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# Elastin and Collagen in the Fetal Sheep Lung. I. Ontogenesis

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**ABSTRACT. The ontogenesis of elastin (desmosine), collagen (hydroxyproline), and DNA concentrations and their rates of increase were studied in fetal sheep lungs from day 60 until term. Elastin increased 13-, 17-, 63- and 11-fold in nondissected parenchyma, dissected (free of tubular structures of >0.1 mm diameter) parenchyma, pleura, and trachea, respectively. Collagen increased 2.1-, 1.8-, 5- and 3-fold, respectively, in the four tissues. A sharp rise in elastin occurred after day 100. The rate of increase in elastin was greater in dissected than in nondissected parenchyma while the reverse was true for collagen. The steepest rise of elastin concentration occurred in the pleura after day 125. DNA concentration peaked on day 125 and was lowest at term. These findings are consistent with 1) the onset of a steep rise in elastin accumulation during the canalicular period, 2) the development of a rigid, mainly collagenous structure of the central airways and blood vessels and a distensible peripheral "gas-exchange tissue," rich in elastin, 3) an important role of elastin in the function of the visceral pleura, and 4) a peak of mitotic activity during the early alveolar period. (*Pediatr Res* 22: 335–338, 1987)**

**Abbreviation**

**RIA, radioimmunoassay**

The morphogenesis of the fetal sheep lung is well documented (1–5) and it is recognized that connective tissue plays an important part in the mechanical behavior of adult lungs (6, 7), but quantitative data on the connective tissue of the developing sheep lung are scant (8, 9). This is true even for elastin (9, 10), a water-insoluble polymer (10), to which a fundamental role in alveolization has been ascribed (11, 12) and which has been considered for some time an important marker of lung maturity (13, 14). One reason for the paucity of data is the absence until recently of reliable methods for the measurement of elastin in perinatal lung (15–18). In the present study we determined elastin by measuring desmosine, a specific cross-linking amino acid, by radioimmunoassay (18). We report the concentrations of elastin, collagen, and DNA in well-defined areas of the fetal sheep lung between day 60 of gestation and term and compare them with the morphological features described by other workers.

## METHODS

*Animals.* Sheep fetuses of gestational ages between 55 and 140 days gestation from freshly killed ewes were obtained from the local abattoir (Table 1). Gestational age was determined from fetal weight, crown-rump length, and from bone age of the limbs (19, 20). Term lungs were obtained from fetuses delivered by hysterotomy in pregnancies with known mating dates. The fetuses had been sham-adrenalectomized at 107 days gestation and cannulated and infused with saline at term (21). Medroxyprogesterone acetate (Depo-Provera, The Upjohn Company, Kalamazoo, MI) was given to these ewes near term to prevent spontaneous delivery. Indices of lung maturation were the same as those of intact term animals (21, 22). Lungs were removed from the thorax, weighed, and frozen. After thawing, the right lung was dissected further: each lobe was transected perpendicular to the lobe bronchus at mid-distance between its origin and its tip and 5 mm proximally and distally yielding two slices of 5 mm thickness each. The pleura was removed from all slices and pooled. The proximal slice of each lobe was dissected under a microscope and "tubular structures" removed. The "dissected" tissue, as well as the distal lung slices ("nondissected" tissue), and the pleura and the trachea (from the bifurcation to 1–2 cm above the right upper lobar bronchus) were refrozen, lyophilized, and dried to constant weight after pulverization. Microphotographs were taken on a grid and light microscopy was performed on dissected tissue. No cartilage was detected in this fraction and there were no tubular structures of >0.1 mm diameter. Lungs of the three 60-day-old fetuses were not separated into lobes and were either dissected or left undissected, yielding three tracheas, two pleuras, two nondissected parenchymas and one dissected parenchyma. In the 125-day group one trachea and one middle lobe were not available for analysis.

*Biochemical analysis.* Approximately 8 mg of dry tissue were weighed twice on a Mettler AC 100 balance and digested twice with papain at 65° C for 24 h. DNA was determined (23). An aliquot was hydrolyzed with 6 M HCl at 110° C for 72 h. Collagen was measured as hydroxyproline (24) without making corrections for hydroxyproline contained in elastin [approximately 1% (25)]. Elastin was determined by measuring desmosine by RIA after cellulose chromatography and acetylation (18). Mean recovery of desmosine standards subjected to three separate chromatographic separations was 104 ± 10% (mean ± SD), 91 ± 6%, and 91 ± 15%, respectively. Inter- and intraassay variability in four RIA assays of standards ($n = 4 \times 12$) were less than 7 and 5%, respectively, in the midrange of the assay.

