The Effect of Intra-amniotic Injection of Triiodothyronine on Pulmonary Maturity in Lambs at 130 Days Gestation

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Summary

Saline or triiodothyronine (T_3) (50–1000 μg) was injected into the amniotic sac of 17 pregnant ewes under general anesthesia at 130 days gestation. Forty-eight h later, the lambs were delivered by hysterotomy. Cord plasma T_3 and cortisol and amniotic fluid T_3 were assayed, and the maturity of the fetal lung was assessed in terms of its pressure-volume response and its surfactant (lamellar body phospholipid) content. With the highest dose of T_3, cord plasma T_3 and cortisol were raised, and lung maturity was enhanced compared with saline-treated controls; the pressure-volume curve showed increased hysteresis on inflation and deflation, and the lung retained air on return to zero pressure. There was also an apparent, but not statistically significant, increase in the lamellar body phospholipid content of the lung. Irrespective of treatment, lungs which were more mature, in terms of their pressure-volume characteristics, tended to contain a higher proportion of lamellar body phospholipid relative to total phospholipid.

Abbreviations

T_4, thyroxine
T_3, triiodothyronine

Pregnant women threatening premature delivery are often treated with glucocorticoid in an attempt to accelerate maturation of the fetal lungs and thus avoid hyaline membrane disease in the neonate (17). However, the treatment is not always effective and early results suggested that such treatment may increase the risk of fetal death in pregnancies complicated by severe hypertension-edema-proteinuria syndromes (17). Hence, other substances which may accelerate the process of lung maturation need to be considered.

The results of several investigations in the lamb, rat, and rabbit strongly suggest that thyroid hormones promote lung maturation and surfactant production (4, 9–11, 22, 23, 25, 27). In addition, it has been shown that T_4 (24) and T_3 levels (6) in cord blood are lower in infants who develop hyaline membrane disease than in those who do not, although there is disagreement (16). One recent uncontrolled study in the human has reported possible beneficial effects of intra-amniotic thyroxine on fetal pulmonary maturity (18), but the number of cases studied was small.

The present investigation was designed to study the effect of intra-amniotically administered thyroid hormone on fetal lung maturation in the sheep using T_3 rather than T_4, T_3 being the more active form of thyroid hormone. The hormone was admin-

istered intra-amniotically rather than maternally, because the sheep placenta is only very slightly permeable to T_3 (8) and it has been shown that thyroid hormone is readily transferred from the amniotic fluid to the fetal circulation (26).

MATERIALS AND METHODS

Treatment of ewes and lambs. Seventeen sheep (13 pure bred Merino ewes and four Dorset Horn/Merino cross-bred ewes) were used. Pregnancies were achieved by single matings after the induction of estrus, and confirmed by test matings. At 116 days gestation, the pregnant ewes were transported from open pasture to the university animal house for acclimatization, where they were maintained in individual pens and fed lucerne chaff. The experiments were conducted in a blind fashion where T_3 (20 μg/ml saline) and saline controls were packaged identically. The code was broken at the end of experimentation on a group of animals so that an ongoing analysis of the results was possible, and modification of the protocol could be carried out until an appropriate dose of T_3 was achieved. At 130 days gestation, under general anesthesia (after 12 h fasting), a lower paramedian incision was made in the abdomen, and the uterus was exposed. The fetal neck was grasped and 20 ml of amniotic fluid was withdrawn. T_3 (50–1000 μg) or saline was injected by the same needle, so that it was introduced as close as possible to the fetal mouth. The incision was closed, and the animals were returned to their cages with only local antibiotic cover to their incision.

After 48 h, the lambs were delivered by hysterotomy under general anesthesia. Prior to delivery of the lamb, 20 ml of amniotic fluid and 10 ml of cord venous blood were taken. The blood was collected into heparinized tubes and after centrifugation, the plasma was stored at -20°C until required for analysis.

