

## MAIN TOPIC

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**Lung liquid, fetal lung growth, and congenital diaphragmatic hernia**

**Abstract** There is now a large body of evidence supporting the role of lung liquid in normal and experimental fetal lung growth. It is clear that tracheal ligation causes retention of lung liquid, increased intratracheal pressure, and lung hyperplasia and that this hyperplastic effect can reverse the experimental pulmonary hypoplasia of clinically relevant entities such as congenital diaphragmatic hernia. Most importantly, this reversal is both structural and physiologic, as these lungs are more compliant and capable of normal gas exchange. In clinical cases of pulmonary hypoplasia, focusing initial therapy on active promotion of lung growth by exploiting a mechanism that may be a part of normal pulmonary development offers some hope of improved outcome in a group of patients that are currently unsalvageable.

**Key words** Congenital diaphragmatic hernia  
Lung growth · Lung liquid · Tracheal ligation

**Introduction**

There is strong evidence that lung liquid is critical to lung growth and that fetal lung-liquid volume must be maintained for normal lung growth to occur. Early investigators believed that lung liquid was derived from the amniotic space [58, 63], however, the amniotic origin of lung liquid has since been disproved [1, 2, 20, 26, 39, 57, 60, 61]. Influx of amniotic fluid into the trachea is, in fact, a very rare event [15, 23, 28, 44]. It is now known that in the normal sheep fetus, lung liquid is actively secreted by the pulmonary epithelium [1, 2, 48, 51, 65], retained in the intrapulmonary space, and maintained at a specific volume and pressure by the regulatory activity of the upper airway, specifically, by changes in the efflux of lung liquid from the

intrapulmonary space to the amniotic space [15]. In studies by Dickson and Harding [15], when lung-liquid volume was experimentally decreased, efflux of lung liquid from the trachea spontaneously decreased until lung-liquid volume and intratracheal pressure (ITP) were restored to normal. Likewise, an increase in lung-liquid volume caused an increase in the efflux of liquid until lung-liquid volume and ITP were restored to normal.

The larynx itself seems to be the major site of this regulation, as decreases in laryngeal resistance by initiation of fetal breathing movements [28–30] and sectioning the recurrent laryngeal nerve [31] both lead to an increase in the rate of efflux of lung liquid in fetal lambs. The present understanding of lung-liquid dynamics, therefore, is that lung liquid is actively secreted by the pulmonary epithelium against the resistance of the upper airway. Lung-liquid volume and ITP are maintained within very precise ranges by the larynx, which through unknown mechanisms regulates the efflux of lung liquid from the trachea to the amniotic space. Disruption of this careful regulation has been shown in both the laboratory and in nature to have dramatic consequences.

Naturally occurring airway occlusions due to absence, atresia, or compression of the larynx, trachea, or bronchus have resulted in large, fluid-filled lungs that histologically appeared to have either normal or slightly distended alveoli [2, 25, 26, 43, 46]. In other experiments of nature, intrauterine airway occlusion resulted in large lungs despite the presence of other anatomic abnormalities that would normally have led to pulmonary hypoplasia. In at least one instance of Fraser syndrome, an infant with bilateral renal agenesis (associated with pulmonary hypoplasia in Potter's syndrome [56]) and concurrent laryngeal atresia had enlarged lungs [67]. In 1941, Potter and Bohlender [57] reported an infant with a left-sided diaphragmatic hernia (CDH) and a left lower accessory lobe without any connection to the airway. The normally connected left lung was hypoplastic, as would be expected in CDH. The accessory lobe, however, which had no bronchial exit for lung liquid, was expanded, fluid-filled, and its architecture was normal. These findings suggested that

maintenance of lung-liquid volume was important to lung growth and development and that disruption might be the cause of pathology.

The first experimental tracheal occlusion was reported in 1948 by Jost and Policard [39], who decapitated fetal rabbits at 19 days' gestation (term = 31 days) and occluded the trachea as part of the operative procedure. On delivery 9 days later, histologic examination of the fetal lungs revealed alveoli that were "larger and more dilated than controls." In several animals the trachea was left in open communication with the amniotic cavity, resulting in alveoli that were "small and collapsed." In 1965, Carmel et al. [11] ligated the tracheas of fetal rabbits to determine whether aspiration of amniotic fluid into the lung was necessary for lung growth. On term delivery, the lungs were described as "large" with increased lung-weight-to-body-weight and lung-volume-to-body-weight ratios. Histology revealed "obvious thinning of the walls of the alveoli and terminal bronchioles with marked dilatation of these structures." The authors concluded that "normal lung development occurs in the absence of amniotic fluid aspiration."

These findings in the rabbit were confirmed by later studies in fetal sheep. In 1969, Berton performed tracheal ligation (TL) in fetal lambs and noted large, distended lungs [7]. In 1971, Lanman et al. [42] performed TL on fetal lambs between 74 and 129 days' gestation (term = 145 days) to determine whether phospholipids present in lung liquid precipitated labor when they reached the amniotic cavity. No change in gestational age at spontaneous delivery of the experimental groups was found. However, wet lung weights were approximately ten times those of controls.

In 1977 Alcorn et al. [4] demonstrated in fetal sheep that TL led to increased lung growth while chronic drainage of lung liquid led to pulmonary hypoplasia. These conclusions were based on lung weights, histologic appearance, and air-space fraction (ASF). Histologically, Alcorn interpreted the "thinning of the future intra-alveolar walls and better defined alveolar formation" as being "generally post-natal events" implying precocious maturation of the ligated lungs. However, this conclusion was not supported by the ratio of type 2 to type 1 pneumocytes, which normally increases with maturity. This ratio was higher in the drained lungs than in the ligated lungs, suggesting that lung-liquid drainage appeared to advance lung maturation while lung-liquid retention did the opposite.

