Intensive Care Med (1988) 14:538–546

Intensive Care Medicine

Effects of positive end-expiratory pressure on hyaline membrane formation in a rabbit model of the neonatal respiratory distress syndrome

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Received: 20 September 1987; accepted: 29 February 1988

Abstract. Sixteen rabbits were anaesthetized and subjected to saline lavage of the lungs to produce surfactant deficiency. This resulted in an arterial oxygen tension of less than 12 kPa on 100% inspired oxygen and an inflection point on the pressure-volume curve at a pressure of 8–12 mmHg. After lavage the animals were randomly assigned to receive either conventional mechanical ventilation (CMV) with a positive end-expiratory pressure (PEEP) of 1–2 mmHg (group I – low PEEP) or CMV with PEEP equal to the inflection point pressure (group II – high PEEP). Mean airway pressures were kept at 14–16 mmHg in both groups by increasing the inspiratory : expiratory time ratios in the low PEEP group. The 5-h protocol was completed by 4 animals in group I and 6 animals in group II, early death usually being associated with a metabolic acidosis. On 100% oxygen, the mean PaO_2 at 2-h post-lavage was 15.2±8.3 kPa in group I and 39.6±21.8 kPa in group II. Group I had much lower end-expiratory lung volumes (3.0±1.5 ml above FRC) than group II (34.9±12.2 ml above FRC). Histological examination of the lungs revealed significantly less hyaline membrane formation in group II ($p = 0.001$). Thus, the prevention of alveolar collapse by the use of high PEEP levels appears to reduce lung damage in this preparation.

Key words: Mechanical ventilation – Positive end-expiratory pressure – Hyaline membranes

The neonatal respiratory distress syndrome (NRDS) is characterized by surfactant deficiency [2, 10] and capillary damage resulting in increased permeability [10]. Regional deficiencies of surfactant produce differences in lung compliance and so cause non-uniformity of expansion in response to a given distending pressure. Thus, during ventilation, intraregional stresses may be produced due to the interdependence of adjacent lung units [13, 14]. These stresses are thought to increase capillary transmural pressure and so cause haemorrhage and the development of hyaline membranes [15]. If the stresses produced by repeated alveolar collapse and re-expansion during conventional mechanical ventilation (CMV) could be avoided, it might be possible to limit or even prevent hyaline membrane formation. It has been postulated that the techniques of apnoeic oxygenation with extracorporeal CO_2 removal [16], or high frequency oscillation (HFO) [5], which minimize lung movement, may prevent such damage.

In a previous study, using a lung lavage model of NRDS [1], it was shown that the use of positive end-expiratory pressure (PEEP) at levels equal to the pressure at the inflection point on the pressure-volume

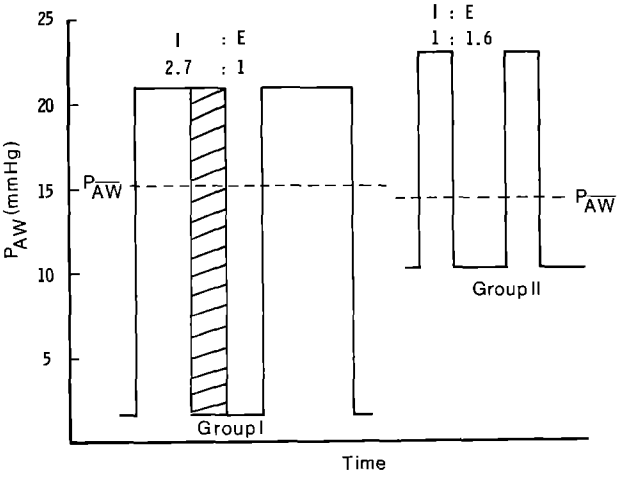

Fig. 1. Illustration of the ventilatory patterns used, showing the mean values of $\overline{P_{AW}}$, peak P_{AW}, PEEP and I:E ratios for the two groups at 1 h post-lavage. (The shaded area represents the inspiratory pause)

(P/V) curve significantly reduced the severity of the hyaline membrane formation when compared to CMV with lower PEEP levels. This was thought to be due to the prevention of alveolar collapse by the higher levels of PEEP. However, the improvement could have been due to a reduction in cardiac output and pulmonary blood flow resulting from the higher mean airway pressure ($P_{\overline{AW}}$) in the high PEEP group. We, therefore, decided to use the same model to compare the effects of CMV with two different levels of PEEP but with a similar $P_{\overline{AW}}$ (Fig. 1). The effects on oxygenation, lung mechanics and severity of hyaline membrane formation were compared in the two groups, changes in the arterial to mixed venous oxygen content difference ($C_aO_2 - C_{\bar{v}}O_2$) being used as an index of changes in cardiac output.