*Data analysis.* Concentrations of desmosine, hydroxyproline, and DNA are given per mg dry weight. The influence on the concentrations of desmosine, hydroxyproline, and DNA of gestational age and site (pleura, trachea, dissected, or nondissected parenchyma and upper middle or lower lobe) were analyzed with general linear model procedures on an IBM 4341 computer using SAS (SAS 1982, SAS Institute Inc., Cary, NC). Tukey's test for multiple comparisons was used as appropriate. $p$ values of >0.05 were considered nonsignificant.

## RESULTS

*Desmosine concentration.* Desmosine concentration increased 13-, 17-, 63-, and 11-fold between day 60 and term in nondis-

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Table 1. *Age and wt of fetal sheep (mean ± SEM)*

| Age (days) (range) | *n* | Body wt (g) | Lung wet wt (g) |
|---|---|---|---|
| 60 (55–65) | 3 | 46 ± 8 | 1.4 ± 0.3 |
| 75 (75) | 3 | 299 ± 28 | 11.9 ± 1.5 |
| 100 (98–103) | 4 | 966 ± 177 | 32.6 ± 4.9 |
| 110 (108–110) | 3 | 1457 ± 75 | 51.7 ± 3.0 |
| 125 (122–127) | 3 | 2888 ± 115 | 81.5 ± 13.9 |
| 137 (135–140) | 3 | 3285 ± 159 | 94.4 ± 5.9 |
| 147 (146–149) | 3 | 6021 ± 578 | 129.5 ± 8.2 |

sected parenchyma, pleura and trachea, respectively ($p < 0.0001$, Fig. 1). Desmosine concentration was higher in pleura and trachea than in parenchyma ($p < 0.0001$) and higher in nondissected than in dissected parenchyma ($p < 0.001$). Desmosine concentration was highest in the trachea up to day 110 and highest in the pleura thereafter due to a more marked increase in the pleura than in any other compartment. The ratio between desmosine in dissected and in nondissected parenchyma was 0.43 on day 75 increasing to 0.74 by day 137 and plateauing at 0.70 on day 147 (interaction age * dissected or nondissected $p = 0.04$ between day 75 and 137 and $p = 0.07$ between day 75 and 147), *i.e.* the rate of increase in desmosine concentration was higher in the dissected than in nondissected tissue (see "Discussion"). No significant differences were found between lobes (not shown).

*Hydroxyproline concentration.* Hydroxyproline concentration increased 2.1-, 1.8-, 5-, and 3-fold between day 60 and term in nondissected parenchyma, dissected parenchyma, pleura, and trachea, respectively ($p < 0.0001$, Fig. 2). Hydroxyproline concentration was higher in pleura and trachea than in parenchyma ($p < 0.0001$) and higher in nondissected than in dissected parenchyma ($p < 0.0001$). Hydroxyproline concentration was highest in trachea up to day 100, similar to pleura on days 110 to 137, and highest in pleura on day 147. The ratio between hydroxyproline concentration in dissected and in nondissected parenchyma decreased steadily from 0.74 on day 75 to 0.59 at term (interaction age * dissected or nondissected, $p < 0.0001$), *i.e.* the rate of increase of hydroxyproline concentration was higher in nondissected than in dissected parenchyma (see "Discussion"). There was a general trend between days 75 and 147 for hydroxyproline concentration to be higher in the upper lobes than in the middle and lower lobes ($p < 0.01$ between upper and middle and upper and lower lobes in dissected tissue and $p = 0.03$ between upper and lower lobes in nondissected tissue). This trend was not observed at all ages and statistically significant only on day 125 if both dissected and nondissected tissue was analyzed together ($p = 0.02$) (Fig. 3).

*DNA concentration.* DNA concentration in parenchyma was highest on day 125 ($p < 0.01$ *versus* days 100, 137, 147 and $p < 0.05$ *versus* days 75 and 100) and lowest on day 147 ($p < 0.01$ *versus* all other ages) (Fig. 4). DNA concentration was highest in dissected parenchyma ($p < 0.0001$ *versus* nondissected parenchyma, pleura, and trachea) and higher in pleura than in trachea ($p < 0.002$). DNA concentration tended to be higher in the lower lobes than in the upper lobes (Fig. 3). This was statistically significant only in nondissected tissue when all ages between day 75 and term were analyzed together ($p < 0.01$).