The hypoplastic effect of lung-liquid drainage was confirmed in 1983 by Fewell et al., whose fetal lamb tracheostomy model yielded decreased lung weight and volume and hypoplastic histology [22]. The hyperplastic effect of tracheal ligation was confirmed in 1984 by Adzick et al. who, based on total lung DNA content, demonstrated that tracheal ligation could prevent pulmonary hypoplasia associated with experimental oligohydramnios [3].

In 1990 Moessinger et al. [47] continually drained fetal lung liquid from the right lung while simultaneously increasing the liquid volume of the left by mainstem bronchus ligation. Lung growth was evaluated by dry and

wet lung weights and DNA content and revealed hypoplasia of the drained right side with hyperplasia of the ligated left side. There was, however, no difference in biochemical indices of lung maturation (total phospholipids, phosphatidylcholine, and disaturated phosphatidylcholine) when comparing tissue from hypoplastic, hyperplastic, and control lungs. This led to the conclusion that while fetal lung-cell multiplication was influenced by local distension with lung liquid, the biochemical maturation of fetal lung surfactant was under systemic control.

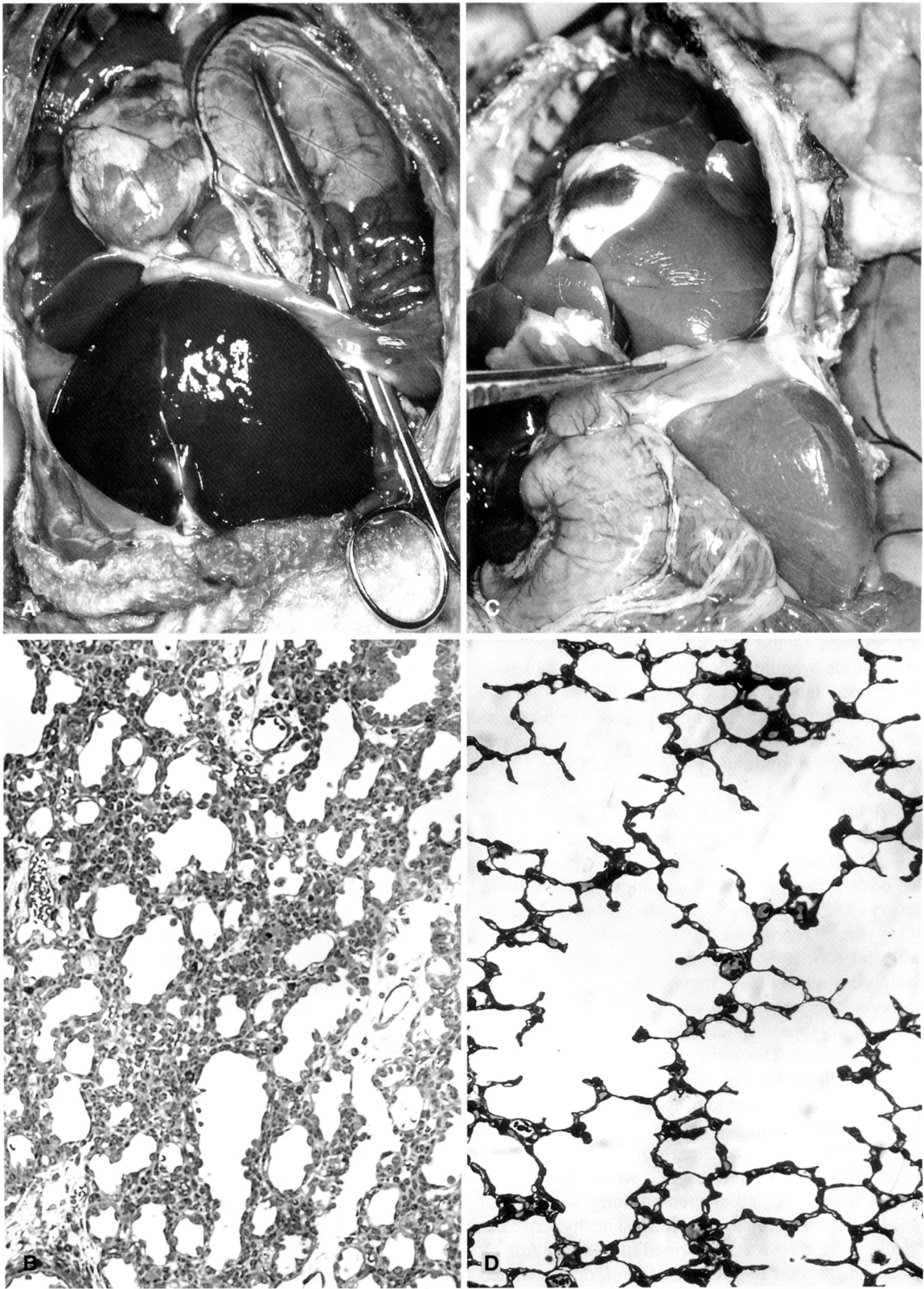
In 1993, Wilson, DiFiore, and Peters [68] recreated the well-documented natural experiment of bilateral renal agenesis (Potter's syndrome) by employing bilateral fetal nephrectomy as the stimulus for pulmonary hypoplasia. This model eliminated the fetal urine contribution to amniotic fluid volume and any renal modulation of pulmonary growth. Histologically, lungs with nephrectomy (Nx) were hypoplastic while those with nephrectomy and TL (Nx/TL) appeared similar to controls. Total lung volume (normalized to body weight), total alveolar surface area (TASA), and total alveolar number (TAN) were elevated in Nx/TL animals over both Nx alone and controls. Total DNA, total protein, and DNA/protein ratios confirmed lung growth by hyperplasia. These data indicated that fetal TL could not only accelerate lung growth beyond even normal limits, but could actually reverse the pulmonary hypoplasia usually seen in anephric sheep.

