Dexamethasone-induced changes in lung function are not prevented by concomitant treatment with retinoic acid

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Received 30 October 2001; accepted in final form 1 February 2002

WITH THE RECENT SUCCESS in increasing the survival of the preterm infant has come an ongoing concern about the potential deleterious effects of the therapeutic interventions used to prolong life. One such intervention, the use of the synthetic glucocorticoid dexamethasone (Dex), was shown in several definitive studies to impair alveolar formation, a process that occurs primarily after birth in many species, including rat and human (15, 17, 32). Although the mechanism(s) is not well characterized, considerably less is known about the effects on lung function. In mature rats treated with Dex for various periods of time during the first 2 postnatal wk, several groups have documented a leftward shift in the static pressure-volume (P-V) deflation curves, consistent with enlarged air spaces and a decrease in elastic recoil. Sahebjami and Domino (27) attributed the decrease in alveolar surface area at 1 mo to a decreased rate of cell proliferation based on decreases observed in total DNA content and concentration. Massaro and colleagues (15) postulated that there is a critical period during which alveolar septation occurs and that Dex treatment during this period blocks septation. More recently, Tschanz and co-workers (33) reported that treatment with Dex induced the maturation of the pulmonary microvasculature, which led to the premature transition of a double to a single capillary network, precluding the emergence of alveolar septa where this single capillary network occurred. The results of these and other related studies suggest that Dex-induced impairment of septation could pose a substantial risk for a population of infants in whom septation is also inhibited by ventilatory support with supplemental O2 and by preterm birth per se (5, 14).

In the context of a mounting concern regarding the potential adverse impact of Dex on lung development in the preterm infant, the observation that this effect was ameliorated by simultaneous treatment with all-trans retinoic acid (RA) was met with considerable enthusiasm. Massaro and Massaro (17) reported, and other investigators (34) have since confirmed, that treatment with both agents during the period of bulk alveolar formation resulted in smaller, more numerous alveoli at 14 days, suggesting that the adverse effects of one therapeutic agent could be offset by treatment with another. Although required for normal fetal and postnatal growth and development, treatment with RA may not be without risk. Known to influence cell proliferation, cell differentiation, and apoptosis, high doses of RA have the potential to influence critical developmental processes in the lung and elsewhere. Furthermore, the potential for adverse effects resulting from treatment with Dex and RA in combination has not been fully explored.

Although the effects of Dex on lung morphology are well characterized, considerably less is known about the effects on lung function. In mature rats treated with Dex for various periods of time during the first 2 postnatal wk, several groups have documented a leftward shift in the static pressure-volume (P-V) deflation curves, consistent with enlarged air spaces and a decrease in elastic recoil. Sahebjami and Domino (27) attributed the decrease in alveolar surface area at 1 mo to a decreased rate of cell proliferation based on decreases observed in total DNA content and concentration. Massaro and colleagues (15) postulated that there is a critical period during which alveolar septation occurs and that Dex treatment during this period blocks septation. More recently, Tschanz and co-workers (33) reported that treatment with Dex induced the maturation of the pulmonary microvasculature, which led to the premature transition of a double to a single capillary network, precluding the emergence of alveolar septa where this single capillary network occurred. The results of these and other related studies suggest that Dex-induced impairment of septation could pose a substantial risk for a population of infants in whom septation is also inhibited by ventilatory support with supplemental O2 and by preterm birth per se (5, 14).

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crease in recoil properties (15, 27, 32). Information regarding the effects of Dex treatment on resting breathing parameters, dynamic compliance (Cdyn), or gas exchange in the mature lung has not been provided by subsequent studies, nor have the combined effects of Dex and RA been explored.

In the present study, we assessed in male and female rats the effects of Dex and RA, alone and in combination, on resting breathing, static and dynamic compliance, and the ventilation required to maintain O\textsubscript{2} saturation at ≥90%. One objective of these studies was to determine the extent to which lung function at 1 mo of age is compromised in rats treated with Dex during the period of bulk alveolar formation, days 4–13 in the rat. A second objective was to evaluate the extent to which concomitant treatment with RA prevented Dex-induced changes in lung function. As anticipated on the basis of the morphometric changes reported to occur in Dex-treated lungs, significant changes in lung function were seen in these animals at 1 mo of age. These changes were not ameliorated by concomitant treatment with RA, however. We also noted that lung recoil was decreased by Dex treatment to a greater extent in female than in male rats, suggesting that earlier studies carried out in male rats only may have underestimated the potential for adverse effects associated with Dex treatment.

METHODS

Animals. In each of three separate experiments, timed-pregnant Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were obtained on days 17–18 of gestation (full term = 22 days). The day of birth was designated day 0. On postnatal day 3, pups were randomized to new litters consisting of eight or nine pups each that were similar with respect to weight, ratio of male to female pups, and percentage of pups born to each of the dams.

Drugs and treatments. We used two injection protocols. Each protocol consisted of the same five treatment groups and differed only with respect to the ages at which Dex and RA treatments were initiated. Pulmonary function studies were conducted on 35 rats treated according to protocol 1 and 31 animals treated according to protocol 2. The O\textsubscript{2} saturation studies were conducted on a total of 25 rats treated according to protocol 1.

In protocol 1, pups in each of two litters received daily injections of RA (500 μg/kg sc; Sigma, St Louis, MO) in cottonseed oil diluent on postnatal days 3–13. Pups in a third litter were injected with equivalent volumes of diluent alone. On postnatal days 4–13, the fourth litter received subcutaneous injections of 0.25 μg of Dex in saline; the fifth litter was treated with saline alone. In addition, one of the litters treated with 500 μg/kg RA on days 3–13 was also treated with 0.25 μg of Dex on days 4–13. In protocol 2, the ages at which treatments with Dex and RA were initiated were reversed. Treatment with Dex began on postnatal day 3, and treatment with RA began on day 4. Dex, RA, and Dex + RA treatments were continued until postnatal day 13. Diluent injections were as described above.

Before the bias-flow, pulmonary function, and O\textsubscript{2} saturation measurements, the rats were anesthetized with a normal saline solution (0.20 ml/100 g body wt) containing 2.5 mg of pentobarbital sodium and 0.5 mg of diazepam. Lidocaine hydrochloride (40 mg/ml) was applied as needed to the tracheal region during the tracheotomy procedure. When supplemental anesthesia was required, the rats were injected intraperitoneally with 0.5–1.0 mg of ketamine in 0.05–0.1 ml.