Methods

Sixteen New Zealand white rabbits, weighing 1.5 to 2.5 kg, were studied. Anaesthesia was induced in a box with 4% halothane in 100% oxygen and 0.12 ml kg^{-1} Hypnorm (Janssen: 0.3 mg fentanyl +10 mg fluanisone in 1 ml) given intramuscularly after 15 min. A 22 gauge cannula was inserted in a left ear vein, and 9 mg doses of pentobarbitone were given every 5 min whilst tracheostomy was performed. A 4 mm I.D. plastic endotracheal tube was inserted into the tracheostomy and secured in place with ligatures to prevent air leaks. Pancuronium 1 mg I.V. was given to produce apnoea and anaesthesia maintained with an I.V. infusion delivering 1 mg pancuronium and 50 µl Hypnorm every hour in 5 ml normal saline.

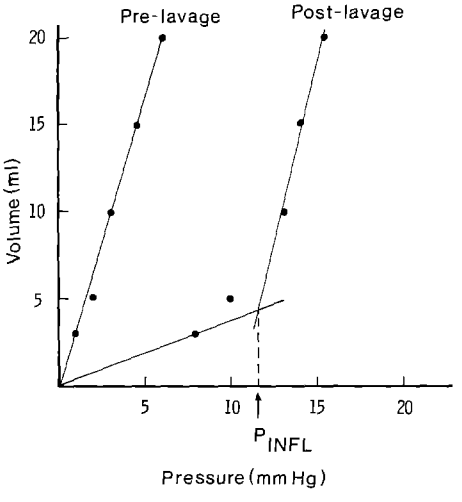

Fig. 2. Typical pressure-volume curves before and after saline lung lavage, demonstrating the presence of an inflection in the post-lavage curve (P_{INFL} is the pressure at the point of inflection)

The animals were initially ventilated with a minute volume of 350 ml kg^{-1} and an inspiratory : expiratory (I : E) ratio of 1 : 1 at 25 breaths per minute, whilst carotid arterial and right ventricular (5F gauge Swan-Ganz catheter, via the internal jugular vein) cannulae were inserted for pressure monitoring and blood sampling. An F_IO_2 of 1 was maintained throughout the experiment. The ECG and rectal temperature were monitored in all animals. After arterial and mixed venous blood samples had been obtained, a pressure/volume (P/V) curve was recorded by injecting a series of 3 to 5 ml aliquots of air into the lungs at 3 s intervals to a total of 20 ml. Lung damage was then produced by washing out the lungs with normal saline (20 ml kg^{-1}), warmed to 37 °C in a manner similar to that described by Lachmann et al. [9]. The saline was syringed directly into the endotracheal tube whilst the airway pressure was monitored to ensure that it remained below 25 mmHg. The saline was aspirated and ventilation reinstituted immediately with a PEEP of 2–3 mmHg. Five minutes later, the arterial blood gases were measured and the lavage repeated until the arterial PO_2 was less than 10 kPa. This was usually achieved by 3 to 6 lavages. Ventilation was continued for a further 15 min following the final lavage when a P/V curve was determined and the inflection point identified (Fig. 2).

The animals were then randomly allocated to receive either a high PEEP, equal to the inflection point pressure, or a low PEEP, of 1–2 mmHg. Mean airway pressures (obtained by electronic damping) were maintained between 14 and 16 mmHg in both groups. In the low PEEP group, this was achieved by increasing the I : E ratio up to 3 : 1. This included an end-inspiratory pause of up to 25% of the total inspiratory time. Normocarbia was maintained in both groups by adjusting the fresh gas flow (up to 1.5 l min^{-1}) and frequency (up to 60 breaths per min), the peak inspiratory pressures being maintained at 24±3 mmHg. Sodium bicarbonate (8.4%) was given as necessary to correct any base deficit which developed.

