# Embryotoxicity of Phenytoin in Adrenalectomized CD-1 Mice (43386)

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*Abstract.* **It has been proposed that the anticonvulsant drug phenytoin (PHT) and glucocorticoids induce orofacial clefting by the same mechanism. Previous work had demonstrated that PHT treatment significantly increased endogenous maternal corticosterone concentrations for approximately 48 hr after dosing in A/J mice. The purpose of the present investigation was to determine whether PHT is embryotoxic in the absence of endogenous maternal glucocorticoids. Maternal adrenal glands were removed on Day 7 of gestation, and the incidence of clefting after PHT treatment was determined. There was a high level of maternal toxicity following adrenalectomy (ADX) and PHT treatment at either 60 or 75 mg/kg. This increased toxicity did not appear to be due to altered maternal drug levels in ADX mice. There was a significant increase in the clefting incidence among offspring of ADX dams treated with PHT at 60 mg/kg. This dose of PHT did not elevate maternal corticosterone levels in ADX dams. These data suggest that PHT is capable of producing clefts in the absence of endogenous maternal corticosterone.** [P.S.E.B.M. 1992, Vol 199]

Phenytoin (PHT) is an anticonvulsant that is a developmental toxicant in humans (1, 2) and mice (3, 4). In mice, it increases the frequencies of resorptions and malformations, particularly orofacial malformations. The mechanism by which PHT causes these effects is unknown. It was recently demonstrated that treatment of A/J mice with a teratogenic dose of PHT produced a sustained increase in maternal plasma corticosterone (5). Since treatment with corticosterone has been shown previously to produce orofacial clefting in A/J (6, 7) and CD-1 mice (8), we hypothesized that PHT might produce clefting, not by a direct effect on the embryos, but rather by an indirect glucocorticoid-mediated effect.

Alterations in glucocorticoid levels have been suggested to play a role in the orofacial clefting produced by other compounds. For example, serum cortisol concentrations were increased following treatment with a teratogenic dose of titanocene dichloride, but not the nonteratogen cisplatinum (9). An increase in maternal plasma corticosterone was also observed following a teratogenic dose of the mycotoxin secalonic acid D (10). Treatment with metyrapone, an inhibitor of corticosterone synthesis, produced a decrease in the incidence of cleft palate following treatment of pregnant mice with polychlorinated terphenyls (11). An increase in maternal corticosterone levels following treatment with haloperidol, 2,4,5-trichlorophenoxyacetic acid, PHT, or vehicle was linearly and highly correlated with an increase in cleft palate incidence among offspring (12).

To determine whether endogenous glucocorticoids play a role in PHT-induced cleft palate, adrenal glands were removed from pregnant CD-1 mice prior to treatment with PHT, and the incidence of orofacial clefting in the offspring was determined.

## Materials and Methods

Mice of the CD-1 strain were originally purchased from Charles River (Wilmington, MA), and a breeding colony was maintained at the National Center for Toxicological Research (Jefferson, AR). Animal care and procedures followed the U.S. Department of Health and Human Services *Guide for the Care and Use of Laboratory Animals* guidelines. All animals were housed in polycarbonate cages with hardwood chips as bedding in rooms in which the temperature (72 ± 3°F), humidity (50 ± 10%), and light:dark cycle (12:12 hr,

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lights on at 7 AM) were held constant throughout the experiments. Nulliparous females were housed overnight with a male; the presence of a vaginal plug the following morning was designated as Day 0 of gestation (GD0).

On GD7, pregnant mice were anesthetized by an intraperitoneal injection of Avertin at 40 mg/kg. Adrenal glands were removed through dorsal incisions. Prior to closure of the incisions, the uterus was examined for the presence of implantation sites. Musculature was then sutured using 6-0 surgical silk, and the incisions were closed with wound clips. Animals were placed in a cage on a warming tray heated to 38°C and allowed to recover from the anesthetic. Cages remained on the warming tray until the morning of GD10, at which time they were returned to the regular animal racks. Since the adrenal gland secretes aldosterone in addition to corticosterone and aldosterone functions in sodium retention in the kidney, normal drinking water was replaced with 0.9% sodium chloride from GD7 to GD18 in all adrenalectomized (ADX) mice.

