years for people, Davis beagles, and female CF_1 mice, respectively, and obtained

$$K = (655 \pm 57) + (119 \pm 1)\,L \quad (3)$$

where L is the normal life expectancy in years, with $\chi^2 = .7$ and $r = .9999$ ($P < .01$ for zero correlation). Hence, the species-dependent response ratios are also linearly related to the respective life expectancies.

Figure 2 shows the dose responses and similar relations describing the competing risk of natural death from old age. As individuals age, they enter regions of increasing risk. At high dose rates, the region of high risk for bone tumors is encountered before the region of high risk for natural death, so that premature death from bone cancer is more probable than death from aging processes. At low dose rates, on the other hand, natural deaths occur before bone tumors develop.

The intersection of these two risk distributions, shown in Fig. 2, explains the practical threshold for bone cancer in people noted by Evans *et al.* (*1*). We speculated that this practical threshold occurs for each species at a dose rate that corresponds to the intersection of t_L and a cancer risk that occurs three geometric standard deviations earlier than the median. For the skeleton of man, dog, and mouse, this yielded calculated practical threshold dose rates of 0.0039 rad/day (0.039 rem/day), 0.011 rad/day (0.11 rem/day), and 0.16 rad/day (1.6 rem/day), respectively, and cumulative doses of 80 rads (800 rem), 50 rads (500 rem), and 110 rads (1100 rem), respectively (with QF = 10).

If it is assumed that the lognormal risk distribution we observed can be extrapolated to the limit and that competing radiogenic risks occur concurrently, then the risk associated with the currently accepted maximum permissible bone burden of 0.1 μCi of ^{226}Ra (0.0082 rad/day) for industrial workers (*1*) can be estimated. If 400 workers remain at the maximum permissible bone burden for 50 years beginning at age 20, we estimate that only one would die from radiogenic bone cancer or other abnormality, 200 would die from nonradiogenic causes, and 199 would still be alive. The time required to reach the median of the bone cancer risk distribution function would be 99 years.

We believe that this analysis provides an improved perspective of the relations among radiation dose, time, and response in animals and man. Further, it elucidates the effect of dose rate on the incidence of biological effects and shows that the cumulative radiation dose alone is not an accurate indicator of risk with respect to the smaller cumulative doses required at lower dose rates to yield a specific bone cancer risk. Dose responses were clearly nonlinear at any time after initial exposure or at a specific dose rate and were satisfactorily represented as lognormal. The resultant interspecies comparison provides response ratios that may be applicable to injury from other radioactive materials or carcinogenic agents.

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Diphenylhydantoin: Pre- and Postnatal Administration Alters Diazepam Binding in Developing Rat Cerebral Cortex

Abstract. *Close correlations between the development of the anticonvulsant effects of diphenylhydantoin and increases in tritiated diazepam binding were observed in rats from fetal day 16 to maturation. In contrast, significant decreases in tritiated diazepam binding were observed in 2- and 3-week-old rats that were exposed in utero to diphenylhydantoin. These changes can be correlated with reported increases in seizure susceptibility after prenatal exposure to diphenylhydantoin.*

Studies on the maturation of the central nervous system and the development of seizures suggest that excitatory and inhibitory systems in the rat develop with a characteristic time sequence (*1*). From these ontogenic studies it appears that diphenylhydantoin (DPH) has a biphasic effect in the brains of maturing rats: excitatory effects in rats of less than 12 days of age and then increasing inhibitory effects that can be correlated to the maturation of inhibitory systems by 17 to 21 days of age (*2*).

We have reported that treatment of adult rats with anticonvulsant, but not subanticonvulsant, doses of DPH significantly increases (*3*) specific high-affinity benzodiazepine binding (*4*) in rat brain tissue. We have now examined the effects of DPH on benzodiazepine binding at various stages of neuronal maturation and compared these effects to the devel-

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opment of the anticonvulsant activity of DPH. Since seizure susceptibility is increased in offspring of mothers treated with DPH during gestation (*2*), we also tested the effects of exposure in utero to DPH on benzodiazepine binding in maturing rats.

Pregnant Sprague-Dawley (Taconic Farms) rats were allowed to give birth, and the pups in each litter, at various postnatal ages (1 day, 7 days, 14 days, 21 days), were separated into two treatment groups that were reduced to five pups per group. One hour before they were killed, littermate pups were injected intraperitoneally with DPH (100 mg/kg) in dilute (*p*H 11) NaOH solution (DPH-treated) or with dilute NaOH solution alone (control). The volume was adjusted so that each pup received 0.04 ml of solution per gram of body weight. Nonlittermate adult male rats (225 to 275 g) from the same supplier were also grouped ($N = 5$) and injected with DPH or dilute NaOH alone. In addition, eight pregnant rats at 16 days of gestation were injected with DPH (100 mg/kg, intraperitoneally) or dilute NaOH solution, and their fetuses were removed 1 hour later.

