

THE EFFECTS OF *IN VIVO* PULMONARY OXYGENATION ON LUNG LIQUID PRODUCTION IN NEAR-TERM FETAL SHEEP

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SUMMARY

Lung liquid (LL) is secreted into the fetal lung lumen, but it must be rapidly absorbed at birth to allow air breathing. *In vitro* studies have implicated oxygen as a possible factor causing the switch from secretion to absorption of lung liquid at birth. We developed a technique of oxygenating the fetal lung using liquid ventilation with haemoglobin (Hb) solutions in chronically catheterized fetal lambs (129–140 days gestation; term, 147 days). In some experiments 2,3-diphosphoglycerate (DPG) was added to increase oxygen delivery. LL secretion rate (J_v) was measured using an indicator dilution method. Eighteen fetuses were divided into four groups and ventilated with liquid under the following conditions: (i) Hb with oxygen, (ii) Hb without oxygen, (iii) Hb with DPG and oxygen and (iv) Hb with DPG without oxygen. There was a significant rise (2.6 mmHg, $P < 0.02$) in fetal arterial P_{O_2} in group iii, but in none of the other groups. In the first 3 h of liquid ventilation there was no difference in J_v between the groups. In group i, during hours 4–6 of liquid ventilation, there was a significant rise in secretion rate from 2.25 ± 0.88 to 3.74 ± 0.85 ml h⁻¹ kg⁻¹ ($P < 0.001$). In group iii, when comparing J_v in the first 3 h of liquid ventilation with that in the following 3 h period of liquid ventilation, a strong trend towards reduction in secretion was observed, falling from 3.03 ± 0.65 to 0.74 ± 0.92 ml h⁻¹ kg⁻¹ (three of the four experiments showed a significant decrease in J_v in hours 4–6). These experiments indicate that oxygen delivered to the fetus using liquid ventilation with haemoglobin solutions leads to increased LL secretion when oxygen delivery is small, and suggest there is a decrease in secretion with greater oxygen delivery to the lung.

INTRODUCTION

The alveolar space of the fetal lung is filled with liquid formed by secondary active chloride transport (Olver & Strang, 1974). Lung liquid (LL) is vital for normal growth and development of the lung, preparing it structurally and biochemically for air breathing (Moessinger *et al.* 1990). Around the time of birth, the secretion of LL ceases and is replaced by sodium-led active reabsorption (Olver *et al.* 1986; Basset *et al.* 1987), which persists throughout life. Failure of the reabsorptive sodium transport at birth appears to be fatal (Hummler *et al.* 1996).

There is now a good understanding of the hormonal mechanisms responsible for the development of the absorptive mechanism in late gestation, which is due to the synergistic action of triiodothyronine and hydrocortisone (Barker *et al.* 1991). During labour, there is a dramatic rise in fetal plasma adrenaline concentration (Brown *et al.* 1983) which, probably acting via cAMP (Walters *et al.* 1990), stimulates the active transport of sodium ions out of the lung by activating apical (luminal) sodium channels which results in rapid LL absorption. There is still some dispute about the precise role of other factors in this process, such as arginine vasopressin (Perks *et al.* 1993; Strang & Barker, 1993) and changes in paracellular

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permeability associated with the onset of ventilation (Egan *et al.* 1975) which can also affect lung liquid movement.

In the period shortly after birth, it is possible to increase the resting capability of the lung to absorb LL using exogenous adrenaline, and also to reveal a secretory process by blocking sodium absorption with amiloride (Ramsden *et al.* 1992). However, these effects decrease over the first months of postnatal life, implying that adrenaline-activated channels become less important in the post-neonatal period. These data suggest the existence of another mechanism which underlies the permanent switch from secretion to absorption of LL which occurs after birth. One theory is that changes in gas tensions after birth are responsible. Oxygen tensions in the fetus are very low (16–24 mmHg) rising to over 80 mmHg postnatally and never subsequently returning to fetal levels. There is a smaller fall in P_{CO_2} . Realizing this, two research groups have subjected fetal lung cells or tissue to postnatal gas tensions *in vitro*. They showed that such a change causes absorption of luminal fluid in fetal rat lung explants (Barker & Gatzky, 1993) and an increase in sodium conductance in fetal rat alveolar cell monolayers (Pitkanen *et al.* 1996).

The aims of the present experiments were to determine whether exposure of the fetal lung epithelium to oxygen *in vivo* would lead to absorption of LL and to attempt to elucidate the time course of any effect.

METHODS

Operative details

Preparation of the fetuses was similar to that described by Walters & Olver (1978) and Brown *et al.* (1983). Briefly, pregnant ewes (Suffolk–Clun Forest cross) were operated on between 118 and 125 days from conception (term, 147 days). Anaesthesia was induced with 0.1–0.2 ml kg⁻¹ of 5% thiopentone i.v. (Intraval) and maintained with 1.5% inspired halothane in a 2:1 mixture of O₂ and N₂O. A mid-line abdominal incision was made and a hysterotomy performed. The fetal head was then delivered and Silastic catheters were placed into the trachea, right carotid artery and right jugular vein. In the trachea, one catheter was inserted towards the carina (i.d. 3 mm, o.d. 6 mm) and another toward the larynx (i.d. 2.5 mm, o.d. 4.6 mm). The wider catheter was chosen to improve lung liquid flow during experiments.