#### Lung liquid secretion rate

In the sheep fetus, the earliest gestational measurements of lung-liquid secretion rate are 1.6 ml/kg-h at 74 days' gestation, increasing to 2.0 ml/kg-h at 84 days [50]. A rate of 2–3 ml/kg-h is maintained from 85 to 115 days' gestation [62]. At 115 days' gestation there appears to be a significant increase in the rate of fluid production, with reported rates of 4.3–5.5 ml/kg-h from 115 to 142 days [9, 41, 45], at which time lung liquid production rates return to their pre-115-day-gestation values of 2–3 ml/kg-h [9, 41]. In one set of experiments in fetal goats, Perks and Cassin documented an exponential increase in secretion rate from 115 to 145 days' gestation with a sixfold increase in lung liquid secretion over that time period [53].

#### Intratracheal pressure

The authors found no record of ITP measurement prior to 105 days' gestation. Dickson and Harding [14] reported in-utero ITP values of 0.75–1.0 mmHg from 105 to 115 days' gestation. At 115 days ITP doubled to 2.0–2.5 mmHg and was maintained at that level until 130 days' gestation, when measurements were discontinued. In-utero ITP data from 130 days to term were supplied by Vilos and Liggins [65], who documented maintenance of ITP at approximately 2.0 mmHg from 120 days to 145 days (full term).



**Fig. 1** A Gross appearance of term animal who underwent creation of left-sided CDH at 90 days' gestation. B Photomicrograph of inflation-fixed lung tissue of same animal. Note histologically immature appearance of lung. C Gross appearance of term animal who underwent creation of left-sided CDH and ligation of trachea at 90 days'

gestation. Note that lung has grown through diaphragm into abdomen displacing abdominal contents. D Photomicrograph of inflation-fixed lung tissue of animal pictured in C. Note histologically mature appearance of lung (reproduced from *J Pediatr Surg* 28, p 1437, with permission)

## Rate of lung growth

In 1981 Alcorn et al. [5] outlined fetal-lamb lung development from 60 days' gestation to term. Data indicated a doubling in dry lung weight from 108 to 133 days' gestation. In 1991 Docimo et al. [19] determined that the greatest increase in lung volume and TAN occurred between 112 and 124 days' gestation, with each of these parameters growing exponentially with respect to body weight.

Thus, in lambs at approximately 112–115 days' gestation there appears to be a significant change in fetal lung-liquid dynamics, which coincides with significant increases in lung growth. At this time the rate of lung-liquid production increases substantially, as does the ITP/intrapulmonary pressure (IPP). More significantly, these changes coincide with the most rapid period of lung growth, with dramatic increases in lung volume and TAN in the 12–14 days following these changes in lung-liquid dynamics.

## Congenital diaphragmatic hernia

The first animal model for fetal DH was created in 1967 by deLorimier et al. [13], who surgically created DH in fetal lambs. The resulting lungs were hypoplastic by histological appearance and lung weights. Confirmatory studies by Burrington and Olley in 1970 [10] and Kent et al. in 1972 [40] revealed similar changes, the latter also demonstrating alterations in hemodynamics, ventilation, and compliance. A model of CDH in fetal lambs using intrathoracic balloon inflation to simulate herniated viscera was first described by Haller et al. in 1976 [27] and later shown by Harrison et al. to produce fatal pulmonary hypoplasia [35]. Subsequent "correction of the hernia" by balloon deflation allowed sufficient lung growth to assure 100% survival [34]. This was followed by in-utero repair of surgically created fetal DH in lambs, again resulting in prevention of gross lung hypoplasia and survival at birth [37, 59]. While these experimental models offered promise, early attempts at antenatal CDH repair in humans have met with limited success [32, 33, 36].

We have learned from the fetal Nx/TL model that the rate of pulmonary growth in the fetus is not fixed and, in fact, can be accelerated in an experimental system known to produce pulmonary hypoplasia. The purpose of our next study was to determine if the pulmonary hypoplasia of experimental DH could be similarly reversed and, if so, whether the resulting lungs would show better function than those of their DH counterparts [18]. It was again our intention to exploit mechanisms of normal fetal lung growth, in particular the critical period between 112 and 124 days' gestation, when maximal secretion rate of lung liquid and maximal ITP/IPP coincide with and perhaps are responsible for the maximal rate of lung growth.

**Table 1** Air-space fraction and alveolar numerical density ( $\times 10^7$  alveoli/cm<sup>3</sup>) in operated and control animals (DH diaphragmatic hernia, TL tracheal ligation)

<i>Airspace fraction</i>			
	<i>Left</i>	<i>Right</i>	<i>Total</i>
Control <sup>a</sup>	.62 ± .02	.63 ± .03	.63 ± .02
DH	.35 ± .08	.47 ± .05	.41 ± .07
DH/TL <sup>a</sup>	.66 ± .01	.65 ± .01	.66 ± .01
<i>Alveolar numerical density</i>			
	<i>Left</i>	<i>Right</i>	<i>Total</i>
Control <sup>b</sup>	2.34 ± .17	2.17 ± .13	2.25 ± .14
DH	1.06 ± .11	1.27 ± .13	1.17 ± .11
DH/TL <sup>b</sup>	2.08 ± .09	2.25 ± .13	2.17 ± .08

<sup>a</sup>  $P = 0.0003$

<sup>b</sup>  $P = 0.0001$

## Materials and methods

The initial arm of the study was designed to determine the structural effects of TL on lung growth and maturation. Eighteen fetal lambs at 90 days' gestation (term = 145 days) were divided into three experimental groups of 6 animals per group: group I: left diaphragmatic hernia alone (DH); group II: left DH and TL performed at the same operation (DH/TL); group III: sham-operated controls undergoing hysterotomy only. Animals were delivered near term by repeat hysterotomy.

