

Elastin and Collagen in the Fetal Sheep Lung. II. Relationship to Mechanical Properties of the Lung

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ABSTRACT. The relationship between elastin and collagen concentration and indices of lung maturation was studied in the lungs of fetal sheep. Fetal sheep of 124 days gestation were infused for 84 h with cortisol, triiodothyronine, prolactin, or epidermal growth factor alone or in combination. Pressure-volume curves with air were performed on the lungs and saturated phosphatidylcholine was measured in lung washes. Desmosine and hydroxyproline were determined in lung tissue in seven hormone-treated fetuses that displayed distensible and stable lungs similar to term lungs [volume of air at 40 cm H₂O (V_{40}) > 1.5 ml/g wet weight and at 5 cm H₂O (V_5) > 0.8 ml/g] and in seven fetuses whose lungs remained nondistensible and unstable (V_{40} < 0.6 ml/g and V_5 < 0.4 ml/g). Alveolar saturated phosphatidylcholine was five times higher ($p < 0.001$) in distensible than in nondistensible lungs, but attained less than 20% of term values. Desmosine and hydroxyproline concentrations in parenchyma, pleura, and trachea of nondistensible, unstable lungs were similar to intact controls of 125 days gestation and those in distensible, stable lungs were similar to controls of 137 days gestation. Desmosine ($p < 0.0001$) and hydroxyproline ($p < 0.001$) concentrations in parenchyma of distensible, stable lungs were higher than those of nondistensible, unstable lungs. We speculate that increased distensibility of the fetal lung in response to treatment with hormones is attributable in part to changes in the composition of connective tissue. (*Pediatr Res* 22: 339-343, 1987)

Abbreviations

SPC, saturated phosphatidylcholine
RIA, radioimmunoassay

METHODS

Animals. Romney-Marsh ewes were mated with Southdown rams at estrus synchronized with vaginal sponges containing 60 mg medroxyprogesterone acetate (The Upjohn Company, Kalamazoo, MI). The first day of pregnancy was assumed to be the second day after withdrawal of the sponges. The estimated gestational age of the fetuses was confirmed after autopsy by radiographic examination of the ossification centers of the fetal limbs (17). On day 124 gestation (day 117 in the saline-infused animal), indwelling catheters were placed into the fetal carotid artery and jugular vein as described previously (18). Infusions were started in the evening of the day of operation (day 121 in the saline-infused animal) and the experiment terminated in the morning of day 128 by killing the fetus and the ewe with overdoses of pentobarbital. Medroxyprogesterone acetate (Depo-Provera, The Upjohn Company), 150-300 mg, was given to all ewes at the beginning of the fetal infusions to prevent premature labor. Infusions were administered alone or in combination at the following rates: cortisol 1 mg/h, triiodothyronine 25 µg/h, prolactin 1.5 mg/day, epidermal growth factor 50 µg/day, and saline

Distensibility and stability are prerequisites for adequate ventilatory function of the lung. There is good evidence that an increase in alveolar surfactant is causally linked to lung "maturation" *i.e.* the process by which the immature, noncompliant lung is transformed into a distensible and stable organ; close correlations between surface active material and lung distensibility and stability have been reported in numerous studies (1) and instillation of surface active material into immature lungs improves pulmonary ventilatory function (2, 3). While it is recog-

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Table 1. Hormonal treatments and indices of lung maturation*

