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Prenatal hormones alter antioxidant enzymes and lung histology in rats with congenital diaphragmatic hernia

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[2]*Division of Pediatric Respiratory Medicine,* [1]*Department of Pediatric Surgery, Department of Pediatrics, and* [5]*Department of Biochemistry, Erasmus University Rotterdam and University Hospital/Sophia Children's Hospital, 3015 GJ Rotterdam, The Netherlands;* [3]*Department of Pediatric Surgery, Pediatric Research Laboratories, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114 and* [4]*Department of Pediatric Surgery, Hospital Clinic St. Joan de Deu, 08034 Barcelona, Spain.*

IJsselstijn, Hanneke, Bella A. Pacheco, Asteria Albert, Wim Sluiter, Patricia K. Donahoe, Johan C. de Jongste, Jay J. Schnitzer, and Dick Tibboel. Prenatal hormones alter antioxidant enzymes and lung histology in rats with congenital diaphragmatic hernia. *Am. J. Physiol.* 272 (*Lung Cell. Mol. Physiol.* 16): L1059–L1065, 1997.— Prenatal administration of dexamethasone (Dex) and thyrotropin-releasing hormone (TRH) synergistically enhances lung maturity, but TRH suppresses the antioxidant enzyme activity. Prenatal hormonal therapy improves alveolar surfactant content and lung compliance in rats with congenital diaphragmatic hernia (CDH). In full term neonatal rats with CDH we studied the effects of prenatal Dex or Dex + TRH on antioxidant enzyme activity at birth, on survival, and on lung morphometry after 4 h of ventilation with 100% O_2. CDH was induced by administration of 2,4-dichlorophenyl-*p*-nitrophenylether (Nitrofen) on gestational *day 10*. Dex + TRH-treated CDH rats had lower activity of glutathione reductase after birth than did sham-treated CDH pups. Dex-treated and sham-treated pups had similar antioxidant enzyme activity. Hormonal treatment did not change survival during ventilation. The average airspace volume increased in Dex-treated CDH pups after ventilation, with a small synergistic effect after addition of TRH. On the basis of our findings, we speculate that prenatal administration of Dex is the best choice to improve lung maturity and airspace volume in CDH patients.

glucocorticosteroids; thyroid-releasing hormone; artificial ventilation; morphometry; newborn animal

THE EFFECTS OF PRENATAL application of corticosteroids and thyroid hormones on lung maturity and on the antioxidant enzyme system have been studied experimentally in several animal models (7, 9, 13, 18). It appeared that dexamethasone (Dex) and thyroid hormone have a synergistic effect on the development of the surfactant system but have a negative effect on antioxidant enzyme activity (18). Clinically, the effects of prenatal hormonal therapy have been studied with respect to mortality and morbidity in preterm children (1, 2). Administration of glucocorticoids to women at risk of preterm delivery was recommended (2), but the addition of thyrotropin-releasing hormone (TRH) to glucocorticoids is still debated because maternal and perinatal risks have been reported (1).

Children with congenital diaphragmatic hernia (CDH) have abnormal morphological development of the lungs (16), which may lead to respiratory insufficiency shortly after birth. Several publications suggest that in CDH the lungs are biochemically immature (12, 16), although normal lecithin-to-sphingomyelin ratios in amniotic fluid of CDH patients have been reported (27). More recent data suggest, however, that infants with CDH do have surfactant deficiency (15). Indications for lung immaturity in CDH have been found in several animal studies of CDH using sheep and rats (10, 25).

In our previously described rat model of CDH (28) and in lambs with prenatal CDH, glucocorticoids improved the biochemical and morphological immaturity at birth and the compliance of the lungs (14, 21, 24, 26). Prenatal administration of Dex in combination with TRH also improved lung compliance (14) and showed the best effects on disaturated phosphatidylcholine and glycogen content of the lungs in rat pups with CDH at birth (26). In this CDH model, the effects of prenatal application of glucocorticoids and TRH on the antioxidant enzyme system and on the tolerance to hyperoxia and barotrauma have not been studied yet. We hypothesized that prenatal glucocorticoids would improve survival and lung morphology after artificial ventilation with 100% O_2, with synergistic effects on lung morphology after the combination of glucocorticoids and TRH. Moreover, we hypothesized that the combination of Dex and TRH might have a negative effect on antioxidant enzyme activity. Therefore, in newborn rats with CDH, we evaluated the effects of prenatal hormonal therapy with Dex or the combination of Dex and TRH on the antioxidant enzyme activity at birth and on survival and lung morphometry after a short period of hyperoxic artificial ventilation.

