Kinetics of Drug Action in Disease States III: Effect of Pregnancy on the Relationship Between Phenytoin Concentration and Antiseizure Activity in Rats

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Received July 8, 1983, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication November 16, 1983.

Abstract □ The purposes of this investigation were to determine the effect of pregnancy on the susceptibility of female rats to experimentally induced seizures and on the relationship between serum phenytoin concentration and antiseizure activity. Pregnant rats (on the 18th day of gestation) were more susceptible than nonpregnant female rats to seizures produced by maximal electroshock or by a body-weight-based dose of pentylenetetrazol. There was no apparent difference between pregnant (20th day of gestation) and nonpregnant rats in the relationship between seizure protection (percent of animals protected) and the serum concentration of total (free plus protein-bound) phenytoin. The relationship between concentration and effect was essentially the same 20 min after an injection of phenytoin and 2 h after the start of a constant-rate infusion preceded by a loading dose of the drug. Since the protein binding of phenytoin is appreciably decreased in late pregnancy, the serum concentration of free phenytoin required for seizure protection tended to be higher in pregnant than in nonpregnant rats. This may be due to the increased susceptibility of pregnant rats to seizure stimuli.

Keyphrases □ Phenytoin—antiseizure activity, effect of pregnancy, maximal electroshock seizure test □ Pregnancy—effect on antiseizure activity, phenytoin, maximal electroshock seizure test

Treatment of epileptic women with phenytoin often requires adjustment of drug dosage during pregnancy for adequate seizure control (1, 2). This appears to be due in part to pregnancy-associated alterations in the pharmacokinetics (3, 4) and possibly in the bioavailability (5) of the drug, and perhaps also to decreased compliance (6). However, the physiological changes associated with pregnancy, including water retention, electrolyte concentration and pH shifts, and altered hormonal concentrations, may provoke seizures in epileptic patients (7–9). It is possible, therefore, that the relationship between phenytoin concentration and antiseizure activity is altered during pregnancy. Such an effect would be in addition to the well-established change in the plasma protein binding of phenytoin in pregnancy (10), which makes it desirable that phenytoin plasma concentration monitoring during pregnancy be based on the concentrations of free rather than total drug.

The purpose of this investigation was to determine the effect of pregnancy on the relationship between serum phenytoin concentration and antiseizure activity in an animal model of experimentally induced seizures. The studies were designed to compare the response of nonmedicated pregnant and nonpregnant female rats to two types of seizure stimuli (electrical and chemical), to relate the antiseizure effect of phenytoin to the serum concentration of both total and free drug, and to compare the concentration–effect relationship after intravenous injection of phenytoin with the corresponding relationship at steady state during intravenous infusion of the drug. Information was also obtained concerning the effect of pregnancy on the distribution of phenytoin between plasma, brain, and cerebrospinal fluid (CSF).

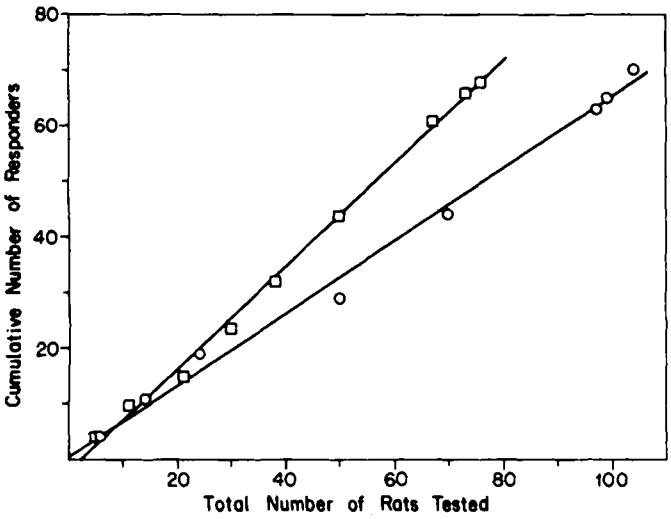

Figure 1—*Seizure response of pregnant (18th day of gestation) (□) and nonpregnant female (○) rats to a maximum electroshock (MES) stimulus. The cumulative number of animals in each group that responded to MES by tonic hind limb extension is plotted against the cumulative number of animals subjected to MES in the course of this investigation. The percentage of pregnant rats responding was 90%; 67% of the nonpregnant female rats responded. The slopes of the regression lines are significantly different,* $p < 0.001$.

