Effect of Short-Term Exposure to Polychlorinated Biphenyls on First-Pass Metabolism of Pentobarbital in Rats

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Abstract □ The enhancement of the first-pass metabolism of orally administered pentobarbital in rats was examined after a 10-day exposure to food contaminated with polychlorinated biphenyls at 25 and 125 ppm. The degree of the first-pass effect and the influence of the polychlorinated biphenyl exposure were quantitated by comparing the areas under the plasma concentration–time curves after oral and intravenous dosing in control and treated animals. By using the clearance model and assuming that pentobarbital was eliminated totally by liver metabolism, the experimentally determined oral availability was predicted adequately from both the oral and intravenous data. The enhanced first-pass effect was principally in the intrinsic clearance term, although liver blood flow rates also appeared to be enhanced in animals treated with polychlorinated biphenyls at 125 ppm.

Keyphrases □ Polychlorinated biphenyls—effect of short-term exposure on first-pass metabolism of pentobarbital, rats □ Pentobarbital—effect of short-term exposure to polychlorinated biphenyls on first-pass metabolism, rats □ First-pass metabolism—pentobarbital, effect of short-term exposure to polychlorinated biphenyls, rats

Induction of liver microsomal enzymes by polychlorinated biphenyls, a class of environmental contaminants, has been reported in both humans and rats (1–11). Enhanced metabolism of drugs that are eliminated *via* these enzymes can have clinical implications because the hepatic clearance of drugs, such as pentobarbital (12–14), that are metabolized mainly by liver enzymes can be altered by exposure to these enzyme inducers. Such exposure may require therapeutic dosage adjustments of affected drugs to maintain comparable clinical effectiveness to the preexposure period.

In this study, the enhancement of the first-pass metabolism of pentobarbital in rats was examined after a 10-day exposure to a polychlorinated biphenyl-contaminated diet. It was determined previously (15) that the extent of enzyme induction due to polychlorinated biphenyl exposure is dose dependent and that a 10-day exposure results in an apparent steady-state level of enzyme induction. Therefore, the purpose of this study was to investigate the dose dependency and predictability of enhanced first-pass metabolism and bioavailability parameters for pentobarbital as a result of hepatic enzyme induction by polychlorinated biphenyls.

EXPERIMENTAL

Male Sprague–Dawley rats[1], 201–225 g, were housed in stainless steel cages in groups of four. All animals were fed standard laboratory rat food[2] for 1 week, and tap water was available *ad libitum*. After this preconditioning period, the rats were divided randomly into groups and fed powdered laboratory rat food containing 0, 25, or 125 ppm of polychlorinated biphenyls[3]. Food preparation was the same as described previously (16).

Four rats from each group were studied at the end of 10 days. The same dose of pentobarbital sodium[4], 30 mg/kg (equivalent to 27.7 mg of pentobarbital/kg), was administered orally to one group of rats and intravenously to another group of rats receiving the same polychlorinated biphenyl pretreatment. The oral dose was introduced *via* gastric intubation, and the intravenous dose was administered *via* the dorsal vein of the penis (17).

Blood samples were collected in microcapillary tubes pretreated with a 10% sodium ethylenediaminetetraacetate solution following the oral dose at 15, 30, 45, 60, 75, 90, 120, 150, and 180 min and similarly following the intravenous dose at time intervals as described previously (15). Because of anticipated faster pentobarbital elimination in the animals pretreated with 125 ppm of polychlorinated biphenyls, the last two time points were replaced for this group by additional samples at 5 and 10 min.

After centrifugation, the plasma samples were kept frozen until analysis. Plasma pentobarbital concentrations were assayed as described previously (15). All animals were sacrificed at the end of the blood sampling study. The liver was removed from each animal, patted dry, and weighed.