The animals were ventilated inside a constant volume box plethysmograph. The changes in box pressure were recorded by a MP45 Validyne transducer and calibrated in terms of volume by clamping the endotracheal tube at the end of the experiment and injecting known volumes of air from a syringe. Every hour the mean, peak and end-expiratory airway pressures and respiratory rate were recorded and arterial and mixed venous blood samples obtained. The end-expiratory lung volume (EELV – the volume of gas in the lungs above the functional residual capacity) was then measured by opening the breathing circuit to atmosphere and recording the change in

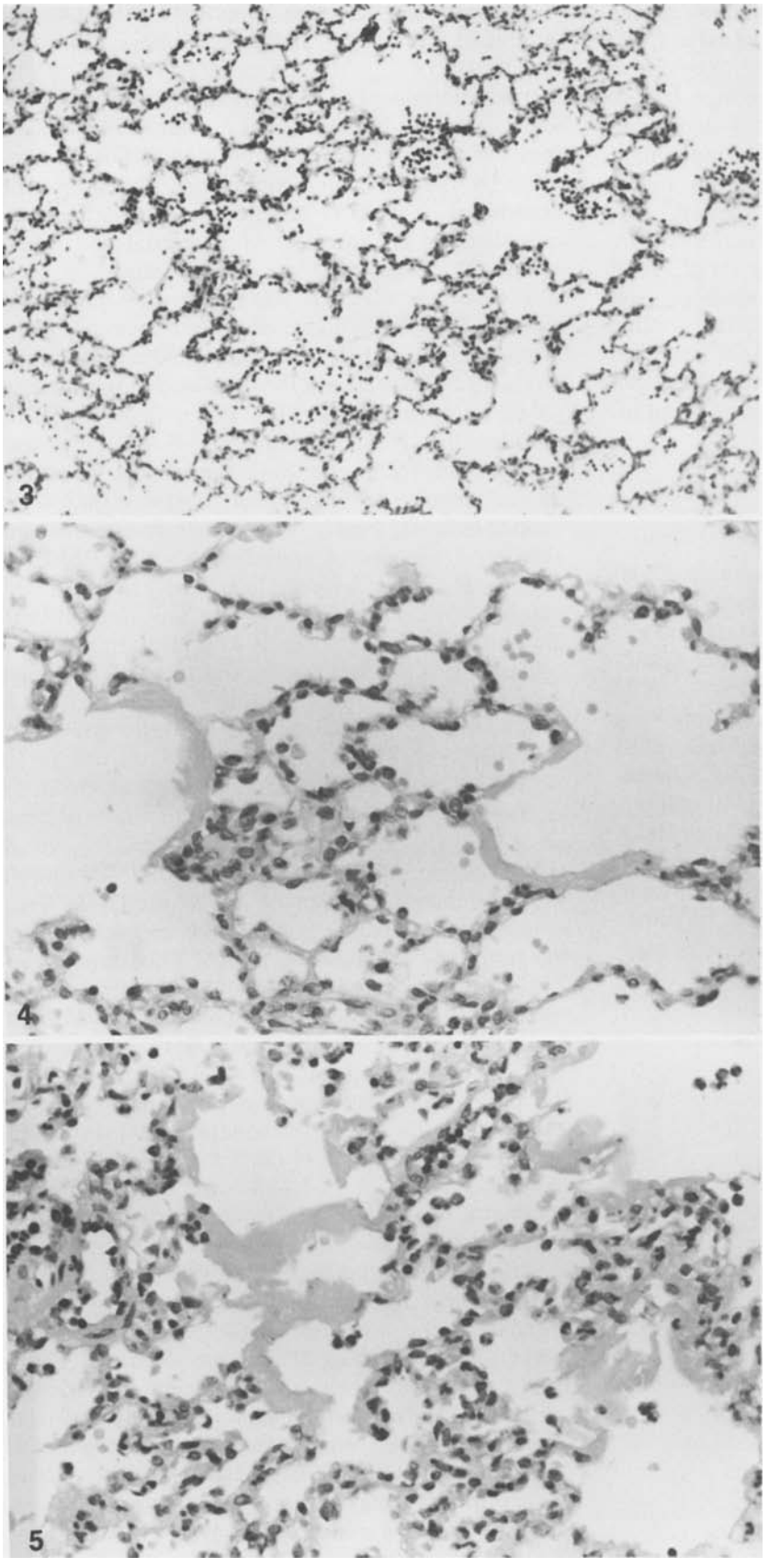

Fig. 3. Lung with no hyaline membrane formation. Haematoxylin and eosin ×20

Fig. 4. Lung with hyaline membranes grade 2. H and E ×50

Fig. 5. Lung with extensive hyaline membrane formation grade 4. H and E ×50

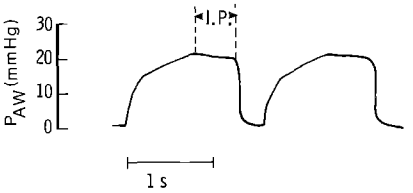

Fig. 6. Airway pressure tracing of a typical animal (low PEEP), showing the inspiratory pause (I.P.)

pressure in the box. This was followed by the injection of aliquots of air into the lungs to establish the P/V curve. Ventilation was then reinstated at peak inflation and the position of the inflection point on the P/V curve checked to ensure that the PEEP settings were correct.

The animals were ventilated in this manner until death, or were sacrificed 5 h after the end of lavage. The lungs were immediately removed and fixed by perfusion of 10% buffered formol saline. Blocks of tissue were than taken from each lung, – dehydrated, cleared and vacuum embedded in paraffin wax. Sections were cut at 4 μm and stained with haematoxylin and eosin. The sections were examined by a pathologist (MSD) who was unaware of the ventilation group to which each specimen belonged. The extent of hyaline membrane formation in each animal was graded on an ascending scale from 0 to 4 (Figs. 3–5), and the extent of neutrophil infiltration was assessed in a similar manner.

Instrumentation