EXPERIMENTAL SECTION

Methods—Inbred female Lewis rats, weighing 175–200 g on receipt, were used in this investigation. Some were mated (3) while others were designated as nonpregnant controls. On the 18th day of gestation, the pregnant animals and their concurrent controls were screened by subjecting them to maximal electroshock (MES), using an apparatus that was adjusted to deliver ~ 150 mA for 0.35 s *via* corneal electrodes (11). Only rats that responded to the stimulus by exhibiting the tonic hind limb extensor component of MES seizure were used in the subsequent experiment. The animals had access to food and water at all times except during the hour of testing to avoid possible alterations in their susceptibility to MES due to starvation or dehydration (11).

One day after the MES screening, the rats had a cannula implanted in the right jugular vein under light ether anesthesia (12). This procedure is known to not alter their seizure pattern (13). On the following day, *i.e.*, on the 20th day of gestation of the pregnant animals, the rats received an intravenous injection of phenytoin, 2–12.5 mg/kg, through the cannula (which was then flushed with saline solution). The drug was dissolved in 10% ethanol–normal saline in various concentrations and 1 mL/kg of the solution was injected over 1 min. The solutions were coded so that the experimenter did not know the

Table I—Effect of Pregnancy on the Tonic-Seizure Response of Rats to Pentylenetetrazol Injection [a]

Type of Rats	Body Weight, g	Number Responding/ Total Number
Nonpregnant female	201 ± 8	5/14
Pregnant 18th day of gestation		
Dosed per actual body weight	263 ± 16	8/9
Dosed per average weight of nonpregnant controls	261 ± 11	3/8

[a] Dose of pentylenetetrazol was 26 mg/kg iv.

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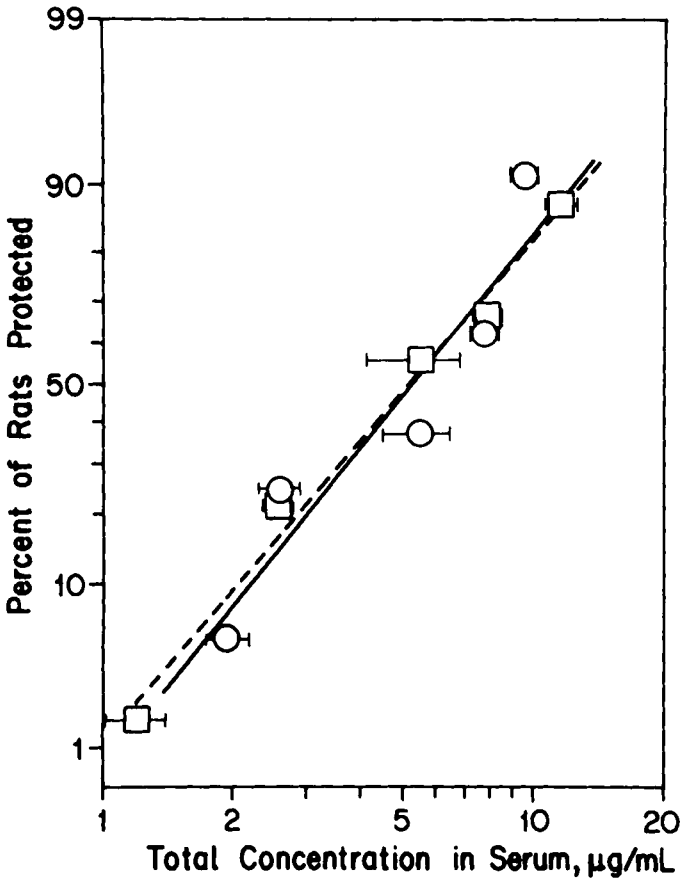

Figure 2—*Relationship between the percent of rats protected from MES-induced tonic seizures (both forelimb and hind limb extension) and serum concentration of phenytoin in pregnant rats at the 20th day of gestation (□) and nonpregnant female (○) rats. The determinations were made 20 min after a rapid intravenous injection of phenytoin, 2–12.5 mg/kg. The data points are based on results from 7–9 rats per point. The horizontal bars represent ± one* SD *of the concentration. The lowest square and the lowest and highest circles represent corrected values for 0 and 100% protection according to Litchfield and Wilcoxon (17). The regression lines and correlation coefficients ($r = 0.993$, $p < 0.001$ for the pregnant rats and $r = 0.940$, $p < 0.02$ for the nonpregnant rats) are based on the probit values for protection and on logarithmic values for serum concentration.*

dose administered. Twenty minutes after the injection, the rats were subjected to MES and the response was recorded. Complete protection was defined as the prevention of tonic seizure, *i.e.*, absence of both forelimb and hind limb extension (14, 15). A blood sample was then obtained through the cannula, and the serum was separated and assayed for phenytoin by HPLC (16).