Pulmonary function studies. Studies were conducted in 30- to 39-day-old rats to measure respiratory airflow and pressure at the airway opening (Pao). A catheter was placed in the esophagus to measure esophageal pressure (Pes). Anesthetized rats in the supine position were tracheotomized in the midcervical region of the trachea. The animals were warmed using a heating pad and a heat lamp, and body temperature was monitored continuously with a rectal probe (model 747 digital thermistor, Omega Engineering, Stamford, CT; model BAT-12, Sensortek, Clifton, NJ). The airway pressure and Pes transducers were calibrated with water manometers at the beginning and end of each day. Pressure and flow signals were amplified (Pes: Gould Universal amplifier, Cleveland, OH; airway pressure and flow: Coulbourn amplifiers, Lehigh Valley, PA) and displayed on a digital storage oscilloscope (model 2214, Tektronix, Beaverton, OR) and on a Graphtec Thermal Array Recorder WR 4000 (Irvine, CA). During data collections, these signals were sampled at 150 samples/s and stored on a computer using Notebook software (Labtech, Wilmington, MA).

Airflow measurements. Airflow patterns during resting breathing were monitored in anesthetized, tracheotomized rats using bias-flow instrumentation described previously (28). The tracheal cannula was connected to a side tap of a bias-flow circuit in which total airflow was measured using a screen-wire pneumotachograph, a pressure transducer (model MP 45, Validyne, Northridge, CA), and a carrier demodulator (model CD 15, Validyne). Airflow past the tracheal connection was maintained at rates well above the peak flows of the rats by the combined use of a high vacuum source (pump) and a needle valve. The bias-flow rate was high enough to prevent rebreathing of expired air, to ensure unidirectional airflow, and to ensure that variations in airflow through the pneumotachograph were generated by the breathing of the rat.

Pao and Pes. An open-ended, saline-filled polyethylene esophageal catheter (PE-100, 0.034 mm ID, 0.060 mm OD) with multiple side holes was connected to a disposable transducer (model 041-500-503, Cobe, Lakewood, CO), inserted into the stomach, flushed with saline to remove air bubbles, and then slowly withdrawn to maximize the negative pressure swings during spontaneous inspiration. The esophageal transducer was placed in a position level with the esophageal catheter. After the animal was connected to the bias-flow circuit, the flow and pressure signals were monitored on a storage oscilloscope and a chart recorder until the breathing pattern during room air breathing appeared visually to have stabilized for ≥1 min. At that time, Pes, Pao, and airflow were sampled for 3 min, and the data were stored on disk. The transpulmonary pressure (Ptp) was calculated as the difference between the esophageal catheter pressure and the Pao.

P-V measurements. P-V measurements were conducted on anesthetized rats using the protocol described previously (2). The rats were mechanically ventilated with 100% O\textsubscript{2} using a small animal ventilator (model SAR 830/P, CWE, Ardmore, PA) at ventilatory rates of 60–75 breaths/min, an inspiratory time (T\textsubscript{i}) of 0.15 s, and tidal volumes (V\textsubscript{T}) of 1.5–2.0 ml. The trachea was then occluded for 5 min to allow for the complete absorption of gas from the lungs. After the lungs were degassed, a second dose of pentobarbital sodium was administered, the chest cavity was opened wide, and the lungs were exposed.
The lungs were inflated with air and deflated in fixed volume increments using a mechanical syringe pump (Harvard Apparatus) with an electronic controller. Lungs were inflated and deflated in increments of 0.4 ml delivered over 10 s at a constant flow rate. Pressure was measured with a differential pressure transducer (model MP 45, Validyne) following a stress relaxation period of 50 s after inflation increments and a stress relaxation period of 20 s after deflation increments. Lungs were inflated to 14 cmH₂O pressure and deflated to 0 cmH₂O three times. P-V curves were constructed from pressures obtained after stress relaxation, and values from the third inflation-deflation loops were compared.

O₂ saturation measurements. Changes in O₂ saturation with decreasing ventilation rates were studied in tracheotomized rats anesthetized as described above and paralyzed with pancuronium (0.2 mg/100 g) injected into the left saphenous vein in a volume of 0.1 ml/100 g. After the right thigh was shaved, a pulse oximeter probe (model NBP-40, Nellcor Puritan-Bennett) was wrapped around the thigh, and pulse oximetry recordings were monitored continuously. Pulse oximeter readings were made during spontaneous breathing before paralysis, after the tracheostomy, and during paralysis. Paralyzed rats were ventilated with a fixed T₁ of 0.15 s at 75 breaths/min. The flow setting on the ventilator was adjusted to deliver the VT necessary to maintain O₂ saturations at >95%. The flow setting was then decreased in 50-ml/min increments (equivalent to a tidal inflation volume change of 0.125 ml), and the change in the pulse oximeter reading was recorded after a 6-min interval. This process was repeated until the O₂ saturation decreased to 75%. After an O₂ saturation of 75% was reached, the flow was increased to the initial setting to verify that a saturation of ~95% could be achieved. The rats were subsequently taken off the ventilator. To measure vital capacity, air was injected at a rate of 0.4 ml/10 s to inflate the lungs to a maximum pressure of ~14 cmH₂O and deflated to <0 cmH₂O.

Lung inflation-fixation. After the P-V measurements were completed, the lungs were inflated in situ with 10% buffered formaldehyde using an intravenous infusion catheter with a 25-gauge flexible tip. An inflation pressure of 17–20 cmH₂O was maintained for ≥30 min, after which time the trachea was ligated, and the lungs were removed and stored in 10% buffered formalin at 4°C. Before paraffin embedding, the lungs were first washed in tap water and then dehydrated in graded alcohols. Lung tissue sections (8 μm thick) were mounted on Superfrost Plus slides and dried for 2 ha t5 5 in an oven. Tissue sections were deparaffinized, rehydrated, stained in an acid orcein-Verhoeff stain, and dehydrated and mounted in Eukitt (28). To visualize lung elastic fibers, tissue sections were paraffinized, rehydrated, stained in an acid orcinol-Verhoff stain for 2 h, counterstained briefly in metanil yellow, dehydrated, and mounted in Permount (28). To visualize lung collagen, tissue sections were paraffinized, rehydrated, mordanted in Zamboni’s fixative for 1 h at 50°C, and washed extensively in running water before they were stained with Gomori’s trichrome stain.

Analysis of P-V data. Before the data were analyzed, tests were performed to determine whether the esophageal catheter imposed any phase lag on the Pes recording. The saline-filled catheter and the transducer used for recording the Pao were connected to holes in a stopper on a small bottle. Pressure inside the bottle was made to vary by connecting the ventilator to a third hole in the stopper. The pressure amplitude was comparable toPes swings during tidal breathing, and nine frequencies ranging from 50 to 90/min were tested. Using Lissajous figures, we determined that pressure from the esophageal catheter lagged that from the airflow opening transducer by 6.7 ms at all frequencies tested. For assessment of Cdyn (see below), the Pao signal was advanced by 6.7 ms to compensate for this lag. All analyses of sampled data were performed using MATLAB version 5. Calibration curves for the pressure signals were obtained by linear regression of the values (in analog-to-digital converter numbers) sampled during calibration trials vs. the known pressure values. These linear curves were then used to convert the sampled pressure signals from various trials into cmH₂O. Airflow was calibrated by injecting and withdrawing a known volume (0.5–2 ml) into the bias-flow circuit using a calibrated syringe (28).