MATERIALS AND METHODS

Animal model. Female Sprague-Dawley rats (Harlan Olac), weighing ~250 g, were mated during 1 h (*day 0* of gestation). Ten of seventeen pregnant rats received 100 mg of 2,4-dichlorophenyl-*p*-nitrophenylether (Nitrofen; Rohm Haas, Philadelphia, PA) on *day 10* of gestation, as described before (28); the remaining seven rats provided control pups. Nitrofen results in a diaphragmatic defect with lung hypoplasia in up to 80% of the offspring (28). Food and water were supplied ad libitum during the whole period of pregnancy. At gestational *day 22* (term = 22–23 days) the dam was anesthetized by inhalation of diethylether, and a caesarean section was performed. The

mean numbers of rat pups in the Nitrofen-exposed and control litters were 13.1 ± 0.8 and 13.6 ± 0.8, respectively.

All animal experiments were performed after approval of the Animal Care and Use Committee of the Erasmus University, Rotterdam, The Netherlands.

Prenatal hormonal therapy. Six different study groups were created: sham-treated CDH rats (2 litters) and controls (2 litters), Dex-treated CDH rats (5 litters) and controls (2 litters), and Dex + TRH-treated CDH rats (3 litters) and controls (3 litters).

On *days 19* and *20*, i.e. 72 and 48 h before delivery, pregnant dams received 0.25 mg/kg Dex sodium-phosphate in 0.2 ml ip of saline, the lowest dose that is known to result in biochemical and morphometrical improvement with the least effects on somatic and pulmonary growth (24). TRH (Calbiochem, La Jolla, CA) was administered intraperitoneally to the pregnant dams as a loading dose (25 μg/kg in 0.5 ml of saline) and by an implanted osmotic minipump (Alzet pump, model 1003D; Alza, Palo Alto, CA) through which TRH was continuously administered intraperitoneally in a dose of 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (18). This pump was implanted intraperitoneally on *day 20* of gestation under short anesthesia with diethylether through a small midline incision using a sterile technique and provided TRH continuously for 48 h. Sham treatment was given using intraperitoneal injections of saline and implantation of an osmotic pump filled with saline.

Measurement of antioxidant enzyme activity. Five to eight of the newborn rats per group were killed for antioxidant enzyme measurement directly after birth. The presence and size of the diaphragmatic defects were assessed in Nitrofen-exposed rats. To obtain a homogeneous study group, Nitrofen-exposed rat pups without CDH ($n = 8$) were excluded. The numbers of rat pups studied per group were $n = 5$ in sham-treated CDH rat pups and controls, $n = 6$ and $n = 5$ in Dex-treated CDH pups and controls, respectively, and $n = 5$ in Dex + TRH-treated CDH pups and controls.

After removal of the heart-lung block, the lungs were stripped of nonpulmonary tissue, weighed, frozen with liquid nitrogen, and stored at −70°C until further processing as previously described (23). All biochemical analyses were performed on lungs from separate rat pups. After thawing, the lungs were diluted 1:15 (wt/vol) in ice-cold phosphate-buffered saline and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) for 15 s at maximum speed. Next, the suspension was sonicated for 10 s on ice. In this crude suspension, concentrations of protein and DNA were estimated as described previously (23). To determine antioxidant enzyme, the crude suspensions were centrifuged at 20,000 *g* for 30 min, and the pellets were discarded. The activities of glutathione peroxidase and catalase were measured as described before (23). Glutathione reductase activity was determined as described by Goldberg and Spooner (11). Superoxide dismutase (SOD) activity was determined by using the SOD-525 method (R & D Systems, Abingdon, UK).

Correction for blood contamination was performed as follows. From three different control litters, the red blood cells of three to five rat pups were collected for measurement of hemoglobin (22) and antioxidant enzyme activity. The antioxidant enzyme activity per milligram of hemoglobin was thus determined. The hemoglobin concentration was also measured in the lung suspensions. The antioxidant enzyme activity resulting from blood contamination was calculated and subtracted from the total antioxidant enzyme activity in the lung suspensions. To facilitate comparison and to exclude differences merely based on differences in lung weight, the activities of glutathione reductase, glutathione peroxidase, catalase, and SOD were all expressed as units per milligram of lung DNA.