In another part of the investigation, pregnant (20th day of gestation) and nonpregnant female rats received an intravenous injection of phenytoin solution, 0.340 mL/min for 1 min, followed immediately by a constant-rate infusion of 0.0136 mL/min for 2 h. The phenytoin concentrations of the solutions ranged from 0.874 to 2.46 mg/mL. At 2 h, the rats were subjected to MES and blood sampling.

Other pregnant (18th day of gestation) and nonpregnant female rats, with a jugular vein cannula implanted 1 d earlier, received a rapid injection of pentylenetetrazol, 26 mg/kg, as a 52-mg/mL aqueous solution. The syringe and cannula were then flushed immediately by withdrawal and reinjection of blood. Some of the pregnant rats received the dose based on their actual body weight while others received a dose based on the average body weight of the nonpregnant controls. Tonic seizures, when present, occurred within a few seconds after the injection and were recorded. The pharmacological activity data were analyzed according to Litchfield and Wilcoxon (17).

Also reported here is a summary of phenytoin distribution data derived from various experiments performed over ~1 year. Briefly, pregnant rats at the 20th day of gestation and nonpregnant female rats received a 14.7-mg/kg injection of phenytoin followed immediately by an infusion of 195 μg/min/kg for 2 h, through a jugular vein cannula. At the end of the infusion, samples of CSF and blood (using EDTA as the anticoagulant for separation of plasma) were obtained and the brain was removed. These fluids and one hemisphere of the

Table II—Effect of Pregnancy on the Median Effective Serum Concentrations of Total and Free Phenytoin for Protection Against Maximal Electroshock-Induced Tonic Seizures

Median Conc., μg/mL	Pregnant Female, Day 20 of Gestation	Nonpregnant Female
Total	5.20 3.58–7.55[a]	5.20 3.55–7.62[a]
Free[b]	1.03 0.710–1.49[a]	0.680 0.464–0.997[a]

[a] 95% Confidence limit. [b] Based on free fraction values obtained in studies on other rats (Table III).

brain were assayed for phenytoin by HPLC (16). The average CSF/plasma concentration ratios of phenytoin in pregnant and nonpregnant rats obtained in these studies were used to estimate the plasma concentrations of free phenytoin in the experiment in which the relationship between total phenytoin plasma concentration and antiseizure activity was determined. This had to be done because of the great difficulty in obtaining CSF from rats after MES.

RESULTS

The results of the screening study are summarized in Fig. 1. The data are plotted cumulatively and reflect the results obtained on successive groups of rats that were screened over approximately 6 months. Sixty-seven percent of the nonpregnant female rats and 90% of the pregnant rats exhibited tonic seizures (with hind limb extension) in response to MES, a significant difference in response rate ($p < 0.001$).

To determine if the apparent difference in the susceptibility of pregnant and nonpregnant rats to MES is limited to the electric stimulus only, other groups of nonmedicated animals were subjected to a chemical stimulus, *i.e.*, pentylenetetrazol injection. The seizure response rate of the pregnant rats was significantly higher than that of the nonpregnant controls [$p < 0.02$ when tested by a statistic comparing two success probabilities (18)] on injection

