Maternal Hyperoxia Greatly Reduces the Incidence of Phenytoin-Induced Cleft Lip and Palate in A/J Mice

Abstract. *The A/J mouse has been used to study the teratogenic effects of phenytoin. The developmental abnormalities produced in offspring of this model are similar to some of the malformations observed in cases of human "fetal hydantoin syndrome." Placing pregnant A/J mice in a hyperoxic chamber after phenytoin injection greatly reduces the incidence of phenytoin-induced cleft lip and palate. These results suggest that phenytoin may affect embryonic development indirectly by altering maternal physiology. This maternally mediated mechanism, and the protection against it afforded by hyperoxia, has general implications for the effects of maternal toxicity on teratogenesis.*

Phenytoin has been used for treating convulsive disorders for over four decades (*1*), but it was not until 1968 that congenital malformations were reported to be associated with the use of phenytoin during pregnancy (*2*). Evidence to date indicates that, in humans and laboratory animals, the ability to metabolize phenytoin is genetically determined (*3*). Similarly, phenytoin teratogenicity in individual species may depend on a genetically determined ability to metabolize the parent compound and its reactive intermediates (*4*).

Phenytoin is teratogenic to A/J mice (*5*). Pregnant animals of this strain receiving intraperitoneal injections of phenytoin (75 mg/kg) on the morning of gestation day 10 have litters with nearly 100 percent cleft lip and palate (*6*). These mice treated at this dose level often develop phenytoin toxicity similar to that produced in humans after intravenous injections for the emergency treatment of status epilepticus or cardiac arrhythmias (*7*) in the form of cardiovascular depression (*8*), cerebellar-vestibular dysfunction (that is, ataxia or disorientation), and other central nervous system disturbances such as hyperactivity and sedation. The effects of phenytoin toxicity are present in A/J mice after injections of 60 to 75 mg/kg, whereas 40 mg/kg (a lower teratogenic dose) does not produce the extreme cerebellar-vestibular components of toxicity, but depression of cardiorespiratory function (*9*) or other physiological alterations (*3*) may occur.

Since phenytoin depresses cardiorespiratory function in the pregnant A/J mouse, it is possible that its teratogenicity to offspring may be related in part to maternal hypoxia. Knowing that hypoxia produced by cardiorespiratory depression can be relieved by elevating the respiratory oxygen (*10*), we evaluated the effectiveness of placing pregnant animals in a hyperoxic environment after phenytoin injections in an effort to reduce maternal toxicity and the associated teratogenesis. We used hyperoxia by procedures similar to those reported by Petter and his co-workers (*10a*) who found that this treatment reduced hereditary limb malformations and associated fetal hemorrhages in rabbits.

Thirty pregnant A/J mice weighing from 22 to 28 g were injected intraperitoneally with phenytoin (60 mg/kg) at 11 a.m. on gestation day 10 (*11*). Phenytoin (Dilantin) was obtained from Parke-Davis, Morris Plains, New Jersey, as a mixture of phenytoin sodium and an aqueous vehicle containing 40 percent propylene glycol and 10 percent ethanol at *p*H 12, adjusted with sodium hydroxide. Immediately after injection, the pregnant animals were divided into two groups and placed in environmental chambers with food (Purina Breeder Chow), water, and bedding material. The 15 animals in the control group were placed in a chamber ventilated with room air, and the remaining 15 animals were placed in an identical chamber ventilated with a constant flow of 50 percent O_2 and 50 percent N_2 bubbled through water. All animals were observed periodically for signs of toxicity until 5 p.m. on gestation day 11, at which time they were returned to their regular cages. Pregnancies were terminated on gestation day 17, and all viable fetuses were examined for external malformations (for example, cleft lip and limb defects) and cleft palate under a binocular dissecting microscope. All resorptions were noted and recorded.

The 15 pregnant animals exposed to hyperoxia therapy after a single phenytoin injection did not exhibit signs of toxicity. In addition, cleft lip and palate were observed in only 25 percent (31 out of 124) of the viable fetuses and no facial hematomas were present (Table 1).

The 15 animals injected with phenytoin but without hyperoxia therapy showed signs of toxicity—irritability, hyperactivity, ataxia, and sedation—and had fetuses with severe teratogenic effects. Most viable fetuses (84 percent; 104 out of 124) had cleft lip with or without cleft palate, and 16 percent had associated subcutaneous hematomas in the malformed orofacial tissues (Table 1). The average litter size was the same in both groups (124 viable fetuses in 15 litters), and in fact there was less embryolethality in the hyperoxia group (3 percent) than in the control group (6 percent). Thus, it seems that the reduction of malformations seen at term in the hyperoxia group is due not to selective killing of malformed embryos but to the effective protection offered by the elevated O_2 concentration.

The highly significant differences ($P < .001$) in the incidence of malformations between the experimental and control groups confirm our hypothesis that hyperoxia protects against phenytoin teratogenicity. This is consistent with Fraser's suggestion (*12*) that cleft lip and palate may result from a combination of genetic and environmental factors. We recently determined that A/J mice exposed to environmental hypoxia (10 percent O_2 and 90 percent N_2) between 11 a.m. on gestation day 10 and 5 p.m. on gestation day 11 have litters with a significantly elevated incidence of cleft lip and palate (*13*).