Experimental systemic availability of pentobarbital was evaluated in rats on the 10-day diet from the ratios of the area under the plasma level–time curves of the oral dosing compared to that from the intravenous dosing schedule:

$$\text{systemic availability} = (AUC_{\text{oral}})_0^\infty/(AUC_{\text{iv}})_0^\infty \qquad \text{(Eq. 1)}$$

where AUC represents the area under the plasma level–time curve. The AUC values were estimated by the trapezoidal method with extrapolation to infinite time from the last data point using standard procedures (18). The total body and intrinsic clearances (Cl_{TB} and Cl_{INT}, respectively) for these animals were evaluated using (19–24):

$$Cl_{TB} = \text{dose}/(AUC_{\text{iv}})_0^\infty \qquad \text{(Eq. 2)}$$

$$Cl_{\text{INT}} = \text{dose}/(AUC_{\text{oral}})_0^\infty \qquad \text{(Eq. 3)}$$

where Cl_{TB} and Cl_{INT} are related by:

$$Cl_{TB} = \frac{QCl_{\text{INT}}}{Q + Cl_{\text{INT}}} \qquad \text{(Eq. 4)}$$

where Q is the liver blood flow rate. The assumption of these equations is that extrahepatic elimination is negligible (19, 20, 23).

The mean values and standard deviations for all parameters for each treatment were calculated and compared statistically to the same parameter for the control. Multiple means were analyzed by a one-way randomized analysis of variance. When analysis indicated that a significant difference existed, the means of each group were compared by a two-tailed Dunnett multiple mean test (25). This test is for multiple mean comparisons to a control.

RESULTS AND DISCUSSION

The plasma level–time curves of pentobarbital sodium administered orally and intravenously at the same dosage (30 mg/kg) to animals on the polychlorinated biphenyl-free control diet are shown in Fig. 1. The bioavailability of pentobarbital in the orally dosed group of animals was

[1] ARS/Sprague–Dawley, Madison, Wis.
[2] Wayne Lab-Blox, Allied Mills, Chicago, Ill.
[3] Aroclor 1254, lot KA601, Monsanto Chemical Co., St. Louis, Mo.
[4] Nembutal Sodium, lot 53-967-AF, Abbott Laboratories, North Chicago, Ill.

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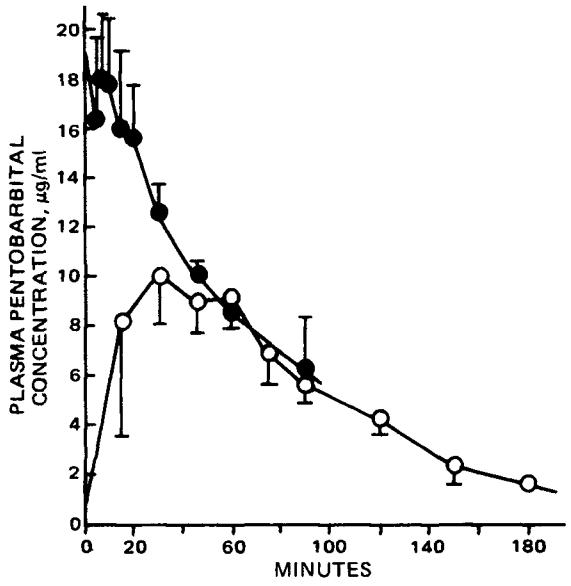

Figure 1—*Time course of plasma pentobarbital concentration* (±SD) *in rats pretreated for 10 days with polychlorinated biphenyl-free control diet. Key:* ●, *obtained after intravenous administration of 30 mg of pentobarbital sodium/kg; and* ○, *obtained after oral administration of 30 mg of pentobarbital sodium/kg.*