Artificial ventilation. After birth, the newborns were weighed, anesthetized with 30 mg/kg ip pentobarbital sodium, paralyzed with 0.08 mg/kg ip pancuronium bromide, intubated with a 24-gauge intravenous catheter (Neoflon; Viggo-Spectramed, Helsingborg, Sweden) with an atraumatic stent, transferred to a multichambered body plethysmograph heated to 37°C, and connected in the supine position to a modified Servo 900B ventilator (Siemens-Elema, Solna, Sweden). Pressure-controlled ventilation was started using the following respirator settings: peak inspiratory pressure (PIP), 25 cmH_2O; positive end expiratory pressure, 3 cmH_2O; frequency, 40 cycles/min; fraction of inspired O_2 concentration, 1.0; and inspiratory-to-expiratory ratio, 1:2. A previously performed study revealed that opening pressures of 25 cmH_2O were needed to obtain a good lung aeration pattern but that continuous ventilation with this peak pressure resulted in a high incidence of pneumothorax (Tibboel, unpublished data). Therefore, PIP was reduced to 17 cmH_2O after 30 min. The incidence of pneumothorax, absent heart action, or other complications related to insufficient ventilation were recorded in ventilated rat pups. In case of death the lungs were processed immediately for histological examination. After 4 h, the surviving pups were killed. Autopsy revealed whether a diaphragmatic defect in Nitrofen-exposed pups was present.

Histological studies. The trachea was cannulated, the thorax was opened, and the lungs were inflated at a constant pressure of 20 cmH_2O with 10% Formalin as described by Burri et al. (6). The distended lungs were removed from the thoracic cavity, and the trachea was ligated to maintain lung inflation during fixation for 24 h. After 24 h, the lung volume (V_L) was measured by the volume displacement method (20). The fixed tissue was dehydrated in a graded alcohol series, embedded in paraffin, and cut in 1-cm slices in the parasagittal plane. Random 1-cm^2 specimens were taken from the lung for histological examination; 8-μm coronal sections were cut and stained with hematoxylin and eosin.

Morphometry was performed using techniques adapted from Weibel (29) and Emery and Mithal (8). Histological sections were examined with the aid of a Nikon microscope (Microphot-FXA; Nikon, Melville, NY) at ×100 magnification via a charge-coupled device color video camera (DXC-151; Sony, Park Ridge, NJ) linked to a Sony color television monitor overlaid with a 42-point equidistant counting grid calibrated with an individual probe length line (Z) where Z = 83 μm. Lungs from CDH rats (sham, $n = 7$; Dex-CDH, $n = 9$; Dex + TRH-CDH, $n = 5$) in each treatment group were subsequently evaluated. The complete coronal sectional area of each lung was analyzed using three different histological sections (6–10 fields per lung per slide) for each animal. Each coronal sectional area encompassed 18–30 fields per lung; the right and left lungs were studied separately, and these data were later combined because there were no significant differences between both lungs.

Volume fractions were established by counting test points falling on airspaces (volume fraction of airspaces, V_{Valv}), alveolar septa (volume fraction of airspace walls), airways (volume fraction of airways), and non-gas-exchanging elements, i.e., all other structures (volume fraction of other elements). The average interairspace wall distance (L_M) was calculated from the formula $L_M = 2 \times Z$/number of transections of septa within the 21 grid lines (or intercept number). The number of airspaces per unit area (N_A) was determined by dividing the airspace number into the area of the counting grid. Airspace number per unit volume (N_V) was calculated from the formula $N_V = K(N_A)^{3/2}/\beta(V_{Valv})^{1/2}$, where K is the

Table 1. *Mean birth weights in newborn rats with and without CDH following prenatal hormonal modulation*

	CDH, g	*n*	Control, g	*n*
Sham	4.55 ± 0.11*	16	5.19 ± 0.06*	24
Dex	3.80 ± 0.07†	40	4.43 ± 0.09	16
Dex + TRH	3.62 ± 0.03	47	4.63 ± 0.09	25

Values are means ± SE; *n*, no. of animals per group; Dex, dexamethasone given intraperitoneally 72 and 48 h before delivery; Dex + TRH, addition of thyrotropin-releasing hormone (TRH) intraperitoneally during 48 h before delivery. *Significantly higher than all other prenatal treatments in the same group ($P < 0.001$). †Significantly higher than Dex + TRH in the congenital diaphragmatic hernia (CDH) group ($P < 0.05$).

coefficient size distribution constant (taken to be 1) and β = 1.55, from the Weibel and Gomez shape constant (29). Total number of airspaces (N_{AT}), total internal surface area (SA), and average airspace volume (AAV) were estimated from the following formulas: $N_{AT} = (N_V)(V_L)$, $SA = 4(V_L)(V_{Valv})/L_M$, and finally $AAV = (V_{Valv})(V_L)/N_{AT}$.

Radial saccular counts, a measure of the complexity of the respiratory acinus, were determined by counting the number of airspaces lying on a line drawn perpendicularly from the center of a terminal or respiratory bronchiole to the closest edge of the acinus (pleural or lobular connective tissue septum) (8).