The hysterotomy was repaired and the catheters were tied into the sutured incision. The catheters were tunnelled subcutaneously to the ewe's flank, where they were exteriorized through a small incision, and the main abdominal incision was closed with sutures. The lung liquid catheters were joined with a sterilized glass T-piece to allow liquid to flow unimpeded out of the lung into the fetal mouth and to allow sampling at a later date through the side arm of the T-piece.

Benzylpenicillin (Crystapen, 600 mg) and gentamicin (Cidomycin, 80 mg) were given in divided doses of 1 part i.v. to the fetus and 3 parts i.m. to the ewe peri-operatively and on the 2 days following the operation. The arterial and venous catheters were flushed and left filled with heparinized saline (500 i.u. ml⁻¹). This was repeated every 2 days. A recovery period of 1 week was allowed before initiating experiments.

Lung liquid volume estimation technique

Lung liquid secretion and absorption rates were determined using an impermeant indicator dilution technique. The experimental procedure was similar to those described by Normand *et al.* (1970) and Walters & Olver (1978). By following the changes in the indicator concentration, after a 30 min mixing period, the rate of lung liquid production or absorption can be calculated. ¹²⁵I-albumin (Amersham) was assayed using a gamma counter (Wallac 1480 Wizard 3') to calculate secretion rate in all except five experiments. In these experiments, haemoglobin was used as an indicator after conversion to cyanomethaemoglobin using Drabkin's solution (Sigma Diagnostics), detectable by spectrophotometric analysis at 540 nm (Philips PU 8720 UV/VIS). The validity of this indicator was established in experiments in which it was used with ¹²⁵I-albumin and near-identical rates of LL formation were obtained.

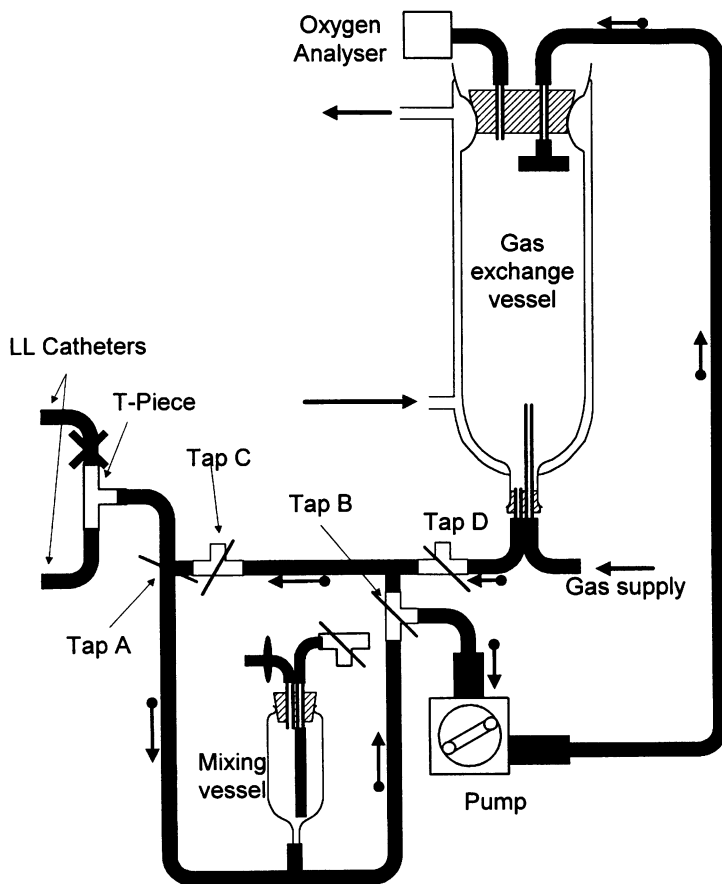


Fig. 1. Diagram of apparatus used for instillation and mixing of lung liquid-haemoglobin mixture (liquid ventilation).

Haemoglobin

Haemoglobin (Hb) was chosen as the oxygen carrier for liquid ventilation. The haemoglobin solution was prepared from whole adult sheep blood taken with sterile equipment and stored in CPDA-1 (Sigma). The blood was spun at 2000 g for 15 min at 4 °C. The plasma was then drawn off and discarded, after which the cells were resuspended in sterile 0.9% NaCl to the original volume of blood in the tube, agitated and again centrifuged. This process was repeated twice.

After the third centrifugation, the supernatant was removed and the cells lysed in distilled water. The water was added to make a solution using 3 parts red cells, 7 parts water, and the solution was then stored at 4 °C, after cellular debris had been removed by further centrifugation. This solution had a haemoglobin concentration of 13.1 g dl⁻¹, measured by comparison with standards (Sigma Diagnostics).

2,3-Diphosphoglycerate

The pentacyclohexylammonium salt of 2,3-diphosphoglycerate (DPG; Sigma) was converted into the sodium salt as follows. A column of packed Dowex-50XS (Bio-Rad), acidified by passing 1 M HCl over it for 4 h, was washed for 2 days with distilled water to remove free acid. A solution of pentacyclohexylammonium DPG was allowed to pass over the column and then the column washed to extract all the DPG. After correcting the pH of the solution to 7.0 with NaOH, it was frozen at -15 °C until the time of the experiment. Haemoglobin, DPG solution and distilled water were mixed to make a solution of osmolarity close to that of the lung liquid from the fetus (Advanced Digimatic Osmometer Model 3DII). The mean \pm S.E.M. osmolarity of the LL taken at the start of the experiments was 300.5 \pm 5.1 mosmol l⁻¹

and that of the Hb-LL solution was 324.0 ± 7.5 mosmol l^{-1} . All solutions except haemoglobin were added through a bacterial filter. The final concentration of haemoglobin was 9.86 g dl^{-1} and of DPG was 0.46 mmol l^{-1} after addition to the lung liquid.