Treatment	Body wt (g)	Lung wet wt (g)	Gestational age (day)	Bone age (day)	SPC ($\mu\text{g/g}$)	V ₄₀ (ml/g)	V ₅ (ml/g)
Distensible lungs							
3013 C + T + P	2675	65.0	126	123	128.6	1.56	1.18
3075 C + T	2675	57.3	126	126	220.0	2.23	1.65
3095 C + T	2710	88.2	128	129	133.7	1.61	1.14
3195 C + E	3075	98.8	125	128	79.8	1.60	0.86
4044A C + T + P	3030	64.4	129	126	34.2	2.02	1.37
4046A C + T + P	2260	57.0	127	121	113.7	2.12	1.41
4148A C + T + P	2600	63.3	128	126	104.0	2.16	1.50
Mean	2718	70.4†	127.0	125.6	115.6‡	1.90§	1.30§
SEM	104	6.2	0.5	1.0	21.7	0.30	0.10
Nondistensible lungs							
3052 T	3035	76.6	128	129	10.6	0.43	0.23
3074 T	3130	80.2	126	129	62.1	0.35	0.23
3094 T	2685	90.4	125	124	10.8	0.31	0.17
3158 C + P	3595	103.3	129	129	16.2	0.42	0.19
3194 C + E	3125	107.0	129	126	3.0	0.56	0.23
4074 T + P	3375	99.7	127	126	40.6	0.47	0.34
4079B S	2535	61.6	128	126	6.7	0.34	0.20
Mean	3069	88.4	127.4	127	21.4	0.41	0.23
SEM	139	6.2	0.6	0.8	8.2	0.03	0.02

* C, cortisol; E, epidermal growth factor; P, prolactin; S, saline; T, triiodothyronine; SPC, alveolar SPC; V₅, volume of air/g lung at 5 cm H₂O (deflation); V₄₀, volume of air/g lung at 40 cm H₂O.

† $p < 0.05$. ‡ $p < 0.01$.

§ $p < 0.001$ for differences between distensible and nondistensible lungs.

0.5 ml/h (Table 1). Quasistatic pressure-volume curves were performed on the left lung as previously described (16). Distensibility and stability were expressed as ml/g wet weight of lung at 40 cm H₂O (V₄₀) and on deflation at 5 cm H₂O (V₅), respectively. After completion of the pressure-volume study, the lung was rinsed three times with 0.9% saline solution using 1.5 times the volume of V₄₀. An aliquot of the effluent of the lung was kept at -20° C until further analysis for SPC.

The 14 lungs analyzed in the present investigation were also used in two larger studies (19, 20) designed to investigate hormonal action on lung maturation. Acceleration of lung maturation was achieved to varying degrees by different hormone infusions (see "Discussion") (19, 20). We chose seven "nonresponders" displaying nondistensible and unstable lungs after the infusion and seven "responders" in which lung distensibility and stability were near term values after the infusion (Table 1). Five of the seven nondistensible and unstable lungs were taken from lungs of fetuses infused with saline ($n = 7$) (20), triiodothyronine alone ($n = 6$), or triiodothyronine and prolactin ($n = 5$) all of which had nondistensible and unstable lungs similar to intact control animals of 128 days gestation (19). One nondistensible and unstable lung was selected from a group of eight fetuses infused with both cortisol and epidermal growth factor which included two "responders" and six "nonresponders" and another lung was selected from a group of six fetuses infused both with cortisol and prolactin of which one fetus displayed a partial response (V₄₀ = 1.3 ml/g) and five were "nonresponders." Four of the seven distensible and stable lungs were taken from a group of nine animals infused with a combination of cortisol, triiodothyronine, and prolactin all of which were "responders." The remaining three distensible and stable lungs were from fetuses infused with cortisol and triiodothyronine ($n = 8$, two "responders") and from fetuses infused with cortisol and epidermal growth factor ($n = 8$, two "responders") (19). In these 14 animals the right lung was subjected to biochemical analysis after dissection into "dissected" parenchyma (removing tubular structures of >0.1 mm diameter) and "nondissected" parenchyma, pleura, and trachea as described (11).

Biochemical analysis. Approximately 8 mg of dry tissue was weighed twice on a Mettler AC 100 balance and digested twice with papain at 65° C for 24 h. An aliquot was hydrolyzed with 6 M HCl at 110° C for 72 h. Collagen was measured as hydroxyproline (11, 21). Elastin was determined by measuring the cross-linking amino acid desmosine by RIA after cellulose chromatography and acetylation (10). Recovery of desmosine standards subjected to chromatography was between 77 and 95% ($n = 4$). Intraassay variability of standards in the RIA was <6% ($n = 10$). SPC was analyzed in lung lavage fluid by the osmium tetroxide method of Mason *et al.* (22) as previously described (23).