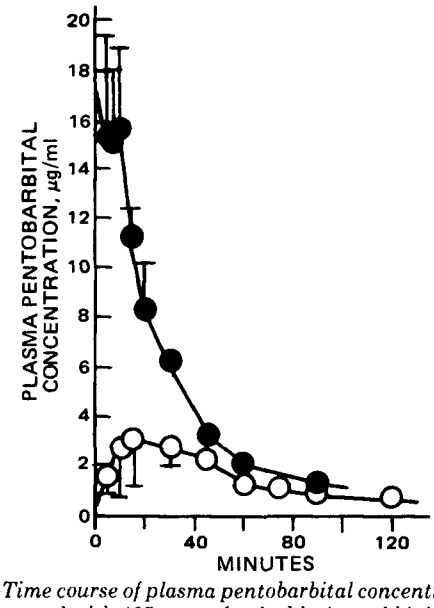

Figure 3—*Time course of plasma pentobarbital concentration* (±SD) *in rats pretreated with 125 ppm of polychlorinated biphenyls in food for 10 days. Key:* ●, *obtained after intravenous administration of 30 mg of pentobarbital sodium/kg; and* ○, *obtained after oral administration of 30 mg of pentobarbital sodium/kg.*

incomplete. Since pentobarbital is well absorbed from the GI tract (26–28) and eliminated almost exclusively (>95%) *via* liver metabolism in the rat (14), the observed reduction in oral bioavailability may be attributed to the first-pass metabolism by the liver, *i.e.*, the first-pass effect.

The disposition profiles of orally and intravenously administered pentobarbital in rats pretreated with 25 or 125 ppm of polychlorinated biphenyls in food for 10 days are given in Figs. 2 and 3. A more pronounced first-pass effect in rats exposed to 25 ppm of polychlorinated biphenyls in food can be discerned from the reduced ratio of the areas under the plasma level–time curves (AUC_{oral}/AUC_{iv}). Rats pretreated with 125 ppm of polychlorinated biphenyls showed an even greater reduction in the *AUC* ratio. This finding indicated an even lower apparent bioavailability of the intact pentobarbital. By comparing AUC_{oral} to AUC_{iv} within a pretreatment group, the effect of clearance in reducing AUC_{iv} when compared to control animals is taken into account.

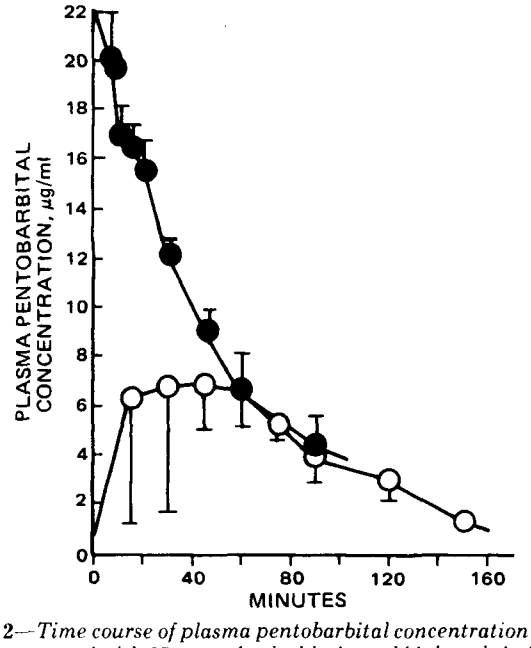

Figure 2—*Time course of plasma pentobarbital concentration* (±SD) *in rats pretreated with 25 ppm of polychlorinated biphenyls in food for 10 days. Key:* ●, *obtained after intravenous administration of 30 mg of pentobarbital sodium/kg; and* ○, *obtained after oral administration of 30 mg of pentobarbital sodium/kg.*

Table I summarizes the pharmacokinetic parameters for intravenously and orally administered pentobarbital in rats pretreated at two levels of polychlorinated biphenyls for 10 days compared to control rats. Pentobarbital was ~76% bioavailable in rats on the control diet. Upon exposure to polychlorinated biphenyls, increases in the degree of the first-pass effect were observed for both the 25- and 125-ppm pretreatments.