Oxygenation apparatus

Figure 1 is a schematic diagram of the oxygenation apparatus. The circuit consisted of three main components: the mixing vessel, the gas exchange vessel and the pump. These components were connected by Silastic tubing and two-way taps. The mixing vessel was connected to a strain gauge and a chart recorder so that the duration and volume of each 'breath' could be measured (see calculation of oxygen delivery). A port in the mixing vessel allowed sampling of LL. The gas exchange vessel contained the Hb solution and was water jacketed to keep the Hb solution at body temperature. Gas rotameters allowed carbon dioxide mixed with oxygen (groups i and iii) or nitrogen (groups ii and iv) to enter the vessel via a port at the base. Before initially allowing the Hb solutions to enter the lungs, the circuit was sampled from tap D and the gas flow to the gas exchange vessel was adjusted to reach appropriate gas tensions in the liquid (non-oxygenated: $P_{O_2} < 35$ mmHg, P_{CO_2} 40–60 mmHg; oxygenated: $P_{O_2} > 250$ mmHg, P_{CO_2} 40–60 mmHg; Ciba-Corning blood gas analyser 840). When these values were reached and the Hb solution had been osmotically matched with the LL, a sample was taken and the final solution volume noted to later calculate the lung volume at this stage using the haemoglobin as a marker. The pump continuously circulated the Hb solution (which had a volume of about 200 ml) through the gas exchange vessel to ensure thorough mixing and to maintain gas tensions.

The side-arm of the T-piece connecting the two LL catheters was connected to the mixing vessel and the gas exchange vessel via tap A. Tap A could be turned either to allow liquid to drain from the lungs into the mixing vessel or to allow liquid to flow from the gas exchange vessel into the lungs. Tap B could be turned either to allow liquid to circulate in the gas exchange vessel via the pump, or to allow liquid to enter the gas exchange vessel from the mixing vessel, via the pump. Taps C and D were used for taking end-tidal and circuit samples, respectively. Circuit samples were taken every hour to ensure the gas tensions had not changed significantly during the experiment.

Following the addition of haemoglobin, 40 min was allowed to elapse for the haemoglobin and isotopes (impermeant tracer) to mix completely into the liquid present in the fetal lungs. Throughout the experiment sterility was maintained, and care was taken to prevent gas within the circuit entering the fetal lungs.

Stability of the fetus

Fetal arterial P_{O_2} and pH and arterial blood pressure measurements were taken during the experiments. None became acidotic or hypercapnic and all maintained their mean blood pressure. At the time at which the Hb-LL was first instilled, there was no acute change in blood pressure.

Experimental procedure

The fetuses were split into four groups and 'ventilated' with the following solutions:

- group i – Hb with oxygen ($n = 6$),
- group ii – Hb without oxygen ($n = 4$),
- group iii – Hb with DPG with oxygen ($n = 5$),
- group iv – Hb with DPG without oxygen ($n = 3$).

In the experiments with oxygen, LL measurement was continued for up to 6 h to look for any late effect of the change in P_{O_2} .

Groups i and ii. LL was drained out of the lungs by gravity into the mixing vessel via tap A. When no more LL could be drained, tap A was closed, and tap B was turned to allow the LL present in the mixing vessel to be pumped into the gas exchange vessel. Once the LL had entered the exchange vessel, tap B was returned to its previous position, and tap A was turned to allow a volume of Hb solution equal to that recovered from the lungs (about 30–40 ml) into the lungs. When this had been instilled, tap A was turned to allow the Hb solution out of the lungs and into the mixing vessel again, and the process continued for the duration of the experiment. The Hb solution was added to and drained from the lungs as quickly as flow would allow, thus performing liquid ventilation.

Care was taken not to instill more liquid into the lungs than had initially been recovered in order to prevent overdistension or excessive pressure within the lung.

Groups iii and iv. The procedure for these groups was identical to that above except that prior to the onset of liquid ventilation LL secretion was recorded for a 40 min control period. This was performed by draining the LL into the mixing vessel via tap A and then elevating the mixing vessel to return the LL to

the lungs. This was repeated as quickly as flow would allow, to ensure good mixing of LL. During the control period, the rest of the circuit was isolated from the mixing vessel by closing tap B. At the end of the control period, liquid ventilation was carried out as above.

Calculation of oxygen delivery

P_{O_2} was measured each hour from the solution entering the fetus. By watching the level of liquid rising in the mixing vessel, a sample ('end-tidal') could be withdrawn at the end of the outflow from the lung, which reflected alveolar P_{O_2} . Number of instillations per minute and instillation volume were obtained by attaching the mixing vessel to a strain gauge (Washington transducer type D) and an amplifier (Washington M400). This in turn was connected to a chart recorder (Kippel and Zonen). From the chart recorder output, breathing rate (breaths min^{-1}) and volume of mixture instilled could be obtained.