Statistical analysis. General linear model procedures were performed on an IBM 4341 computer using SAS (SAS 1982, SAS Institute Inc., Cary, NC). *T* tests were used as appropriate. Parenchyma, pleura, and trachea were analyzed separately. Variables taken into account in parenchyma were lobe (upper, middle, lower lobe), distensibility (distensible or nondistensible) and dissection (dissected or nondissected). Dissected and nondissected parenchyma was analyzed both in combination and separately. *p* values of >0.05 were considered nonsignificant.

RESULTS

Mean body weight, mean lung weight, and mean bone age were slightly lower in the "responders" than in the "nonresponders" (Table 1). Elastin and collagen concentrations of distensible and stable lungs were higher than those of nondistensible lungs in each compartment, *i.e.* in dissected and in nondissected parenchyma of all three lobes as well as in pleura and trachea (Figs. 1 and 2). Distensible and nondistensible lungs differed most in respect to parenchymal elastin and least in respect to elastin in pleura and trachea (Figs. 1 and 2). Differences between distensible and nondistensible lungs were more marked in dissected than in nondissected parenchyma. The difference between distensible and non-distensible lungs was greater for elastin than for collagen concentration (Figs. 1 and 2). Alveolar SPC in distensible lungs was five times higher in distensible lungs than in nondistensible lungs (Table 1) but attained less than 20% of

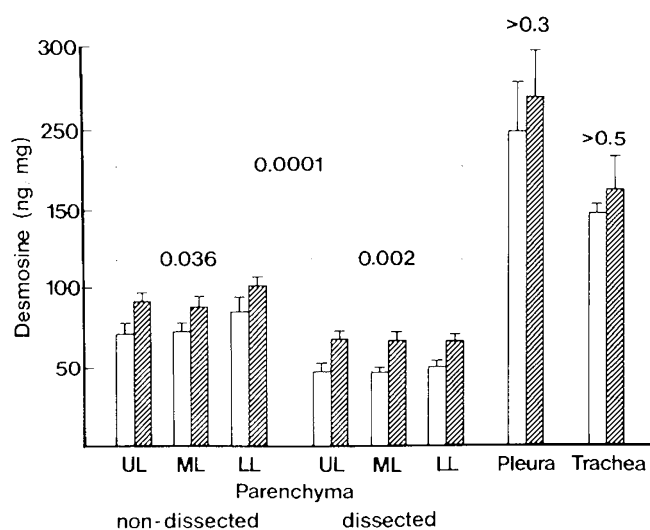


Fig. 1. Desmosine concentrations of nondistensible, unstable lungs ($V_{40} < 0.6$ ml/g lung wet weight and $V_s < 0.4$ ml/g, $n = 7$, open bars) and of distensible, stable lungs ($V_{40} > 1.5$ ml/g and $V_s > 0.8$ ml/g, $n = 7$, hatched bars) of fetuses subjected to various hormone treatments before sacrifice at 128 days of gestation. UL, upper lobe; ML, middle lobe; LL, lower lobe. Dissected parenchyma, devoid of tubular structures of >0.1 mm diameter. Numbers indicate level of statistical difference between nondistensible and distensible lungs (all three lobes combined). Brackets with probability values indicate whether dissected and nondissected parenchyma was subjected to separate or to combined statistical analysis (mean \pm SEM).