In earlier studies (*14*) we described the normal development of the mouse primary palate during critical stages of formation (six to ten tail somites) and demonstrated that the surface epithelia of the medial and lateral nasal prominences undergo a complex series of changes critical for their approximation and fusion; we also showed that phenytoin interferes with these epithelial activities in the A/J

Table 1. Amelioration of phenytoin-induced cleft lip and palate and facial hematomas by hyperoxia. Mean and standard error (S.E.) were computed from the data for each litter.

Ventilation of chamber	Number of litters	Cleft lip and palate (%)		Hematomas (%)	
		Range	Mean ± S.E.	Range	Mean ± S.E.
Air	15	66 to 100	85 ± 3.2	0 to 50	16 ± 4.2
50% O_2, 50% N_2	15	0 to 57	25 ± 4.6	0	0
P values (two-tailed)			.001*		.006†

*Differences between the percentages of cleft lip and palate in the two groups were analyzed by means of the Mann-Whitney test (*20*). †A generalization of this test (*21*) was used to calculate the *P* value for hematomas.

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mouse embryo, and we related this interference to the pathogenesis of cleft lip and palate (*14*). In addition, this treatment retards the schedule of embryonic development by 4 to 6 hours (*15*) and produces hypoplasia of the lateral nasal prominences (*16*). These manifestations of abnormal development may result from interference with critical interactions between mother and embryo. Since hyperoxia is so effective in reducing the teratogenicity of phenytoin, it is tempting to speculate that the drug, and its alkaline vehicle in this case, by depressing maternal cardiorespiratory function, indirectly affects embryonic development. This maternally mediated mechanism of teratogenesis can be partly compensated for by increasing the O_2 tension, which restores oxygen delivery to the mother and embryo. The results of the present study are supported by evidence reported by Millicovsky and his co-workers (*17*) that injection of substances that acutely alter maternal cardiovascular function can severely affect the structure and function of the embryonic cardiovascular system and produce craniofacial, limb, and trunk defects. It may be that phenytoin injection decreases uterine circulation, since an earlier study has shown that brief reductions in uterine blood flow at critical times in pregnancy may disrupt embryonic cardiac function and severely compromise the craniofacial region of the developing embryo (*18*). This finding substantiates our hypothesis that the depression of cardiorespiratory function elicited by phenytoin in A/J mice may be responsible in part for the facial clefts seen in the term fetuses. The results of our study demonstrate that a drug does not have to reach the embryo to produce malformations. This concept has general implications for the possible roles of maternal toxicity on teratogenesis.

Although we have shown here that respiratory hyperoxia (50 percent O_2 and 50 percent N_2) reduces the teratogenic effect of phenytoin, it is necessary to determine if elevated O_2 concentrations help the embryo by compensating for depression in maternal cardiorespiratory function or by reducing other effects of phenytoin (*3*) in the maternal-placental-embryonic complex (*19*).

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Measles Virus Nucleotide Sequences: Detection by Hybridization in situ

Abstract. *A tritium-labeled probe that detects measles virus nucleotide sequences was hybridized in situ to cells infected with measles virus and to sections of brain tissue from patients with subacute sclerosing panencephalitis and from patients with multiple sclerosis. The measles virus genome was detected in many cells in subacute sclerosing panencephalitis where this virus would have been missed by methods such as immunofluorescence. Measles virus sequences were also found in two foci in one of four cases of multiple sclerosis. This refined method of hybridization in situ, which can be useful in the search for covert virus infections of man, provides evidence that viruses may be involved in multiple sclerosis.*

In a number of diseases of animals caused by viruses or virus-like agents, symptoms appear long after the infectious process is initiated (*1*). The relevance of these slow infections to diseases of humans was recognized when diseases like kuru and Creutzfeldt-Jakob disease were shown to be transmissible (*2*). Subsequently, the consistent isolation of viruses and the demonstration of viral antigens and particles in affected organs linked a measles-like virus and a papovavirus, respectively, to subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy (PML) (*3, 4*). There are considerable indirect and epidemiological data suggesting that more prevalent human illnesses, including multiple sclerosis (MS), may also be slow infections, with measles virus as the most likely infective agent (*5*), but reproducible evidence of the kind just cited has not been found.

If the measles virus genome is present in MS, but focally distributed and restricted in genetic expression, as is the case in visna, a slow infection of sheep (*6, 7*), then infectious virus, antigens, or particles would not be detected, and viral genetic sequences might not be discovered by conventional hybridization methods. The visna provirus was identified in infected tissues by hybridization in situ (*7*); as now refined, this method is capable of detecting less than a single copy of viral sequences in individual cells (*8*). We therefore used this method to assay brain tissues of patients with MS and with SSPE, the latter serving as a positive control. We can detect the measles virus genome in cells in tissue sections from cases of SSPE in which viral antigens were undetectable by immunofluorescence. We have found the measles virus genome in one of four cases of MS.

The virus-specific tritium-labeled probe used in these studies was prepared by reverse transcription of full-length measles RNA purified from a heat-resis-

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