Groups of four to eight pregnant mice were sacrificed at 24-hr intervals following adrenalectomy such that maternal blood was collected daily from GD8 to GD16. Other groups of four to eight intact (non-ADX), untreated pregnant mice were also sacrificed at 24-hr intervals from GD10 through GD15. All animals were sacrificed between 8:00 and 9:00 AM by cervical dislocation followed immediately by decapitation. Animals were sacrificed in a separate room using procedures shown to have no significant stress effect on maternal plasma corticosterone levels (5). Trunk blood was collected in heparinized tubes and centrifuged to obtain plasma, which was frozen at −20°C until analyzed for corticosterone using a commercially available radioimmunoassay (RIA) kit (ICN Biomedicals, Inc., Costa Mesa, CA). The antibody in this kit was specifically raised against rodent corticosterone and has been shown previously not to cross-react with PHT or its major metabolite, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (5). The intra-assay variation in our laboratory is approximately 5%, and the interassay variability is approximately 20%.

In other groups of ADX or intact mice, PHT (Aldrich Chemical Co., Milwaukee, WI) in distilled water adjusted to approximately pH 11 with NaOH was administered by intraperitoneal injection on the morning of GD10 at 0 (vehicle, pH 11 distilled water), 25, 60, or 75 mg/kg. Other groups of intact or ADX mice were left untreated. Animals were sacrificed by cervical dislocation on GD18. Gravid uteri were removed, and the positions of live or dead fetuses and resorbed implants were noted. All live fetuses were weighed, anesthesized by cooling on ice, and decapitated; fetal sex was determined by internal examination of gonads. Fetal heads were fixed in Bouin's fluid, and the integrity of the palate was determined.

To determine whether maternal corticosterone levels were increased in ADX mice treated with PHT, groups of six to 13 mice were ADX on GD7 and treated with 60 or 75 mg/kg of PHT on the morning of GD10. Mice were sacrificed by cervical dislocation followed by decapitation 4, 24, or 48 hr after PHT dosing. Blood was collected, and plasma was prepared by centrifugation. Corticosterone concentrations were determined by RIA as described.

Plasma PHT levels were determined in these samples as described previously (13). Briefly, an aliquot of plasma (50–200 μl) was mixed with an aliquot of 5-(*p*-methylphenyl)-5-phenylhydantoin, which was used as an internal standard. PHT was extracted from plasma using ethyl acetate and dried under a stream of nitrogen. The residue was reconstituted in 100 μl of methanol, and aliquots were injected onto a μBondapak $C_{18}$ reverse-phase high-performance liquid chromatography column (3.9 mm × 30 cm; Waters Associates, Milford, MA) with a guard column filled with $C_{18}$ reverse-phase packing material (Rainin Instrument Co., Woburn, MA). The high-performance liquid chromatography system consisted of a model 510 pump (Waters Associates) equipped with a Rheodyne injector (model 7125). Absorbance at 217 nm was monitored using a Tracor 970A variable wavelength detector. Quantitation of PHT was done using a standard curve made up by adding known amounts of PHT and internal standard to blank plasma samples and carrying these samples through the entire procedure (14); a Hewlett Packard 3380A integrator was utilized.

To determine when palatal closure occurs in this strain of mice, groups of four to eight untreated pregnant mice were sacrificed by cervical dislocation on the morning (8:00–9:00 AM) of GD14 or GD15 or on the afternoon (3:00–4:00 PM) of GD14. All live fetuses were anesthetized by cooling on ice prior to decapitation. Fetal heads were fixed in Bouin's fluid, and the integrity of the palate was determined.