Data analysis. All data are presented as means ± SE unless stated otherwise. Because data were normally distributed, differences among the groups were tested by two-way analysis of variance and one-way analysis of variance with the Student-Newman-Keuls and Bonferroni-Dunn tests. χ^2 Tests were used for proportions. Statistical significance was assumed at the 5% level.

RESULTS

In both CDH and controls, prenatal hormonal modulation resulted in lower birth weights compared with sham-treated pups (Table 1; $P < 0.001$). At birth, CDH rat pups had lower lung-to-body weight ratios than controls (Table 2; $P < 0.001$), but hormonal modulation did not alter these ratios. The protein-to-DNA ratios were not different between the study groups (Table 2).

In controls, prenatal hormonal treatment with the combination of Dex and TRH led to a significantly lower activity of glutathione reductase ($P < 0.001$) and to a higher activity of glutathione peroxidase ($P = 0.002$); the activities of catalase and SOD were not impaired (Fig. 1). In CDH, only the activity of glutathione reductase was significantly lower in the Dex + TRH group ($P = 0.01$). The activity of glutathione peroxidase did not increase as was the case in controls (Fig. 1). Prenatal treatment with Dex alone led to a higher activity of glutathione peroxidase and catalase in controls ($P = 0.002$ and $P = 0.03$, respectively). A similar trend to a higher activity of glutathione peroxidase and catalase was present in CDH, but these differences did not reach the level of significance.

Table 2. *Lung-to-body weight ratios and lung protein-to-DNA ratios in newborn rats with and without CDH at birth*

	Lung-to-Body Weight Ratio, mg/g		Protein-to-DNA Ratio, μg/μg	
	CDH	Control	CDH	Control
Sham	15.9 ± 0.8	26.6 ± 1.3*	4.5 ± 0.2	4.5 ± 0.1
Dex	13.8 ± 0.7	28.5 ± 0.5*	4.6 ± 0.1	4.9 ± 0.2
Dex + TRH	16.7 ± 1.1	28.2 ± 0.6*	4.6 ± 0.3	4.8 ± 0.2

Values are means ± SE; $n = 6$ in the Dex-CDH group, $n = 5$ in all other groups. *Significantly different from CDH, same treatment; $P < 0.001$.

Intubation was successful in 50–70% of control rats irrespective of prenatal hormonal treatment. In the CDH rat pups, intubation was successful in 70% (7 of 10) of the sham group; this was 26 (9 of 34) and 28% (12 of 43) in the Dex and Dex + TRH groups, respectively ($P < 0.01$). Birth weights from rat pups that could not be intubated successfully did not differ from those that could be intubated (data not shown). The mortality rate or the incidence of pneumothorax were not significantly influenced by prenatal hormonal modulation (Fig. 2).

Lung morphometry of CDH rat pups showed a significant increase of the AAV with a decrease in the number of airspaces in the Dex group compared with sham-CDH group (Table 3). The total internal SA was unchanged. These significant effects were stronger in the Dex + TRH group (Table 3). Representative pictures showing the effects of prenatal hormonal modulation on lung histology after artificial ventilation in CDH are shown in Fig. 3; there was no evidence of septal disruption or diffuse alveolar damage in any of the treatment groups.

DISCUSSION

In this study, we evaluated the effects of prenatal hormonal treatment on a number of parameters in a rat model of CDH. The major findings after artificial ventilation were that the survival rate was not influenced by hormonal therapy and that an increase in airspace volume without changes in the total internal SA was observed after prenatal Dex, with stronger effects after the addition of TRH. In addition, it is noteworthy that prenatal Dex + TRH resulted in decreased glutathione reductase activity in CDH rat pups and controls at birth. Hormonal treatment led to a significantly higher glutathione peroxidase activity in controls but not in CDH.

In the present study, we confirmed the finding that prenatal hormonal modulation reduces birth weight, both in healthy full-term rats and in CDH rats (9, 13, 14, 18, 24), and found no effect on lung-to-body weight ratios and lung protein-to-DNA ratios. That prenatal hormonal therapy has no effect on lung-to-body weight ratios and protein-to-DNA ratios suggests that lung growth is reduced to the same extent as body weight, which is in agreement with earlier studies (9, 13).

