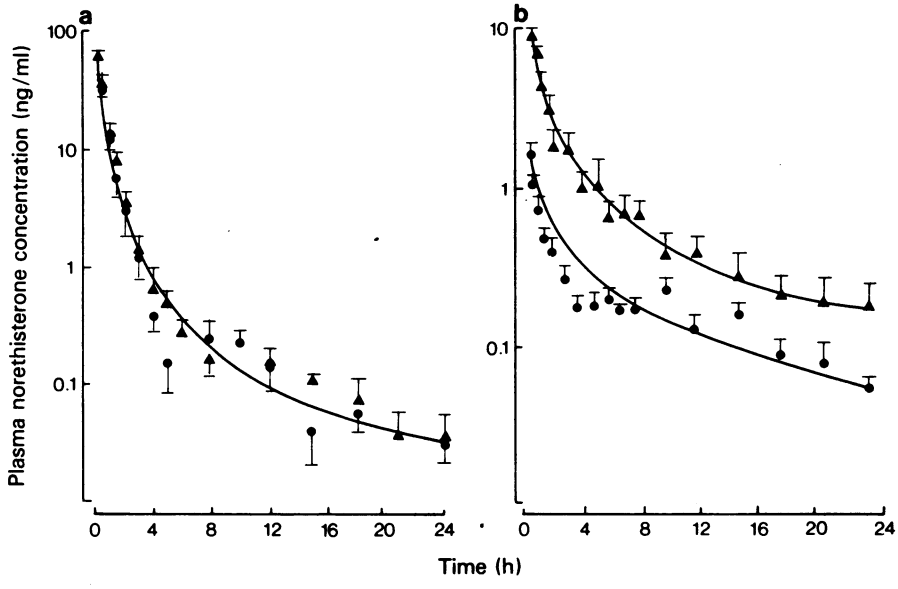

Figure 2 Plasma norethisterone concentrations (ng/ml) in control (▲) and phenobarbitone pretreated (●) rabbits after (a) intravenous and (b) oral administration of norethisterone (85 μg/kg). Each point is the mean of at least 4 experiments; vertical lines show s.e. mean.

barbitone treated female rats were previously derived (Yates, Hiley, Roberts, Back & Crawford, 1978).

Metabolism of norethisterone in the gastrointestinal tract

Analysis by t.l.c. of the ether extract from each portal sample showed that there was no increase in the ring A reduced or polar metabolites formed by the gastrointestinal tract in the first 10 min following administration of norethisterone into the duodenum of phenobarbitone pretreated rats (Figure 5). The non-ether extractable radioactivity (conjugates) in portal plasma at 2 min was considerably greater in phenobarbitone-treated rats (Figure 6). At 10 min, 25% of the steroid present in portal plasma of treated rats was conjugates compared to 5% in controls. In contrast, in carotid arterial plasma there was no significant difference in the appearance of conjugates in controls or treated rats (Figure 6).

Discussion

Comparison of $AUC_{i.v.}$ and AUC_{oral} for EE_2 in rabbits shows that only 4% of an oral dose reaches the systemic circulation. Therefore, assuming complete absorption, EE_2 has a first pass effect of 96%. The rapid β-phase half life of EE_2 in the rabbit (1.41 ± 0.45 h) is also indicative of rapid removal of the steroid from the circulation.

A consistent feature of the plasma concentration time curves for EE_2 was the occurrence of a secondary peak at approximately 5 h. We have also shown that this peak is present in women (Back *et al.*, 1979). These data are compatible with EE_2 undergoing enterohepatic circulation since it is known that EE_2 is

Table 3 Pharmacokinetic parameters of norethisterone in control and phenobarbitone-treated rabbits