### Statistics

The proportions of resorbed implants within litters, as well as the proportions of live fetuses with orofacial clefting, were transformed using the arcsin transformation. The data were analyzed using a two-way analysis of variance followed by Student's *t* test on the effect of adrenalectomy or a one-way analysis of variance on the effect of dose, using Bonferroni's correction for multiple comparisons. Fetal weight data were also analyzed in this manner. Data are expressed as means ± SE. Since multiple comparisons were being made, the $P < 0.02$ level of significance was used unless noted.

Differences in corticosterone levels between ADX and intact pregnant mice were tested for each day of gestation by Student's *t* test. Differences in PHT concentrations between control and treated intact or ADX dams at 4, 24, or 48 hr after dosing were tested by

Student's $t$ test. The $P < 0.05$ level of significance was used for these experiments.

## Results

The results in Figure 1 demonstrate that following adrenalectomy on GD7, maternal plasma corticosterone levels were very low until GD15, at which time they began to increase. The levels continued to increase to at least GD16. On GD10, there was no difference in the corticosterone levels between intact and ADX pregnant mice. However, in intact mice, the level of this hormone increased approximately 3-fold between GD10 and GD11. The concentration then remained fairly constant until GD14, when the level nearly doubled and stayed constant to GD15. The corticosterone concentration was significantly higher in intact mice on GD11 through GD14 than in ADX dams.

In this strain of mice, the palate was fused in 54 of 96 (56.3%) fetuses by the morning of GD14. By that afternoon (3:00–4:00 PM), 93.5% (29 of 31) of fetuses had fused palates. By 9:00 AM on the morning of GD15, 100% (42 of 42) of fetuses had fused palates. The data in Figure 1 demonstrate that maternal corticosterone levels in ADX mice were below the limit of sensitivity of the RIA (2.5 μg/100 ml) during the period of palatal development.

When mice were left untreated following adrenalectomy, there was very little maternal toxicity (Table I). Except for PHT treatment at 60 mg/kg, ADX dams gained more weight between GD10 and GD18 than did intact mice. Vehicle injection produced no maternal deaths, but did result in the complete resorption of six litters, an incidence that is similar to that seen at 60 or 75 mg/kg of PHT. When PHT was injected into ADX dams, there was a dose-related increase in maternal deaths.

There was a significant increase in the resorption frequency when PHT was administered at 75 mg/kg to intact mice when compared with untreated dams and 60 mg/kg of PHT (Table II). The drug produced a significant increase in the resorption frequency among ADX mice treated with PHT at either 60 or 75 mg/kg when compared with other ADX groups. There were no significant increases in the resorption frequencies of ADX mice when compared with the corresponding intact group, except in the 60 mg/kg of PHT group.

No cleft palates were present in the offspring of intact mice that were either untreated or received the vehicle or PHT at 25 mg/kg (Table III). There was a significant increase in the cleft palate incidence at 75 mg/kg in intact mice and at 60 mg/kg in ADX animals. The incidence was very low in ADX mice treated with PHT at 25 or 75 mg/kg.

Lower average fetal weights were associated with adrenalectomy in all groups; however, these differences were not statistically significant (Table III). Among the ADX groups, PHT at 60 mg/kg significantly decreased weight compared with the untreated control group. Among the intact groups, PHT at 75 mg/kg produced a decrease in weight that was significantly lower than that in all groups except the 60 mg/kg group.

When the data of the first experiment were analyzed (Table III), the increased cleft palate incidence at 60 mg/kg of PHT in ADX mice was unexpected. We then repeated the 0, 60, and 75 mg/kg doses in ADX mice (Table IV). The observations of the first experiment were essentially repeated, in that the vehicle did not produce clefts in ADX mice, but PHT at 60 mg/kg did increase the incidence of cleft palate when com-

