Biochemical Pharmacology, Vol. 25, pp. 2623–2629. Pergamon Press, 1976. Printed in Great Britain.

PHENOBARBITAL-INDUCED PROLONGATION OF HALF-LIFE AND ALTERATION OF DISTRIBUTION OF A PHENOTHIAZINE DRUG METABOLITE IN THE RAT

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(*Received* 22 *September* 1975; *accepted* 25 *June* 1976)

Abstract—Male rats received a repeated oral treatment with perazine which led to an accumulation of the metabolite *N*-[γ-(phenothiazinyl-10)-propyl]ethylenediamine (PPED). When phenobarbital was administered concomitantly, the distribution of PPED and partly also of other perazine metabolites was altered, since higher concentrations were attained in liver and lower ones in kidney and brain. The PPED precursor desmethyl perazine was converted to PPED to a smaller extent when rats had been pretreated with phenobarbital; the same effect was exerted by SKF 525-A. Following a single oral dose of PPED, its elimination from liver and kidney was retarded in phenobarbital-treated rats, whereas *p,p'*-DDT slightly enhanced the decline of PPED tissue levels. PPED did not abolish phenobarbital- and DDT-induced increases of cytochrome P-450 and ethylmorphine demethylation in rat liver microsomes. It is concluded that phenobarbital treatment increases PPED binding to liver cell constituents and thus reduces its availability for distribution to extrahepatic organs and for metabolism.

Shortening of the *in vivo* half-life of drugs as a consequence of prolonged pretreatment with other foreign compounds, for instance barbiturates, is a well-known phenomenon. What shall be reported here, is an observation of the opposite effect: retarded elimination of a compound from tissues, especially from liver, in phenobarbital-treated rats. The compound which exhibited this unusual feature was *N*-[γ-phenothiazinyl-10)-propyl]-ethylenediamine (PPED), a metabolite of the neuroleptic drug perazine, from which it is formed by demethylation and partial degradation of the piperazine ring [1]. PPED is characterized by a slow distribution within the rat organism and a slow elimination from it leading to its accumulation during chronic treatment with perazine, also in the absence of perazine accumulation [2]. From the excretion of urinary metabolites by patients during and following perazine ingestion, it can be concluded that it also accumulates in the human organism [3].

Since the formation as well as the elimination of PPED were assumed to be dependent on cytochrome P-450, it seemed interesting to study the effect of known enzyme inducers on the kinetics of the drug metabolite. Phenobarbital (PB) and *p,p'*-DDT were found to influence the half-life of PPED in opposite directions; in addition, PB also affected the distribution within the organism of PPED and of other perazine metabolites present after repeated perazine administration. Some of the results have been the subject of a preliminary communication [4].

MATERIALS AND METHODS

Drugs and metabolites. Perazine dimalonate and γ-(phenothiazinyl-10)-propylamine (PPA) hydrochloride were gifts from Chemische Fabrik Promonta (Hamburg, W. Germany). Desmethyl perazine (DMP) dimalonate was synthesized as described by Breyer and Villumsen [5].

N-[γ-(Phenothiazinyl-10)-propyl]-ethylenediamine (PPED) was prepared from γ-(phenothiazinyl-10)-propylchloride [6] and ethylenediamine in analogy to the procedure used for the synthesis of the 2-chloro analogue [7]. The dimalonate was recrystallized from absolute ethanol, m.p. 151.5–152.5°. $C_{17}H_{21}N_3S \cdot C_6H_8O_8$ (507.5): calculated: C 54.43, H 5.75, N 8.28, S 6.32; found: C 53.95, H 5.72, N 8.27, S 6.11.