For analysis of resting breathing, a data segment, lasting ~30 s, that was free of visual artifacts or obvious changes in breathing pattern was identified in each 3-min data set. The beginning and end of inspiration were determined from the airflow signal by custom software that also permitted visual validation of these points for every breath. For each breath analyzed, Vt was calculated as the numerical integral of inspiratory airflow and equivalent respiratory rate as the reciprocal of 60/breath duration (the latter in units of minutes). Equivalent minute ventilation was evaluated as Vt multiplied by the equivalent respiratory rate.

To determine Cdyn, we assumed that flow (dV/dt), volume (V), and Ptp (Ptp = Pao – Pes) were related as follows: Ptp = R(dV/dt) + V/Cdyn, where R is dynamic resistance. R and 1/Cdyn were calculated using multilinear regression with V and dV/dt as independent variables and Ptp as the dependent variable. Ten to 20 breaths of resting breathing data were used for this analysis.

Analysis of P-V curves. An exponential function of the form

\[ V = V_{max} - Ae^{-Kp} \]

was fitted to data points obtained from each of the third deflation loops of the P-V curves (6, 13). In this equation, V is lung volume, P is static recoil pressure, V_{max} represents the volume extrapolated to infinite pressure, and A represents the difference between V_{max} and volume extrapolated to P = 0. K, a constant related to the incremental compliance (dV/dP), is an index of the shape of the curve. Data points obtained at volumes <50% of total lung capacity typically deviate from a single exponential curve and were not included in the analysis. The best-fit exponential function was obtained by an iterative least-squares fit of ln(V) to ln(P).

Initially, V_{max} was estimated visually. Then, for each of 100 values of V_{max} in the range 0.5–1.5 times this initial estimate, A and K were estimated by a least-squares fit. The values of V_{max}, A, and K that minimized the sum of squared errors were chosen as the best fit.

Statistical analyses. Values are means ± SD. Group means were considered to be significantly different when P < 0.05. Effects of treatment, order of treatment, gender, and the interaction between treatment and treatment order or gender were evaluated by a two-way analysis of variance (ANOVA) using the Student-Newman-Keuls test for multiple pairwise comparisons. The Kruskal-Wallis one-way ANOVA on ranks was used to evaluate differences in Cdyn among groups; Dunnett’s test was used to compare Cdyn in each of the treatment groups with controls. The significance of differences among treatment groups for all other parameters was assessed by a one-way ANOVA, and Tukey’s test was used for multiple pairwise comparisons.

RESULTS

Resting breathing. Bias-flow measurements were conducted on anesthetized, tracheotomized rats to determine Vt, respiratory rate, and minute ventilation.
(V) during resting breathing (Table 1). Because alveolar number and gas exchange surface area are reported to be significantly decreased in rats treated with Dex, but not in those treated with Dex + RA, we anticipated that resting breathing parameters might be altered in rats treated with Dex alone, but perhaps not in those treated with Dex + RA.

The effects of two different Dex and RA treatment protocols were compared in these studies. In protocol 1, which was identical to that used by Massaro and Massaro (17), the RA treatment was initiated on postnatal day 3 and treatment with Dex was started on day 4. Rats treated according to protocol 2 were injected with Dex first, on postnatal day 3, and with RA on day 4. The objective was to determine whether any effects of RA on Dex-treated rats were influenced by the order of administration of these two drugs. Analysis by a two-way ANOVA indicated that the temporal sequence of the Dex and RA injections had no effect on VT, VT normalized to body weight (VT/wt), V, VT normalized to body weight (VT/wt), VT/wt, breaths per minute, body weight, or VT/Ti. Because the values obtained from these two treatment protocols were similar, the data obtained from experiments conducted according to protocols 1 and 2 were pooled for subsequent analyses of the resting breathing data.

Measurements of resting breathing parameters are presented in Table 1. Because the effects of treatment with saline and cottonseed oil did not differ significantly, these two control groups were combined for subsequent analyses. Analysis by a one-way ANOVA indicated that VT/wt was significantly larger in Dex + RA-treated rats than in controls (P < 0.05). Differences among other groups were not statistically significant, however. Although respiration rates were 6–12% lower in the RA-, Dex-, and Dex + RA-treated groups than in controls, neither respiration rate nor VT/wt differed significantly. Average VT/Ti did not differ among groups.

We also observed a gender effect on resting breathing that was independent of treatment (Table 2). Analysis of the effects of treatment and gender on resting breathing parameters by a two-way ANOVA indicated that V, VT/Ti, respiration rate, and body weight were each significantly greater in male than in female rats. Mean values for VT/wt were significantly greater in female than in male rats. The effects of treatment with RA, Dex, or Dex + RA on resting breathing parameters did not vary significantly with gender. Furthermore, in no instance was the interaction between gender and treatment significant.

P-V. P-V curves were measured to determine whether treatment with RA or Dex alone, or in combination, significantly altered these relationships. After measurements of resting breathing were performed, the lungs were degassed, the rib cage was opened, and the lungs were inflated to ~14 cmH2O pressure and deflated to baseline pressure in fixed volume increments. The P-V loops were repeated three times, and lung volumes at 5.0 and 13.5 cmH2O pressure during the third inflation loop were compared among treatment groups.

A comparison of the actual lung volumes at 5 and 13.5 cmH2O pressure and lung volumes normalized for weight in rats treated according to protocol 1 vs. protocol 2 indicated that lung volumes at these pressures did not differ significantly with the order of treatment with RA and Dex. The data obtained from the two treatment protocol groups were then combined for the evaluation of the effects of treatment and gender by a two-way ANOVA.