Previous observations on the dose-dependent nature of DPH in benzodiazepine binding (*3*) led us to use a relatively large (100 mg/kg) dose of DPH (*5*) in order to demonstrate most clearly the enhanced binding effects of DPH. The 1-hour time point was chosen to correlate with peak anticonvulsant effects of DPH at this dose in maximal electroshock seizure tests (*5*). No animals died during the treatment period, but young DPH-treated pups were observed to "twitch" more frequently than vehicle-injected littermates, whereas animals aged 14 days or more appeared to be sedated and moderately ataxic after this DPH treatment.

Cortical membrane fractions prepared from the cerebral cortex of pups (or from whole brains of 16-day fetuses) were used to assess the specific binding of [^{3}H]diazepam (*6*). Specific [^{3}H]diazepam binding, affinities, and total number of binding sites at different postnatal ages in cerebral cortical membranes from control animals were comparable to previously reported values (*7*).

When pregnant mothers were injected intraperitoneally with DPH, a statistically significant decrease in [^{3}H]diazepam binding was observed in the brains of their 16-day (gestational age) fetuses (Table 1). By postnatal day 21, pups injected with DPH 1 hour before they were killed showed a significant increase in [^{3}H]diazepam binding which approached the magnitude of increase seen in cortical membranes of adult rats (Table 1). The alteration in specific benzodiazepine binding in animals injected with DPH can be attributed to a 19 percent increase in the total number of benzodiazepine binding sites without a significant change in their affinity for [^{3}H]diazepam (Table 1).

Table 1. Specific binding of [^{3}H]diazepam to cerebral cortical membranes of rats at various ages injected intraperitoneally with DPH (100 mg/kg) or vehicle and killed 1 hour later. Tissues from individual rats or from 16-day fetuses were assayed at 0.5 n*M* concentrations of [^{3}H]diazepam; the dissociation constant (K_D) and total binding were calculated by Scatchard analysis on pooled samples. A two-tailed Student's *t*-test was used for the statistical analysis of differences between specific binding after the addition of 0.5 n*M* [^{3}H]diazepam (N.S., not significant).

Age (days)	Ratio of protein to wet weight of tissue*	Control			DPH				*P*
		Binding at 0.5 n*M* [^{3}H]diazepam (fmole/mg tissue)	Apparent K_D (n*M*)	Total number of sites (fmole/mg tissue)	Binding at 0.5 n*M* [^{3}H]diazepam (fmole/mg tissue)	Apparent K_D (n*M*)	Total number of sites (fmole/mg tissue)	Percentage change from control at 0.5 n*M*	
				Fetuses†					
16	0.012 ± 0.001	0.18 ± 0.0	4.6	1.5	0.13 ± 0.01	6.7	1.3	−28	< .02
				Pups‡					
1	0.021 ± 0.002	1.7 ± 0.0	5.2	19.3	1.6 ± 0.1	3.6	19.4	− 6	N.S.
7	0.025 ± 0.002	1.8 ± 0.1			1.6 ± 0.1			−13	N.S.
14	0.045 ± 0.000	4.5 ± 0.1	5.8	59.9	4.6 ± 0.1	6.9	70.1	+ 2	N.S.
21	0.047 ± 0.002	5.1 ± 0.1	6.6	75.8	6.1 ± 0.2	7.1	90.3	+20	< .02
				Adults‡					
> 55	0.056 ± 0.002	4.9 ± 0.2	5.5	75.4	6.4 ± 0.2	5.8	103.8	+31	< .01

*Measured in milligrams. †Four fetuses per treatment group. ‡Five animals per treatment group.

Table 2. Specific binding of [^{3}H]diazepam to cerebral cortical membranes from offspring of rats injected subcutaneously with DPH (20 mg/kg) from days 14 to 20 of gestation; control pregnant rats received vehicle (dilute NaOH solution, *p*H 11) for the same period. Tissues from individual pups were assayed at 0.5 n*M* concentrations of [^{3}H]diazepam; K_D and total binding were calculated by Scatchard analysis on pooled samples for all rats in treatment group. The statistical test was as described in Table 1.

Age of pup (days)	*N*	Control			DPH				*P*
		Binding at 0.5 n*M* [^{3}H]diazepam (fmole/mg tissue)	Apparent K_D (n*M*)	Total number of sites (fmole/mg tissue)	Binding at 0.5 n*M* [^{3}H]diazepam (fmole/mg tissue)	Apparent K_D (n*M*)	Total number of sites (fmole/mg tissue)	Percentage change from control at 0.5 n*M*	
1	10	0.95 ± 0.05			1.04 ± 0.05			+ 9	N.S.
7	10	1.7 ± 0.1			1.7 ± 0.1			0	N.S.
14	8	4.6 ± 0.1	5.7	59.4	4.2 ± 0.1	4.8	52.8	−10	< .05
21	10	6.6 ± 0.2	4.6	75.2	5.2 ± 0.1	5.0	62.2	−21	< .01
28	10	6.2 ± 0.1	6.4	91.3	6.1 ± 0.1	6.6	91.0	− 2	N.S.
35	9	5.5 ± 0.1	5.5	71.1	5.4 ± 0.1	5.5	62.4	− 2	N.S.