## Results

In all DH animals (group I) the left chest was occupied by stomach, spleen, and small and large bowel and the lungs were markedly reduced in size. In contrast, in all animals with DH/TL (group II) the herniated viscera were completely reduced from the chest by enlarged lungs, which had grown through the diaphragmatic defect into the abdominal cavity (Fig. 1).

On delivery, the DH/TL group showed a positive ITP of  $6.14 \pm 0.55$  mmHg; there was no detectable ITP in the DH or control groups ( $P = 0.0001$ ). This small change in pressure resulted in large changes in the volume of liquid in the lungs. Mean lung-liquid volume in the DH/TL group was  $256.7 \pm 28.9$  ml, versus  $4.0 \pm 0.71$  ml in the DH group and  $12.0 \pm 2.3$  ml in controls ( $P = 0.0001$ ). In all groups, lung liquid was clear and translucent.

Histologically, DH lungs appeared structurally immature when compared to controls, with marked thickening of alveolar walls and decreased air-space. In contrast, DH/TL lungs were similar to controls with normal-appearing alveoli and thin alveolar septae (Fig. 1). Air-space fraction (ASF—percentage of lung occupied by air as opposed to tissue) in the normal lung is approximately 66%. In the DH group this ratio was reversed, with approximately 35% air and 65% tissue ( $P = 0.0001$ , Table 1), while ASFs of the DH/TL group were virtually identical to those of controls. ASF values for the control group in this study were similar to those previously reported [4, 22]. Alveolar numerical density (AND = number of alveoli per cm<sup>3</sup> lung tissue) was



**Table 2** Lung-volume-to-body-weight ratio (ml/kg), total alveolar surface area ( $\times 10^4$  cm<sup>2</sup>) and total alveolar number ( $\times 10^9$ ) in operated and control animals (DH diaphragmatic hernia, TL tracheal ligation;  $P = 0.0001$ )

Lung-volume-to-body-weight ratio ml			
	Left	Right	Total
Control	22.2 $\pm$ 2.2	31.7 $\pm$ 3.3	53.9 $\pm$ 5.5
DH	6.04 $\pm$ 0.7	16.3 $\pm$ 2.1	22.3 $\pm$ 2.8
DH/TL	75.6 $\pm$ 7.3	80.0 $\pm$ 5.3	155.5 $\pm$ 11.4
Total alveolar surface area			
Control	3.94 $\pm$ .71	5.62 $\pm$ 1.0	9.56 $\pm$ 1.7
DH	0.74 $\pm$ .12	2.27 $\pm$ .33	3.01 $\pm$ .45
DH/TL	15.65 $\pm$ 2.3	17.3 $\pm$ 2.2	33.96 $\pm$ 4.3
Total alveolar number			
Control	1.13 $\pm$ .13	1.54 $\pm$ .21	2.67 $\pm$ .35
DH	0.16 $\pm$ .03	0.51 $\pm$ .12	0.67 $\pm$ .15
DH/TL	5.13 $\pm$ .84	5.78 $\pm$ .96	10.9 $\pm$ 1.6

significantly lower in the DH animals than in controls ( $P = 0.0001$ ). Once again, DH/TL increased AND up to but not beyond control values (Table 1). Because the ligated lungs had normal ASF and normal AND, their alveoli must be of normal size. Therefore, these lungs were not emphysematous, but rather had matured normally and maintained normal lung architecture.

When normalized to body weight, DH animals had decreased mean lung volumes when compared to controls. In contrast, the DH/TL group showed markedly increased lung volumes; seven times those of the DH group and three times those of controls ( $P = 0.0001$ , Table 2). In the DH animals, TASA was lower than that of controls. TASA was increased in the DH/TL group tenfold over both DH and control animals ( $P = 0.0001$ , Table 2). Values for TASA in the control group of this study were similar to those previously reported [19]. In DH animals, the TAN was lower than control values. DH/TL animals demonstrated a tenfold increase in TAN above the DH/TL animals and four times over controls ( $P = 0.0001$ , Table 2). The TANs of the control group in this study were similar to those previously reported [22].

Lung DNA, a rough marker for total nuclear material, was markedly increased in the DH/TL group when compared to either DH animals or controls ( $P = 0.0001$ ). Lung protein, a rough marker for total cytoplasmic and other extra-nuclear material, was again increased in the DH/TL group over both DH animals and controls ( $P = 0.0001$ ). Despite these increases, the DNA/protein ratio was similar in all three groups ( $P = \text{NS}$ ), indicating that cells in the ligated lungs had a normal ratio of nucleus to cytoplasm, were therefore of normal size, and had therefore grown by cell division and not by hypertrophy.

To determine how well these structurally normal lungs would function, animals were divided into two experimental groups of 5 each. In group IV, a DH was created at 90 days' gestation as before. In group V, a DH was again created at 90 days, however, given our observation that performing TL at this time caused the left lung to grow

through the hernia defect into the abdomen (Fig. 1), we had concerns about potential mechanical problems in ventilating the intra-abdominal portion of the lung. To attenuate this explosive growth, TL was performed 20 days later at 110 days' gestation rather than simultaneously with the DH. This timepoint was selected to coincide with the marked increase in lung-liquid secretion rate, ITP, and lung growth at this gestational age in normal fetal lambs. Animals were delivered near term and ventilatory support maintained over a wide range of rates and pressures. Support was adjusted in response to arterial blood gas results.