The relationship between the systemic availability after oral dosing and the total body clearance for exclusively liver-metabolized drugs was discussed by various investigators (18–24) and is defined by Eqs. 1–4. By rearrangement of Eqs. 1–4, an expression predicting the systemic availability can be obtained using Cl_{TB} values from the intravenous data:

$$\text{systemic availability} = 1 - Cl_{TB}/Q \qquad \text{(Eq. 5)}$$

Table I compares the predicted oral bioavailability for pentobarbital (calculated by Eq. 5 using the Cl_{TB} values from the intravenous data and the blood flow rates, which were calculated from the liver weights and the literature blood flow of 2 ml/min/g of liver) with the experimentally determined oral pentobarbital bioavailabilities for the variously treated animals. The predicted systemic bioavailability of pentobarbital was calculated from the individual Cl_{TB} and calculated Q values for individual animals and not from the mean Cl_{TB} and mean liver blood flow rates. Excellent agreement between the predicted and experimental bioavailability is seen. This result again illustrates the usefulness of intravenous data in assessing apparent systemic availabilities when the drug of interest is eliminated only by liver metabolism (18–24).

Rearrangement of Eqs. 1–4 yields a third expression for computing bioavailability from the liver blood flow rate and intrinsic clearance estimated from oral data:

$$\text{systemic availability} = Q/(Q + Cl_{INT}) \qquad \text{(Eq. 6)}$$

The percent systemic availability values calculated from Eq. 6 were 76.7 ± 2.1, 71.6 ± 3.1 (NS), and 47.8 ± 5.2 ($p < 0.01$) for the control and the 25- and 125-ppm polychlorinated biphenyl-pretreated rats, respectively. Again, these values were calculated from individual animal Q and Cl_{INT} values. These numbers showed excellent agreement with those estimated from the experimental *AUC* ratios.

The total body and intrinsic clearances in Table I were obtained from the dose and the areas under the intravenous and oral plasma level–time curves, respectively, using Eqs. 2 and 3. The induction effects due to polychlorinated biphenyl exposure at the 25- and 125-ppm levels as demonstrated by the Cl_{TB} values increased only by 31 and 193% relative to the controls, respectively. However, the Cl_{INT} values indicate ~47 and

Table I—Pharmacokinetic Parameters [a] for Intravenously and Orally Administered Pentobarbital in Rats Pretreated at Two Levels of Polychlorinated Biphenyls for 10 Days Compared to the Control Animals

Parameter	Control	25-ppm Pretreatment	125-ppm Pretreatment
Cl_{TB} [b], ml/min/kg of body weight	19.3 ± 3.8	25.3 ± 4.0 (NS)	56.5 ± 6.4 ($p < 0.01$) [c]
Cl_{INT} [d], ml/min/kg of body weight	24.4 ± 1.0	35.8 ± 6.7 (NS)	107.3 ± 20.8 ($p < 0.01$) [c]
Liver weight/body weight, %	4.2 ± 0.3	4.3 ± 0.3 (NS)	5.2 ± 0.6 ($p < 0.01$) [c]
Liver blood flow rate [e], ml/min/kg of body weight	82.7 ± 6.2	86.5 ± 6.0 (NS)	106.0 ± 12.2 ($p < 0.01$) [c]
Systemic availability calculated from AUC_{oral}/AUC_{iv} ratios, %	76.0 ± 8.7	71.2 ± 24.2 (NS)	53.9 ± 9.5 ($p < 0.05$) [c]
Systemic availability calculated from Eq. 5, %	77.6 ± 5.0	69.8 ± 2.9 (NS)	49.5 ± 8.3 ($p < 0.01$) [c]

[a] All values are means ± SD ($n = 4$). [b] Calculated from dose/AUC_{iv}. [c] Results are significantly different from the control group of animals at the level indicated. [d] Calculated from dose/AUC_{oral}. [e] Calculated from the literature value of 2.0 ml/min/g of liver.

340% increases over the control values at the 25- and 125-ppm pretreatment levels, respectively. Therefore, the degree of enzyme induction due to exposure to polychlorinated biphenyls is underestimated if only Cl_{TB} values are considered. This point was adequately discussed previously (15).