N-[γ-(Phenothiazinyl-10)-propyl]-*N'*-methyl-ethylenediamine (PPMED): γ-(Phenothiazinyl-10)-propylchloride (0.3 g) was dissolved in 2 ml of dry dimethylsulfoxide and added within 10 min to 3 ml of *N*-methylethylenediamine (EGA Chemie, Steinheim, W. Germany) kept at 95°. After further 20 min at 95–100° the mixture was distributed between 30 ml of 1 N NaOH and 15 ml of chloroform. The organic phase was washed with another portion of NaOH and extracted with 20 ml of 1 N HCl. After washing with 5 ml of chloroform, the aqueous phase was alkalinized with 4 ml of 25% ammonia and extracted with three 7-ml portions of benzene. The residue obtained upon evaporation of the combined organic phases was chromatographed on fourteen 20 × 20-cm plates coated manually with 0.4 mm of Silica gel GF_{254} (E. Merck, Darmstadt, W. Germany). The solvent was isopropanol–chloroform–25% ammonia–water (16:8:1:1, by vol.). Thus, separation of the desired compound from the non-desired isomer was achieved. The u.v. absorbing band at R_f 0.35 was removed, suspended in 1 ml of 2 N ammonia and extracted three times with 2 ml of diisopropyl ether. The combined extracts from all plates were reacted with 100 mg of malonic acid [7], and the precipitate was crystallized once from ethanol and twice from methanol. Pale yellow needles, m.p. 153.5–155°. $C_{18}H_{23}N_3S \cdot C_6H_8O_8$ (521.6): calculated; C 55.26, H

5.99, N 8.06, S 6.15: found; C 55.58, 55.12, H 6.08, 5.88, N 8.17, S 6.07.

Phenobarbital (PB)-Na was purchased from E. Merck (Darmstadt, W. Germany), chlorophenothane (*p,p'*-DDT) from EGA Chemie (Steinheim, W. Germany). Diethylaminoethyl diphenylvalerate hydrochloride (SKF 525-A) was donated by Smith, Kline & French Laboratories (Philadelphia, U.S.A.).

Organic solvents were of ordinary grade and were distilled before use.

Treatment of animals. Male Wistar rats (S. Ivanovas, Kisslegg, W. Germany) weighing 240–300 g were used. They had free access to tap water and a standard laboratory chow (Altromin R, Altromin GmbH, Lage, W. Germany) except 12 hr before and 2 hr after oral treatment with PPED, when they were fasted. Perazine and PPED were administered by gavage as aqueous solutions of their dimalonates (5 ml/kg body wt). For i.p. injection, DMP dimalonate was dissolved in water, neutralized with the 2-fold molar quantity of NaOH and made up to an isotonic solution (2 ml/kg) by addition of water and 0.9% NaCl. PB-Na was either administered by gavage of an aqueous solution (5 ml/kg) at a dose of 50 mg/kg or via the drinking water as a 0.1% solution. DDT (100 mg/kg) was injected i.p. in olive oil (2 ml/kg); the control group received olive oil. SKF 525-A (50 mg/kg) was injected i.p. as an isotonic solution (2 ml/kg); controls were given 0.9% NaCl i.p. All doses are specified in terms of free base or free acid. Details on the treatment schedules are given under Results.

At the times indicated, the rats were killed by decapitation, the organs removed and cooled to −20°. They were extracted within 2 weeks of sacrifice.

Measurement of drug metabolites in tissues. In most of the experiments, the extraction was carried out according to method B of Breyer [2] using dichloroethane. When studying the influence of DDT on the elimination of PPED, benzene was substituted for dichloroethane: 5 g of liver tissue was homogenized with 20 ml of 10% NaCl using an Ultra-Turrax blendor (Janke & Kunkel, Staufen, W. Germany), and after addition of 0.2 ml of 10% sodium deoxycholate solution and 3.5 ml of 25% ammonia, shaken with 25 ml of benzene. Following centrifugation for 12 min at 4000 rev/min, a measured aliquot of the benzene phase was transferred to a round-bottom flask and evaporated. The residue was transferred to a conical centrifuge tube and evaporated again. A pair of kidneys was processed analogously using 10 ml of 10% NaCl, 0.1 ml of 10% sodium deoxycholate, 1.2 ml of 25% ammonia and 15 ml of benzene.

Thin-layer chromatography was performed as described previously [2] except that in all cases chloroform–isopropanol (10 : 1) was used for washing the plates prior to development with isopropanol–chloroform–25% ammonia–water (16:8:0.8:0.8, by vol.). In this solvent, PPMED had the same R_f value (0.30) as PPED. Subsequent separation of these two metabolites by rechromatography in acetone–isopropanol–1 N ammonia (18:14:8, by vol.), was necessary when the animals had received perazine. R_f values of PPED and PPMED were 0.56 and 0.30, respectively. In the analysis of brain extracts, the rechromatography was omitted and the sum of PPED + PPMED was determined.

Recovery experiments were carried out with all compounds by adding adequate quantities to tissues from untreated rats. For DMP and PPED, recoveries were also determined using livers from PB-treated animals. The recovery of DMP from brain was taken from Breyer [2]. Data shown in Tables 1 and 2 and in Fig. 5 are corrected for recovery.