In order to prevent gasping, the lambs were sacrificed by intravenous pentobarbitone at the moment of cord clamping and before delivery of the fetal head. The lambs so delivered were drained immediately of tracheal fluid through an endotracheal tube. The left side of the chest wall was then removed, and two wedges of tissue were excised from the left lobe, one from the apical margin of the upper lobe (about 2.5 g) and one from the basal margin of the lower lobe (about 4.5 g). A thin section of lung, obtained by cutting transversely through the center of each wedge, was fixed for light microscopy. The remainder of each lung sample was rinsed in ice-cold 0.25 M sucrose and used as soon as possible for the determination of lamellar body phospholipid and total lung phospholipid.

The left main bronchus was then carefully dissected and occluded with a suture and the right lung, which remained *in situ*, was used for pressure-volume studies.

Hormone assays. T_3 in amniotic fluid or cord venous plasma, and cortisol in cord venous plasma were assayed by double

antibody radioimmunoassay using reagents prepared by Diagnostic Products Corp., Los Angeles, CA.

Light microscopy. Tissue was fixed in 10% formalin in cacodylate buffer and processed according to standard procedures used for human autopsy specimens. Criteria used to judge maturity included development of the terminal air sac, flattening of the alveolar cells, thinning of the blood-air barrier, and the absence of mesenchymal tisues among the pneumatocytes.

Lung and lamellar body phospholipid determinations. The lamellar body fraction was isolated by isopycnic density gradient centrifugation, as described previously (7), with the following modifications. Five per cent (w/v) lung homogenates were prepared in 1.2 M sucrose, using 1.25-g portions of chopped lung, and the homogenate (5 ml) was overlaid with a discontinuous gradient, consisting of 2 ml 0.85 M sucrose, 2 ml 0.75 sucrose, and 2.5 ml 0.25 M sucrose, prior to centrifugation at $125 \times g$ and $80,000 \times g$. The band of material which came to equilibrium at the 0.25 M/0.75 M sucrose interface was collected as the lamellar body fraction. Electron microscopy (7) confirmed that this fraction consisted mainly of lamellar bodies or membranous structures apparently of lamellar body origin, whereas fragments of plasma membrane predominated in the small band of material which floated at the 0.75 M/0.85 M sucrose interface (Duck-Chong C. G., and B. Duncan, unpublished data). The phospholipid content of the lamellar body fraction and the whole homogenate (diluted 1:5 with 0.9% NaCl) was determined as described previously (7).

Pressure-volume measurements. The trachea was attached to a pressure-volume apparatus as described by Avery and Mead (3). The lungs were twice slowly inflated and deflated with air to 30 cm H$_2$O pressure; then pressure-volume readings were commenced. Readings were taken at 5-cm pressure intervals or less allowing 2 min of equilibration at each pressure. The area of the pressure-volume curve was then expressed in arbitrary planimeter units.

RESULTS

At the time of delivery of the lambs, that is, 48 h after treatment, T$_3$ was present in the amniotic fluid of all T$_3$-treated animals, but not detectable in the amniotic fluid of saline-treated controls. In those who received 100–1000 μg T$_3$, significant increases in cord venous plasma T$_3$ concentrations were noted (Table 1). At the highest dose of T$_3$, but not at lower doses, there was also a significant increase in the concentration of cortisol in the plasma, due mainly to the fact that one of the T$_3$-treated fetuses had a serum cortisol of 160 nmol/liter. There was also an increase in the maturity of the fetal lungs, as indicated by a 6-fold increase in the area of the pressure-volume curve (Table 1). One atypical individual in the control group had a high cord plasma T$_3$ level and unusually mature lungs, but a plasma cortisol level comparable with that of the other animals in the control group. This fetus was also comparable in size to the others in the control group.

Figure 1 illustrates the difference in the pressure-volume response characteristics of "immature" and "mature" lungs. To allow the shapes of the different curves to be compared directly, the data were normalized by expressing volumes as a percentage of total lung volume for each lung. More mature lungs show marked hysteresis and retention of air on return to zero pressure. Only one of seven animals in the control group showed a mature response, compared with three of four in the group treated with 1000 μg T$_3$ (Fig. 1). The difference between these two proportions is significant ($p = 0.04$).