The following formulae were used to estimate average oxygen delivery for an experiment:

$$\text{Volume instilled per minute} = R(V_I - V_D),$$

$$\text{Oxygen delivery} = (R(V_I - V_D)) \times (O_{\text{Hb}}(S_I - S_O)),$$

where V_I is the average volume (ml) of each instillation, V_D the dead space of the airways (estimated from data in Olver *et al.* 1981) and tubing and R the number instillations per minute. O_{Hb} is the volume of oxygen carried in 1 ml of the solution, calculated from the haemoglobin concentration and oxygen carrying capacity of 1 g haemoglobin (1.34 ml Geigy tables). S_I and S_O are the relative saturations of the instilled and 'end-expiratory' liquid respectively, derived from the liquid gas tensions and the haemoglobin dissociation curve of the solution.

The dissociation curve of the Hb-DPG solution (Fig. 2) was measured as follows. A glass tonometer containing a small volume of the haemoglobin in lung liquid with DPG at a concentration of 0.32 mmol l^{-1} was evacuated using a high vacuum pump (F2M2, Edwards, UK). By adding small volumes of air back into the tonometer, the saturation could be measured spectrophotometrically at a given oxygen tension.

Fetal monitoring

In addition to frequent blood gas analysis, fetal arterial blood pressure and heart rate were continuously monitored (Washington 400 MD4R) throughout the experiment.

Postmortem findings

As several experiments were carried out on each fetus, autopsy did not occur until some time after the experiment. Fetuses were killed with an overdose of i.v. pentobarbitone. Fetal weight was compared with average weights for the gestation period to ensure that it corresponded with the calculated gestation given by the supplier.

Statistics

The data were analysed using Microsoft Excel 5.0. A P value of 0.05 using Student's t test was used to define statistical significance.

RESULTS

A total of 18 experiments were performed on fetuses ranging from 129 to 140 days gestation.

Three non-oxygenation and five oxygenation experiments were performed in the presence of DPG. Four non-oxygenation and six oxygenation experiments were performed in the absence of DPG.

Fetal arterial gas partial pressures

Mean arterial and venous P_{O_2} and P_{CO_2} values for each protocol are shown in Table 1. In the experiments in which oxygen was used with haemoglobin and DPG (group iii), there was a significant rise ($P < 0.02$) in fetal arterial oxygen after the liquid ventilation was started. This lasted throughout the oxygenation period. No significant change was found in the arterial or venous P_{O_2} or P_{CO_2} levels in any of the other groups (i, ii or iv).

Table 1. Mean (\pm S.E.M.) gas tensions and pH from arterial and venous samples taken during the experiments

Group	n	Before haemoglobin			After haemoglobin			
		P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH	
Arterial								
i	O ₂ , no DPG	6	22.0 \pm 0.4	46.2 \pm 2.9	7.37 \pm 0.01	24.6 \pm 1.1	46.0 \pm 1.1	7.37 \pm 0.01
ii	no O ₂ , no DPG	4	17.5 \pm 3.9	39.7 \pm 1.5	7.39 \pm 0.01	23.3 \pm 0.6	36.0 \pm 2.6	7.39 \pm 0.01
iii	O ₂ , DPG	6	21.1 \pm 1.0*	48.6 \pm 1.0	7.42 \pm 0.00	23.8 \pm 0.4*	48.4 \pm 0.8	7.40 \pm 0.01
iv	no O ₂ , DPG	4	20.7 \pm 2.0	43.3 \pm 3.25	7.38 \pm 0.02	21.7 \pm 3.7	45.1 \pm 0.8	7.40 \pm 0.02
Venous								
iii	O ₂ , DPG	5	16.2 \pm 1.3	53.0 \pm 0.9	7.40 \pm 0.01	18.4 \pm 0.4	53.3 \pm 1.1	7.39 \pm 0.01
iv	no O ₂ , DPG	3	16.0 \pm 1.9	49.6 \pm 2.6	7.38 \pm 0.02	17.2 \pm 2.8	50.2 \pm 1.5	7.37 \pm 0.01

*O₂ signifies oxygenation, and 'no O₂' non-oxygenation experiments. *Significantly different ($P < 0.02$) P_{O_2} in group iii before and after oxygenation (Student's paired *t* test).

Table 2. Oxygenation parameters and calculated oxygen delivery for the experiments

Group	Breath volume (ml)	Breathing rate (breaths min ⁻¹)	Lung liquid haematocrit	Instilled liquid P_{O_2} (mmHg)	Expiratory liquid P_{O_2} (mmHg)	Minute volume (ml)	Change in saturation (%)	Oxygen delivery (ml min ⁻¹)	
i	O ₂ , no DPG	—	—	0.26 \pm 0.02	453.7 \pm 28.1	35.0 \pm 3.6	—	—	
ii	no O ₂ , no DPG	—	—	0.15	28.4 \pm 1.9	28.8 \pm 3.6	—	—	
iii	O ₂ , DPG	30.1 \pm 3.1	0.25 \pm 0.02	0.3 \pm 0.0	364.7 \pm 43.8	37.7 \pm 2.8	6.1 \pm 0.7	59 \pm 4	0.54 \pm 0.08
iv	no O ₂ , DPG	22.6 \pm 2.15	0.33 \pm 0.07	0.3 \pm 0.0	33.9 \pm 3.4	22.6 \pm 4.5	7.3 \pm 0.8	8 \pm 3	0.07 \pm 0.02

Saturation change estimated from dissociation curve. Minute volume is corrected for dead space.