PB plasma levels were measured by spectrophotometry [8].

Experiments in vitro. Liver microsomes were prepared by pooling 3 g of liver each of two rats and homogenizing them in a Potter–Elvehjem homogenizer with 24 ml of 0.25 M saccharose containing 20 mM Tris–HCl pH 7.5 and 5.4 mM EDTA-Na_2 [9]. Following centrifugation at 1000 and 10,000 *g* for 10 min each, the microsomes were sedimented at 100,000 *g* within 60 min. They were washed with the above buffer and suspended in it to a concentration of about 10 mg protein/ml. Their protein content was determined according to Lowry *et al.* [10] using bovine serum albumin as standard, and cytochrome P-450 was measured spectrophotometrically [11].

Ethylmorphine demethylation was determined in an incubation system containing 2 mg/ml microsomal protein, 125 mM saccharose, 64 mM Tris–HCl pH 7.5, 36 mM KCl, 10 mM $MgCl_2$, 2.7 mM EDTA, 1 mM NADP, 8 mM trisodium isocitrate, 40 μg/ml isocitrate dehydrogenase and 16 mM ethylmorphine in a volume of 5 ml. After a 5-min pre-incubation period at 37°, the reaction was started by the addition of the substrate and was carried on with shaking under air. Samples of 1.5 ml were drawn 0, 5 and 10 min after the start for formaldehyde determination according to Nash [12].

Statistical calculations were performed using Student's *t*-test.

RESULTS

The structures of perazine and of its metabolites measured in rat organs are shown in Fig. 1.

Recoveries. The percentages of DMP, PPMED and PPED recovered from tissues increased steadily with the quantity added (Figs. 2–4). For PPED, no difference in recovery was found whether dichloroethane or benzene served as extractant (Fig. 4b). From livers of rats pretreated with phenobarbital (PB) the same percentages of DMP and PPED were recovered as from those of untreated animals (Figs. 2b and 4b). The recovery of 1–10 μg of PPA from kidney or from 2 g of liver was 90 ± 3% (mean ± S.E.M., n = 4).

Metabolite accumulation during repeated administration of perazine. When rats were killed 24 hr after the last of 14 daily oral perazine doses (see Table 1), the compound present in tissues in the highest concentration was PPED, while the other metabolites occurred in small quantities only. Perazine was not detected in measurable amounts. PPMED has previously [1] been described as an unidentified product. Its structure has now been elucidated by mass spectrometry.*

From Table 1 it appears that organ levels of perazine metabolites differed characteristically whether

* Krauss, Prox and Breyer, to be published.