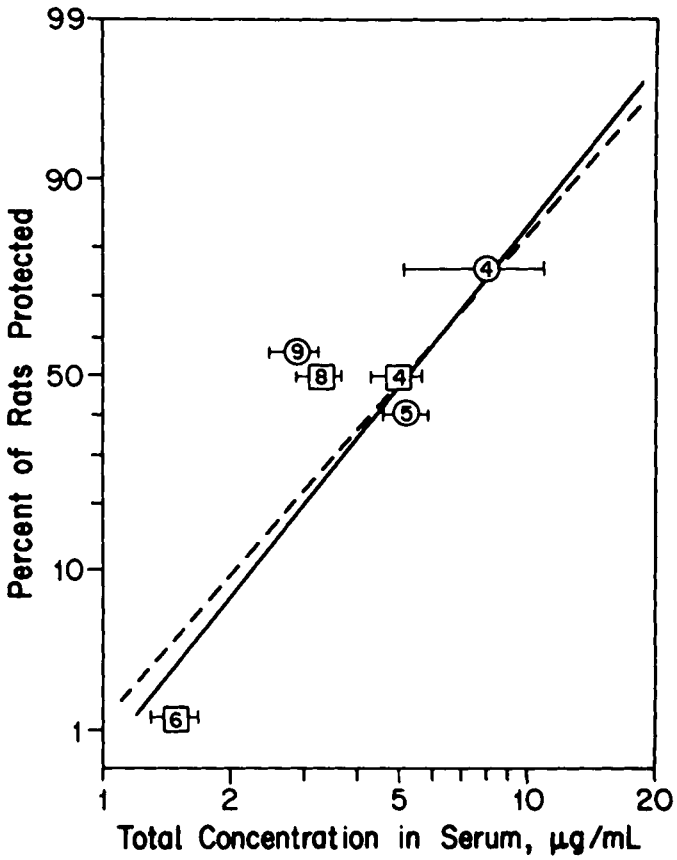

Figure 3—*A plot similar to Fig. 2, except that the results shown here were obtained after a 2-h intravenous infusion of phenytoin. The number of rats per groups are indicated by the number in the symbols. The regression lines are those obtained in the experiment shown in Fig. 2. The lowest square is a corrected value.*

Table III—Effect of Pregnancy on the Distribution of Phenytoin Between Plasma, Cerebrospinal Fluid, and Brain in Rats [a]

Variable	Pregnant Female, Day 20 of Gestation		Nonpregnant Female
Number of animals	22		19
Phenytoin conc., μg/mL or g			
Plasma	14.5 ± 2.4	N.S.	14.0 ± 3.7
CSF	2.78 ± 0.47	$p < 0.001$	1.78 ± 0.42
Brain	26.7 ± 7.3	$p < 0.005$	19.8 ± 5.0
Concentration ratio [b]			
CSF/Plasma	0.193 ± 0.027	$p < 0.001$	0.130 ± 0.019
Brain/Plasma	1.83 ± 0.36	$p < 0.001$	1.43 ± 0.19
Brain/CSF	9.51 ± 1.58	$p < 0.001$	11.1 ± 1.0

[a] Results (mean ± *SD*) obtained after intravenous injection of phenytoin, 14.7 mg/kg, followed by an intravenous infusion of 195 μg/min/kg for 2 h. [b] None of the ratios are significantly correlated with the plasma phenytoin concentration.

of both groups with the same relative (mg/kg) dose (Table I). On the other hand, pregnant rats injected with the convulsant in a dose based on the average weight of the nonpregnant controls exhibited essentially the same seizure response rate as the control animals.

The results of the experiments to determine the relationship between serum concentration of total phenytoin and seizure protection are shown in Fig. 2. The determinations were made 20 min after the intravenous injection of phenytoin. Essentially identical results were obtained with pregnant and nonpregnant rats (Table II).

The results of a more-limited series of experiments, performed under steady-state conditions (constant rate intravenous infusion of phenytoin), are summarized in Fig. 3. For both pregnant and nonpregnant rats the average concentration–response data at two of three phenytoin concentrations were essentially identical to those obtained when phenytoin was administered by rapid injection.

The steady-state distribution of phenytoin between brain and CSF was significantly altered during pregnancy (Table III). The decreased brain/CSF concentration ratio and the increased CSF/plasma concentration ratio of phenytoin in the pregnant rats are consistent with decreased binding of the drug in the brain and plasma, respectively.

The steady-state concentration ratio of phenytoin, CSF/plasma, is a measure of the free fraction of the drug in plasma under *in vivo* conditions (16). The average free fraction value of phenytoin, which is independent of concentration over a wide range (19), was substantially higher in pregnant than in nonpregnant rats (Table III). Using these free fraction values, the experimental concentration data summarized in Fig. 2 were converted to free phenytoin concentrations to determine the relationship between serum concentration of free phenytoin and seizure protection (Fig. 4). The thus estimated median effective serum concentration of free phenytoin was 1.03 μg/mL in the pregnant rats and only 0.680 μg/mL in the nonpregnant animals, while the median effective serum concentration of total (free plus protein-bound) drug was identical in both groups (Table II).