The actual lung volumes and lung volumes normalized to 100 g varied significantly with treatment at each of the pressures evaluated (Table 3). At 5 cmH2O, a pressure likely to be encountered during resting breathing, the actual lung volumes and lung volumes normalized to 100 g were significantly greater in Dex- and Dex + RA-treated rats than in the saline and cottonseed oil controls or RA-treated rats (P < 0.0001). Lung volumes measured at 13.5 cmH2O pressure were also significantly greater in Dex- and Dex + RA-treated rats than in each of the other three groups (P < 0.001). Accordingly, static recoil pressure at a given

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**Table 1. Resting breathing parameters**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>VT (Vt/wt)102, ml/g</th>
<th>VT/wt, ml per min/g</th>
<th>Breaths/min</th>
<th>VT/Ti, ml/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed oil</td>
<td>12</td>
<td>0.4516 ± 0.1005</td>
<td>0.441 ± 0.103</td>
<td>98 ± 16</td>
<td>2.67 ± 0.47</td>
</tr>
<tr>
<td>Saline</td>
<td>9</td>
<td>0.4270 ± 0.0909</td>
<td>0.448 ± 0.088</td>
<td>106 ± 18</td>
<td>2.82 ± 0.42</td>
</tr>
<tr>
<td>RA</td>
<td>12</td>
<td>0.4411 ± 0.0788</td>
<td>0.419 ± 0.081</td>
<td>95 ± 15</td>
<td>2.84 ± 0.62</td>
</tr>
<tr>
<td>Dex</td>
<td>13</td>
<td>0.5062 ± 0.0950</td>
<td>0.428 ± 0.054</td>
<td>88 ± 18</td>
<td>2.97 ± 0.64</td>
</tr>
<tr>
<td>Dex + RA</td>
<td>13</td>
<td>0.5226 ± 0.1456</td>
<td>0.481 ± 0.108</td>
<td>94 ± 13</td>
<td>2.97 ± 0.51</td>
</tr>
</tbody>
</table>

Values are means ± SD. **Significantly greater than controls (P < 0.05).**

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**Table 2. Effects of gender on resting breathing parameters**

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Wt, g</th>
<th>VT × 10−1, ml</th>
<th>VT (Vt/wt) × 102, ml/g</th>
<th>V, ml/min</th>
<th>V/wt, ml·min−1·g−1</th>
<th>Breaths/min</th>
<th>VT/Ti, ml/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29</td>
<td>132 ± 23§</td>
<td>5.86 ± 1.02</td>
<td>0.455 ± 0.098</td>
<td>58.4 ± 12.6†</td>
<td>0.448 ± 0.087</td>
<td>100 ± 18§</td>
<td>3.057 ± 0.587‡</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>113 ± 18</td>
<td>5.40 ± 0.80</td>
<td>0.491 ± 0.117*</td>
<td>49.4 ± 8.9</td>
<td>0.444 ± 0.095</td>
<td>91 ± 13</td>
<td>2.586 ± 0.348</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly greater than males (P = 0.032). Significantly greater than females: †P = 0.004; §P < 0.01; ‡P = 0.021.
lungs were smaller in rats treated with Dex or Dex + RA than in controls and RA-treated rats. Lung volumes obtained at 5 and 13.5 cmH₂O pressure did not differ significantly in Dex- vs. Dex + RA-treated rats, however.

Lung volumes measured at 5.0 or 13.5 cmH₂O pressure did not vary significantly with gender. When normalized for body weight, however, lung volumes in female rats exceeded those in male rats at both pressures. Analysis by a two-way ANOVA indicated that at 5.0 cmH₂O pressure the mean lung volume per 100 g was 3.32 ± 1.50 ml in female rats vs. 2.91 ± 1.19 ml in male rats (P = 0.030), while at 13.5 cmH₂O pressure, mean lung volumes per 100 g were 4.17 ± 1.72 ml in female rats vs. 3.76 ± 1.19 ml in male rats (P = 0.044). The interaction between gender and treatment was not significant at 5.0 or 13.5 cmH₂O pressure.

Cdyn. Cdyn of the lung was calculated from changes in airflow and Pes in anesthetized rats during resting breathing. Two-way ANOVA indicated that although the effects of protocol and treatment were significant, there was no gender effect on Cdyn. The effect of treatment on Cdyn was significant at P = 0.010. In ascending order, Cdyn was as follows: 2.066 ± 0.412, 2.203 ± 0.589, 3.107 ± 1.817, and 3.227 ± 1.255 (SD) ml⋅cmH₂O⁻¹⋅kg⁻¹ for RA, control, Dex, and Dex + RA, respectively. Cdyn was significantly greater in Dex + RA-treated rats than in controls (P = 0.011) or RA-treated rats (P = 0.010) and in Dex-treated rats than in controls (P = 0.024) and RA-treated rats (P = 0.020). Cdyn was also greater in the rats treated according to protocol 1 (n = 14) than protocol 2 (n = 27, P = 0.012). Cdyn values in rats treated according to protocols 1 and 2 were 3.11 ± 1.63 and 2.28 ± 0.63 (SD) ml⋅cmH₂O⁻¹⋅kg⁻¹, respectively.

Because Cdyn is equal to the change in lung volume produced by a 1-cmH₂O change in pressure, compliance might be expected to increase as overall lung volume increases. Linear regression analysis of Cdyn normalized for weight vs. lung volume at 13.5 cmH₂O pressure demonstrated a significant dependence of compliance on lung volume (P = 0.017), indicating that the increased compliance seen in the Dex- and Dex + RA-treated lungs was due, at least in part, to the larger lung volumes in these treatment groups (Fig. 1).

Static compliance. We then compared an additional index related to static compliance among the treatment groups. The slope of the line connecting the mean volumes of the third inflation-deflation loops at 2–11 cmH₂O pressure was used as an index of the average static compliance in the middle two-thirds of the P-V curve. Analysis by a two-way ANOVA indicated that there was no influence of protocol or gender on outcome. Treatment was a significant factor, however. The mean slope was greater for Dex- and Dex + RA-treated lungs than for RA-treated or control lungs (P < 0.05), indicating that at any given pressure change the change in lung volume was greater in the Dex- and Dex + RA-treated lungs than in the other groups. Dex- and Dex + RA-treated lungs did not differ significantly, however (Table 4).

Lung volumes at the end of the third deflation loop were also compared among treatment groups to determine whether the amount of air trapping varied with treatment. Although neither protocol nor gender influenced these values, treatment did influence air trapping. At 0 cmH₂O pressure, lung volumes in the Dex and Dex + RA treatment groups were ~75% greater than in controls and RA-treated lungs (P < 0.05; Table 4).

To evaluate the effects of treatment with RA and/or Dex on the shape of the static P-V curve and, therefore, on the variation of compliance with pressure, we fit an exponential curve to the P-V data obtained from the third deflation curve of each rat (Fig. 2). The position on the y-axis was higher for the Dex- and Dex + RA-treated rats than for the RA-treated rats or controls, as expected, on the basis of the greater lung volumes in these treated rats.