This change in the total number of binding sites is qualitatively different from the changes in the apparent affinity observed after the addition of γ-aminobutyric acid (GABA) to homogenates or treatment of animals with agents affecting GABA in brain (*8*). In addition, we found that the direct, in vitro, addition of GABA ($10^{-5}M$) to cortical membranes from DPH-treated animals enhanced [^{3}H]diazepam binding at each age tested (data not shown). These data and data obtained by using [^{3}H]diazepam binding and electrophysiological techniques in the adult rat (*3*) indicate that increases in [^{3}H]diazepam binding after DPH administration are qualitatively different and independent from effects of GABA on [^{3}H]diazepam binding.

The biphasic effect of a single injection of DPH on benzodiazepine binding (decrease and then increase) in developing rats can be correlated with the effects of DPH in experimentally induced seizures during maturation. In rats under 12 days of age DPH has excitatory effects as measured by increased susceptibility to electroshock and chemically induced seizures, whereas after this period DPH decreases susceptibility to seizures (*2*). Thus, a single injection of DPH induces changes in benzodiazepine binding during development that parallel the effects of DPH on the development of seizures in the maturing rat.

We also studied the effect of exposure in utero to DPH on benzodiazepine binding in maturing rats. Twelve pregnant rats received one subcutaneous injection of DPH (20 mg/kg in dilute NaOH, *p*H 11) or vehicle (dilute NaOH, *p*H 11) daily for 7 days (days 14 through 20 of gestation). All rats delivered on day 22 of gestation within a 24-hour interval. Pups from treated and control mothers were counted and weighed before they were killed at 1, 7, 14, 21, 28, and 35 days after birth. No significant differences in offspring survival (there were no stillbirths or deaths during the treatment period), size of litter (6 control mothers, 62 pups; 6 DPH-treated mothers, 65 pups) or weight of offspring were noted between treatment groups.

The effects on [^{3}H]diazepam binding in rats exposed in utero to DPH (Table 2) were different from those in rats treated postnatally with DPH (Table 1) at every age tested. For example, [^{3}H]diazepam binding to cortical membranes from 21-day-old (postnatal age) rats treated in utero with DPH was significantly decreased compared to that in rats of the same age that received DPH 1 hour before they were killed.

The decrease of specific benzodiazepine binding in offspring exposed in utero to DPH can be attributed to a 17 percent decrease in the total number of benzodiazepine binding sites without a significant change in their affinity for [^{3}H]diazepam (Table 2). These decreases in [^{3}H]diazepam binding sites in rats exposed in utero to DPH do not persist, but return almost to control values at 28 and 35 days of age. Similar schedules of exposure to DPH in utero result in decreases in seizure threshold up to 3 weeks after birth, with susceptibility returning to control levels thereafter (*2*).

Thus alterations in benzodiazepine binding can be closely correlated with the convulsant and anticonvulsant effects of DPH at various stages of central nervous system maturation, with decreases in binding being associated with increased seizure susceptibility and enhanced binding with anticonvulsant effects of DPH. Whether these changes in binding are causal or secondary to the convulsant and anticonvulsant actions of DPH is not known. Since changes in benzodiazepine binding have been reported in several animal models of epilepsy (*9*) and in animals that have received certain convulsant agents (*8*) or been subjected to electroshock (*10*), the binding changes may provide a biochemical marker for seizure mechanisms.

The potency of various benzodiazepines against pentylenetetrazole-induced seizures is closely correlated to their effects on [^{3}H]diazepam binding (*11*). In addition to DPH (*3*), other compounds with anticonvulsant activity also alter benzodiazepine binding (*8*). Whether other active anticonvulsant compounds also affect benzodiazepine binding is unknown.

In the present study, the changes in binding in rats exposed to DPH in utero were the opposite of those in rats injected with DPH postnatally. In contrast, exposure in utero to benzodiazepines (*12, 13*) or phenobarbital (*12*) fails to alter consistently the number of benzodiazepine binding sites that develop postnatally. Thus, postnatal alterations in benzodiazepine binding after prenatal exposure may not be generalized to all compounds with anticonvulsant activity. However, the effects of some other drugs have been shown to depend on whether an animal is exposed to them pre- or postnatally. For example, the effects on behavior and striatal [^{3}H]spiroperidol binding in rats subjected to prenatal and early postnatal exposure to the antipsychotic drug haloperidol are the opposite of those in adult rats subjected to long-term treatment with haloperidol (*14*). These data suggest that the fetal central nervous system is especially vulnerable to exposure to at least some psychoactive compounds.

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