	Control	*Phenobarbitone*
Intravenous	$n = 5$	$n = 4$
$T_{\frac{1}{2}\alpha}$ (h)	0.40 ± 0.10	0.46 ± 0.05
$T_{\frac{1}{2}\beta}$ (h)	7.4 ± 2.1	8.5 ± 0.7
AUC (ng ml^{-1} h)	40.6 ± 2.5	33.71 ± 4.84
Vd(1)	99.2 ± 27.2	126.5 ± 19.0
Peak height (ng/ml)	61.4 ± 4.3	41.6 ± 3.4**
Plasma clearance (ml min^{-1} kg^{-1})	35.5 ± 2.1	45.7 ± 6.8
Oral	$n = 4$	$n = 5$
$T_{\frac{1}{2}\alpha}$ (h)	0.71 ± 0.10	0.52 ± 0.10
$T_{\frac{1}{2}\beta}$ (h)	10.4 ± 1.2	10.69 ± 0.50
AUC (ng ml^{-1}h)	21.9 ± 4.86	4.58 ± 0.35**
Peak height (ng/ml)	9.7 ± 2.7	1.7 ± 0.2**
Oral clearance (ml min^{-1} kg^{-1})	72.6 ± 10.4	313 ± 22.1***

Values are given as mean ± s.e. mean.
Student's *t* test was used to determine statistical significance: ** $P < 0.01$; *** $P < 0.001$.

subject to extensive conjugation and biliary excretion in the rat, rabbit and man (Steinetz, Meli, Giannina & Beach, 1967; Beach, Steinetz, Giannina & Meli, 1967; Cargill, Steinetz, Gosnell, Beach, Meli, Fujimoto & Reynolds, 1969; Fotherby, 1974; Helton & Goldzieher, 1977).

The end result of hepatic enzyme induction is essentially to increase the intrinsic clearance of a drug, which if it has a low extraction ratio will produce a lowering in AUC mainly due to a shortening of plasma half life; this will happen after both intravenous and oral administration. On the other hand, if the extraction ratio is high, increases in intrinsic clearance will have a relatively small effect on hepatic extraction, causing virtually no change in the intravenous profile and a smaller AUC and peak height but identical half life after oral administration (Wilkinson & Shand, 1975).

The low systemic availability of EE_2 after oral administration in the rabbit is evidence that the steroid is highly extracted. Indeed, the plasma clearance of 52.5 ml min^{-1} kg^{-1} approaches total hepatic blood flow (Neutze, Wyler & Rudolph, 1968). Phenobarbitone pretreatment had no effect on EE_2 plasma concentrations after intravenous administration but produced a lowering of AUC (by 43%) and peak concentration (by 40%) and an increase in the apparent oral clearance (of 77%) after oral administration. These changes failed to reach an acceptable level of significance because of the great interanimal variation in plasma levels after oral administration. It is not entirely unexpected that with such a high initial extraction, and therefore relatively low plasma concentrations of EE_2, phenobarbitone does not give a further clear reduction in systemic availability. An alternative explanation for the lack of effect of phenobarbitone could be that some or all of the EE_2 which gets into the systemic circulation after oral administration has bypassed the portal circulation and liver by absorption into the lymphatics. Absorption into the lymph system has been proposed for several steroids e.g. testosterone undecanoate (Nieschlag, Mauss, Coert & Kicovic, 1975).

Norethisterone in the rabbit has a systemic availability of 54% which indicates that it has at best an intermediate extraction ratio (0.46). However, the data following phenobarbitone pretreatment best fit a highly extracted drug since there was no significant change in $AUC_{i.v.}$ after induction whereas after oral administration plasma concentrations of norethisterone were significantly reduced. In phenobarbitone-treated rabbits the AUC_{oral} was only 21% of control, the peak concentration 17% of control and the β-phase half life remained unchanged. Since it would have been expected that for a drug of intermediate extraction, enzyme induction would produce some change in half life it is probable that phenobarbitone is not acting solely or even primarily on the liver to give the observed changes but is acting also on the gastrointestinal tract.

In order to examine this further we initiated studies in the rat. Norethisterone was administered by three routes to control groups of rats and rats pretreated with phenobarbitone. The advantage of hepatic portal administration is that incomplete absorption and intestinal metabolism are eliminated from consideration. The systemic availability of norethisterone as determined by a comparison of $AUC_{i.v.}$ and AUC_{portal} was 23% which gives an extraction ratio of 0.77; hence in the rat norethisterone is highly extracted. Plasma clearance was not significantly different from the previously determined value for total hepatic blood flow in the female rat (Yates *et al.*, 1978). On theoretical grounds, phenobarbitone (as an hepatic enzyme inducer) should have no effect on plasma norethisterone concentrations following systemic intravenous administration, but should give a reduction in AUC (with no effect on half life) after bolus portal administration. However, with phenobarbitone pretreatment we were unable to show any significant