There was no difference in the concentrations of desmosine, hydroxyproline, or DNA between lungs of male and female fetuses.

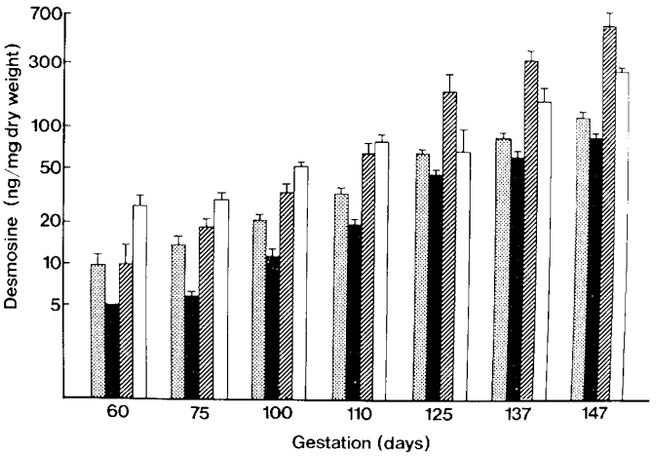


Fig. 1. Elastin (desmosine) concentrations in nondissected lung parenchyma (*stippled bars*), dissected parenchyma (*solid bars*), pleura (*hatched bars*), and trachea (*open bars*) between day 60 of gestation and term in fetal sheep. Note logarithmic scale (mean ± SEM).

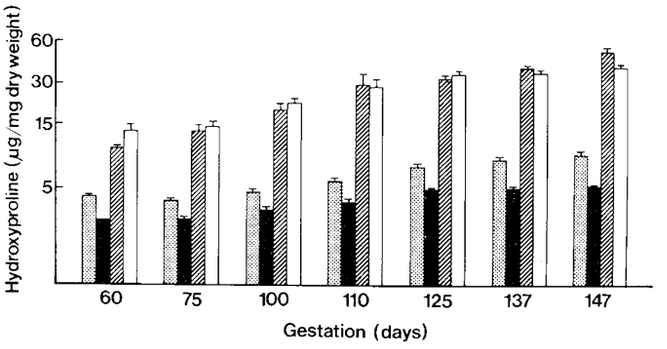


Fig. 2. Collagen (hydroxyproline) concentrations in fetal sheep lung between day 60 of gestation and term. *Symbols* and *scale* as in Figure 1 (mean ± SEM).

## DISCUSSION

The present study describes distinctive time courses of accumulation of elastin and collagen in various structural components of the lungs of fetal sheep. Lung parenchyma was separated into "nondissected" tissue containing "gas-exchange tissue" as well as large conducting airways and vessels rich in collagen and elastin and into "dissected" tissue containing only "gas-exchange tissue" and probably, particularly in early gestation, future small conducting airways (see "Methods"). As would be expected from macroscopic and microscopic observations, the concentrations of elastin and collagen were highest in pleura and trachea and lowest in dissected parenchyma at all gestational ages while the reverse was true for DNA concentration representing cellularity (1–3).

In all compartments, the rate of accumulation of elastin was greater than that of collagen which is in accord with biochemical findings in parenchyma in lungs of rats and foals (15, 16, 18, 26). Histological and biochemical studies have linked the period of alveolization with a period of particularly rapid accumulation of elastin in all species so far studied (2–5, 15–18, 27–30). In the rat, the steep rise in elastin concentration occurs during the period of alveolization (15–18, 28, 29). In the sheep, this event appears to commence earlier, between days 100 and 110 (Fig. 1), *i.e.* during the late canalicular stage of morphogenesis (2, 3). Other dissimilarities in ontogenesis between the rat and the sheep have been reported, particularly the absence of a distinct saccular period in the sheep (2) and differences in the morphogenesis of