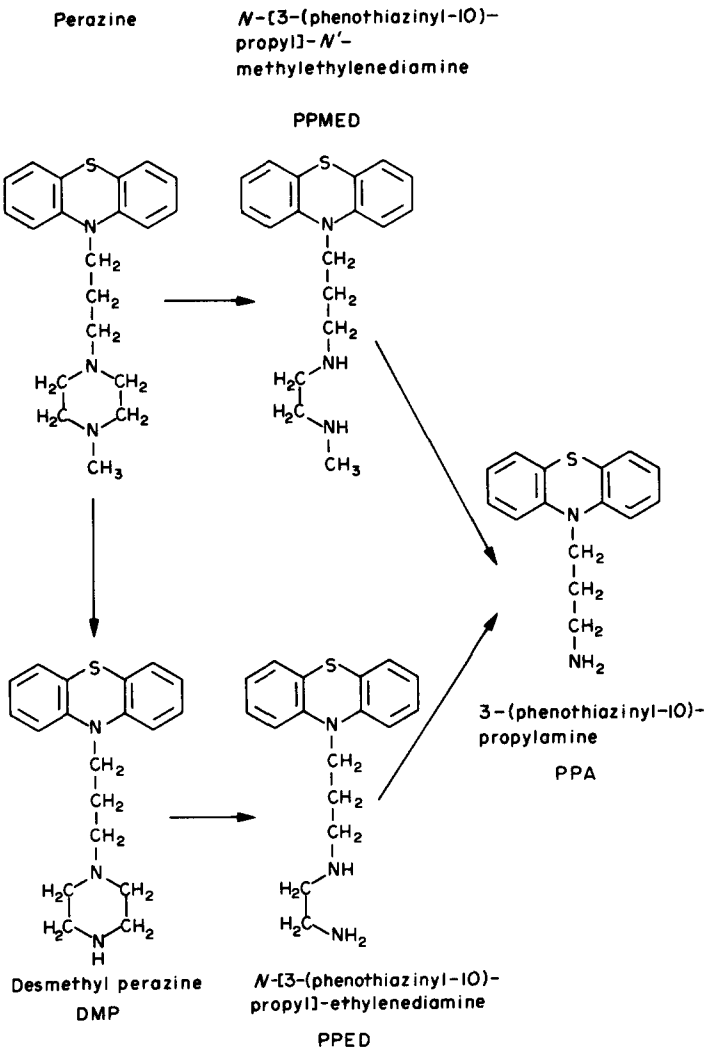

Fig. 1. Structural formulas of perazine and its metabolites occurring in rat tissues. The arrows indicate *in vivo* metabolic pathways.

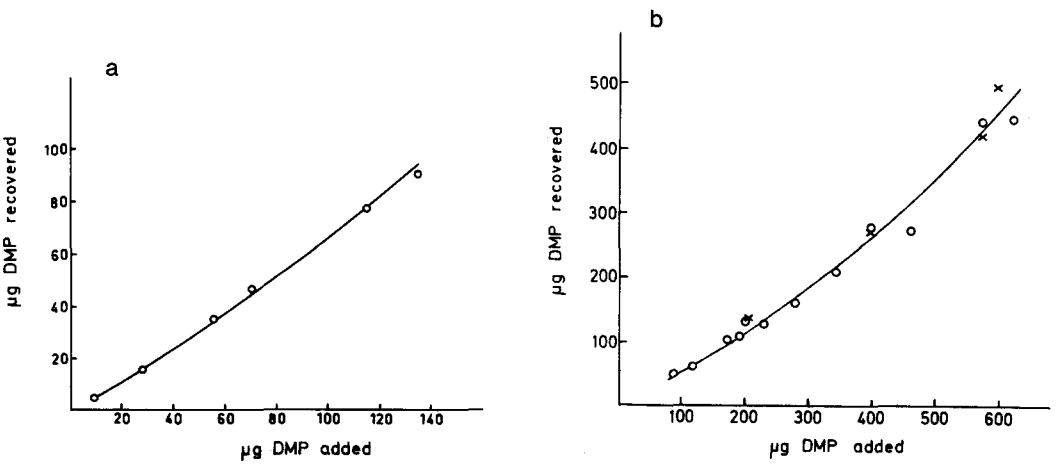

Fig. 2. Recovery of DMP from rat organs using dichloroethane as extractant. (a) Experiments on 2 g of liver or two kidneys; (b) experiments on 5 g of liver from untreated rats (open circles) and from rats that had received 5 daily oral doses of 50 mg/kg phenobarbital (crosses).

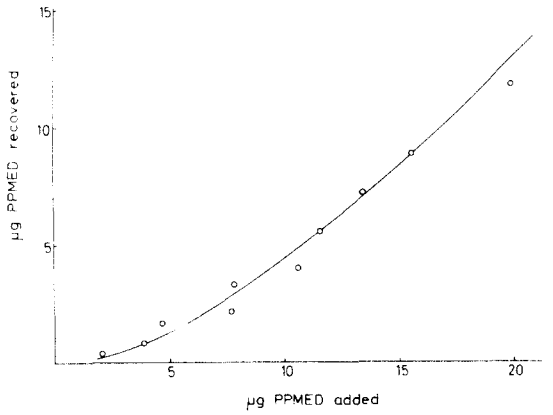

Fig. 3. Recovery of PPMED from 2 g of rat liver or two kidneys using dichloroethane as extractant. The isolated compound was rechromatographed as described for the separation of PPMED and PPED.

the animals had received PB in addition or not. The PPED concentration in liver was significantly increased by the barbiturate, while the levels in kidney and brain were lowered. As a consequence, a highly significant reduction of kidney/liver and brain/liver ratios could be stated. Similar changes were observed for the distribution of DMP, PPMED and PPA, though in view of the poor recoveries of PPMED and the low absolute concentrations of PPA the results with these two compounds have to be regarded with reservation.