The effects of prenatal hormonal therapy on antioxidant enzyme activity at birth have previously been studied in full-term spontaneously born healthy rats (9, 13, 18). Prenatal Dex administered 48 and 24 h before the expected time of delivery did not change the activities of catalase, glutathione peroxidase, and SOD (9, 13). We found that in control pups the activities of catalase and glutathione peroxidase increased signifi-

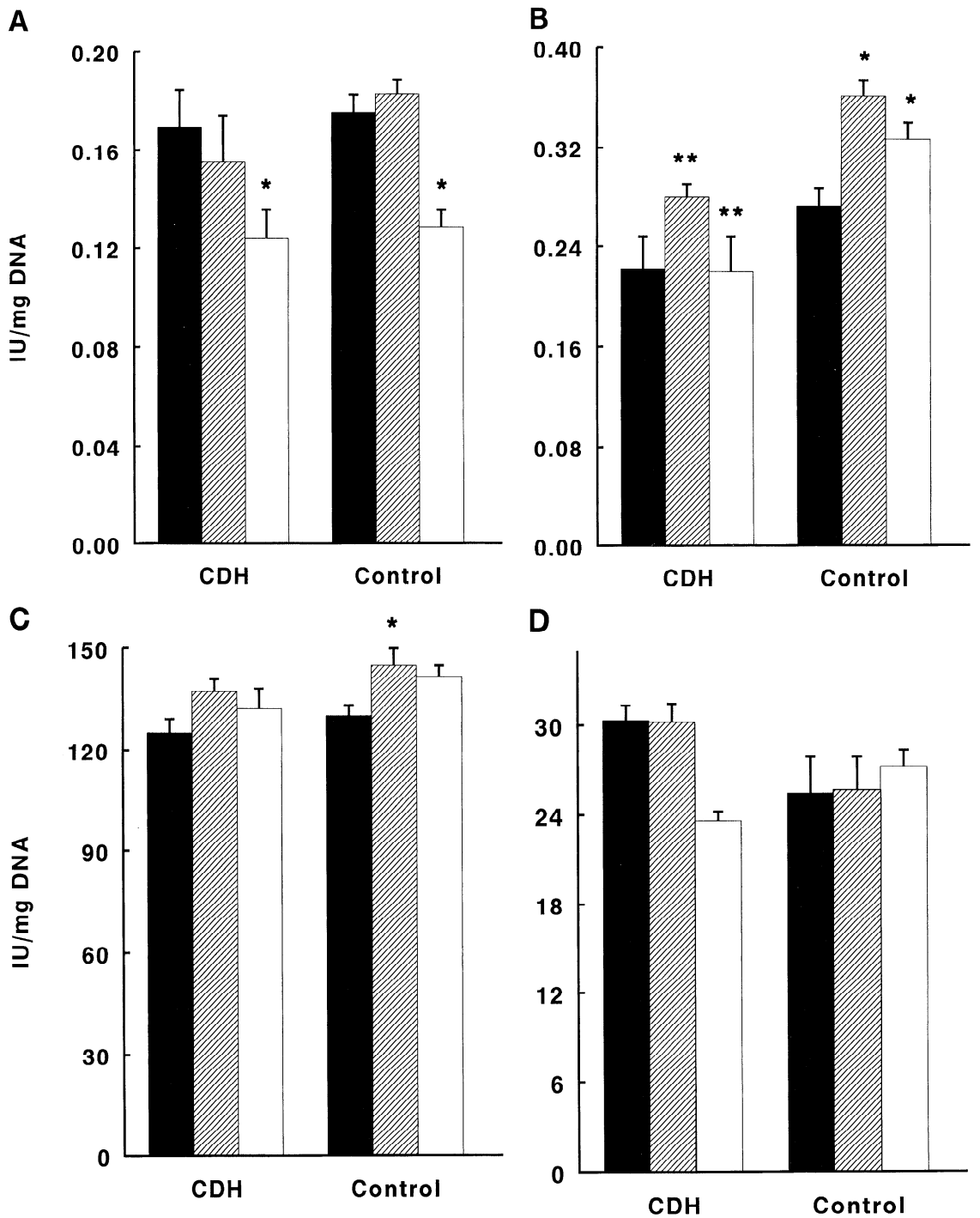


Fig. 1. Antioxidant enzyme activity after birth in lungs of newborn rats with congenital diaphragmatic hernia (CDH) after prenatal hormonal modulation. All data are expressed as IU/mg DNA (mean and SE). Five to six animals per group were studied. Filled bar, sham treatment; hatched bar, dexamethasone (Dex) treatment; open bar, Dex + thyrotropin-releasing hormone (TRH) treatment. *A*: glutathione reductase. *B*: glutathione peroxidase. *C*: catalase. *D*: superoxide dismutase. *Significant difference compared with all other treatments in the same group, $P < 0.05$. **Significant difference compared with the same treatment in controls, $P < 0.05$.

cantly after prenatal treatment with Dex 72 and 48 h before delivery by caesarean section. Our timing and dosage of Dex administration, which was based on a previous experiment in rat pups with CDH (24), may probably explain the differences compared with other studies.