Analysis of lung phospholipids showed an apparent increase in lamellar body phospholipid in response to T$_3$ treatment (Table 2), but there was considerable variation between individuals and the differences were not statistically significant. Since data expressed per g lung may be influenced by changes in tissue composition, for example, changes in its water or glycogen content, the lamellar body phospholipid content of the lung was also expressed as a percentage of its total phospholipid content. Lamellar body phospholipid levels, whether expressed in terms of tissue wet weight or relative to total lung phospholipid, were significantly higher in the upper lobe than in the lower lobe in controls and those treated with lower doses of T$_3$, but the difference between lobes was not significant in those treated with 1000 μg T$_3$ (Table 2).

When data for lung phospholipids were related to the area of the pressure-volume curve for individual lambs, a direct correlation between the percentage lamellar body phospholipid content of the lung and "maturity" of the pressure-volume response was apparent, irrespective of treatment (Fig. 2). All four lungs with pressure-volume areas greater than 100 U contained more than 8% lamellar body phospholipid, whereas of the nine lungs with pressure-volume areas less than 100, all but one contained less than 8% lamellar body phospholipid. The difference between these two proportions is highly significant ($p < 0.001$). This relationship was independent of the sex of the fetuses.

All lungs studied by light microscopy showed histological maturity.

DISCUSSION

These data show that fetal lung maturity can be enhanced by the intra-amniotic administration of T$_3$ in the sheep, and thus lend support to other studies in which the effect of thyroid hormones on lung maturation has been investigated (4, 22, 25, 27).

The finding of histological maturity in all lambs at 132 days gestation irrespective of treatment is in agreement with Kikkawa *et al.* (15), who could find no difference by light microscopy between lambs of 121 days gestation and term. The finding also indicates that histological maturity (as defined in "Materials and Methods") is not synonymous with functional maturity and that the enhanced lung compliance observed in some T$_3$-treated fetuses did not result from gross changes in lung morphology.

Using electron microscopy, it has been shown that beyond 120 days gestation in the lamb there is a marked increase in the number of lamellar bodies present in Type II cells of the alveolar epithelium (1, 5). These lamellar bodies are the source of surfactant, the phospholipid-rich substance which lowers surface tension within the lungs and prevents alveolar collapse on expiration. The ultracentrifugation technique used in this study allowed quantitation of the amount of lamellar body (or surfactant) phospholipid in the lung. There was an overall correlation between the lamellar body phospholipid content of the lung and the maturity of the lung with respect to its pressure-volume response (Fig. 2). However, while increased surfactant availability
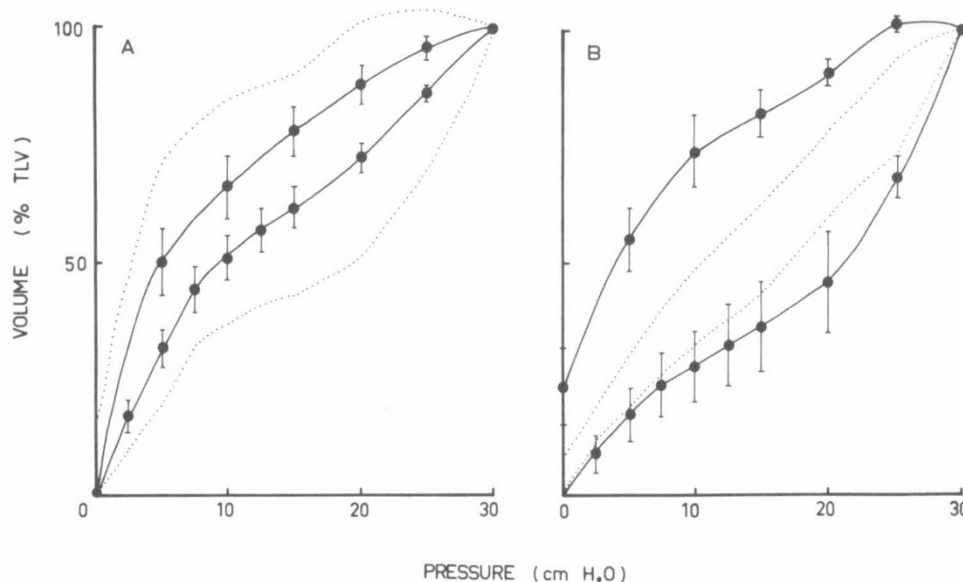
Table 1. *Effect of intra-amniotic administration of T$_3$ on cord venous T$_3$ and cortisol levels and on lung pressure-volume curves in lambs delivered 48 h after treatment**