Table 3. Effects of treatment on lung volume at 5 and 13.5 cmH₂O pressure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Wt, g</th>
<th>5 cmH₂O</th>
<th>5 cmH₂O/100 g</th>
<th>13.5 cmH₂O</th>
<th>13.5 cmH₂O/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed oil</td>
<td>11</td>
<td>118 ± 16</td>
<td>2.90 ± 0.90</td>
<td>2.49 ± 0.77</td>
<td>3.66 ± 0.84</td>
<td>3.15 ± 0.83</td>
</tr>
<tr>
<td>Saline</td>
<td>10</td>
<td>128 ± 24</td>
<td>2.68 ± 0.60</td>
<td>2.14 ± 0.55</td>
<td>3.49 ± 0.58</td>
<td>2.81 ± 0.67</td>
</tr>
<tr>
<td>RA</td>
<td>9</td>
<td>134 ± 23</td>
<td>2.70 ± 0.48</td>
<td>2.85 ± 0.55</td>
<td>3.66 ± 0.55</td>
<td>2.82 ± 0.66</td>
</tr>
<tr>
<td>Dex</td>
<td>13</td>
<td>117 ± 27</td>
<td>4.78 ± 1.14</td>
<td>4.22 ± 1.21</td>
<td>5.91 ± 1.24</td>
<td>5.21 ± 1.31</td>
</tr>
<tr>
<td>Dex + RA</td>
<td>14</td>
<td>118 ± 0</td>
<td>4.61 ± 0.89</td>
<td>3.99 ± 0.96</td>
<td>5.82 ± 1.04</td>
<td>5.01 ± 1.25</td>
</tr>
</tbody>
</table>

Values are means ± SD, V, lung volume. *Significantly greater than RA, saline, and cottonseed oil controls (P < 0.001).
Table 4. Effects of treatment on slope and lung volume at 0 cmH₂O pressure (third deflation curve)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>V at 0 cmH₂O, ml</th>
<th>Slope of 3rd P-V Loop, ml/cmH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>20</td>
<td>1.552 ± 0.586</td>
<td>0.145 ± 0.037</td>
</tr>
<tr>
<td>RA</td>
<td>8</td>
<td>1.586 ± 0.432</td>
<td>0.176 ± 0.055</td>
</tr>
<tr>
<td>Dex</td>
<td>9</td>
<td>2.746 ± 1.085*</td>
<td>0.235 ± 0.055*</td>
</tr>
<tr>
<td>Dex + RA</td>
<td>12</td>
<td>2.424 ± 0.794*</td>
<td>0.229 ± 0.061*</td>
</tr>
</tbody>
</table>

Values are means ± SD. P-V, pressure-volume. *Significantly greater than controls and RA (P < 0.05).

Values for K, a shape constant that is independent of absolute volume changes, were calculated from the exponential fit to the third deflation curves as described in METHODS. Analysis by two-way ANOVA of the values for K, a measure of elastic recoil, indicated that there was no effect of protocol on outcome. There was, however, a significant effect of gender on values of K that were greater in female than in male rats: 0.1527 ± 0.0434 and 0.1432 ± 0.0442, respectively (P = 0.037). Thus the deflation curves for female rats were steeper at low pressures, independent of the overall differences in lung volumes.

K also differed as a function of treatment (P < 0.001). Values for K were higher for Dex-treated rats than for controls (P < 0.001) or RA-treated rats (P < 0.001) and also higher for Dex + RA-treated rats than for controls (P = 0.006) or RA-treated rats (P = 0.006; Fig. 2). Although values for K were lowest in the RA-treated rats, they did not differ significantly from controls, nor did K values differ significantly between Dex- and Dex + RA-treated rats.

There was also a significant interaction between the effects of gender and treatment (P = 0.026; Fig. 3). Among the female rats, K was greater for Dex-treated rats than for controls (P < 0.001) or RA-treated rats (P = 0.006) and greater for Dex + RA-treated rats than for controls (P = 0.024). Among the male rats, K was greater for rats treated with Dex (P = 0.036) and Dex + RA (P = 0.004) than for RA-treated rats. In addition, the effect of Dex on K was significantly greater in female than in male rats (P = 0.006).

Hysteresis. In view of the significant changes in lung structure and P-V curves associated with Dex treatment, we anticipated that the mechanical efficiency of the Dex-treated lungs might also be impaired. We initially assessed hysteresis as the volume difference between the third inflation and deflation curves at 6 cmH₂O pressure to determine whether this difference was increased in Dex-treated rats. Neither gender nor protocol had a significant influence on this measure of hysteresis. The effect of treatment on hysteresis was significant, however (P < 0.001). Values were as follows: 0.81 ± 0.39, 0.88 ± 0.32, 1.45 ± 0.35, and 1.52 ± 0.41 (SD) ml for controls (n = 10) and rats treated with RA (n = 9), Dex (n = 11), and Dex + RA (n = 14), respectively. Volume differences at 6 cmH₂O were significantly greater for Dex- and Dex + RA-treated rats than for controls or RA-treated rats (P < 0.001). Dex- and Dex + RA-treated rats did not differ significantly, however.

We also considered the possibility that differences in maximum lung volumes (Vₘₐₓ), which were significantly larger in the Dex- and Dex + RA-treated rats, may have contributed to the observed differences in this measure of hysteresis. Therefore, we determined for each treatment group the mean hysteresis ratio (HR), a value that provides a measure of the viscoelastic properties of the lung that is independent of lung volume (1, 9). The HR is defined as the area enclosed by the P-V loop divided by the maximum possible hysteresis for that loop: (Vₘₐₓ - Vₘᵓᵣᵦ) × Pₘₐₓ, where Vₘᵓᵦ is minimum lung volume and Pₘₐₓ is maximum pressure.
Recruitment-derecruitment of alveoli and small airways, pulmonary surfactant, and tissue elasticity are potential contributing factors to the HR. Because substantial derecruitment and increased hysteresis occur at end-expiratory pressures <0 cmH2O (3), we excluded from this analysis any lungs that were deflated to <0 cmH2O pressure at the end of the first or second P-V loop.

The values for HR differed significantly with treatment (P = 0.002) and with gender (P = 0.007). The mean HR values were greater for Dex-treated rats than for controls (P = 0.004) and RA-treated rats (P = 0.009). HR values were also greater for Dex + RA-treated rats than for controls (P = 0.003) and RA-treated rats (P = 0.006). The interaction between gender and treatment was also significant (P = 0.005; Fig. 4). As shown for the K values, the values for HR were also significantly larger in female rats treated with Dex than in male rats (P < 0.001).

**O2 saturation.** In view of the reported decrease in the volume density of lung parenchyma associated with postnatal Dex treatment, we anticipated that gas exchange would be impaired in the lungs of animals treated with Dex and that this impairment would be ameliorated to some extent by treatment with Dex + RA. To investigate these possibilities, the effects of Dex + RA on the gas exchange properties of the lung were assessed by measuring the percent O2 saturation at decreasing airflow rates (ml/breath) in paralyzed rats ventilated at 75 breaths/min. All animals included in this study were treated according to protocol 1. Gender did not influence outcome.

O2 saturation and heart rate assessed during resting breathing indicated that although resting O2 saturations were somewhat higher and resting heart rates were slightly lower in the Dex-treated rats, these differences were not significant (Table 5). Vital capacity normalized to body weight was significantly greater in Dex- and Dex + RA-treated rats than in the controls or RA-treated rats (P < 0.001). In the paralyzed, ventilated animals, we found that the ventilation rates normalized for body weight that were required to maintain O2 saturation at ≥90% did not differ significantly among the treatment and control groups.