Table 4 Effects of phenobarbitone (40 mg/kg twice daily for 5 days) on liver weight and liver enzyme activity in female rats

	Control $n = 8$	*Phenobarbitone* $n = 8$	*% change*	*P*
Body wt. (g)	232 ± 4.9	224 ± 3	+8	NS
Liver wt. (g/100 g body wt.)	3.09 ± 0.12	3.86 ± 0.23	+25	<0.01
Microsomal protein (mg/g liver)	30.81 ± 3.08	27.46 ± 2.85	−10	NS
Cytochrome P-450 (nmol mg protein)	0.47 ± 0.07	1.15 ± 0.18	+144	<0.01
Cytochrome c reductase (nmol/mg^{-1} protein min^{-1})	50.39 ± 8.7	85.30 ± 8.84	+70	<0.01
Pentobarbitone sleeping time (h)	5.51 ± 0.18	1.29 ± 0.16	−77	<0.001

Values are given as mean ± s.e. mean.
Student's *t* test was used to determine statistical significance.

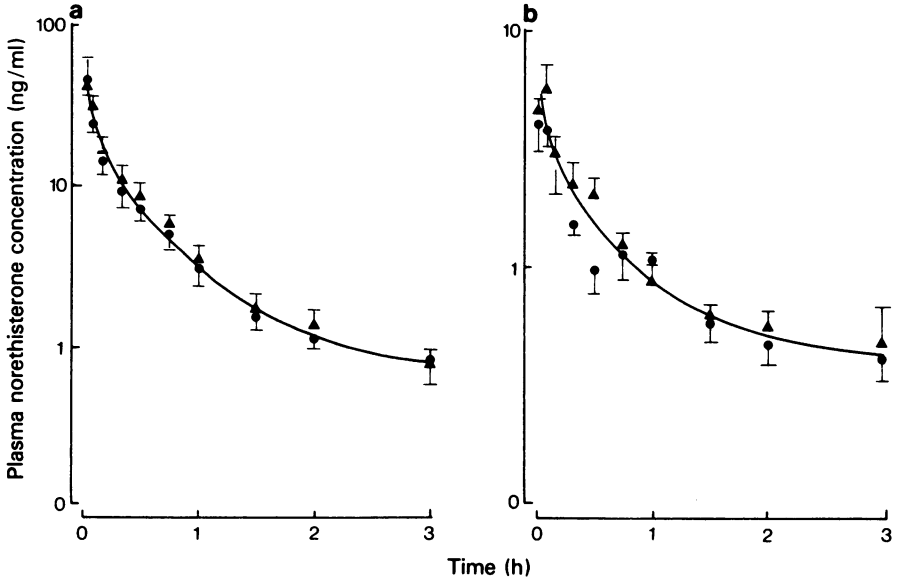

Figure 3 Plasma norethisterone concentrations (ng/ml) in control (▲) and phenobarbitone pretreated (●) rats after (a) intravenous administration and (b) a rapid bolus injection into the hepatic portal vein of norethisterone (50 μg/kg). Each point is the mean of at least 4 experiments; vertical lines show s.e. mean.

changes in plasma norethisterone concentrations after either route of dosing. The extraction ratio was only increased by 1.3% (from 0.77 to 0.78). It is important to note that in female rats, phenobarbitone did not significantly increase total liver blood flow (Yates *et al.*, 1978).