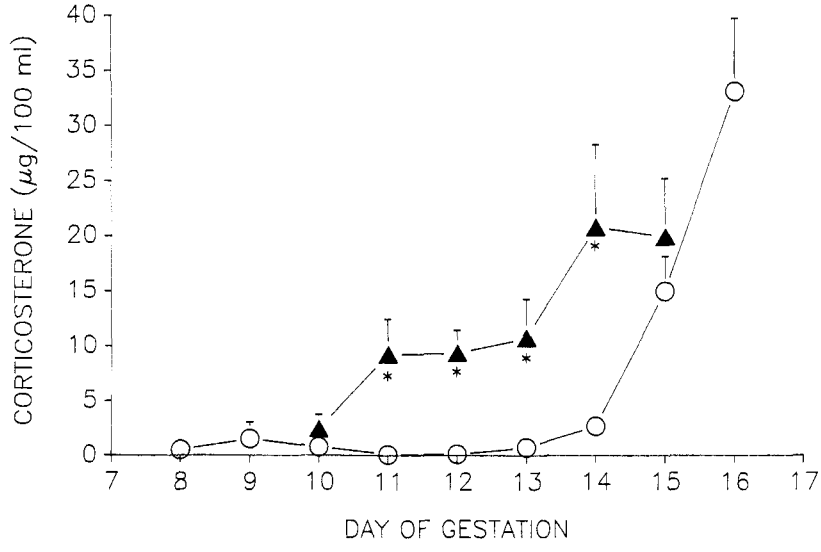


**Figure 1.** Endogenous maternal corticosterone levels in intact or ADX pregnant mice are shown as a function of gestational day. Animals were ADX on GD7, and groups of mice were sacrificed at 24-hr intervals. Intact mice were untreated. Sacrifice always occurred between 8:00 and 9:00 AM. Each point is the mean of four to eight mice. Data are shown as means ± SE. If the SE bar is not apparent, it fell within the symbol. Open circles represent ADX mice; filled triangles represent intact animals. * $P < 0.05$ from corresponding intact group.

**Table I.** Maternal Toxicity of PHT in Intact or ADX CD-1 Mice—Replicate 1

| | Untreated | Vehicle | PHT | | |
|---|---|---|---|---|---|
| | | | 25 mg/kg | 60 mg/kg | 75 mg/kg |
| No. dams treated | | | | | |
| Intact | 9 | 16 | 13 | 12 | 19 |
| ADX | 18 | 18 | 13 | 23 | 37 |
| No. dead dams | | | | | |
| Intact | 0 | 0 | 0 | 0 | 0 |
| ADX | 0 | 0 | 1 | 7 | 16 |
| Maternal weight gain GD10-GD18 | | | | | |
| Intact | ND[a] | 14.3 ± 2.0 | 15.1 ± 1.5 | 19.5 ± 1.5 | 10.3 ± 1.6 |
| ADX | ND | 18.1 ± 0.9 | 21.4 ± 1.5 | 16.2 ± 1.4 | 13.8 ± 1.5 |
| No. litters totally resorbed | | | | | |
| Intact | 0 | 1 | 1 | 0 | 3 |
| ADX | 1 | 6 | 1 | 5 | 8 |
| No. litters with live pups | | | | | |
| Intact | 9 | 15 | 12 | 12 | 16 |
| ADX | 17 | 12 | 11 | 11 | 13 |
| Percentage of litters with live pups | | | | | |
| Intact | 100 | 93.8 | 92.3 | 100 | 84.2 |
| ADX | 94.4 | 66.7 | 84.6 | 47.8 | 35.1 |

[a] ND, not determined.

**Table II.** Embryolethality in Intact or ADX CD-1 Mice after PHT Treatment—Replicate 1[a]

| | Untreated | Vehicle | PHT | | |
|---|---|---|---|---|---|
| | | | 25 mg/kg | 60 mg/kg | 75 mg/kg |
| No. litters examined | | | | | |
| Intact | 9 | 15 | 12 | 12 | 16 |
| ADX | 17 | 12 | 11 | 11 | 13 |
| No. implants | | | | | |
| Intact | 93 | 116 | 105 | 136 | 177 |
| ADX | 191 | 155 | 130 | 127 | 143 |
| No. resorptions or dead fetuses | | | | | |
| Intact | 4 | 13 | 16 | 11 | 68 |
| ADX | 20 | 16 | 8 | 36 | 40 |
| No. litters with resorptions or dead fetuses | | | | | |
| Intact | 2 | 8 | 8 | 4 | 13 |
| ADX | 11 | 7 | 5 | 11 | 13 |
| Percentage of resorptions/litter (±SE) | | | | | |
| Intact | 1.6 ± 0.4* | 9.5 ± 0.7*,† | 11.5 ± 0.6*,† | 3.1 ± 0.8* | 31.0 ± 1.2† |
| ADX | 6.9 ± 0.3* | 4.2 ± 0.5* | 2.2 ± 0.3* | 28.1 ± 0.5[b],† | 26.1 ± 0.4† |