**Histological observations** The rats used in these studies were treated according to protocol 1. Relative to the lungs of control and RA-treated rats, lungs of the Dex- and Dex + RA-treated rats had markedly enlarged air spaces at 1 mo of age. Alveolar walls appeared somewhat thinner in the rats receiving Dex and somewhat thicker in the rats treated with RA. Although no dramatic differences were observed among the control and treatment groups with respect to the intensity of the collagen stain, there appeared to be slightly more collagen in the peribronchial and perivascular regions in the RA-treated rats than in the other groups (Fig. 5). Abundant elastic fibers were seen in the lung parenchyma of all the rats examined, although the concentration of elastic fibers appeared to be slightly decreased in the lungs of the rats treated with Dex + RA relative to the other groups (Fig. 6). In addition, the structure of elastic fibers in the Dex + RA-treated rats was often abnormal, in that the degree of tortuosity was increased and there was a greater incidence of tangled fibers than in the other groups.

**DISCUSSION**

The results of these studies demonstrate that treatment with Dex during the period of bulk alveolar formation alters specific parameters of lung function at 1 mo of age. Despite the fact that RA ameliorates the Dex-induced inhibition of alveolar septal formation in the neonatal rat, we found that treatment with Dex + RA did not afford significant protection against the

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**Fig. 3.** Effect of Dex on K was significantly greater in female than in male rats (P = 0.006). Numbers of male and female rats in each of the treatment groups are as follows: 9 males and 12 females in control, 4 males and 5 females in RA, 7 males and 6 females in Dex, and 8 males and 6 females in Dex + RA. *Significantly greater than control females (P < 0.001); ‡‡significantly greater than RA-treated females (P = 0.006); †significantly greater than control females (P = 0.004); ††significantly greater than RA-treated males (P = 0.036); ‡‡significantly greater than RA-treated males (P = 0.004); and ††significantly greater than Dex-treated males (P = 0.006).

**Fig. 4.** Effect of Dex treatment on hysteresis ratio was significantly greater in female than in male rats (*P < 0.001). Number of rats in each treatment group is as follows: 4 males and 6 females in control, 2 males and 2 females in RA, 3 males and 5 females in Dex, and 5 males and 2 females in Dex + RA.
observed changes in lung function. We also found that Dex decreased lung recoil and increased the HR to a greater extent in female than in male rats, implying that the female rat is more sensitive than the male rat to specific deleterious effects of Dex.

In order that we might relate our findings to morphometric observations in the rat model made by other investigators, we followed the treatment protocol devised by Massaro and Massaro (17), one that has formed the basis for other recent studies (34). In the clinical setting, RA and Dex are not necessarily administered sequentially; thus we also sought to determine whether the order of treatment influenced the outcome. With one exception, this did not appear to be the case.

The substantial increase in lung volume at 1 mo of age was one of the more striking effects observed in the rats treated with Dex or Dex + RA. The magnitude of this increase was unexpected, however, since several earlier reports failed to demonstrate an effect of Dex on lung volume. After inflation-fixation at 20 cmH₂O pressure, lung volumes determined by volume displacement measurements at 14 days (15, 34) and 4–60 days (33) were not significantly increased as a result of Dex treatment. In contrast, we found that at normal physiological (5 cmH₂O) and high (13.5 cmH₂O) pressures, lung volumes in the Dex- and Dex + RA-treated rats were significantly larger than in controls or RA-treated rats. In related studies conducted by other investigators, volumes of excised lungs determined from the deflation loop of P-V curves were also significantly greater than controls at 60 (32) and 100 days (27). This apparent discrepancy is likely due, at least in part, to the fact that lung inflation was constrained by the intact chest wall during inflation-fixation in the studies that found no difference in volume. This effect likely

![Fig. 5. Representative photomicrographs of sections of lungs from 1-mo-old rats stained for collagen using Gomori’s trichrome stain. A: saline control; B: RA; C: Dex; D: Dex + RA. Alveoli are smaller and more numerous in sections from rats treated with saline or RA than with Dex or Dex + RA. Compared with blood vessels of similar size, perivascular regions of the RA-treated rat lung contained more collagen (arrow) than perivascular regions of the control lung. Green, collagen; black, nuclei; red, red blood cells. Original magnification ×100. Scale bar, 100 μm.](image)

### Table 5. Effects of treatment on resting HR, VC, and O₂ saturation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight, g</th>
<th>Resting HR, beats/min</th>
<th>Resting O₂ saturation, %</th>
<th>VC/100 g, ml/100 g</th>
<th>V at 90% O₂ Saturation/wt, ml·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed oil</td>
<td>108 ± 20</td>
<td>212 ± 16</td>
<td>94.4 ± 2.3</td>
<td>1.889 ± 0.198</td>
<td>1.090 ± 0.1487</td>
</tr>
<tr>
<td>Saline</td>
<td>104 ± 6</td>
<td>211 ± 20</td>
<td>94.0 ± 1.6</td>
<td>1.636 ± 0.626</td>
<td>1.197 ± 0.0973</td>
</tr>
<tr>
<td>RA</td>
<td>105 ± 6</td>
<td>201 ± 17</td>
<td>95.4 ± 1.7</td>
<td>1.711 ± 0.379</td>
<td>1.075 ± 0.1992</td>
</tr>
<tr>
<td>Dex</td>
<td>106 ± 10</td>
<td>195 ± 21</td>
<td>95.4 ± 1.1</td>
<td>3.360 ± 0.437*</td>
<td>1.009 ± 0.0932</td>
</tr>
<tr>
<td>Dex + RA</td>
<td>96 ± 7</td>
<td>195 ± 19</td>
<td>95.4 ± 2.3</td>
<td>3.781 ± 0.280*</td>
<td>1.121 ± 0.0970</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5. HR, heart rate; VC, vital capacity. *Significantly greater than cottonseed oil, saline, and RA (P < 0.001).
would occur even if the diaphragm had been pierced (15), because lung expansion at the high pressures used would surely fill the thoracic cavity and expand the chest wall beyond its resting position. Furthermore, underestimation of true lung expansion (i.e., unimpeded by the chest wall) at a given Pao would be larger when lung compliance is greater, e.g., in Dex- and Dex + RA-treated rats compared with controls. The grossly enlarged air spaces seen by others immediately after treatment with Dex on days 4–13 implied a decrease in the gas exchange surface area per unit of lung. If this were the case, an alteration of breathing pattern should be required in order for the Dex-treated rats to maintain PO₂ within the normal range. An increase in VT, rather than an increase in respiration rate, is the more efficient compensatory response to a decrease in gas exchange surface area when the contribution of dead space to total lung volume remains constant. In fact, when examined at 1 mo of age, VT/wt was significantly greater in rats treated with Dex + RA than in controls. Although respiration rates were lower in rats treated with Dex than in controls, rates did not vary significantly among treatment groups. VT/Ti did not vary among the five groups, suggesting that the neurogenic drive for respiration was not altered by treatment with Dex in the presence or absence of RA.