For all animals in groups IV (DH alone) and V (DH/TL), the highest  $\text{paO}_2$  and lowest  $\text{paCO}_2$  attained were averaged within each group. The DH animals exhibited poor gas exchange with a mean highest  $\text{paO}_2$  of  $51.0 \pm 13.7$  mmHg and a mean lowest  $\text{paCO}_2$  of  $143.6 \pm 9.9$  mmHg. DH/TL animals had improved function, achieving a mean highest  $\text{paO}_2$  of  $360.4 \pm 71.5$  mmHg ( $P = 0.003$ ) and a mean lowest  $\text{paCO}_2$  of  $41.6 \pm 3.7$  mmHg ( $P = 0.0001$ ). There were no significant differences between pre- and postductal blood gases. Normal values for newborn lambs are  $\text{paCO}_2 = 41 \pm 5.0$  mmHg [8] and  $\text{paO}_2 = 259 \pm 38$  mmHg [38]. Mean compliance for the DH group was  $0.184 \pm .036$  ml/cmH<sub>2</sub>O and for the DH/TL group  $0.646 \pm .062$  ml/cmH<sub>2</sub>O, 3.5 times that of the DH group ( $P = 0.0001$ ).

## Discussion

The above study [18] was undertaken to determine whether the structural and physiologic effects of pulmonary hypoplasia in fetal DH could be reversed by TL. Our data demonstrated seven principal findings: (1) experimental fetal DH produced hypoplastic lungs that were not capable of adequate gas exchange with conventional ventilation; (2) fetal TL in DH was capable of reversing these effects by accelerating lung growth beyond even normal levels based on alveolar number, alveolar surface area, and lung-volume-to-body-weight ratios; (3) this growth was associated with a normal maturation pattern as evidenced by histologic appearance, ASF, and AND determinations; (4) based on DNA/protein ratios, it appeared that cell size was normal and that lung growth was achieved by cell proliferation rather than hypertrophy; (5) the mechanical consequence of TL in DH was reduction of the herniated abdominal viscera from the chest by enlarged lungs; (6) the physiologic consequences of TL in DH were more compliant lungs, which were more efficient at gas exchange than their DH counterparts; and (7) fetal TL produced increased ITP, which may be responsible for the pulmonary growth observed. These findings also confirm the conclusion from our nephrectomy/TL study that TL can actually reverse the effects of a stimulus known to produce pulmonary hypoplasia.

While the specific mechanisms responsible for lung growth are unknown, we believe increased ITP/IPP may be involved. The ITP values of 6–7 mmHg consistently

found in the DH/TL group are well above the 1.8–2.0 mmHg values seen in normal fetal lambs of similar gestational age in utero [65] and concur with the 6.4 mmHg ITP found in ligated animals by Alcorn et al. [4]. In the normal sheep fetus, outflow of lung liquid is impeded by the upper airway, giving rise to elevated pressures within the trachea [17, 23, 31, 65]. At approximately 115 days' gestation, there are significant increases in lung-liquid secretion rate and ITP that coincide with the period of maximal lung growth (see previous discussion). Conversely, chronic drainage of fetal lung liquid with decreased ITP leads to pulmonary hypoplasia [4, 22]. These data all support our hypothesis that TL corrects pulmonary hypoplasia by enhancing normal mechanisms of fetal lung growth.

If increased intrabronchial pressure is involved in lung growth, what are the consequences of these changes in pressures? It is known that activation of pulmonary stretch receptors by increased ITP inhibits fetal breathing movements by increasing afferent activity in the vagus nerve [55]. This demonstrates that increased ITP and pulmonary "stretch" are already recognized in fetal lambs. It is also known that small pressure changes produce large volume changes in liquid-filled lungs of near-term fetal lambs [42]. This is consistent with our observation that airway distending pressures of 4.0–5.0 mmHg above normal produce appreciable increases in airway lung-liquid volumes. We believe this volume increase may translate into tension on the cells lining the airways. Several studies in vivo and in vitro have shown that cells respond to mechanical tension by increased mitosis and uptake of [<sup>3</sup>H]thymidine [12, 24, 64]. That this is occurring in the current study is supported by the stable DNA/protein ratios, which suggest that lung growth is achieved by cell proliferation rather than hypertrophy. This hypothesis is further supported by similar findings demonstrated in other organ systems, such as vascular smooth muscle [52] and bladder [54]. In fact, soft-tissue growth seen with tissue expanders and the pronounced growth of gastrointestinal tissue proximal to intestinal atresias may both be examples of a ubiquitous mechanism for growth.

We hypothesize that mechanical alveolar distension may promote pulmonary growth biochemically, through elaboration of local growth factor(s). Moessinger et al.'s study [47] of right-lung liquid drainage and left-lung bronchial occlusion would refute the concept of a systemic growth factor. If this were the case, hypoplasia of the drained right lung in this study should have been prevented by concurrent left mainstem bronchus ligation, and it was not. In our model, TL may increase concentrations of local factors by delaying lung-liquid drainage. On a molecular level, alveolar distension may involve alterations in the regulation of particular gene sequences. These are areas for future investigation that we are pursuing.