[a] Differences between groups were analyzed by transforming the litter proportional data using the arcsin transformation. Two-way analysis of variance (ANOVA), followed by a *t* test or one-way ANOVA were then used as described in Materials and Methods. Groups with the same symbol (*, †) across a line are not significantly different from each other.
[b] $P < 0.02$ when compared with corresponding intact group.

pared with intact mice. No live offspring were produced at the 75 mg/kg of PHT dose in the second experiment.

Maternal plasma corticosterone levels were determined at 4, 24, or 48 hr following PHT treatment (Fig. 2). In ADX animals, corticosterone levels were below the limit of sensitivity of the RIA at all time points following either dose and are not depicted in Figure 2. In intact mice, corticosterone levels were very high 4 hr after PHT dosing. These concentrations decreased until 48 hr, at which time they had returned to control levels. There were no differences in corticosterone concentrations of intact mice between 60 and 75 mg/kg of PHT at any of the three time points examined.

Maternal plasma PHT levels were determined in intact and ADX mice at 4, 24, or 48 hr after dosing. There were no differences in PHT concentrations between intact and ADX mice treated with the higher dose at any of the three time points examined (Fig. 3B). The PHT level was significantly higher in intact mice treated at 60 mg/kg compared with the ADX group treated with the same dose; there were no significant differences at 4 or 48 h after the 60 mg/kg dose (Fig. 3A). PHT levels 4 hr after dosing were significantly higher after 75 mg/kg than after 60 mg/kg in either intact or ADX mice; there were no differences between

**Table III.** Embryotoxicity in Intact or ADX CD-1 Mice after PHT Treatment—Replicate 1[a]

| | Untreated | Vehicle | PHT | | |
|---|---|---|---|---|---|
| | | | 25 mg/kg | 60 mg/kg | 75 mg/kg |
| No. live fetuses | | | | | |
| Intact | 89 | 103 | 89 | 125 | 109 |
| ADX | 171 | 139 | 122 | 91 | 103 |
| No. with clefts | | | | | |
| Intact | 0 | 0 | 0 | 3 | 16 |
| ADX | 0 | 0 | 3 | 36 | 3 |
| No. litters with clefts | | | | | |
| Intact | 0 | 0 | 0 | 3 | 9 |
| ADX | 0 | 0 | 2 | 9 | 2 |
| Percentage with clefts/litter ± SE | | | | | |
| Intact | 0 ± 0* | 0 ± 0* | 0 ± 0* | 0.7 ± 0.2*,† | 13.3 ± 1.2† |
| ADX | 0 ± 0* | 0 ± 0* | 0.4 ± 0.2* | 39.8 ± 2.7[b],† | 0.5 ± 0.2[b],* |
| Fetal weight/litter ± SE (g) | | | | | |
| Intact | 1.41 ± 0.03* | 1.39 ± 0.05* | 1.46 ± 0.04* | 1.29 ± 0.03*,† | 1.22 ± 0.04† |
| ADX | 1.27 ± 0.04* | 1.23 ± 0.03*,† | 1.19 ± 0.06*,† | 1.02 ± 0.06† | 1.15 ± 0.04*,† |

[a] Differences between groups were analyzed by transforming the litter proportional data using the arcsin transformation. Two-way ANOVA, followed by a *t* test or one-way ANOVA, were then used as described in Materials and Methods. Groups with the same symbol (*, †) across a line are not significantly different from each other. Fetal weight data were not transformed prior to analysis.
[b] $P < 0.02$ when compared with corresponding intact group.