DISCUSSION

Pregnancy is obviously not a disease, but, like many diseases, it is associated with profound changes in physiological status. Thus, an investigation of the effect of pregnancy on the relationship between drug concentration and pharmacological activity is appropriate for inclusion in this series of reports concerning the kinetics of drug action in disease states.

Some women experience epileptic fits only during pregnancy. This condition, known as true gestational epilepsy (7), reflects an increased susceptibility of at least some individuals to the altered physiological conditions associated with pregnancy. Consistent with these observations, there is an increased incidence of electroencephalograms classified as slow or high-voltage fast in pregnant women, reflecting a type of cortical activity that is known to occur more frequently in conditions in which the incidence of convulsions is higher than in the general population (20).

In the present study, a greater percentage of pregnant than nonpregnant rats responded with seizures to an electroshock stimulus. To determine if this difference also occurs in rats after exposure to a chemical stimulus, other groups of animals were given a pentylenetetrazol injection. The design of such a study raises the problem of proper normalization of dosage (3, 21): should the pregnant animals receive the same absolute (mg) or relative (mg/kg) dose of the convulsant drug? Pharmacokinetic evidence suggests that pentylenetetrazol distributes in body water (22). However, animals have seizures within seconds after intravenous injection, *i.e.*, possibly before the distribution of pentylenetetrazol from blood to the total body water space is complete. Significantly, the *relative* volumes of both body water and blood remain essentially constant during pregnancy in rats (23, 24). These considerations suggest that the dose of pentylenetetrazol for pregnant rats should be based on their actual body weight. Under these conditions, the relative number of pregnant rats with tonic seizures after pentylenetetrazol injection was significantly higher than that of nonpregnant controls. No such difference was found when pregnant and nonpregnant rats received the same absolute dose of the convulsant. Thus, while the results of this experiment appear to be consistent with those of the electroshock study, *i.e.*, increased susceptibility to seizures during pregnancy, definitive studies with pentylenetetrazol require determination of effective concentrations of the convulsant in the brain, CSF, or plasma. Such studies are now in progress.

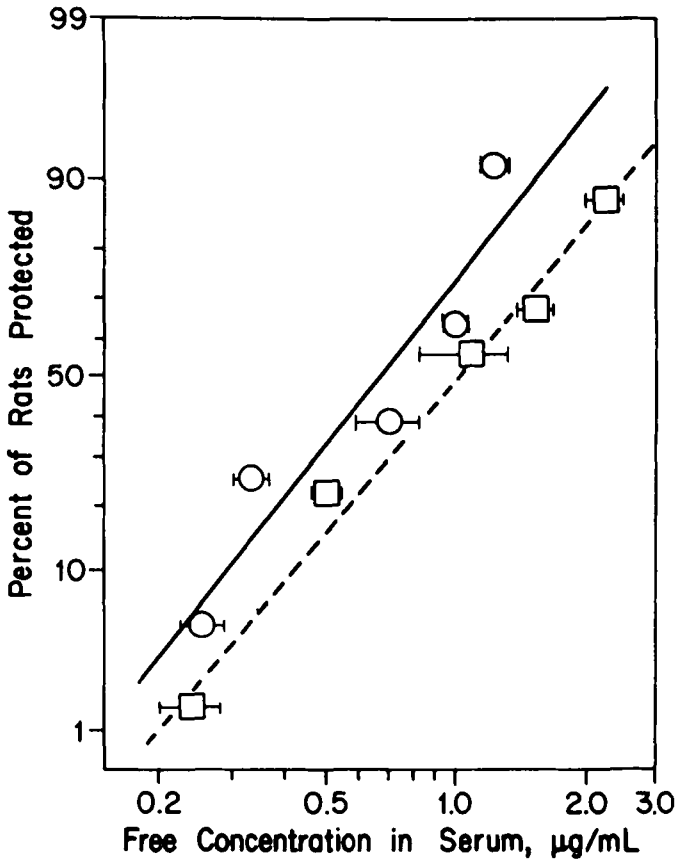

Figure 4—*A plot of the data in Fig. 2, except that serum phenytoin concentrations are corrected for protein binding (determined in other rats) to show the relationship between protection from MES-induced tonic seizures and the estimated serum concentration of free (unbound) phenytoin.*