While the parameters for evaluation of lung growth are clear, those for evaluation of lung maturation are more variable. Alcorn et al.'s study of TL and chronic tracheal drainage [4] used the ratio of type 2 to type 1 pneumocytes to evaluate maturation and found that this ratio was higher

in drained, hypoplastic lungs and lower in ligated, hyperplastic lungs. By these criteria, drained lungs were hypoplastic and hypermature while ligated lungs were hyperplastic and immature. Pringle et al.'s DH 1984 model [59] also showed an increase in type 2 alveolar cells in the hypoplastic lungs of DH animals when compared to lungs from animals repaired in utero and controls.

Moessinger et al.'s study of concomitant right lung drainage and left mainstem bronchus ligation [47] evaluated lung maturation biochemically measuring total phospholipids, phosphatidylcholine, and disaturated phosphatidylcholine (SPC) content per unit of tissue DNA. While drained lungs were hypoplastic and ligated lungs hyperplastic, they were both of normal biochemical maturation when compared to controls. Fewell et al.'s fetal tracheostomy model [22] also demonstrated normal SPC/tissue DNA values in drained, hypoplastic lungs, indicating normal biochemical maturation. Our study evaluated lung maturation by quantitative morphometrics and supports Moessinger's conclusion that ligation leads to hyperplasia and normal maturation. We are presently evaluating the tissue by electron microscopy and biochemical indices to make more direct comparisons with these previous studies possible.

Perhaps the most significant finding in this study is that TL, in addition to correcting the structural effects of pulmonary hypoplasia in DH, also markedly improved the lung's capacity for gas exchange. We are presently undertaking detailed morphometric studies of the pulmonary vasculature of these animals, however, their excellent physiologic function would suggest that the vascular and alveolar development have kept pace with one another.

Another very interesting finding of our study, not previously discussed, involves four animals in which an attempted TL was incomplete, with a pinhole tract being observed that allowed communication between the intrapulmonary system and the amniotic space. In these animals, all morphometric parameters, histologic analyses, and DNA-protein ratios were virtually identical to those of the lungs from the DH group; moreover, there was no detectable ITP on delivery. This would suggest that in order to stimulate hyperplasia, complete tracheal occlusion is required and that loss of the increased ITP created by tracheal occlusion results in no significant hyperplastic effect. Regulation of the hyperplastic effect would therefore be achieved by regulating the time period of complete tracheal occlusion, but not by modulating the amplitude of positive ITP by variable partial tracheal occlusion. These hypotheses are supported by Moessinger et al.'s 1990 study [47] where in two animals complete left bronchus ligation had failed, resulting in a persistent pinhole tract in the left bronchus. In these animals total lung DNA was unchanged from controls, indicating that this partial TL eliminated the hyperplastic effect of complete tracheal occlusion.

Before pursuing clinical studies, it will be important to determine in the laboratory whether increased ITP can be combined with extracorporeal membrane oxygenation (ECMO) to accelerate postnatal lung growth. We have already demonstrated that lung growth does occur postna-

tally in CDH patients, however, the rate is too slow to improve lung function within the time limits of present ECMO support [6]. The results of our DH/TL study show that during a 25-day period (TL at 110 days' gestation; delivery at 135 days' gestation) lung growth was sufficient to reduce the DH and improve physiologic function. If increased ITP could produce a similar rate of growth postnatally, ECMO could support these infants during the process. Studies indicate that several days prior to delivery, secretion of lung liquid decreases [16, 41] and that during spontaneous labor, the fetal sheep lung changes from a secretory to an absorptive state [9]. Furthermore, this absorptive state can be manipulated pharmacologically [9, 49, 66]. These data need to be considered when designing studies of lung liquid and postnatal lung growth.

If it is determined that intervention must take place in the fetal period, tracheal occlusion may easily be adapted to a laparoscopic approach. Occlusion of the airway by an endoscopically delivered balloon apparatus or by external ligation of the fetal trachea with a surgical clip should result in less morbidity than present antenatal techniques. Pilot studies in "fetoscopic surgery" have been reported [21], and we have been successful in accessing the airway of a 95-day gestation fetal sheep using laparoscopic equipment.