Formation of PPED from a single dose of DMP. In order to study influences exerted on the metabolic production of PPED, rats were injected i.p. with DMP, an immediate precursor of PPED, and the concentrations of DMP and PPED in liver were determined after 2 hr (Table 2). Pretreatment with PB according to various schedules in no case led to an increase of the PPED concentration. Instead, DMP was considerably augmented in the livers of rats treated by gavage in spite of a 16–17 per cent increase in relative liver weight. Following PB application via the drinking water, DMP levels were not elevated, and PPED levels were depressed. These differential influences may be partly explained by a difference in the PB plasma concentrations.

The changes observed after injection of SKF 525-A were those to be expected and were equal to those brought about by PB, since PPED occurred in smaller quantities, while more DMP was still present. The difference in DMP levels between unpretreated rats and those injected with NaCl serving as controls for the SKF group was due to the fact that the exper-

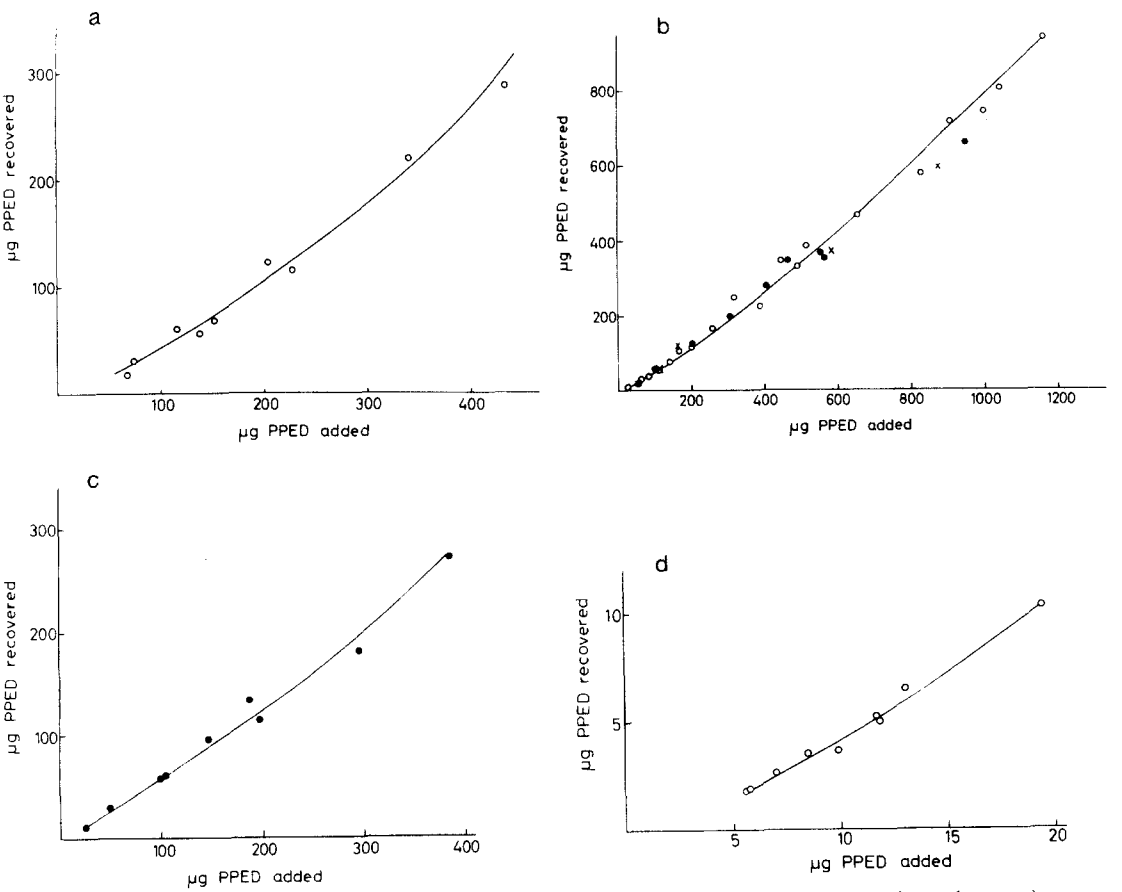

Fig. 4. Recovery of PPED from rat organs using as extractant dichloroethane (open circles and crosses) or benzene (filled circles). (a) Experiments on 2 g of liver or two kidneys. The isolated compound was rechromatographed as described for the separation of PPMED and PPED; (b) experiments on 5 g of liver from untreated rats (open and closed circles) and from phenobarbital-treated rats (crosses; see legend to Fig. 2b); (c) experiments on two kidneys; (d) experiments on two brains.