We have previously shown (Back *et al.*, 1978a) that the rate of delivery of norethisterone to the liver is an important determinant of systemic availability. Increasing the time of portal injection from 15 s to 2 min (thereby decreasing the rate of delivery) resulted in a 40% decrease in AUC. It was postulated that the initial disappearance of norethisterone from the portal vein may be due to a selective uptake process by the liver as has been suggested for propanolol (Shand & Rango, 1972). In the present study, con-

Table 5 Pharmacokinetic parameters of norethisterone in control and phenobarbitone-treated rats following intravenous or hepatic portal (bolus) administration

	Control	*Phenobarbitone*
Intravenous	$n = 6$	$n = 4$
$T_{\frac{1}{2}\alpha}$ (min)	5.10 ± 1.20	3.40 ± 1.50
$T_{\frac{1}{2}\beta}$ (h)	0.75 ± 0.11	0.71 ± 0.15
AUC (ng ml^{-1} min)	788 ± 120	746 ± 121
Vd (ml)	1292 ± 350	1517 ± 267
Peak height (ng/ml)	43.0 ± 3.7	47.1 ± 12.3
Plasma clearance (ml min^{-1} 100 g^{-1} body wt.)	7.08 ± 1.05	7.23 ± 0.95
Portal	$n = 5$	$n = 4$
$T_{\frac{1}{2}\alpha}$ (min)	5.60 ± 2.40	5.60 ± 1.10
$T_{\frac{1}{2}\beta}$ (h)	0.94 ± 0.22	1.12 ± 0.25
AUC (ng ml^{-1} min)	187 ± 37	171 ± 20
Peak height (ng/ml)	5.75 ± 1.24	4.30 ± 0.90

Values are given as mean ± s.e. mean.
Student's *t* test was used to determine statistical significance. There was no significant difference in any parameter: $P > 0.05$.

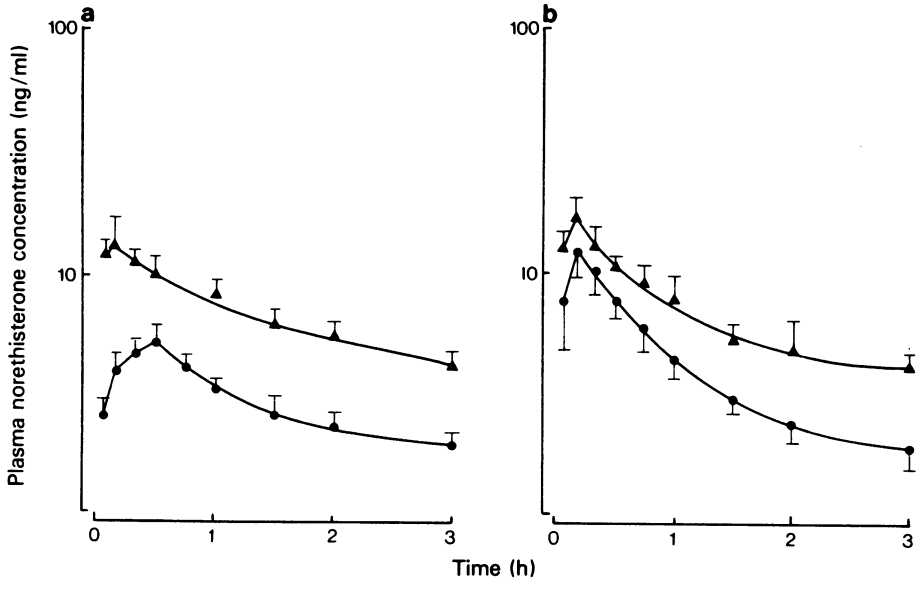

Figure 4 Plasma norethisterone concentrations (ng/ml) in control (▲) and phenobarbitone pretreated (●) rats after (a) oral administration and (b) infusion (over 5 min) into the hepatic portal vein of norethisterone (500 μg/kg). Each point is the mean of at least 4 experiments; vertical lines show s.e. mean.

Table 6. Pharmacokinetic parameters of norethisterone in control and phenobarbitone-treated rats following oral or portal (infusion for 5 min) administration

	Control	*Phenobarbitone*
Oral	$n = 5$	$n = 5$
$T_{\frac{1}{2}\beta}$ (h)	1.79 ± 0.04	1.49 ± 0.36
AUC (ng ml^{-1} min)	1256 ± 152	540 ± 94**
Peak height (ng ml)	15.1 ± 3.3	5.50 ± 0.91*
Oral clearance (ml min^{-1} 100 g^{-1} body wt.)	43.7 ± 6.2	109 ± 19.4**
Portal (infusion)	$n = 4$	$n = 4$
$T_{\frac{1}{2}\beta}$ (h)	1.71 ± 0.09	1.55 ± 0.08
AUC (ng ml^{-1} min)	1218 ± 166	743 ± 165*
Peak height (ng ml)	18.3 ± 3.1	12.8 ± 3.2

Values are given as mean ± s.e. mean.
Student's t test was used to determine statistical significance: * $P < 0.05$; ** $P < 0.01$.