Rodriquez and co-workers (18) reported, in full-term normal rats, a negative effect of prenatal Dex in combination with TRH on the activities of catalase, glutathione peroxidase, and SOD compared with sham-treated rat pups. Our observation that only the activity of glutathione reductase decreased after prenatal Dex + TRH is not in accordance with their findings. The dosage and timing of TRH was similar in both studies, and it is not clear whether the difference in Dex administration may explain the dissimilarity. However,

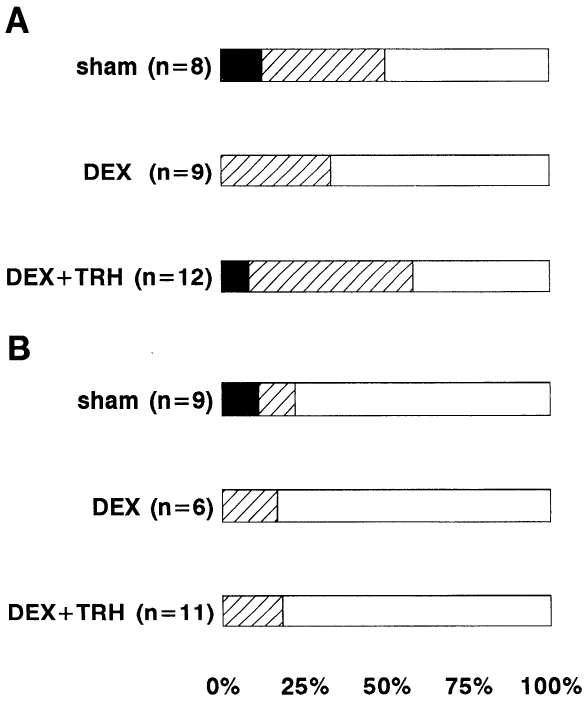


Fig. 2. Incidence of pneumothorax and mortality in ventilated rat pups with CDH (*A*) and controls (*B*) after prenatal hormonal modulation. Number of animals that died from pneumothorax (solid bars) or by other causes (hatched bars) and the survivors (open bars) after 4 h of ventilation are indicated for each group. No significant differences were found. Dex, dexamethasone given intraperitoneally 72 and 48 h before delivery. Dex + TRH, addition of TRH intraperitoneally during 48 h before delivery.

both their study and ours showed negative effects of prenatal Dex in combination with TRH on antioxidant enzyme activity. A lower activity of glutathione reductase may result in a lower production rate of reduced glutathione, which is the substrate for the reduction of organic peroxides and hydrogen peroxide catalyzed by glutathione peroxidase. Catalase can convert hydrogen peroxide to water but is not able to catalyze the reduction of organic peroxides. The decreased activity of glutathione reductase after prenatal treatment with Dex and TRH may thus result in accumulation of organic peroxides (17).

In the present study, up to 75% of CDH pups in the groups with prenatal hormonal therapy died during the procedure of intubation. This may be due to their low birth weights and not to structural changes induced by prenatal hormonal therapy, because controls, which all had a birth weight of >4 g, could be intubated easily irrespective of the prenatal hormonal therapy. Although the number of ventilated newborns studied was small, the results of the present study indicate that prenatal glucocorticoids and TRH did not affect the mortality rate or incidence of pneumothorax. Previous studies in full-term healthy rat pups exposed to hyperoxia showed that Dex improves survival (9), whereas TRH or the combination of Dex and TRH has a negative effect on the survival rate (19). However, these effects in nonventilated rat pups were observed after at least 1 wk.