Partial pressures of the Hb-LL mixture

The P_{O_2} of the instillate and the mixture recovered at the end of the 'expiration' was measured once an hour during all the experiments as shown in Table 2. Data for the liquid instillations were taken in five oxygenation (group iii) and three non-oxygenation experiments (group iv). For each, the number of instillations and average instillation volume was determined from the mixing vessel weight transducer. All of these data are shown in Table 2 along with the calculated oxygen delivery.

Parameters relating to the liquid ventilation other than gas tensions and delivery were not significantly different in the oxygenated and non-oxygenated groups. In the experiments without DPG (groups i and ii) oxygen delivery cannot be estimated, as the precise number and volumes of the breaths were not measured.

As small amounts of oxygen can diffuse through the Silastic tubing walls in the circuit, oxygen delivery was 0.074 ± 0.022 ml min⁻¹ in the 'non-oxygenation' experiments with DPG. This was much less than the 0.536 ml min⁻¹ in the oxygenated experiments with DPG.

Haemoglobin characteristics

The dissociation curve of adult sheep haemoglobin in lung liquid with a starting pH of 6.3 is shown in Fig. 2. Also shown is the curve with 2,3-diphosphoglycerate at a concentration of 0.32 mmol l⁻¹. In the experimental conditions the concentration of DPG was higher

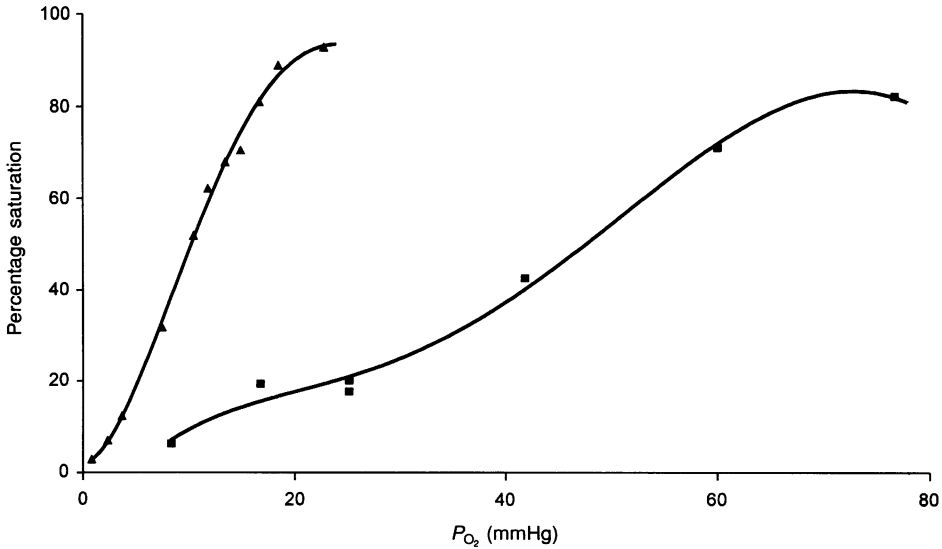


Fig. 2. Dissociation curve of haemoglobin with and without DPG. Starting pH 6.3. ▲, no DPG; ■, with 0.32 mmol l^{-1} DPG.

(0.46 mmol l^{-1}), the pH was lower and CO_2 was present. The oxygen delivery in Table 2 is therefore an underestimate and the true values may be substantially higher.

Estimate of oxygen consumption

The lung's oxygen consumption can also be calculated. The whole fetus has an oxygen consumption of $0.23 \text{ mmol min}^{-1} (\text{kg wet weight})^{-1}$ (Dejours, 1981). For a 3.1 kg fetus, the average weight at 132 days (Olver *et al.* 1981), the mean oxygen consumption for the whole fetus is $0.713 \text{ mmol min}^{-1}$ or $15.69 \text{ ml min}^{-1}$. The wet weight of the lung will be 30.25 g when luminal fluid is removed (based on data in Stephens *et al.* 1996). Thus for the lung, assuming it has the same oxygen consumption as the average for the whole fetus, it is $0.00696 \text{ mmol min}^{-1}$ or $0.153 \text{ ml min}^{-1}$. The oxygen supplied to the tissue by the apparatus is additional to that already supplied by the pulmonary circulation.

Lung liquid secretion measurements

J_v was measured for the first 3 h after the start of liquid ventilation for all the groups. There was no difference in secretion rate between any of these groups. These results are shown in Table 3.

In groups i and iii oxygenation was continued beyond 3 h to look for any delayed effect. In group i there was a significant rise ($P < 0.001$) in secretion rate at this low oxygen delivery from 2.25 ± 0.88 to $3.74 \pm 0.85 \text{ ml h}^{-1} \text{ kg}^{-1}$ (Table 4). An example experiment is shown in Fig. 3A.

In groups iii and iv, J_v was measured in a control period (before the addition of haemoglobin), in the first 3 h of liquid ventilation and then in a further 3 h period in the same conditions.

In group iii, one experiment only lasted for hours 1–3, thus $n = 5$ for hours 1–3, and $n = 4$ for hours 4–6. In three of the four longer experiments in group iii, there was a significant decrease in the rate of LL secretion, and in one experiment there was no significant difference.