Table 1. Influence of PB on the concentrations of perazine metabolites in the organs of male rats given daily oral doses of 50 mg/kg perazine for 14 days*

Metabolite	Additional treatment	Concentration (μg/g) Liver	Kidney	Brain	Ratio Kidney/liver	Brain/liver
DMP	—	8.5 ± 0.7	7.7 ± 0.8	1.0 ± 0.06	0.95 ± 0.05	0.13 ± 0.02
	PB	9.0 ± 0.9	6.0 ± 0.8	0.7 ± 0.05‡	0.66 ± 0.05‡	0.08 ± 0.01†
PPED	—	89 ± 7	78 ± 5	3.7 ± 0.1§	0.88 ± 0.04	0.043 ± 0.003
	PB	130 ± 5‡	60 ± 4†	2.0 ± 0.2‡§	0.47 ± 0.04‡	0.015 ± 0.001‡
PPMED	—	6.0 ± 0.7	4.4 ± 0.4		0.75 ± 0.05	
	PB	6.0 ± 0.4	2.8 ± 0.2‡		0.48 ± 0.04‡	
PPA	—	2.6 ± 0.3	1.3 ± 0.1		0.53 ± 0.08	
	PB	3.8 ± 0.4†	0.8 ± 0.1‡		0.20 ± 0.02‡	

* Perazine was administered at 8 a.m. One group received in addition 50 mg/kg PB by gavage daily at 8 p.m. starting 2.5 days before the first perazine dose. Animals were sacrificed 24 hr after the last perazine dose, and 2 g of liver, two kidneys or two brains were analysed. Mean ± S.E.M., n = 8–10 for liver and kidney, n = 4–5 for brain.

† $P < 0.05$; ‡ $P < 0.005$ in comparison to the respective controls.

§ PPED + PPMED.

iments with SKF were carried out three years later than those with PB. Apparently the properties of the rats concerning foreign compound metabolism changed within this time (see below), but the direction of influences remained the same, as could be concluded from comparison with preliminary experiments carried out earlier.

Elimination of PPED following a single oral dose. Since the PB-induced augmentation of PPED in perazine-treated rats could not be explained by enhanced formation, its elimination was expected to be retarded. In fact, the decline of PPED in liver proceeded slower when the rats received a continuous PB treatment before and after the PPED dose (Fig. 5a). The half-life was prolonged from 1.3 to 3.7 days. The elimination from kidney, which in accordance with earlier observations [2] was slower than that from liver in control animals, was also impaired, the approximate half-life being increased from 2.4 to 3.6 days and thus resembling that in liver. An indication of the opposite effect was obtained when rats had been pretreated with DDT (Fig. 5b), since from 2 to 4 days after dosage PPED levels in liver and kidney declined faster than in oil-treated controls.

Extrapolation of the PPED concentrations in liver to zero time resulted in nearly identical values for

Table 2. Influence of PB and SKF 525-A on the concentrations of DMP and PPED in livers of male rats 2 hr after i.p. injection of 25 mg/kg DMP*

Additional treatment	Interval (hr)	Plasma PB (μg/ml)	Concentration in liver (μg/g) DMP	PPED	n
—	—	—	57 ± 4	28 ± 1	10
PB, gavage	12	33 ± 1	108 ± 6‡	27 ± 1	6
PB, gavage	36	5 ± 1	86 ± 8‡	25 ± 1	6
PB, drinking water	36	1 ± 1	61 ± 3	21 ± 1‡	5
NaCl	0.5	—	35 ± 2	24 ± 1	5
SKF 525-A	0.5	—	99 ± 6‡	19 ± 1†	5

* Two groups were pretreated with PB by orally administering 50 mg/kg at 8 p.m. for 3 days, the last dose being given either 12 or 36 hr before DMP. One group received 80 mg/kg PB *per os* and subsequently 0.1% PB in the drinking water for 6.5 days. Water was substituted for PB solution 36 hr before DMP injection. SKF 525-A (50 mg/kg) was injected i.p. 30 min prior to DMP. Five g of liver were used for analysis. Mean ± S.E.M.

† $P < 0.02$; ‡ $P < 0.005$ in comparison to the respective controls.