Table 7. Estimation of hepatic total drug clearance (Cl_H) and intrinsic clearance (Cl_{Int}) in control and phenobarbitone-treated rats using previously determined values for liver blood flow

	Portal (bolus)			*Portal (infusion)*		
	Control	*Pheno*	*% change*	*Control*	*Pheno*	*% change*
Liver blood flow (ml min^{-1} 100 g^{-1} body wt.)	4.97	5.41	+8.9	4.97	5.41	+8.9
Extraction ratio	0.77	0.78	+1.3	0.845	0.906	+7.2
$Cl_{(H)}$ (ml min^{-1} 100 g^{-1} body wt.)	3.83	4.22	+15.3	4.20	4.90	+16.7
$Cl_{(Int)}$ (ml min^{-1} 100 g^{-1} body wt.)	16.64	19.8	+10.2	27.09	52.14	+92.5

Data derived following either bolus administration or infusion into the hepatic portal vein.

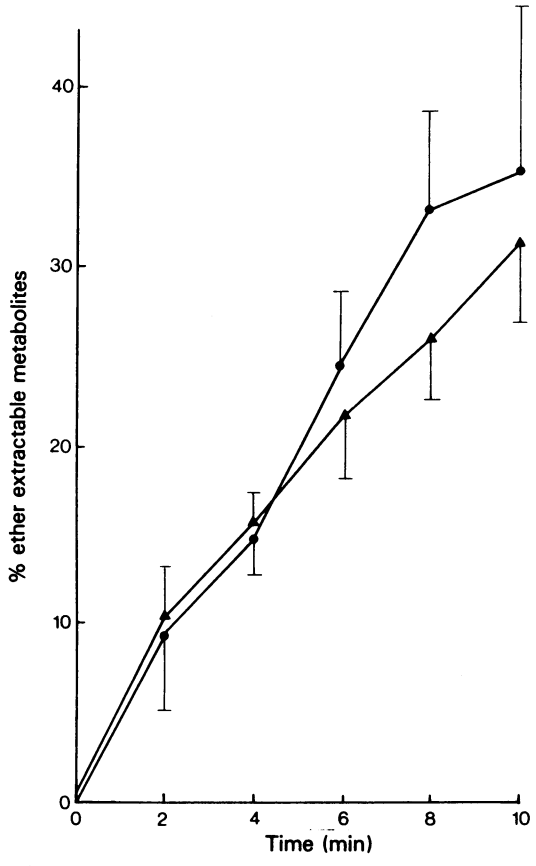

Figure 5 The effect of phenobarbitone pretreatment on the appearance of ether extractable metabolites of norethisterone in the hepatic portal vein of the rat over a period of 10 min following administration of norethisterone (50 μCi/kg; 125 μg/kg) into the duodenum: (▲) control; (●) phenobarbitone pretreated. Each point is the mean of 3 experiments; vertical lines show s.e. mean.

stant infusion of norethisterone over 5 min into the hepatic portal vein of phenobarbitone-treated rats gave plasma concentration time curves showing a lower peak concentration and AUC with unchanged β-phase half life, in comparison with controls. This finding is in agreement with the Wilkinson & Shand model (1975) for a highly extracted drug. It therefore appears that enzyme induction will only influence the plasma concentration after portal administration if the amount of drug initially arriving at the liver is below a threshold level. Intrinsic drug clearance increased by 92.5% (from 27.09 to 52.14 ml min^{-1} 100 g^{-1} body wt) following phenobarbitone treatment in rats in which norethisterone was infused into the portal vein compared to an increase of only 10.2% (from 16.64 to 19.18 ml min^{-1} 100 g^{-1} body wt) in rats in which the steroid was given as a bolus injection.