The relationship of liver blood flow to systemic availability and the clearance terms in Eqs. 5 and 6 provides a means of estimating liver blood flow rates from the experimentally determined bioavailability data. Rearrangement of Eqs. 5 and 6 and Eqs. 1–3 give:

$$Q = \frac{D}{(AUC_{iv})_0^\infty - (AUC_{oral})_0^\infty} \qquad \text{(Eq. 7)}$$

The blood flow rates calculated from Eq. 7 for the present data are given in Table II.

The estimated values compare favorably with the blood flow rates calculated using the literature value of 2 ml/min/g of liver (29). The agreement between the experimental and predicted liver blood flow for the control, 25-, and 125-ppm data supports the possibility of increased liver blood flow with enlarged livers without an increase in the flow per gram of liver.

The hepatic clearance of pentobarbital, the primary route of elimination in rats, can be approximated by the Cl_{TB} value (19, 20, 23). By converting clearances from milliliters per minute per kilogram of body weight to milliliters per minute per gram of liver in individual rats, the relationship between the hepatic clearance (equivalent to the total body clearance in this case) and the intrinsic clearance can be depicted as shown in Fig. 4. The lines represent the relationship between these parameters drawn by substituting a literature blood flow rate of 1.2 ml/min/g of liver (30) and 2 and 3 ml/min/g of liver (29) for Q in Eq. 4. Close agreement between the data points and the prediction using 2 ml/min/g of liver (29) for Q is readily seen and again supports the possibility of increased liver blood flow in polychlorinated biphenyl-treated rats with enlarged livers without an increase in the flow per gram of liver.

The clearance model suggests that substances that are highly extracted by the liver and, therefore, have a high intrinsic clearance relative to the blood flow display a higher sensitivity to blood flow changes. For instance, *d*-propranolol has a high intrinsic clearance (219 ml/min). The enhanced clearance subsequent to phenobarbital treatment is mainly (57%) due to the increase in the hepatic blood flow rate (31). On the other hand, antipyrine has a lower intrinsic clearance (54 ml/min); upon phenobarbital induction, only ~15% of the enhanced clearance results from increased blood flow. At the high level of polychlorinated biphenyl pretreatment, the intrinsic clearance of pentobarbital is increased due to the increased activity of the metabolic enzymes. A higher sensitivity to blood flow changes, reflected by the slightly higher blood flow, therefore is expected to contribute to the enhanced clearance with the 125-ppm pretreatment relative to the 25-ppm pretreatment.

In conclusion, this study showed that hepatic enzyme induction precipitated by environmental factors can significantly affect the bioavailability of drugs that are eliminated largely *via* facile hepatic metabolism. The reduction in the apparent oral bioavailability of pentobarbital in rats increased with the level of polychlorinated biphenyl exposure. As anticipated, the reduction in the apparent bioavailability or the increase in the first-pass effect was more pronounced at the 125-ppm pretreatment level than at the 25-ppm pretreatment level. Although inter- and intraspecies differences do prevail with such dose–effect relationships and extrapolation of the current findings to human subjects may not be valid, the potential clinical implications should not be overlooked. Accidental

Table II—Calculated Liver Blood Flow Rates in Rats Pretreated with Two Doses of Polychlorinated Biphenyls in Food for 10 Days Compared to Control Animals

Liver Blood Flow Rate	Control	25-ppm Pretreatment	125-ppm Pretreatment
Calculated from literature value of 2 ml/min/g of liver	82.7 ± 6.2	86.5 ± 6.0 (NS)	106.0 ± 12.2 ($p < 0.01$)
Calculated from Eq. 7	77.4 [a]	85.8 [a]	121.5 [a]
	1.84 [b]	1.99 [b]	2.33 [b]

[a] Milliliters per minute per kilogram of body weight. [b] Milliliters per minute per gram of liver.