## References

- Adams FH, Fujiwara T (1963) Surfactant in fetal lamb tracheal fluid. *J Pediatr* 63: 537–542
- Adams FH, Fujiwara T, Rowshan G (1963) The nature and origin of the fluid in the fetal lamb lung. *J Pediatr* 63: 881–888
- Adzick NS, Harrison MR, Glick PL, Villa RL, Fikbeiner W (1984) Experimental pulmonary hypoplasia and oligohydramnios: relative contributions of lung fluid and fetal breathing movements. *J Pediatr Surg* 19: 658–663
- Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM (1977) Morphologic effects of tracheal ligation and drainage in the fetal lamb lung. *J Anat* 123: 649–660
- Alcorn DG, Adamson MT, Maloney JE, Robinson PM (1981) A morphologic and morphometric analysis of fetal lung development in the sheep. *Anat Rec* 201: 655–667
- Beals DA, Schloo BL, Vacanti JP, Reid LM, Wilson JM (1992) Pulmonary growth and remodeling in infants with high risk congenital diaphragmatic hernia. *J Pediatr Surg* 27: 997–1002
- Berton JP (1969) Effets de la ligature de la trachée chez le foetus de mouton à la fin du premier et au deuxième tiers de la gestation: accumulation de liquide dans les ramifications bronchiques primitives et anasarque foeto-placentaire. *CR Assoc Anat* 145: 122–131
- Brooks DL, Tillman PC, Niemi SM (1984) Ungulates as laboratory animals. In: Fox JG, Cohen BJ, Loew FM (eds) *Laboratory animal medicine*. Academic Press, New York London, p 283
- Brown MJ, Olver RE, Ramsden CA, Strang LB, Walters DV (1983) Effects of adrenaline and spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb. *J Physiol (Lond)* 344: 137–152
- Burrington JD, Olley PM (1970) Experimental diaphragmatic hernias in fetal lambs. *Clin Res* 18: 198
- Carmel AJ, Friedman F, Adams FH (1965) Fetal tracheal ligation and lung development. *Am J Dis Child* 109: 452–456
- Curtis ASG, Seehar GM (1978) The control of cell division by tension or diffusion. *Nature* 274: 52–53
- DeLorimier AA, Tierney DF, Parker HR (1967) Hypoplastic lungs in fetal lambs with surgically produced congenital diaphragmatic hernia. *Surgery* 62: 12–17
- Dickson KA, Harding R (1991) Fetal breathing and pressures in the trachea and amniotic sac during oligohydramnios in sheep. *J Appl Physiol* 70: 293–299
- Dickson KA, Harding R (1987) Restoration of lung liquid volume following its acute alteration in fetal sheep. *J Physiol* 385: 531–543
- Dickson KA, Maloney JE, Berger PJ (1986) Decline in lung liquid volume before labor in fetal lambs. *J Appl Physiol* 61: 2266–2272
- Dickson KA, Maloney JE, Berger PJ (1987) State-related changes in lung liquid secretion and tracheal flow rate in fetal lambs. *J Appl Physiol* 62: 34–38
- DiFiore JW, Fauza DO, Slavin R, Peters CA, Fackler JC, Wilson JM (1994) Experimental fetal tracheal ligation reverses the structural and physiological effects of pulmonary hypoplasia in congenital diaphragmatic hernia. *J Pediatr Surg* 29: 248–257
- Docimo SG, Crone PK, Davies P, Reid L, Retik AB, Mandell J (1991) Pulmonary development in the fetal lamb: morphometric study of the alveolar phase. *Anat Rec* 229: 495–498
- Enhoring G (1964) Surface tension of amniotic fluid. *Am J Obstet Gynecol* 88: 519
- Estes J, MacGillivray TE, Hedrick MH, Adzick NS, Harrison MR (1992) Fetoscopic surgery for treatment of congenital anomalies. *J Pediatr Surg* 27: 950–954
- Fewell JE, Hislop AA, Kitterman JA, Johnson P (1983) Effect of tracheostomy on lung development in fetal lambs. *J Appl Physiol* 55: 1103–1108
- Fewell JE, Johnson P (1983) Upper airway dynamics during breathing and during apnea in fetal lambs. *J Physiol* 339: 495–504
- Folkman J, Moscona A (1978) Role of cell shape in growth control. *Nature* 273: 345–349
- Goodlin R, Lloyd D (1968) Fetal tracheal excretion of bilirubin. *Biol Neonat* 12: 1
- Griscom NT, Harris C, Wohl ME, Vawter GF, Eraklis AJ (1969) Fluid-filled lung due to airway obstruction in the newborn. *Pediatrics* 43: 383–390
- Haller AJ, Signer RD, Galladay ES, Inon AE, Harrington DP, Shermeta DW (1976) Pulmonary and ductal hemodynamics in studies of simulated diaphragmatic hernia of fetal and newborn lambs. *J Pediatr Surg* 11: 675–680
- Harding R, Bocking AD, Sigger JN (1986) Influence of upper respiratory tract on liquid flow to and from fetal lungs. *J Appl Physiol* 61: 68–74
- Harding R, Bocking AD, Sigger JN (1986) Upper airway resistances in fetal sheep: the influence of breathing activity. *J Appl Physiol* 60: 160–165
- Harding R, Johnson P, McClelland ME (1980) Respiratory function of the larynx in developing sheep and the influence of sleep state. *Resp Physiol* 40: 115–179
- Harding R, Sigger JN, Wickham PJD, Bocking AD (1984) The regulation of flow of pulmonary fluid in fetal sheep. *Resp Physiol* 57: 47–59
- Harrison MR, Adzick NS, Flake AW, Jennings RW, Estes JM, MacGillivray TE, Chueh JT, Goldberg JD, Filly RA, Goldstein RB, Rosen MA, Cauldwell C, Levine AH, Howell LJ (1993) Correction of congenital diaphragmatic hernia in utero. VI. Hard-earned lessons. *J Pediatr Surg* 28: 1411–1418
- Harrison MR, Adzick NS, Longaker MT, et al (1990) Successful repair in utero of a fetal diaphragmatic hernia after removal of viscera from the left thorax. *N Engl J Med* 322: 1522–1524
- Harrison MR, Bressack MA, Churg AM, deLorimier AA (1980) Correction of congenital diaphragmatic hernia in utero. II. Simulated correction permits fetal lung growth with survival at birth. *Surgery* 88: 260–268
- Harrison MR, Jester JA, Ross NA (1980) Correction of congenital diaphragmatic hernia in utero. The model: intrathoracic balloon produces fatal pulmonary hypoplasia. *Surgery* 88: 174–182
- Harrison MR, Langer JC, Adzick NS, Golbus MS, Filly RA, Anderson RL, Rosen MA, Callen FW, Goldstein RB, deLorimier