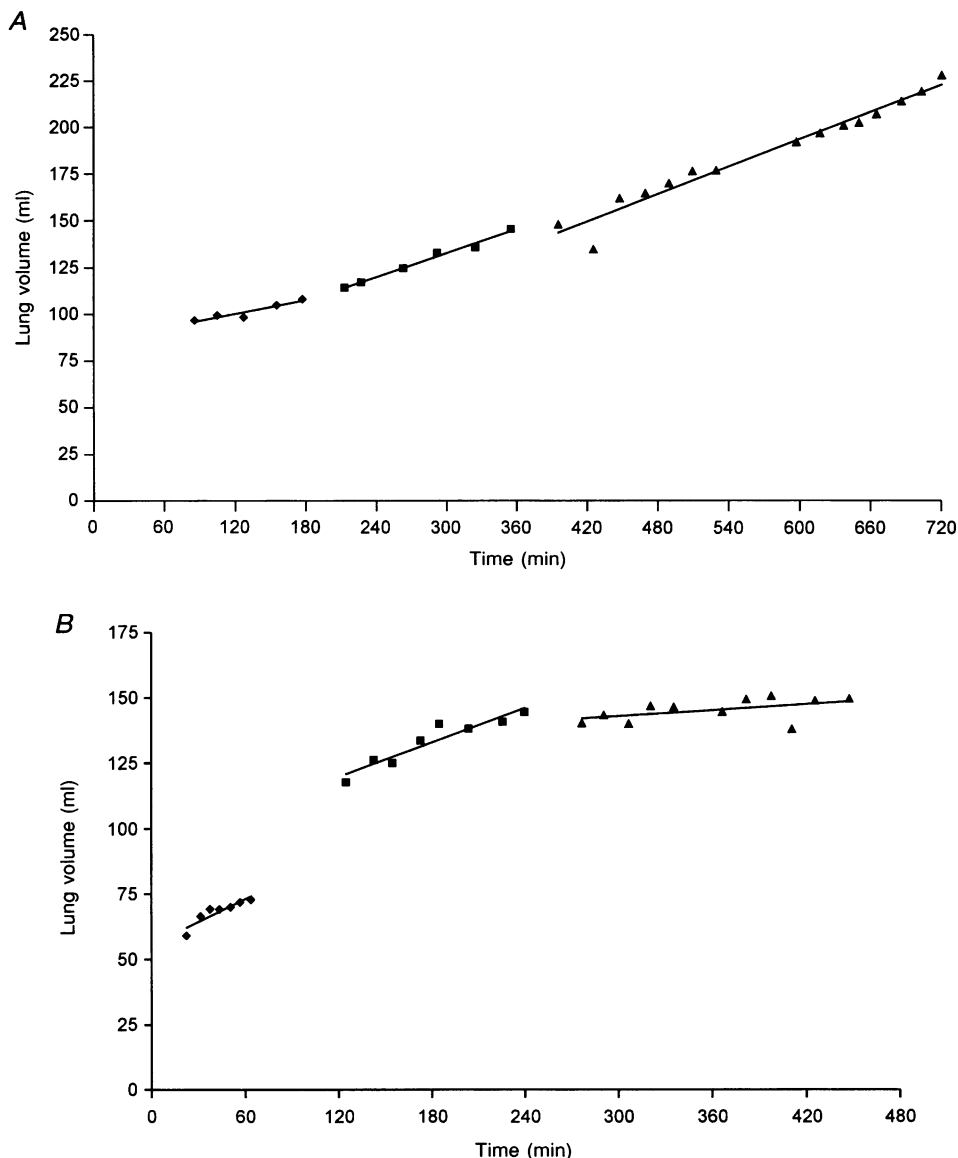


Fig. 3. Examples of two experiments with lung volume measured during oxygenation without DPG (A) and with DPG (B). Haemoglobin oxygenation starts at time 0 in A and at time 85 min in B.

Mean data from the whole of group iii demonstrated a non-significant reduction in secretion ($P = 0.07$, Student's paired t test) with secretion rates measured at $3.03 \pm 0.65 \text{ ml h}^{-1} \text{ kg}^{-1}$ during the first 3 h of liquid ventilation and $0.74 \pm 0.92 \text{ ml h}^{-1} \text{ kg}^{-1}$ during the last 3 h of liquid ventilation. An example experiment is shown in Fig. 3B. However, the secretion rate after 3 h was significantly different when comparing group i (oxygenated, without DPG) with group iii (oxygenated, with DPG).

Table 3. Secretion rate (J_v) and oxygen delivery for the first 3 h of the different protocols

Group	Gestation (days)	n	J_v (ml h ⁻¹ kg ⁻¹)	Oxygen delivery (ml min ⁻¹)
i O ₂ , no DPG	134.5	6	2.25 ± 0.88	—
ii no O ₂ , no DPG	140	4	2.75 ± 1.44	—
iii O ₂ , DPG	133.2	5	2.99 ± 0.49	0.536 ± 0.09*
iv no O ₂ , DPG	133.2	3	3.94 ± 5.36	0.07 ± 0.02

*Significantly different ($P < 0.05$) from oxygen delivery in all other groups.