Following oral administration of norethisterone, rats which had been pretreated with phenobarbitone showed a significant reduction in peak concentration and AUC compared to controls. The question was whether or not the reduced plasma concentrations produced by phenobarbitone were the result of an effect on the liver or the gastrointestinal tract or both. Clearly, after oral administration the rate of drug delivery to the liver is such that phenobarbitone will increase intrinsic drug clearance. In addition, however, the result of the portal withdrawal studies indicate that phenobarbitone increases the metabolism of norethisterone in the gastrointestinal tract. Whilst little change in the 'ether extractable' metabolites was seen, the 'non-ether extractable' (conjugates) were significantly increased in the portal vein following phenobarbitone treatment. Since there was no difference in the percentage of conjugates in the carotid arterial plasma of phenobarbitone-treated and control rats the increase in conjugates in portal plasma of treated rats cannot be due to recirculation of conjugates formed by the liver. Hence phenobarbitone has increased conjugation of norethisterone in the gastrointestinal tract.

An interesting feature of the plasma concentration-time profiles of norethisterone in phenobarbitone-treated rats was the apparent delay in reaching peak concentration. Phenobarbitone-treated rats peaked at 30 min; control rats at 10 min. Further evidence of some delay in absorption was obtained in the portal withdrawal studies in which the radioactivity appearing in the portal vein of phenobarbitone rats in the first 2 min was only 28% of control. Riegelman, Rowland & Epstein (1970) studied the interaction between griseofulvin and phenobarbitone and were able to demonstrate that the concomitant administration of these drugs resulted in reduced absorption of griseofulvin.

Phenobarbitone is also known to increase bile flow in rats (Hart & Adamson, 1968; Klaassen, 1969) and this may result in changed conditions in the gastrointestinal tract due to an increase in bile salts.

It is clear that the interaction between phenobarbitone and the contraceptive steroid components is complex. The findings of this study do suggest, however, that in women where systemic availability of norethisterone is 64% (Back, Breckenridge, Crawford, MacIver, Orme, Rowe & Smith, 1978c) and EE_2 is 42% (Back *et al.*, 1979) after oral administration, that phenobarbitone will produce considerable changes in plasma concentrations of both steroids.

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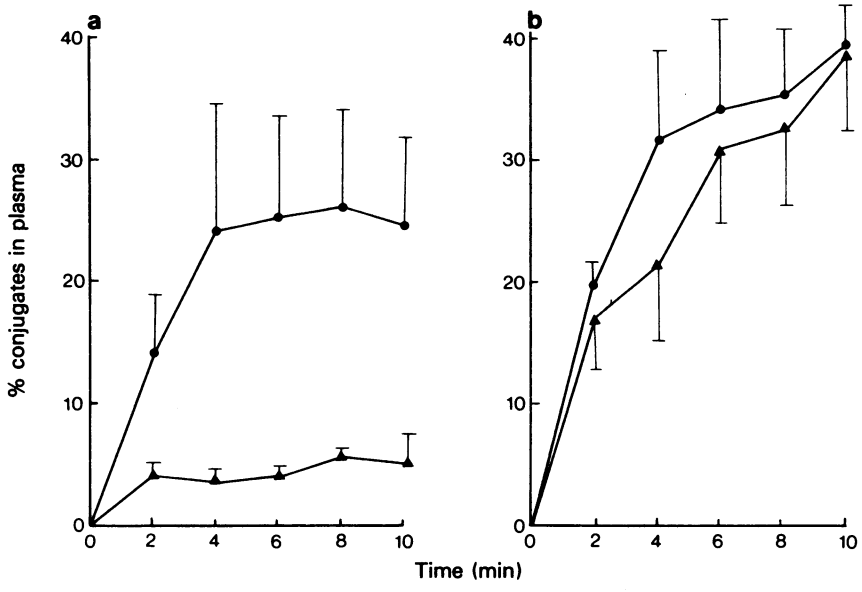

Figure 6 The effect of phenobarbitone pretreatment on the appearance of non-ether extractable metabolites (conjugates) of norethisterone in (a) the hepatic portal vein and (b) the carotid artery of the rat over a period of 10 min following administration of norethisterone (50 μCi/kg; 125 μg/kg) into the duodenum: (▲) control; (●) phenobarbitone pretreated. Each point is the mean of 3 experiments; vertical lines show s.e. mean.

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