**Table IV.** Effects of PHT in ADX CD-1 Mice—Replicate 2[a]

| | Vehicle | PHT | |
|---|---|---|---|
| | | 60 mg/kg | 75 mg/kg |
| No. dams treated | 10 | 9 | 26 |
| No. dead dams | 1 | 1 | 9 |
| No. litters totally resorbed | 3 | 1 | 17 |
| No. litters with live pups | 6 | 7 | 0 |
| No. implants | 66 | 75 | — |
| No. resorptions or dead | 5 | 15 | — |
| Percentage of resorptions/litter ± SE | 6.96 ± 0.8 | 20.1 ± 0.4 | — |
| No. live fetuses | 61 | 60 | — |
| No. with clefts | 0 | 15 | — |
| Percentage with clefts/litter ± SE | 0 ± 0 | 12.0 ± 3.3[b] | — |
| Fetal weight/litter ± SE (g) | 0.94 ± 0.07 | 0.89 ± 0.05 | — |

[a] Differences between groups were analyzed by transforming the litter proportional data using the arcsin transformation. Two-way ANOVA, followed by a *t* test or one-way ANOVA, were then used as described in Materials and Methods. Fetal weight data were not transformed prior to analysis.
[b] $P < 0.05$ when compared with vehicle control.

the dose groups at 24 or 48 hr in either intact or ADX mice.

## Discussion

Results of the present study demonstrate that CD-1 mice can undergo adrenalectomy during early gestation and are able to maintain pregnancy to at least GD18. Following adrenalectomy, maternal corticosterone levels are quite low until GD15. The corticosterone present in the maternal animal subsequent to GD15 has been shown to be of fetal origin (15). Our results suggest that adrenalectomy does not induce a precocious fetal corticosterone secretion earlier in gestation, and there was no difference in maternal levels between intact and ADX mice on GD15. Fusion of the palatal shelves occurs late on GD14 in most fetuses in this strain of mice, so the rise in endogenous maternal corticosterone occurs shortly after palatal development is complete. Throughout the period of palatal fusion (GD10–GD14), corticosterone levels in intact dams are significantly higher than those in ADX dams. Thus, maternal adrenalectomy provided appropriate conditions for determination of the role of endogenous corticosteroids in cleft palate production.

The results of PHT administration at 60 mg/kg demonstrate that the anticonvulsant is capable of producing orofacial clefting in the absence of maternal glucorticoids. It has been suggested that PHT and glucocorticoids may share a common mechanism in producing clefts (16, 17), and we have previously reported that a teratogenic dose of PHT increased maternal plasma corticosterone levels for 48 hr in A/J mice (5). In the present investigation, PHT produced a pronounced increase in maternal corticosterone concentrations in intact CD-1 mice 4 hr after dosing. These levels were still increased at 24 hr but decreased to control levels by 48 hr. This suggests that there may be a difference in the corticosterone response to PHT among mouse strains (5). Additionally, despite the elevated corticosterone levels in intact mice at 60 mg/kg, there was a higher clefting incidence in ADX mice treated with the same dose of the anticonvulsant.