In view of the enlarged air spaces and the approximate doubling of lung volumes in both groups of rats treated with Dex, we considered the possibility that work of breathing might be increased in these animals, as is the case in patients with pulmonary emphysema (4). We did not have any direct evidence to suggest that pulmonary resistance, the primary determinant of the work of breathing, varied among treatment groups. However, the larger lung volumes in the Dex-treated rats would require the chest wall to operate at larger volumes, which would also be expected to increase the work of breathing. In addition, the reduced density of lung parenchyma may provide less support for intrapulmonary airways, leading to a reduction of airway diameters during expiration and an increase in the work of breathing.

Cdyn, a measure of the change in lung volume vs. the change in Ptp, was significantly greater in Dex- and Dex + RA-treated rats than in controls or RA-treated rats. We also found that the order of treatment with Dex and RA influenced outcome. Although Cdyn was significantly greater in the rats treated according to protocol 1 than in rats in which the order of treatment was reversed (protocol 2), the importance of this observation is tempered by the limited number of rats included in this comparison. The stringent criteria used in determining the acceptability of these Cdyn data resulted in the rejection of many data sets. We selected for analysis only those segments having consistent flow and pressure waveforms of <30-s duration. Furthermore, if for any given rat two or more such segments

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Fig. 6. Representative photomicrographs of sections of lungs from 1-mo-old rats stained for elastin using an acid orcein-Verhoeff stain. A: saline control; B: RA-treated rat; C: Dex-treated rat; D: Dex + RA-treated rat. Elastic fibers (arrows) were often thicker in lung sections from RA-treated rats than in lungs from rats in control, Dex, or Dex + RA group. Tangled and tortuous fibers were seen more frequently in sections from Dex + RA-treated rats than in sections from the other groups. Purple/black, elastic fibers; black, nuclei; yellow, red blood cells. Original magnification ×1,000. Scale bar, 10 μm.
(from the 3-min record of sampled data) were dissimilar, the data set was also excluded. As a result, for each protocol there were relatively few animals in each of the four groups. Thus, despite the significant difference between these two protocols ($P = 0.012$), we believe that further studies are warranted.

The slope of the third inflation-deflation loop of the P-V curve, a measure related to static compliance, was also significantly greater in Dex- and Dex + RA-treated rats than in controls or RA-treated rats. The use of the static deflation curve of a P-V loop to describe the elastic recoil properties of the lung has several limitations, however, since the P-V relationship is non-linear, the slope of the deflation curve varies with its position along the pressure axis. In addition, this approach does not permit standardization for differences in absolute lung volume. To avoid these concerns, we calculated $K$ using an analytic method that involved fitting the deflation curve by an exponential function over a range of volumes between functional residual capacity and total lung capacity. $K$ is related to elastic recoil but is independent of absolute lung volume. $K$ is a measure of the rate at which the P-V deflation curve plateaus with increasing pressures; a curve that plateaus at lower pressures has a larger $K$. We found that the mean value for $K$ was significantly greater in the Dex- and Dex + RA-treated groups, indicating that the reduction in elastic recoil as a result of Dex treatment was not uniform, being greater at lower pressures and volumes.

The decrease in lung recoil seen in the Dex- vs. the RA-treated rats could be explained, in part, by a difference in the volume density of parenchymal elastic fibers. Although the in vivo effects of postnatal RA administration on lung elastin have not been rigorously investigated, available evidence suggests that lung elastin synthesis during alveolarization may be further upregulated by treatment with RA during the early neonatal period. Results of in vitro studies indicate that endogenous RA stores play a critical role in elastogenesis in the neonatal rat lung fibroblast (19) and that steady-state tropoelastin mRNA expression is upregulated two- to threefold in neonatal rat lung fibroblasts cultured in the presence of RA (12).

There is a paucity of information regarding the effects of Dex administration on elastin synthesis in the postnatal lung. When administered in late gestation, Dex has been shown to increase tropoelastin mRNA in fetal rat lung cells in vivo and in vitro (22, 25, 31). We are aware of only one study that has addressed the effects of antenatal Dex treatment on postnatal lung elastin synthesis. In this report, desmosines (elastin cross links) were significantly decreased on postnatal days 10 and 15 in the lungs of rats treated with Dex on day 17 of gestation (29). In a related study, Tschanz and colleagues (33) observed fewer lipid interstitial fibroblasts (LIF) in the developing lung during Dex treatment. Normally the predominant lung fibroblast subset during alveolarization, these cells are the primary source of parenchymal elastic fibers. Within 6 days after the cessation of Dex treatment, postnatal day 21, these authors noted that the number of LIF increased at a time when cells in this subset would normally have undergone developmental apoptosis, and, as a result, LIF constitute ~5% of the total lung fibroblast population (30). The return of LIF after Dex treatment suggests that, under these circumstances, elastin synthesis might occur in the lung parenchyma beyond the first 2.5 wk of life, consistent with our observations that the concentration of elastic fibers did not appear to be diminished in the lungs of Dex-treated rats.

Treatment with RA alone or in combination with Dex during the period of alveolar formation is reported to increase the collagen content of the alveolar walls in rats exposed to hyperoxia (34). In contrast, Dex alone is reported to suppress collagen synthesis and degradation (7, 8, 24). In the present study, we found no compelling histological evidence of differences in lung collagen at 1 mo. These observations suggest, as others have shown, that when treatment is stopped, rates of collagen turnover return to, or exceed, control levels (7, 29).

Hysteresis, defined as the degree to which, at a given pressure, the lung volume during deflation exceeds the volume during inflation, is a measure of the mechanical efficiency of the lung. The area encompassed by the P-V loop represents the hysteresis of the lung. In the present study, hysteresis, measured as the difference in volume at 6 cmH$_2$O pressure between the inflation and deflation curves of the third P-V loop, was significantly greater in the Dex- and Dex + RA-treated rats than in RA-treated rats or controls. However, others have shown that the width of quasi-static P-V hysteresis loops increases with increasing $V_t$ (20). Because lung volumes were significantly larger in rats that received Dex alone or Dex + RA, the larger lung volumes in the rats treated with Dex are likely to have contributed to the increased hysteresis observed in these two treatment groups.

Other investigators have compensated for the differences in lung volumes associated with Dex treatment by expressing volume as a percentage of maximal lung volume. Using this approach, Massaro and colleagues (15) observed that hysteresis was significantly greater in saline-filled lungs from 20-day rats (treated on days 4–13 with 0.1 µg/kg Dex vs. 0.25 µg/kg in the present study) than in controls at 3 and 4 cmH$_2$O pressure. In a similar study, Thibeault et al. (32) observed no significant differences with respect to lung hysteresis in Dex-treated rats.