| Dose of T$_3$ (μg) | Cord venous plasma | | Area of pressure-volume curve (planimeter U) | |
	T$_3$ (nmol/liter)	Cortisol (nmol/liter)		
0	0.40 ± 0.03	44 ± 4 (7)†	29 ± 10	(6)†
0‡	1.34	40 (1)	162	(1)
50	0.42 (0.35, 0.49)	59 (53, 65) (2)	39 (38, 40)	(2)
100–200	0.82 ± 0.06***	63 ± 17 (3)	27 (22, 31)	(2)
1000	2.00 ± 0.58**	89 ± 25* (4)	182 ± 72*	(4)

* Values represent individual data or the mean ± SEM. The asterisks indicate the significance of the difference between that value and the corresponding value for the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Student's *t* test.

† The number of animals is indicated in these parentheses.

‡ Atypical individual; data excluded from statistical analysis.

Fig. 1. Pressure-volume curves of lungs of fetal lambs 48 h after treatment with either saline (*A*) or 1000 μg T$_3$ (*B*). Volumes are expressed as a percentage of total lung volume (*TLV*) at a pressure of 30 cm H$_2$O. In *A*, the *solid line* represents the mean of data for the six lambs who showed a typical immature response (area < 100 planimeter U, no air retention on deflation). In *B*, the *solid line* indicates the mean for the three lambs who showed a typical mature response (area > 100 planimeter U, with air retention on deflation). The bars indicate ± 1 SEM. *Dotted lines* indicate data for a single lamb in each group, whose lungs were unusually mature (*A*) or immature (*B*), compared with the others in the group.

Table 2. *Effect of intra-amniotic administration of T$_3$ on lung phospholipids**

Dose of T$_3$ (μg)	Lamellar body phospholipid (mg/g lung)		Lamellar body phospholipid†/Total lung phospholipid	
	Upper lobe	Lower lobe	Upper lobe	Lower lobe
0	0.63 ± 0.10	0.48 ± 0.09‡	6.9 ± 1.0	5.3 ± 0.9‡ (6)*
0*	1.61	1.30	15.8	11.1 (1)
50–200	0.86 ± 0.19	0.58 ± 0.13	8.6 ± 1.6	5.7 ± 0.9‡ (4)
1000	0.97 ± 0.29	0.81 ± 0.21	11.1 ± 3.4	9.0 ± 2.2 (4)

* See footnotes to Table 1.

† Lamellar body phospholipid (mg/g lung) expressed as a percentage of total lung phospholipid (mg/g lung).

‡ The difference between this value and the corresponding value for the upper lobe is significant ($p < 0.05$, paired *t* test).

possibly contributes to the more mature lung function observed in some of these fetuses, other factors such as tissue elasticity or the degree of alveolation (1, 20) may also be important.

There was a tendency for the upper lobe of the lung to be more mature than the lower lobe, especially in fetuses whose lungs had not been matured by T$_3$ treatment (Table 2). This finding is in agreement with the results of others (5, 12), who measured surface tension, pressure-volume characteristics, or the amount of total lung phospholipid, or disaturated phosphatidyl-choline per mg DNA. They found a similar marked difference in the degree of maturity of the upper and lower lobes in lambs of 120–130 days gestation, which tended to disappear as the lungs matured.