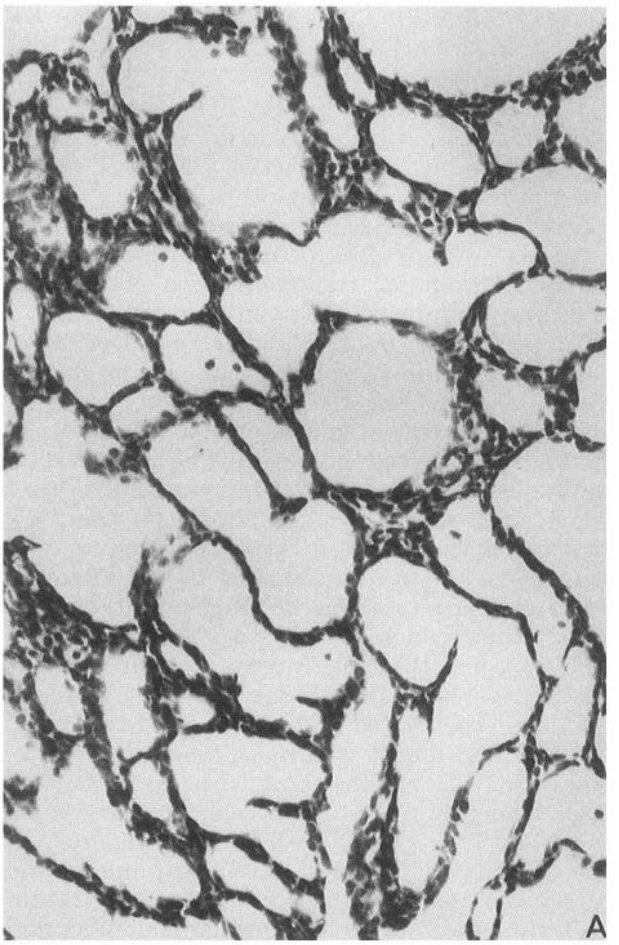
Lung morphometry showed higher AAV in Dex-treated and Dex + TRH-treated CDH pups than in the sham-CDH group, which is in accordance with previous findings in nonventilated CDH rats (24). Artificial ventilation may explain that the AAV in the present study was three times higher than that in lungs of nonventilated pups (24). In neonatal rats and lambs with CDH, prenatal treatment with glucocorticoids significantly improved lung compliance (14, 21). The addition of TRH to Dex further improved alveolar stability and lung morphology in nonventilated rat pups with CDH (14). The increased lung compliance may result in an easier dilatation of airspaces and thus explain the observed higher AAV. That we did not observe evidence of septal disruption or diffuse alveolar damage suggests that prenatal hormonal therapy has beneficial effects on lung expansion that may have implications for the treatment of CDH patients: PIP may be lowered as soon as the lungs have been inflated properly, thus reducing the risk of volutrauma.

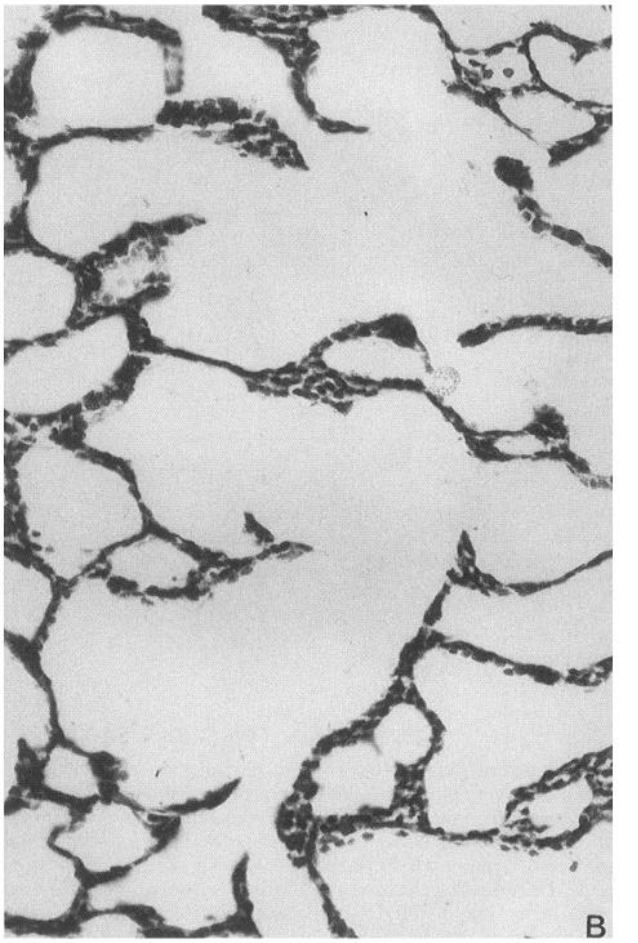
The V_{Valv} and the total internal SA did not change after prenatal hormonal therapy as a result of a lower N_{AT} in the Dex-treated and Dex + TRH-treated CDH rats. The unchanged internal SA together with the increase in V_{Valv} is consistent with the premise that prenatal hormonal therapy accelerates lung maturation but not lung growth (24). Larger airspaces with a decreased number of alveoli have been found in premature rhesus monkey fetuses after prenatal treatment with betamethasone (3). In addition, a similar finding has been ascribed to inhibited septation in rats at the age of 28 days after exposure to hyperoxia in combination with postnatal Dex administration (4). Because septation in the rat starts around the third or fourth