In the present study, we minimized the contribution of lung volume by comparing HR values, a measure of hysteresis that normalizes for lung volume. When the contribution of lung volume was minimized by comparing mean values for HR, we again saw a significant increase in HR in Dex- and Dex + RA-treated rats relative to controls and RA-treated rats, indicative of differences in the mechanical properties of lungs in the Dex treatment groups independent of lung volume. Recruitment-derecruitment, a factor that contributes to lung hysteresis, depends on end-
expiratory pressure and has been shown to increase at \( \leq 4 \) cmH\(_2\)O end-expiratory pressure. Thus, had we used a higher end-expiratory pressure for a cutoff value at the end of the deflation curves, the HRs may well have been smaller (10).

Surfactant and the viscoelastic properties of lung parenchyma and connective tissue contribute to lung hysteresis. Because these studies were conducted in air-filled lungs, the relative contributions of each of these parameters could not be determined. It seems unlikely that differences in lung surfactant concentrations contributed to the treatment-related differences in pulmonary mechanics observed in the present study. Although Dex has been shown to increase the synthesis of surfactant phospholipids and proteins in animal studies, the doses were on the order of 100 \( \mu \)g/kg to 20 mg/kg, doses substantially higher than those used in the present study (23). However, studies conducted in preterm infants have also shown that Dex treatment is associated with an increase in the concentrations of surfactant proteins A and D in tracheal aspirates (35).

RA has also been shown to influence the synthesis of surfactant proteins. Metzler and Snyder (21) reported that, in human fetal lung explants cultured in the presence of RA, synthesis of surfactant protein B increased whereas synthesis of surfactant proteins A and C decreased. Although increased surfactant could offset the inactivation of endogenous surfactant by proteases and serum proteins associated with an inflammatory response, it is unlikely that a modest increase in surfactant phospholipids or proteins would have a detectable influence on hysteresis in an otherwise normal lung.

In addition to surfactant, the HR can also be influenced by lung connective tissue (9). The possible contribution of parenchymal and connective tissue to HR was addressed in a study by Fedullo and colleagues (9), in which they evaluated HR in emphysematous hamster lungs filled with saline, thus eliminating any contribution of surfactant to the HR. These investigators found a significant increase in HR in emphysematous lungs vs. controls and concluded that tissue resistance secondary to cellular and connective tissue was decreased in emphysematous lungs.

As discussed above, treatment with RA, Dex, or Dex + RA could also affect lung recoil properties via specific effects on elastin and collagen synthesis. The difference in the volume density of lung parenchyma is also likely to be a contributing factor. Although we saw considerable variability with respect to air space size, the air spaces were generally much larger in the Dex- and Dex + RA-treated lungs than in the other groups. The results of P-V studies conducted by other investigators in saline-filled lungs, which eliminated the contribution of surfactant, indicated that static deflation curves (where volume was expressed as percent maximum lung volume) in Dex-treated rats differed significantly from controls (15, 27). Although not definitive, these observations are also consistent with the possibility that lung recoil is decreased as a result of treatment with Dex during the period of alveolar formation.

Our observations of gender differences with respect to HR and K were unexpected, as was the finding that the effects of Dex treatment on K and HR were significantly greater in the female than in the male rats. Several key morphometric studies have focused exclusively on the effects of Dex on the male rat lung (17, 33). This decision was justified by previous observations that alveolar formation is more complex in the female rat and mouse (16, 18). There is substantial evidence in the literature to support the concept of sexual dimorphism during lung development. Lung maturation and surfactant production are delayed in the male in a variety of species, including human. Massaro and colleagues (16, 18) showed that alveoli are smaller and more numerous in female rats and mice, a phenomenon that appears to be estrogen dependent. The response to prenatal glucocorticoid therapy is also gender dependent in several species, including human, a disparity found to be independent of glucocorticoid receptor number or binding affinity in preterm sheep lungs (11). The fact that Dex treatment increased K and HR to a greater extent in female than in male rats indicates a greater decrease in lung recoil, despite the fact that, in female rats, alveoli are reported to be smaller and more numerous (16, 18), properties that would tend to increase lung recoil. Altered connective tissue synthesis could also be a factor in the decreased lung recoil seen in the female rats; however, there is no evidence in the literature to support this concept. Another unanticipated result was that, despite enlarged air spaces and a decrease in volume density of parenchyma, there was no evidence of impaired gas exchange in Dex-treated rats. Although it would have been preferable to make direct arterial blood gas measurements in these studies, the relatively small size of these animals precluded the repeated removal of blood samples. The apparent absence of impaired gas exchange in Dex-treated animals could be attributable to any of several factors. First, as observed in the present study and similar studies conducted by others, gas exchange could be facilitated by the marked decrease in alveolar wall thickness in the Dex-treated rats vs. controls. Another possible explanation relates to the density of capillaries in the lung parenchyma. Although the absolute tissue volume was significantly decreased in Dex-treated rats during the first 2 wk of life, Tschanz et al. (33) showed that mean absolute values for capillary blood volume and capillary surface area were consistently greater in Dex-treated rats than in controls on days 4, 7, 10, 13, and 36. This increased vascularity was not statistically significant, perhaps because of the small number of rats studied at each age (\( n = 4 \)). When these morphometric data are considered in light of our observations, they suggest that, during the period of Dex treatment and at 1 mo of age, the absolute pulmonary capillary volume is not diminished and may, in fact, be increased as the result of Dex treatment. The observed increases in capillary blood volume and surface area did not persist beyond day 36, however, suggesting that as the lung matures, gas
exchange may not be improved as the result of Dex treatment during the neonatal period. The changes in lung function observed in these studies resulted from treatment with Dex and RA, alone and in combination, throughout the entire period of postnatal alveolar formation, albeit at doses substantially lower than those used clinically. Although the results of these studies cannot be directly extrapolated to the clinical setting, they demonstrate the complexity of the physiological responses to, and interactions between, two potent therapeutic agents. Once bound to their cognate receptors, Dex and RA can influence transcription of genes that control a myriad of functions, e.g., cell division, connective tissue synthesis, growth factor production, and surfactant synthesis. It is, therefore, not surprising that the effects of these drugs alone, and in combination, on lung function could not be predicted by considering a single parameter such as alveolar size. The failure of RA to ameliorate Dex-induced changes in lung function serves to underscore the importance of following studies conducted at the microscopic level with related studies conducted at the macroscopic level to fully understand the implications to organ system function. Finally, although there may be adequate justification for focusing attention on only one gender to facilitate the interpretation of results, our observations emphasize the importance of the inclusion of both genders in studies likely to have an impact on therapeutic interventions.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-40369 and HL-62877.

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