PHT has been reported to be an alternative ligand

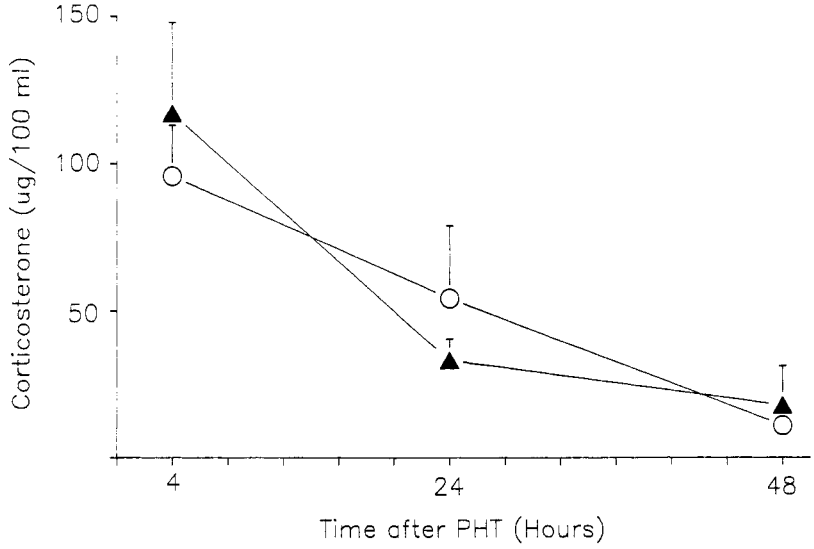


**Figure 2.** Endogenous maternal corticosterone levels in intact pregnant mice are shown as a function of time after dosing with PHT. Animals were treated with PHT at either 60 (open circles) or 75 (filled triangles) mg/kg on the morning of GD10 and were sacrificed at 4, 24, or 48 hr after dosing. Corticosterone was below the limit of detection of the RIA for all ADX mice. Each point is the mean of six to 13 mice, and data are presented as means ± SE. If the SE bar is not apparent, it fell within the symbol.

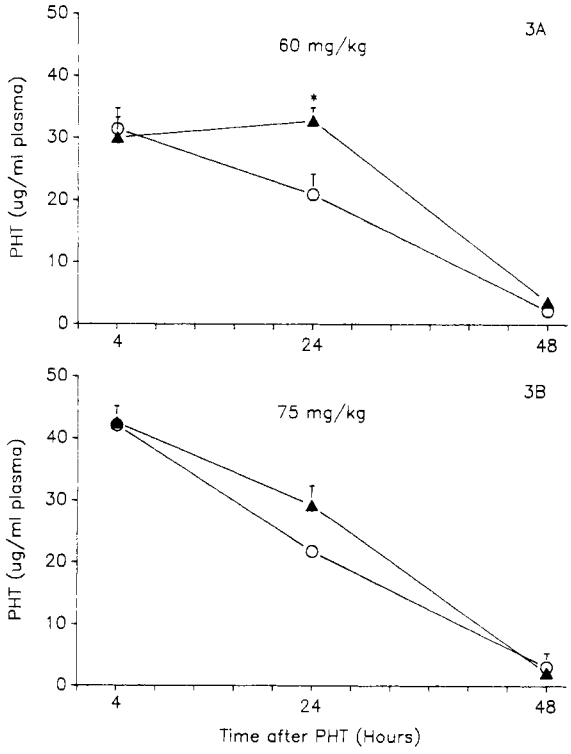


**Figure 3.** Maternal plasma PHT levels in intact or ADX mice are shown as a function of time after PHT dosing. Animals were treated with PHT at either (A) 60 or (B) 75 mg/kg on the morning of GD10; in some mice, adrenalectomy had been performed on GD7. Each point is the mean of five to 10 mice. Data are shown as means ± SE. If the SE bar is not apparent, it fell within the symbol. Open circles represent ADX mice; filled triangles represent intact animals.

for the glucocorticoid receptor (18) and to interfere with the production of arachidonic acid, which is necessary for prostaglandin synthesis (16). Recent evidence using rodent embryos cultured *in vitro* demonstrated that the addition of arachidonic acid was able to attenuate effects of PHT on the neural tube and facial arches (19) and that inclusion of indomethacin, an inhibitor of prostaglandin synthesis from arachidonic acid, produced nearly the same incidence of defects as PHT treatment alone (20). Cortexolone, a glucocorticoid receptor antagonist, reduced PHT-induced abnormalities in embryo culture, suggesting as one possibility that PHT functions via the glucocorticoid receptor. Although the results of the present study could be interpreted to support this hypothesis, further work is necessary to clarify the role of PHT binding to the glucocorticoid receptor in drug-induced clefting.