In lambs given a single dose of 1000 μg T$_3$, the cord plasma T$_3$ level at the time of delivery approached that noted in lambs at term (21). However, as the half-life of T$_3$ in fetal lamb serum is known to be only about 4–6 h (8), the concentration of T$_3$ in the circulation must have been considerably higher earlier in the 48-h treatment period. Similarly, cortisol levels higher than those

reported may have resulted from treatment with 1000 μg T$_3$. This raises the possibility that the enhanced lung maturity, which was observed only in response to a very high dose of T$_3$, may have been mediated by a rise in cortisol. If this were so, then no advantage would be gained by using thyroid hormone instead of corticosteroid to enhance fetal lung maturity. However, it is likely that both hormones exerted an effect in such cases, since maturation of the lung is under multihormonal control (11, 19),
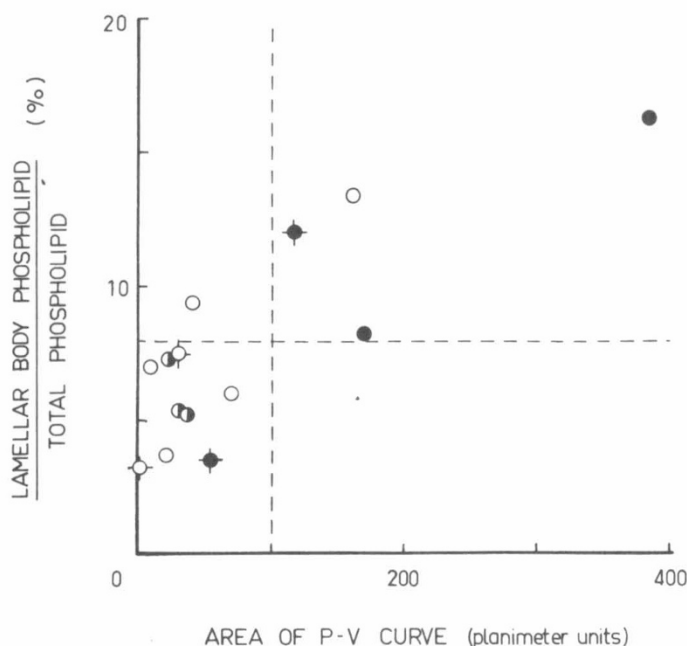

Fig. 2. The relationship between lung lamellar body phospholipid, expressed as a percentage of total lung phospholipid, and the area of the pressure-volume (*P-V*) curve for individual lambs treated with saline (○), 50–200 μg T$_3$ (◐), or 1000 μg T$_3$ (●). The phospholipid data represent the mean of the values obtained for the upper and lower lobe of the lung. The data points derived from Dorset Horn/Merino Cross bred lambs are marked with a *cross*.

and thyroid hormones and glucocorticoid seem to act in different ways (4, 10).

The use of agents such as thyroid hormones must always be approached with caution, because they affect many tissues. A beneficial effect on the lung may be offset by harmful effects on some other system. For example, brain development is dependent on thyroid hormones. Administration of T_4 in large doses to neonatal rats resulted in structural and functional changes in the brain (13). The effects appeared to be due to premature switching to a more mature nerve cell production. Those neurones which had not made synaptic connections at that time died, with a resultant overall reduction in brain cell number.

It has never been established that the apparent enhancement of lung maturity in response to thyroid hormones does, in fact, represent a beneficial effect on the fetus in terms of improved respiratory performance and survival following premature delivery. In one study involving rabbits (27), a significantly higher number of deaths occurred *in utero* in T_4-treated fetuses compared with their littermate controls. However, the survival of the prematurely delivered fetuses was not assessed.

In another study in the rat, in which T_3 or T_4 was administered to the mother, the lungs of some fetuses (delivered 2 days prematurely and maintained for 60 min in a humidicrib) showed widespread atelectasis, although the lungs of their littermates examined at the time of delivery appeared to have matured in response to the treatment (14). In the sheep, treatment of three fetuses by intravenous infusions of T_3 or T_4 at the rate of 1–12 mg/day did not lead to enhanced survival on premature delivery (2), and two other lambs given higher doses died *in utero*. In another study, administration of 150 μg T_3 intravenously to the lamb *in utero* at 119 days gestation resulted in immediate fetal death (22).

Clearly, further investigation is needed to establish whether the apparent enhancement of lung maturity, observed in this and other studies, results in improved respiratory performance and survival on premature delivery, or whether toxic side effects outweigh the potential benefits of the treatment.

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