Table 4. Secretion rate of control, the first 3 h and the subsequent 3 h

Group	Gestation (days)	n	J_v		
			Control (ml h ⁻¹ kg ⁻¹)	Hours 1–3 (ml h ⁻¹ kg ⁻¹)	Hours 4–6 (ml h ⁻¹ kg ⁻¹)
i O ₂ , no DPG	134.5	6	—	2.25 ± 0.88	3.74 ± 0.85*
iii O ₂ , DPG	133.2	4	3.72 ± 0.91	3.03 ± 0.65	0.74 ± 0.92†

*Significantly different ($P < 0.001$) from hours 1–3. †Significantly different ($P < 0.05$) from hours 4–6, group i; this value not significantly different ($P = 0.07$) when comparing with 1–3 h, group iii.

DISCUSSION

Introduction

The results from these experiments apparently show that exposure of the fetal lung to small amounts of oxygen in late gestation sheep increases the production of LL while exposure to larger amounts leads to a reduction in secretion in some fetuses with a delayed effect. The validity of these findings depends on whether oxygenation was adequate and whether the experimental technique alters lung physiology.

Adequacy of oxygenation

Our experiments showed no significant change in fetal arterial P_{O_2} (P_{a,O_2}) until the oxygen delivery to the fetus was over 0.5 ml min⁻¹, when a small rise in P_{a,O_2} (2.6 mmHg) was noted. In a fetus which has an intact circulation, there are right-to-left shunts at atrial and ductal levels and only 10% of its cardiac output perfuses the lungs. Thus it is theoretically impossible to achieve a high systemic P_{O_2} even if there were massive oxygen delivery to the lungs with a normal fetal circulation.

Lung ventilation with gases *in utero* is also inefficient at raising the P_{a,O_2} . Cassin *et al.* (1964) used exteriorized lambs with intact circulations and found that ventilation with air increased the P_{a,O_2} from 20.0 ± 1.4 to only 27.8 ± 2.5 mmHg. By occluding the ductus, Giraud *et al.* (1995) reported a larger increase in P_{a,O_2} (from about 13.5 to 33 mmHg) with *in utero* gas ventilation. Ogundipe *et al.* (1993) ventilated *in utero* lambs and found an even bigger change in P_{a,O_2} from 18 ± 1 to 86 ± 29 mmHg but this required cord occlusion.

In the context of these other observations, the small change in pre-ductal arterial P_{O_2} with our *in utero* liquid ventilation is all that would be expected. Furthermore our calculations

indicate that the fetal lung's maximum oxygen consumption was greatly exceeded in group iii.

Estimating the average alveolar P_{O_2} is difficult in this preparation, but it is likely to be similar to the P_{O_2} of the solution recovered at the end of the 'expiration', as shown in Table 2. Alveolar P_{O_2} could also be calculated from pulmonary and systemic blood flow rates in the fetus and the increase in the oxygen content of the systemic blood. Assuming that the increase in oxygen content of the systemic blood is entirely due to an increase in oxygen in the pulmonary vein, the oxygen content of the pulmonary vein blood can be estimated if one assumes that the proportion of cardiac output supplying the lung does not change (7% according to Rudolph & Heymann, 1974). Making these assumptions the pulmonary venous blood will be fully saturated. However, it may be that there is an increase in pulmonary blood flow brought about by the increase in pulmonary oxygen. Whether there is a change in the amount of shunt cannot be determined in this preparation.

Changes in secretion rate

The instillation of Hb and DPG or the mixing procedure itself might alter secretion rate; however, there have been no reports of the use of DPG or Hb in the lung before. On the other hand, an increase in intraluminal pressure in the lung can either reduce secretion or promote absorption. A large increase in the pressure disrupts the integrity of the lung and as a result, isotope is lost into the fetal blood.

In order to minimize the possibility of excessive pressure within the lung, care was taken to control the volume of Hb-LL that was instilled into the lung during each cycle and it was limited to a volume equal to that initially drained from the fetus at the beginning of the experiment. Thus the volume of liquid contained in the lung at 'end inspiration' never exceeded functional residual capacity. Such a volume would not lead to a pressure higher than that in the fetal lung before the start of the experiment. Epithelial disruption would be reflected by an increase in the isotope counts measured in the blood taken during the experiments, but no such rise was identified in the experiments.

In these experiments no significant changes were found between the four groups up to the end of the third hour. All of these groups had a mean J_v within the normal range for gestational age. Two groups had J_v measured before and after the haemoglobin, and these groups (iii and iv) had a similar J_v before and after the intervention. It is therefore extremely unlikely that any of the experimental techniques of mixing the haemoglobin solution or DPG in LL themselves led to a change in J_v . Group iv was however small ($n = 3$), so small differences in the rates between this and other groups might not have been readily apparent.

The groups were well matched apart from group ii, which had a greater mean age. At this gestation (140 days), adrenaline would be expected to stop LL secretion and cause an immediate absorption. Any effect of stress on the fetus caused by the technique would be expected to be particularly obvious in this group. The protocol for this group (no oxygen, no DPG) was designed to see if the mixing of the Hb solution itself altered secretion rate, and so a normal secretion rate is good supportive evidence that the fetal lung behaves normally under experimental conditions.

Significant changes were found in group i (J_v increased) and three of the four experiments in group iii that went on for 6 h (J_v decreased), but only after 3 h of exposure to oxygen. Reduced secretion was shown in fetuses of average gestation 133 days in group iii. Here a rise in plasma adrenaline caused by the procedure cannot be implicated in producing the reduced secretion, since experiments performed at 140 days using an identical technique (group ii),

when adrenaline should lead to an absorption, showed no change in secretion rate from the normal.