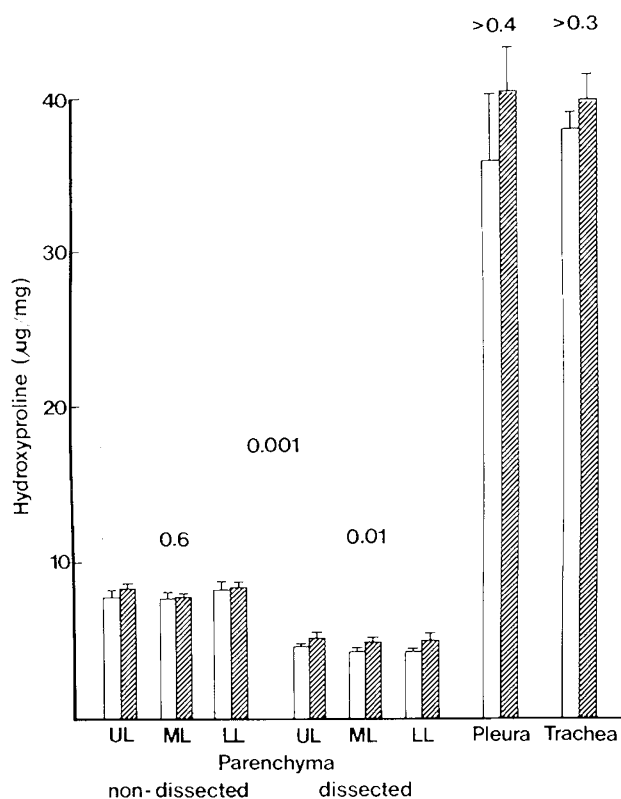


Fig. 2. Hydroxyproline concentrations of nondistensible lungs ($n = 7$, open bars) and of distensible lungs ($n = 7$, hatched bars) of fetuses subjected to various hormone treatments before sacrifice at 127 days of gestation. Symbols and definitions as in Figure 1 (mean \pm SEM).

term values (16, 23). Despite preterm values for elastin, collagen, and alveolar SPC, V_{40} and V_s of distensible and stable lungs were in the range of term animals (16, 23) (Table 1).

DISCUSSION

The purpose of this study was to investigate the relationship between connective tissue maturation and maturation of the mechanical properties of fetal lungs. Although maturation was accelerated by various hormonal treatments the study was not designed to relate particular hormones to changes in connective tissue composition but rather to determine whether a relationship existed between composition and mechanical properties regardless of the means by which maturation was advanced. We measured the concentrations of elastin and collagen and alveolar surfactant (SPC) in lungs of sheep fetuses in which development of distensibility and stability was advanced by hormone treatment between days 124 and 128 of gestation and compared them with values obtained from fetuses of similar age in which hormone treatment was without effect. Given the fact that all ewes were date-mated and that mean body weight, mean lung weight, and mean bone age of the "nonresponders" were slightly lower than those of the "responders" it is unlikely that errors in determining gestation length accounted for the observed differences in the mechanical and biochemical properties of the lungs. We selected lungs that had been exposed to a number of different treatments to diminish the possibility of finding differences in elastin and collagen concentrations that were due to specific hormonal effects which had nothing to do with the maturation of the mechanical properties of the lungs.

At around 125 days gestation, distensibility and stability of the fetal sheep lung are very low (19, 20, 24, 25). Distensibility, stability, and alveolar surfactant concentrations appear not to increase substantially until a few days before birth (24). At that time, lung maturation proceeds rapidly and in parallel with increasing fetal plasma cortisol levels suggesting a regulatory role of cortisol in ovine lung maturation (24).

The present study revealed that elastin and collagen concentrations in all compartments of nondistensible and unstable lungs, *i.e.* dissected and nondissected parenchyma, pleura, and trachea, were similar to those of untreated fetuses of 125 days gestation (11). In distensible and stable lungs, elastin and collagen concentrations of each compartment were similar to those of 137-day-old fetuses (11). This suggests that the changes in the collagen and elastin caused by hormonal treatment represented maturation rather than a nonphysiological response unrelated to maturation. However, this conclusion must be tentative until more detailed studies of the composition of the connective tissue, including the proteoglycan matrix, have been made.

Although the levels of alveolar SPC, the major constituent of surfactant in fetal sheep lung (26), were five times higher in distensible and stable lungs than in nondistensible and unstable lungs, they were still at least six times lower than in control animals (16, 23). Similar levels of alveolar SPC to those in distensible and stable lungs of the present study were found both in nondistensible and unstable lungs of adrenalectomized fetal sheep infused with ACTH at term and in lungs of cortisol-infused adrenalectomized fetuses which were moderately distensible (mean $V_{40} = 1.2$ ml/g) but unstable (16). This lack of correlation between distensibility and stability and alveolar SPC levels in lungs exposed to different treatments points to structural differences causing differences in the mechanical properties of the lung. The present observation of increasing elastin and collagen concentrations being associated with maturation of the mechanical properties of the lung offers further support to this contention. Unlike surfactant, which can be added or removed from lung (2, 27), connective tissue is an integral part of the organ; accordingly, a direct relationship between connective tissue maturation and the mechanical properties of the lung is difficult to establish.