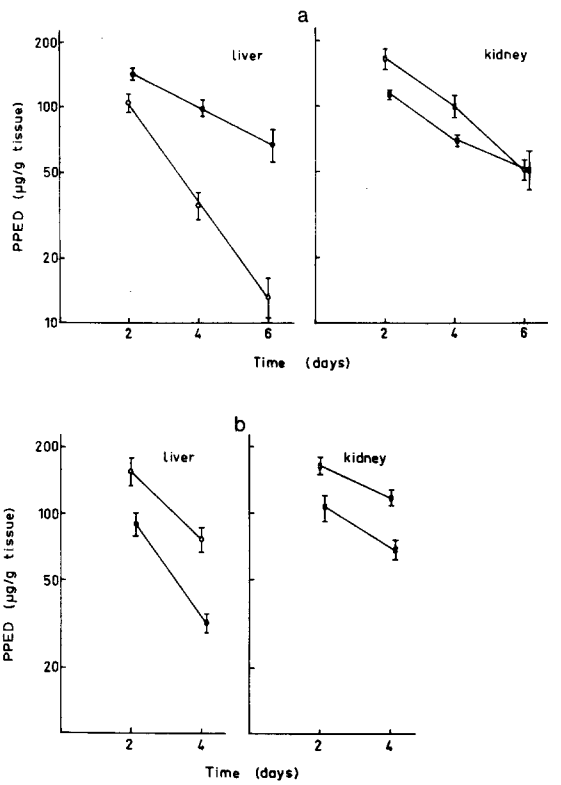

Fig. 5. Concentrations of PPED in liver and kidney following administration of 100 mg/kg by gavage to rats fasted for 12 hr. (a) Comparison of unpretreated animals (open symbols) to those receiving 50 mg/kg PB daily at 8 p.m. starting 2.5 days before PPED dosage (filled symbols); (b) comparison of rats injected with olive oil (open symbols) to those treated with 100 mg/kg *p,p'*-DDT in olive oil (filled symbols) 8 days before PPED dosage. Vertical bars represent standard errors, n = 4–7.

Table 3. Influence of PB and DDT on cytochrome P-450 concentration and ethylmorphine demethylation in liver microsomes from male rats treated with PPED*

Additional treatment	Time after PPED (days)	Cytochrome P-450 (nmoles·mg protein^{-1})	HCHO formation from ethylmorphine (nmoles·mg protein^{-1}·min^{-1})
—	2	0.77; 0.99	12.1; 15.7
PB	2	1.59; 2.05	20.7; 24.6
PB	4	2.05	25.2
oil	2	0.98; 1.11	14.2; 16.1
DDT	2	1.95; 1.64	22.3; 23.3

* Livers of two rats were pooled for the preparation of microsomes. For details on the treatment see legend to Fig. 5.

all four groups when allowance was made for the increase in relative liver weight (21 per cent with PB, 15 per cent with DDT).

The distribution of PPED between liver and kidney was changed by PB in the same way as following metabolite accumulation from perazine (Table 1); DDT, however, did not exert such an influence.

Besides PPED, liver and kidney tissue contained PPA. Its concentration was measurable 2 days after PPED dosage and amounted to about 1 per cent that of PPED.

In order to exclude the possibility that PPED led to a destruction of cytochrome P-450 or to an inhibition of its activity, measurements were carried out on liver microsomes prepared from the rats used for the kinetic study (Table 3). PB and DDT treatment augmented cytochrome P-450 as well as ethylmorphine demethylation above the values of the respective controls.

A comparison of Figs. 5a and b shows that PPED was eliminated more slowly from rats injected with olive oil than from untreated controls. This difference was most probably not due to the oil treatment, but to a change in the properties of the rats, since the experiments with oil and DDT were carried out 2 years later than all the others. Preliminary experiments with DDT performed at the same time as those with PB resulted in PPED levels in rat liver of 49 and 58 μg/g 2 days after PPED dosage and of 9.8 ± 1.1 μg/g (n = 4) after 4 days. These values are considerably lower than those measured later (Fig. 5b) of 90 ± 11 and 32 ± 3 μg/g after 2 and 4 days, respectively.

DISCUSSION

When under the influence of an inducer of microsomal drug metabolism the concentration of a drug metabolite in the organism is increased, the most probable explanation for this effect has to be sought in an enhanced formation of the compound. In the present investigation, where an increased PPED accumulation from perazine in the livers of rats was brought about by PB, this explanation did not hold.