There was no direct comparison between the oxygenated groups (i and iii) and those not oxygenated (ii and iv). Although such experiments would rule out a late effect of Hb *per se* on secretion, it is difficult to imagine how the Hb itself could cause a different effect in the high and low oxygen delivery experiments. A synergistic effect of DPG with Hb (but not through oxygen delivery) remains a theoretical possibility.

Implications of increased secretion rate with a small increase in oxygen delivery

With a small increase in oxygen delivery, there was a small increase in J_v , particularly in the period from 4 to 6 h. This is in accord with the experiments of Hooper & Harding (1989). In addition to a range of metabolic effects, they showed that lung liquid secretion was reversibly altered from 9.1 ± 1.5 to 3.0 ± 0.5 ml h⁻¹ in response to a fall in fetal P_{O_2} from 22.9 ± 1.0 to 13.8 ± 0.5 mmHg brought about by obstructing maternal uterine artery flow in pregnant ewes. They concluded that as liquid secretion was an active process, the removal of a nutrient (oxygen) reduced the cellular activity in that tissue.

No other experiments relate hypoxia or small increases in oxygen supply to lung liquid flow. However, there is some evidence that the lung physiology is particularly prone to alteration in nutrient supply. Using the reduced uterine blood flow model, and measuring incorporation of labelled thymidine, Hooper *et al.* (1991) found a larger reduction in DNA synthesis in some organs (muscle, thymus and lung) than others caused by a reduction in fetal arterial P_{O_2} from 20.1 ± 1.1 to 12.3 ± 1.6 mmHg. Also in fetal rats, maternal exposure to 10% oxygen for 1 week resulted in a decreased lung growth, but did not alter lung maturation (Faridy *et al.* 1988).

These findings suggest that the lung is unable to grow normally with a reduction in oxygen delivery. Increasing oxygen delivery might allow increased metabolism in the lung, as seen in the higher J_v in group i.

Implications of reduced secretion rate with a larger increase in oxygen delivery

The reduction in secretion rate in the fetuses exposed to oxygen, haemoglobin and DPG after 3 h exposure may indicate that the oxygen is capable of inducing absorptive processes in the lung at higher P_{O_2} levels. The time course for these changes (after 3 h) suggests that any such process may involve gene expression or protein synthesis. These results are compatible with those *in vitro* studies in which a rise in environmental oxygen led to maturation of elements in the liquid absorption process. There are, however, certain important differences.

Working with distal lung explants from the fetal rat, Barker & Gatzky (1993) examined the effects of several different potential stimuli on the wet/dry weight ratio of the explants. Significant differences in wet/dry ratio, attributable to different gas tensions, were found only in explants grown in stripped fetal bovine serum. At 20 days only, the explants grown in more physiological fetal bovine serum had a mean wet/dry weight ratio greater than in the explants cultured in fetal gases with respect to neonatal gases, but no statistical support is given for the difference. There is no evidence for a physiologically relevant change (i.e. at 22 days, in unstripped serum). The preparation is also difficult to compare with other models, particularly *in vivo* preparations. Fetal rat distal lung explants actually show stimulated secretion in response to β -adrenergic stimuli (Krochmal-Mokrzan *et al.* 1993) indicating that they have an appreciable component of proximal airway tissue.

Pitkanen *et al.* (1996) reported changes in cultured monolayers of rat fetal distal lung epithelium mounted in an Ussing chamber associated with alterations in oxygen levels. By increasing the oxygen content of the gas surrounding the culture vessel the current blocked by amiloride (10^{-4} M) increased slowly, becoming evident at 18 h and still increasing up to 48 h. There was no increase in the amiloride blockable current at 8 h. They were also able to show an increase in mRNA related to oxygen exposure using quantitative scanning densitometry on hybridized Northern blots. Although there was no increase at 18 h, there was a rise at 48 h in mRNA for the α , β and γ subunits of the epithelial sodium channel.

These findings seem to indicate that oxygen may be responsible for sodium-led reabsorption in response to postnatal levels of oxygen and perhaps a permanent change in gene transcription in the alveolar epithelium. However, the culture of these cells is itself likely to dramatically alter their properties. The resting resistance was much lower than those found by other groups (Barker *et al.* 1995). Furthermore, the epithelium in these experiments is not typical of fetal lung epithelia. In a similar preparation, before incubation, 90% of the cells harvested were type II alveolar epithelial cells (O'Brodovich *et al.* 1993). This contrasts with the findings of Crapo *et al.* (1982) who, studying the morphology of adult rat lung, found only 14.2 ± 0.7 % of cells in the lung were type II cells. Also the cellular oxygen tensions in this culture model may be very different to the levels *in vivo*. It may be that this experiment is demonstrating a hyperoxic response of the lung epithelium, which includes increased permeability and expression of amiloride blockable sodium channel (Yue *et al.* 1995).

So it is likely that the cultured epithelium is not representative of the *in vivo* epithelium at birth. Even so the findings of these groups broadly supports the results presented in this communication.

Conclusions

This report indicates that oxygen delivered to the fetus using liquid ventilation with haemoglobin and DPG leads to an increase in secretion with a small increase in oxygen delivery, and a decrease in secretion with a large increase in oxygen delivery to the lung. These results confirm the findings of *in vitro* oxygenation of fetal lung cells, but further studies are need to demonstrate the precise mechanisms involved.

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