Pulmonary distensibility and stability determined by single-loop quasistatic pressure-volume curves in the group of "responders" was similar to values obtained in normal term fetuses (16, 23, 24) although elastin and collagen concentrations and alveolar SPC levels were substantially lower. This raises the question of how well these pressure-volume curves define pulmonary maturity and whether the lungs of the "responders" are mechanically less mature than normal term lungs in respect to criteria that we did not evaluate such as dynamic compliance and the behavior in response to repeated inflations.

The several-fold lower surfactant levels in lung washes of "responders" than in washes from normal term lungs is unlikely to be due solely to less surfactant being required to cover the airspace walls in view of the finding that alveolar surface area increases less than 50% between days 120 and 141 gestation (28). Higher intraalveolar reserves of surfactant (situated in the sub-phase) and increased washout of intracellular SPC when rinsing with saline may contribute to the larger quantities of alveolar SPC measured in term lungs.

Because pressure-volume curves give little information on events at the alveolar level we can only speculate what the underlying mechanism of increasing distensibility and stability in the lungs of "responders" might be. In lungs of adult rabbits inflated with air and fixed intravascularly (thereby preserving as much as possible the air-tissue interface), morphometric and morphological analysis suggested that changes in lung volume are associated with recruitment/derecruitment of air spaces, balloon-like changes in alveolar size, simultaneous changes of alveolar size and shape (accompanied by septal pleating) and crumpling of the alveolar surface (29). In a similar study, the same authors suggested a model for the lung consisting of a flimsy meshwork subjected mainly to surface forces suspended within a wider-meshed fibrous skeleton that is stabilized by interdependence (27). This model offers a compromise between the bubble-on-a-tube model (30) recognizing surface forces only and the interdependence model stressing the existence of a framework formed by the walls separating individual airspaces (31). Kitterman *et al.* (24) published microphotographs showing that distensible lungs of fetal sheep at term have larger airspaces than nondistensible term lungs fixed intratracheally at the same pressure (thereby abolishing surface forces) suggesting that the walls of the airspaces become more distensible during maturation. Mitzner *et al.* (32) concluded from their morphometric studies in lungs of fetal monkeys exposed to betamethasone (see below) that some form of recruitment increased distensibility of these lungs. We speculate that increasing elastin and collagen concentrations may reflect increased subdivision of airspaces and thus the creation of more recruitable airspaces (8, 28), increasing length of elastin and collagen fibers which coil up at low lung volumes and permit more stretching of the airspace walls when the lung is inflated (27) and finally, a stronger structural meshwork that helps to prevent the lung from collapsing (33).

Our observations differ from those of Johnson *et al.* (13), Mitzner *et al.* (14), and Beck *et al.* (15) in several ways. While we found an increase in both SPC and the elastin/collagen ratio (which is one of the salient features of connective tissue development of the fetal lung (1, 10, 11) (Figs. 1 and 2), Johnson and coworkers (13–15) found no evidence of changes in surfactant and a slight decrease in the elastin/collagen ratio in distensible lungs of fetal rhesus monkeys that had been exposed to betamethasone. They suggested that the latter finding signifies pathological development, and indeed, multiple developmental anomalies including retarded growth of the lungs and reduced alveolar stability were found in monkeys that had been exposed to a similar regimen of betamethasone (34). There could be several reasons for the discrepancies between their findings in monkeys and our results in sheep. None of our fetuses was exposed to glucocorticoids alone, but all received triiodothyronine, prolactin or EGF in addition (Table 1). The monkeys were exposed to betamethasone at an earlier stage of lung development

than our sheep fetuses. Bunton and Plopper (35) have shown that glucocorticoids have age-specific effects on fetal lung development in the monkey but their animals displayed apparently normal accelerated morphological development of the lungs when glucocorticoids were given at a gestational age similar to that in the study of Beck *et al.* (15).

We conclude that, under the conditions of these experiments, altered mechanical properties of the fetal lungs are associated with changes in the concentrations of elastin and collagen that are in the same direction as those occurring during normal maturation. This finding suggests that these components of connective tissue have a significant role in the functional maturation of the lung.

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