The entry of phenytoin into the central nervous system (CNS) is rapid and may be blood-flow limited (8, 13, 25–27). Once in the CNS, the drug acts without any apparent delay (13). The major metabolites of phenytoin are pharmacologically inactive (28, 29). Under postdistribution conditions, phenytoin concentrations in plasma ultrafiltrate, brain ultrafiltrate, and CSF are essentially equal (25), and the anticonvulsant effect of the drug is, therefore better correlated with the plasma concentrations of free rather than total (free plus bound) phenytoin (30).

Pregnancy had no apparent effect on the relationship between the serum concentration of total phenytoin and antiseizure activity in rats subjected to maximal electroshock (Fig. 1). However, since the plasma or serum protein binding of phenytoin is significantly decreased during pregnancy in rats (3, 19), pregnant animals apparently require higher serum concentrations of free phenytoin than do nonpregnant controls for equivalent protection against MES-induced seizures.

The original design of this study included determination of phenytoin protein binding in the serum of every phenytoin-treated animal tested for protection against MES seizure. The protein binding determinations were made by equilibrium dialysis before it was found that the results thus obtained were of questionable value due to extensive *in vitro* lipolysis in plasma from pregnant rats (31, 32). Since it is difficult to obtain CSF from rats after application of MES, *in vivo* phenytoin free fraction values (*i.e.*, the ratio of phenytoin concentrations in CSF and plasma) were determined in separate groups of animals from the same source. It should be noted that the CSF-

plasma concentration ratios of phenytoin were obtained in a relatively large number of pregnant and nonpregnant rats (Table III), that the interindividual varition of these values is quite small, and that the free fraction value of phenytoin is independent of concentration over a wide range (19). As is evident in Fig. 4 and Table II, the serum concentrations of free phenytoin required for seizure protection tended to be higher in pregnant than in nonpregnant rats. While a statistical analysis of these hybrid data is not considered appropriate, we state for the record that the median effective concentrations of free drug in the serum of pregnant and nonpregnant animals were not statistically significantly different.

The phenytoin concentration–antiseizure effect relationship was also determined, in a more limited study, in rats that were infused with phenytoin to steady state. The data thus obtained are generally quite similar to those obtained 20 min after a rapid injection of phenytoin (Fig. 4), consistent with the rapid entry of phenytoin into the CNS and with the lack of pharmacological activity of phenytoin metabolites.

As already shown by other investigators (8, 25, 33, 34), it was found in this investigation that the total concentrations of phenytoin are higher in the brain than in the plasma (Table III). This appears to be due to extensive protein binding of the drug in the brain rather than to partitioning into brain lipids (35). The results of the present investigation are also consistent with previous findings of an increased brain–blood phenytoin concentration ratio during pregnancy (34). The reason for this change in drug distribution becomes apparent on examining the concentration ratios relative to CSF determined in this investigation. Since the concentration of proteins in normal CSF is very low, phenytoin in CSF exists almost totally in the unbound form. The increased CSF–plasma phenytoin concentration ratio during pregnancy reflects the decreased plasma protein binding of the drug. By the same reasoning, the decreased brain–CSF phenytoin concentration ratio during pregnancy indicates decreased protein binding of phenytoin in the brain. It appears that the decreased plasma protein binding of the drug is due to lower albumin concentrations and the presence of circulating endogenous binding inhibitors during pregnancy (19, 32); endogenous inhibitors may be responsible also for the decreased binding of phenytoin in the brain.

In an earlier study on Sprague–Dawley rats treated chronically with phenytoin, it was found that pregnant animals had increased serum concentrations of total phenytoin and exhibited increased protection against MES-induced convulsions (36). The present investigation has shown that the relationship between *total* phenytoin serum concentration and seizure protection is essentially the same in pregnant (20th day of gestation) and nonpregnant inbred Lewis rats. Since the protein binding of phenytoin in plasma or serum is appreciably decreased during pregnancy, this suggests that pregnant rats require higher serum concentrations of free phenytoin (the pharmacologically active form of the drug) than do nonpregnant animals for the same degree of seizure protection. This apparent difference may be related to the observed increased susceptibility of unmedicated rats to an experimental seizure stimulus during late pregnancy.

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