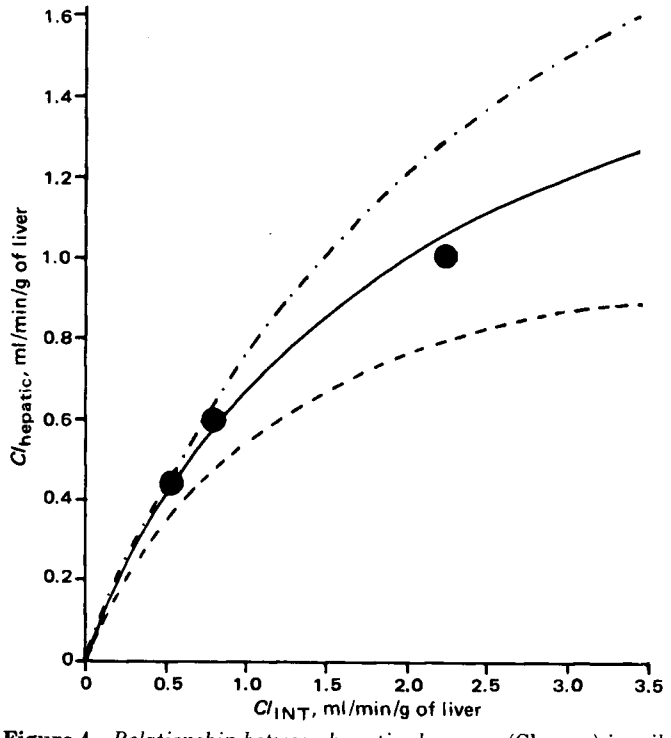

Figure 4—*Relationship between hepatic clearance* ($Cl_{hepatic}$) *in milliliters per minute per gram of liver and intrinsic liver clearance* (Cl_{INT}) *in milliliters per minute per gram of liver. The points are experimentally determined values from oral and intravenous studies, and the lines are for liver blood flow values of 1.2 (– –), 2 (——), and 3 (· – · – ·) ml/min/g of liver.*

exposure of humans to enzyme inducers such as polychlorinated biphenyls may lower the oral bioavailability of facile liver-metabolized drugs and require dosage adjustments.

The apparent agreement of the bioavailability estimation from intravenous or oral data with that determined experimentally from *AUC* values tends to support the validity of the clearance model in assessing systemic availability due to the first-pass effect for drugs exclusively metabolized by the liver. Estimation of the liver blood flow for the control and polychlorinated biphenyl-treated animals showed excellent agreement with literature values. This finding suggests that the blood flow rate on a liver weight basis does not change with induction but that the total hepatic blood flow may be affected by enzyme inducers.

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Effects of Antibiotics on Platelet Functions in Human Plasma *In Vitro* and Dog Plasma *In Vivo*

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Abstract □ The effects of 31 antibiotics on platelet aggregation in human plasma in the presence of adenosine 5′-diphosphate were studied. The marked activity of tetracycline hydrochloride led to a study of its effects on various platelet functions *in vivo* in dogs.

Keyphrases □ Antibiotics—effects on platelet functions □ Platelet aggregation—effect of antibiotics

Many compounds, including some antibiotics (1–6), interfere with platelet activity under certain conditions. The present investigation was concerned with whether antibiotics in current clinical use affect platelet aggregation, either *in vitro* in human plasma or *in vivo* in dog plasma. When such an effect was found, the mechanism was studied; the investigation was broadened to include other platelet functions such as platelet adhesiveness, platelet factor 3 liberation, platelet mobility in an electric field, and the aggregation of platelets filtered in Sepharose gel.

EXPERIMENTAL

The *in vitro* experiments were carried out with plasma from apparently healthy subjects. The aggregation results correspond to the mean values of at least three trials.

The *in vivo* experiments were carried out on five dogs with similar physical characteristics. The results correspond to the mean values of three trials.

Plasma Samples—Human plasma samples were obtained by forearm venipuncture. The blood was collected in silanized tubes containing 3.8% sodium citrate (1:9) to obtain both platelet-rich plasma and platelet-poor plasma for the aggregation studies.

Dog plasma samples were obtained in a similar manner from mongrel dogs weighing 16–20 kg.

Platelet-rich plasma was obtained by sedimentation or centrifugation of samples at 800 rpm for 10 min for human plasma and for 8 min for dog

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