In a small study, the cleft palate incidence of Swiss-Webster mice ADX on Day 10 of gestation and given PHT at 50 mg/kg on GD11–GD13 was not different from that of controls (21). In the first replicate in the present investigation, cleft palate was increased at 60 mg/kg of PHT, while a significant decrease in the frequency of cleft palate was observed in surviving ADX fetuses dosed at 75 mg/kg. There are several differences between the present study and the earlier work of Harbison and Becker (21), which may account for the discordant results. These include differences in dosage, time of administration, time of adrenalectomy, and strain of mouse used. Additionally, maternal toxicity produced by the drug was not reported in the earlier study.

The elevated maternal toxicity observed in PHT-treated ADX mice makes interpretation of the data difficult. The similarity of the resorption frequencies in the first replicate in ADX mice dosed with 60 or 75 mg/kg of PHT suggests that the increased clefting incidence observed at the lower dose is not due simply to an increased loss of abnormal embryos at the higher

dose of the drug. In intact animals, PHT was not lethal to the dams, even at the highest dose; however, dams treated with this dose were sedated and ataxic for 24 to 48 hr. The same effects were observed for a shorter period of time at 60 mg/kg in intact animals. Ataxia was also produced by PHT at these doses in ADX dams. More than one half of the ADX animals treated with either of these doses of PHT produced no viable offspring, suggesting a toxic interaction between adrenalectomy and the drug. Two-way analysis of variance of the data in Tables I–III indicated that there was an interaction of the ADX state and the dose of PHT, but there was no significant effect of adrenalectomy alone. This is supported by maternal weight gain data; ADX females actually gained slightly more weight between GD10 and GD18 than did corresponding intact mice. There appears to be greater toxicity of PHT in ADX mice than in comparably treated intact animals, and 75 mg/kg is extremely toxic in ADX mice.

The increased toxicity of PHT in ADX mice was not a result of higher maternal plasma drug levels; there were no differences between intact and ADX mice treated with the higher dose of the drug. The increased clefting incidence observed in ADX mice treated with 60 mg/kg also does not appear to be due to altered maternal plasma drug levels. The only significant difference observed was an increased drug concentration in intact mice 24 hr after dosing. Additionally, there were no differences in drug levels following dosing with 60 or 75 mg/kg at either 24 or 48 hr after dosing. Further work is necessary to clarify the reason for the increased toxicity of PHT in ADX mice.

Mechanisms other than glucocorticoid effects have been suggested to explain the embryotoxicity of PHT, including drug-induced decreases in folate levels. Chronic PHT treatment has been shown to alter normal folate metabolism in mouse dams, but it does not affect embryonic folate levels (13). The weight gain data in Table I suggest that PHT treatment had no effect on weight gain in either intact or ADX mice; this implies that there were no differences in food consumption or dietary folate intake between control and treated mice in the present study. It seems unlikely that a drug-induced folate deficiency might be responsible for the results of the present study.

Additionally, metabolism of PHT to a reactive intermediate, possibly via the prostaglandin synthetase pathway, has been suggested to be involved in drug-induced defects (22). It has been shown that the anticonvulsant can be metabolized via this pathway *in vitro* to a reactive intermediate that binds covalently to macromolecules. *In vivo* pretreatment with an inhibitor of this pathway, with an antioxidant, or with a free radical spin trapping agent decreased the incidence of clefting following PHT treatment (23). The balance of PHT metabolism between this pathway and the hepatic P-450 system is currently unknown. Further work is needed to determine this balance and whether an alteration in maternal glucocorticoid level has any effect on the metabolic flux through the prostaglandin synthetase pathway.

Adrenalectomy of pregnant mice early in gestation allows them to maintain pregnancy until at least GD18. PHT is more toxic in ADX mice than in intact animals, and there is an increase in maternal lethality and resorption of entire litters. Administration of 60 mg/kg of PHT to ADX mice produced a significant increase in the clefting incidence when compared with comparably treated intact animals, indicating that the drug is able to produce clefting in the absence of endogenous maternal glucocorticoids. Further work will be necessary to determine the mechanism of PHT-induced embryotoxicity.

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