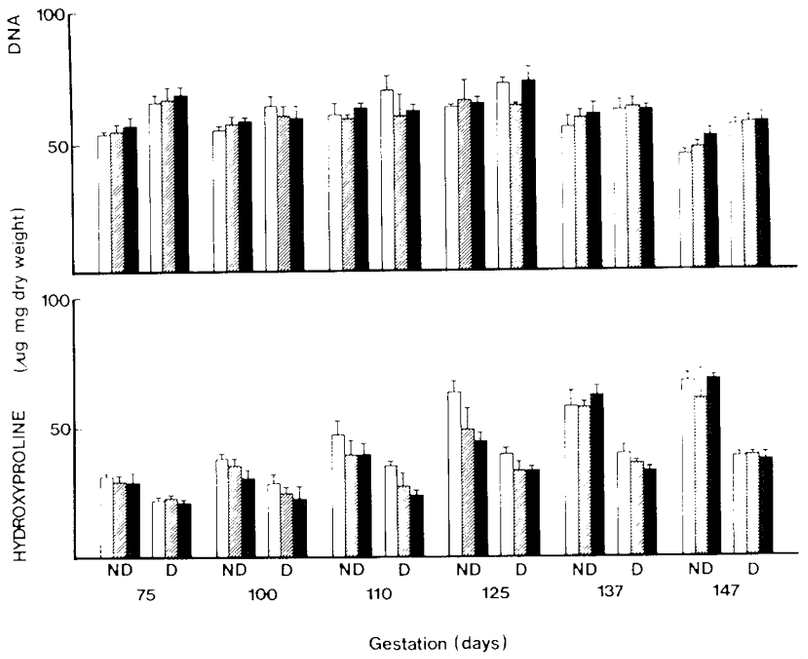


Fig. 3. DNA concentration and hydroxyproline concentration in upper lobes (*open bars*), middle lobes (*hatched bars*), and lower lobes (*solid bars*) in nondissected (*ND*) parenchymal lung tissue and in dissected (*D*) parenchymal lung tissue (*i.e.* free from tubular structures of >0.1 mm diameter) (mean ± SEM).

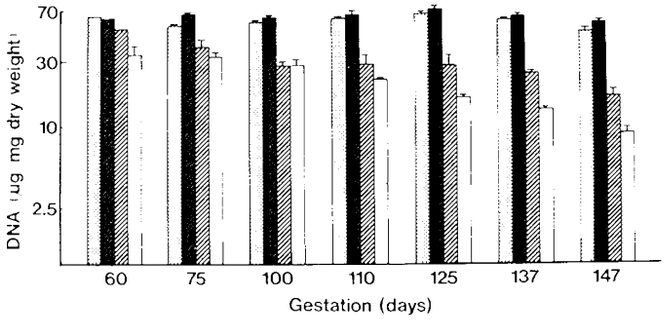


Fig. 4. DNA concentrations in fetal sheep lung between Day 60 of gestation and term. *Symbols* and *scale* as in Figure 1 (mean ± SEM).

the alveolar septa (3). However, the observed peak in DNA concentration on day 125 is comparable to the peak in $^3$[H]-thymidine labeling during the early alveolar period in the rat lung (31).

There was a general but inconsistent trend for collagen concentration to be higher in the upper lobes than in the lower lobes and a reverse trend for DNA concentration. This confirms our impression gained at dissection of the presence of a tough fibrous structure extending between the pleura and the central airways in many upper lobes (32). Conversely, in many lungs, the lower lobe was particularly easy to dissect, which is consistent with a higher DNA and lower collagen concentration. Preliminary studies showed no clear-cut differences in collagen concentrations between control and peripheral parts of lung lobes (Schellenberg J-C, Liggins GC, unpublished data) suggesting that the mid-lobe sections used in this study are a reasonable reflection of the structure of the whole lobe.

The fetal lung appears to have little importance *in utero* but assumes a vital function immediately after birth. Maturational events preparing the lung for its postnatal role take place in the latter part of pregnancy (33). This is well documented for surfactant which is synthesized and secreted at increasing rates toward the end of gestation (7, 33). Since other components that are essential for normal neonatal lung function may also increase more rapidly toward term, we suggest that the mechanical function of elastin and collagen after birth may be deduced in part from their rates of accumulation before birth. The rate of elastin accumulation was greater in dissected than in nondissected parenchyma while the reverse was true for the rate of collagen accumulation, findings that are consistent with the development of a rigid collagenous structure of the central airways and vasculature and of a distensible peripheral "gas-exchange tissue" relatively rich in elastin (34). The high rate of increase in elastin and collagen concentration in the pleura was maximal between day 125 of gestation and term. This is consistent with a major role for elastin in visceral pleura and hence of an important contribution of the visceral pleura itself to neonatal lung mechanics, perhaps by stabilizing lobe shape as has been suggested recently in adult dogs (35).

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