Table 3. *Morphometric analysis of lungs after 4 h of artificial ventilation in newborn rats with CDH. Effect of prenatal hormonal modulation*

	Sham	Dex	Dex + TRH
Volume fraction of airspaces	0.63 ± 0.01	0.61 ± 0.02	0.62 ± 0.01
Volume fraction of airspace walls	0.093 ± 0.003	0.113 ± 0.007*	0.125 ± 0.007†
Volume fraction of airways	0.089 ± 0.009	0.123 ± 0.012*	0.142 ± 0.011†
Volume fraction of other elements	0.187 ± 0.009	0.156 ± 0.008*	0.118 ± 0.009‡§
Average airspace volume, $\mu m^3 \times 10^5$	3.06 ± 0.12	4.53 ± 0.30†	5.74 ± 0.48‡§
Total no. of airspaces, $\times 10^5$	4.80 ± 0.16	3.31 ± 0.14‡	2.81 ± 0.29‡
No. of airspaces/unit volume, μm^{-3}	2.11 ± 0.07	1.45 ± 0.06‡	1.23 ± 0.12‡
Total internal surface area, $\mu m^2 \times 10^{10}$	3.60 ± 0.21	3.71 ± 0.20	3.73 ± 0.17
Interairspace distance, μm	16.8 ± 0.75	16.1 ± 0.98	15.4 ± 0.60
Radial saccular count	4.45 ± 0.12	4.06 ± 0.08†	4.45 ± 0.08§

Values are means ± SE; $n = 7$ for Sham group, $n = 9$ for Dex group, and $n = 5$ for Dex-TRH group. *$P < 0.05$; †$P < 0.002$; ‡$P = 0.0001$ compared with sham-treated CDH; §$P \leq 0.01$ compared with Dex-treated CDH by the Bonferroni-Dunn test.





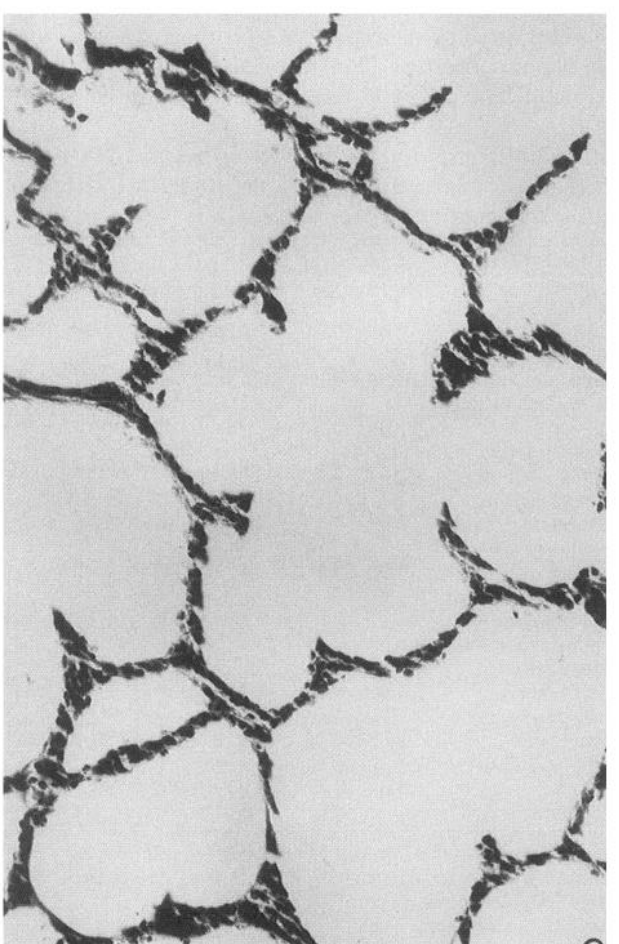


Fig. 3. Representative pictures from lungs of CDH rats after 4 h of artificial ventilation. Lungs were inflated at a constant pressure of 20 cmH_2O with 10% Formalin. *A*: sham-treated CDH. *B*: Dex-treated CDH. *C*: Dex + TRH-treated CDH. Magnification ×200.

postnatal day (5), it seems unlikely that inhibited septation explains the present findings.

In conclusion, prenatal Dex had a positive effect on the AAV in ventilated newborn rats with CDH, and only a small synergistic effect was observed after the addition of TRH. However, the activity of glutathione reductase decreased significantly after prenatal treatment with Dex and TRH. We speculate that our findings of hormonal therapy in rat pups extend to CDH patients as well. If that hypothesis is correct, antenatal administration of Dex as a monotherapy will offer better prospects for randomized trials in prenatally diagnosed children with CDH than the combination of Dex and TRH.

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