- AA (1990) Correction of congenital diaphragmatic hernia in utero. V. Initial clinical experience. *J Pediatr Surg* 25: 47–57
37. Harrison MR, Ross MA, deLorimier AA (1981) Correction of congenital diaphragmatic hernia in utero. III. Development of successful surgical techniques using abdominoplasty to avoid compromise of umbilical blood flow. *J Pediatr Surg* 16: 934–942
  38. Hershenson MB, O'Rourke PP, Schena JA, Crone RK (1987) Effect of halothane on critical levels of oxygen transport in the anesthetized newborn lamb. *Anesthesiology* 67: 18–23
  39. Jost PA, Policard A (1948) Contribution experimentale a l'etude du developpement prenatal du poumon chez le lapin. *Arch Anat Microscop* 37: 323–332
  40. Kent GM, Olley PM, Creighton RE, Dobbinson T, Bryan MH, Symchych P, Zingg W, Cummings JW (1972) Hemodynamic and pulmonary changes following surgical creation of a diaphragmatic hernia in fetal lambs. *Surgery* 72: 427–433
  41. Kitterman JA, Ballard PI, Clements JA, Mescher EJ, Tooley WH (1979) Tracheal fluid in fetal lambs: spontaneous decrease prior to birth. *J Appl Physiol* 47: 985–989
  42. Lanman JT, Schaffer A, Herod L, Ogawa Y, Castellanos R (1971) Distensibility of the fetal lung with fluid in sheep. *Pediatr Res* 5: 586–590
  43. MacGregor AR (1960) Pathology of infancy and childhood. Livingstone, London, pp 172–173
  44. Maloney JE, Adamson TM, Brodecky V, Cranage S, Lambert TF, Ritchie BC (1975) Diaphragmatic activity and lung liquid flow in the unanesthetized fetal sheep. *J Appl Physiol* 39: 423–428
  45. Mescher EJ, Platzker ACG, Ballard PL, Kitterman JA, Clements JA, Tooley WH (1975) Ontogeny of tracheal fluid, pulmonary surfactant, and plasma corticoids in the fetal lamb. *J Appl Physiol* 39: 1017–1021
  46. Milles G, Dorsey DB (1950) Intra-uterine respiration-like movements in relation to development of the fetal vascular system: a discussion of intra-uterine physiology based upon cases of congenital absence of the trachea, abnormal vascular development and other anomalies. *Am J Pathol* 26: 411
  47. Moessinger AC, Harding R, Adamson TM, Singh M, Kiu GT (1990) Role of lung fluid volume in growth and maturation of the fetal sheep lung. *J Clin Invest* 86: 1270–1277
  48. Olver RE (1977) Fetal lung liquids. *Federation Proceedings* 36: 2669–2775
  49. Olver RE, Ramsden CA, Strang LB, Walters DV (1986) The role of amiloride-blockable sodium transport in adrenaline-induced lung liquid reabsorption in the fetal lamb. *J Physiol* 376: 321–340
  50. Olver RE, Schneeberger EE, Walters DV (1981) Epithelial solute permeability, ion transport and tight junction morphology in the developing lung of the fetal lamb. *J Physiol* 315: 395–412
  51. Olver RE, Strang LB (1974) Ion fluxes across the pulmonary epithelium and the secretion of lung liquid in the fetal lamb. *J Physiol (Lond)* 241: 327–357
  52. Owens GK, Geisterfer AA, Yang W, Komoriya A (1988) Transforming growth factor, beta-induced growth inhibition and cellular hypertrophy in cultured vascular smooth muscle cells. *J Cell Biol* 107: 771
  53. Perks AM, Cassin S (1985) The rate of production of lung liquid in fetal goats, and the effect of expansion of the lungs. *J Dev Physiol* 7: 149–160
  54. Peters CA, Vasavada S, Dator D, Carr MC, Shapiro E, Lepor H, McConnell J, Retik AB, Mandell J (1992) The effect of obstruction on the developing bladder. *J Urol* 148: 491–496
  55. Ponte J, Purves MJ (1973) Types of afferent nervous activity which may be measured in the vagus nerve of the sheep foetus. *J Physiol* 229: 51–76
  56. Potter EL (1946) Bilateral renal agenesis. *J Pediatr* 29: 68–76
  57. Potter EL, Bohlender GP (1941) Intrauterine respiration in relation to development of the fetal lung. *Am J Obstet Gynecol* 42: 14–22
  58. Preyer W (1885) *Specielle physiologie des embryo*. Grieben, Leipzig, p 149
  59. Pringle KC, Turner JW, Schofield JC, Soper RT (1984) Creation and repair of diaphragmatic hernia in the fetal lamb: lung development and morphology. *J Pediatr Surg* 19: 131–140
  60. Ross BB (1963) Comparison of fetal pulmonary fluid with fetal plasma and amniotic fluid. *Nature* 199: 1100
  61. Setnikar I, Agostini E, Taglietti A (1959) Fetal lung: source of amniotic fluid. *Soc Exp Biol Med* 101: 842
  62. Shermeta DW, Oesch I (1981) Characteristics of fetal lung fluid production. *J Pediatr Surg* 16: 943–946
  63. Snyder FF, Rosenfeld M (1937) Direct observation of intrauterine respiratory movements and the role of carbon dioxide and oxygen in their regulation. *Am J Physiol* 119: 153–166
  64. Squier CA (1980) The stretching of mouse skin in vivo: effect on epidermal proliferation and thickness. *J Invest Dermatol* 74: 68–71
  65. Vilos GA, Liggins GC (1982) Intrathoracic pressures in fetal sheep. *J Dev Physiol* 4: 247–256
  66. Walters DV, Olver RE (1978) The role of catecholamines in lung liquid absorption at birth. *Pediatr Res* 12: 293
  67. Wigglesworth JS, Desai R, Hislop AA (1987) Fetal lung growth in congenital laryngeal atresia. *Pediatr Pathol* 7: 515–525
  68. Wilson JM, DiFiore JW, Peters CA (1993) Experimental fetal tracheal ligation prevents the pulmonary hypoplasia associated with fetal nephrectomy: possible application for congenital diaphragmatic hernia. *J Pediatr Surg* 28: 1433–1440