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Hyperoxia and hypoxia in pregnancy: Simple experimental manipulation alters the incidence of cleft lip and palate in CL/Fr mice

(facial development/genetic defect/maternal physiology/oxygen)

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ABSTRACT **This paper describes alterations in the incidence of cleft lip and palate in CL/Fr mice subsequent to experimental manipulation of maternal respiratory oxygen levels during a critical period of pregnancy. Only a few previous studies have shown that the incidence of some "genetically determined" malformations in mammals can be decreased by environmental procedures. In addition to demonstrating a decreased incidence of cleft lip and palate subsequent to maternal hyperoxia on gestational days 10 and 11 in a genetically susceptible strain, the results of the present study show that hypoxia at this time increases the incidence of cleft lip and palate.**

The mother, placenta, and embryo form a functional system. This biological association makes all three components vulnerable to physiological and pathological changes in any part of the system. The significance of this interdependence is demonstrated by the experimental reduction of the incidence of defects with genetic predisposition by manipulation of the maternal environment. Erway *et al.* (1, 2) demonstrated that abnormal otolith development in the *pallid* mouse (a homozygote for a mutant gene) can be prevented by giving pregnant females a diet containing high levels of manganese. Supplementation with this element was also effective in reducing otolith malformations and agenesis in the analogous *pastel* mink (3). Hereditary brachydactyly in rabbits, an autosomal recessive trait, is associated with hemorrhagic necrosis that destroys the tissues in limbs of fetuses. Petter *et al.* were able to prevent this malformation by subjecting pregnant rabbits of this strain to hyperoxia (4) or by supplementing their diet with folic acid plus vitamin B-12 or with folinic acid (5). Millicovsky and Johnston (6) recently reported that maternal hyperoxia greatly decreases the incidence of phenytoin (Dilantin)-induced cleft lip and palate in A/J mice.

The CL/Fr mouse has an incidence of spontaneous cleft lip (which is almost invariably associated with cleft palate) of 35–40% in near-term fetuses in our colony. Our observations of gravid uteri and concepti of CL/Fr mice often revealed uterine and placental cyanosis and facial hematomas in the fetuses. Based on the hypothesis that these vascular disturbances may result from deficient circulatory function in the maternal–placental–embryonic complex, we designed the present study to evaluate the potentials of hyperoxia and hypoxia to alter the incidence of cleft lip and palate in this strain. We studied the effects of hypoxia because an increase in facial malformations in response to this treatment would provide further evidence that maternal factors may mediate birth defects. To our knowledge, a decrease *and* an increase in the incidence of a genetic malformation in response to variation in the maternal environment has not been demonstrated heretofore.

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MATERIALS AND METHODS

Fifteen pregnant animals were divided into three groups of five each and housed in environmental chambers which contained food, water, and bedding material and permitted control of respiratory gases. The animals were kept in these chambers from 1700 on gestational day 10 until 1700 on day 11 (plug day = 0), the time during which the primary palate of CL/Fr embryos in our laboratory undergoes critical stages of development. One group was exposed to 10% O_2/90% N_2 (hypoxia), one group was exposed to room air (approximately 22% O_2/78% N_2), and the third group was exposed to 50% O_2/50% N_2 (hyperoxia). Then, the animals were returned to their regular cages, and sacrificed on day 18 of gestation (approximately 24–48 hr before term). At this time, laparotomies were performed, and the fetuses were examined for viability, presence of cleft lip and other external malformations, and cleft palate.

Differences in the percentage of cleft lip and palate among groups were analyzed by using the Jonckheere variation of the Mann–Whitney test (7). The hypoxia and hyperoxia experiments were repeated 6 months after the initial study, with four pregnant animals exposed to hypoxia and five exposed to hyperoxia.

RESULTS

Examination of fetuses on day 18 of gestation revealed a highly significant ($P < 0.001$) difference in the percentage of normal (without cleft lip and palate) fetuses among the three groups. In this study, affected animals in all groups had cleft lip associated with cleft palate. Only 11% (range, 0–29%; SEM, 6.6) of the fetuses from the group exposed to hypoxia were normal, 63% (37–87%; SEM, 8.1) of those exposed to air were normal, and 87% (78–100%; SEM, 4.1) of those exposed to hyperoxia were normal. The proportion of fetuses without cleft lip and palate in the group placed in the chamber ventilated with room air was the same as that of untreated control litters of the CL/Fr colony in our laboratory for the past 2 years. Resorptions resulted from exposure to hypoxia but not from exposure to room air or hyperoxia. Results from our repeat experiments with hypoxia and hyperoxia confirmed that both maternal treatments alter the incidence of cleft lip and palate as indicated by our initial results. Only 19% (0–33%) of the fetuses from the repeat hypoxia group were normal, and 86% (83–90%) of the fetuses from the repeat hyperoxia group were normal.

DISCUSSION

The causes of spontaneous congenital defects have puzzled mankind for many centuries (8). A "multifactorial/threshold" hypothesis (9, 10) suggests that malformations may result from interactions between genetic predisposition and environmental factors. It is currently believed that this concept may also apply to orofacial malformations (11). Previous studies have demonstrated that orofacial malformations may result from alterations in maternal physiologic status (12–14). This study demonstrates that the incidence of cleft lip and palate in genetically predisposed CL/Fr mice can be decreased or increased by altering the O_2 concentration in the maternal environment during a critical gestational period.

The CL/Fr mouse should be valuable in our efforts to understand the complex interactions of the maternal–placental–embryonic system in teratogenesis. Because the incidence of cleft lip and palate in CL/Fr mice is increased by hypoxia, this strain might serve to identify other environmental factors that may be teratogenic in humans with genetic susceptibility for this malformation. Potentially of greater significance is the fact that the incidence of cleft lip and palate is significantly decreased by hyperoxia, strongly indicating that this animal should also be useful in evaluating environmental measures that may be applicable in the prevention of human congenital malformations in general.

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