The concentration of DMP, the immediate precursor of PPED, in the liver was not augmented. When this precursor was injected, PB administered according to various schedules proved unable to enhance PPED formation, even in the presence of elevated DMP levels. This was the more surprising since the inhibiting effect of SKF 525-A on the biotransformation of DMP to PPED pointed to a dependence of this reaction on cytochrome P-450.

The enhanced PPED accumulation in livers of rats receiving PB in addition to perazine was apparently due to two effects the mechanisms of which are not clear: to an altered distribution of PPED between the liver and extrahepatic tissues and to retardation of its elimination. The effect on distribution was not confined to PPED, since lower kidney/liver and partly also brain/liver ratios under the influence of PB were observed for the other perazine metabolites, too. The data for PPMED and PPA, however, were not as well validated as those for PPED and DMP.

Alterations of brain/plasma concentration ratios caused by PB have been described for phenazone [13] and phenytoin [14].

Since tricyclic psychoactive drugs are excessively bound to tissue constituents, PB must be assumed to exert an influence on binding processes. Apparently this is not directly related to its effect on the drug metabolizing enzyme system, since DDT, a similarly efficient inducer [15], did not affect the distribution of PPED between liver and kidney. Nor can the enhanced formation of smooth endoplasmic membranes due to PB explain the change in PPED localization, for DDT also leads to an augmentation of the endoplasmic reticulum [16, 17]. Moreover, fractionation studies on rat liver containing PPED showed a preferential localization of the substance in mitochondria, while a much smaller quantity was associated with the microsomal fraction [2].*

Similar considerations apply to the conspicuous retardation of PPED elimination caused by PB. Even when high concentrations of PPED were present in tissues, rats did not excrete measurable quantities of unchanged PPED into urine [2]; minute quantities only were removed in bile, as could be shown with a radiotracer method.† Hence the elimination must be dependent on metabolism; in fact, PPA was detected in organs of rats given PPED, and PPA sulfoxide was excreted in urine [18]. Therefore, an enhancing action as it was exerted by DDT on the decline of PPED tissue levels would have been expected with PB, particularly since PPED did not inhibit the increase in cytochrome P-450 and the activating influence on ethylmorphine demethylation of these two inducers. The continued presence of PB, which when given acutely may inhibit the metabolic oxidation of other foreign compounds [19], does not seem to explain satisfactorily the findings, since after administration of a barbiturate for several days, other substrates of the mixed function oxidase are in most cases eliminated more rapidly even in the presence of the inducer. This results from measurements in man [20, 21], in dogs [22, 23] and in rats [24, 25]. In a number of epileptic patients, phenytoin blood levels were lower during treatment with phenytoin alone than when the patients received the same phenytoin dose and PB in addition [26, 27]. In the majority of

* Breyer and Rassner, unpublished results.

† Breyer, Jahns *et al.*, manuscript in preparation.

cases, however, the opposite effect or no influence was observed [26]. In the present study, qualitatively equal changes were induced by PB on the PPED formation from DMP irrespective of whether moderately high PB levels still occurred in the organism or not.

Comprehensive studies by Kunz *et al.* [28] in mice revealed PB-induced changes of activity for a great number of hepatic enzymes, among these also mitochondrial enzymes. Electron microscopy did not disclose structural alterations in mitochondria [29]. Following DDT injection, a few biochemical parameters only were studied, but in these already differences were noted in comparison to the PB effects. It is thus conceivable that the differential effects of PB and DDT on the kinetics of PPED are due to differential influences on liver mitochondria. The action of PB may be explained on the basis of enhanced binding of PPED and other perazine metabolites to liver mitochondria. Consequently, they are less available for metabolism in the endoplasmic reticulum and for distribution to other organs.

Since PPED and its ring-substituted analogues originating from prochlorperazine, perphenazine, trifluoperazine and fluphenazine [7] exhibit unusual kinetic properties being eliminated slowly from liver, but still distinctly slower from extrahepatic tissues [2, 30], it is not too surprising that the influences of additional treatments not always follow the usual pattern. It seems to be the first time that PB given chronically could be shown to prolong the half-life of a chemically unrelated xenobiotic in the organism. In addition, the investigation gave an example of the potential of an "inducer" not only to influence the rate of elimination, but also the distribution among tissues of a foreign compound.

Acknowledgements—The authors wish to thank Miss I. Greiner for the phenobarbital measurements and Mr. G. Joachim for skilful technical assistance. They are grateful to the companies mentioned above for the gifts of the substances. The work was supported by the Deutsche Forschungsgemeinschaft.

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