The ventilator was designed and made in the Department. It consisted of a 'T'-piece system with two solenoid valves, one on the inspiratory limb and the other operating a Bennett balloon valve on the expiratory limb. An end-inspiratory pause could be generated by closing the inspiratory valve before the expiratory valve opened. The system was designed as a constant flow generator but actually delivered a slightly decreasing flow pattern on inspiration, due to the retention of fresh gas within the ventilator and fresh gas supply line during the expiratory phase of the cycle (Fig. 6). PEEP was applied using a magnetic valve (Instrumentation Industries). Arterial, right ventricular and airway pressures were measured by Druck transducers and a Validyne MP 45 transducer was used to monitor volume changes within the plethysmograph. All the signals were recorded on a Lectromed M19 heated stylus recorder. Blood gases and pH were measured on an ABL 2 automated analyzer (Radiometer, Copenhagen) whilst arterial and mixed venous oxygen contents were determined using a Lex-O_2-Con analyzer (Lexington Instruments).

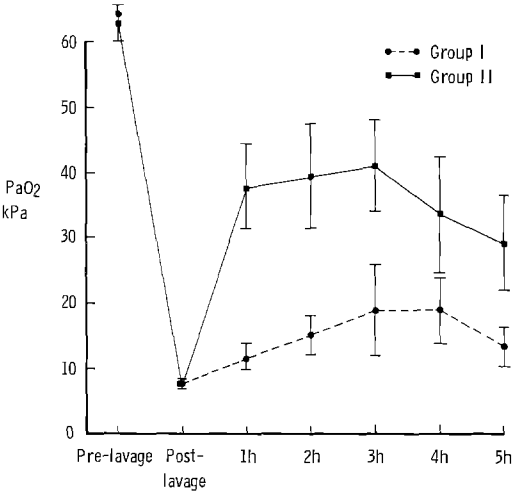

Fig. 7. Arterial oxygen tensions at each stage of the experiment for the two groups of rabbits. Group I = low PEEP, group II = high PEEP

The Mann-Whitney U test for non-parametric data was used to compare data from the two groups at each stage of the experiment, and to compare hyaline membrane and neutrophil scores. Changes with time within each group were examined using an one-way analysis of variance.

Results

There were no significant differences in the mean weights and the pre-lavage recordings of blood gases, airway pressures, and the arterio-venous O_2 content difference ($CaO_2 - C\bar{v}O_2$) in the two groups of rabbits. Initial P/V curves (inflation to 20 ml) were similar in both groups, showing a mean compliance of 4.3 ± 1.6 ml mmHg^{-1} in the low PEEP group and 4.9 ± 1.0 ml mmHg^{-1} in the high PEEP group. Lung lavage produced a significant fall in PaO_2 ($p < 0.001$) which was comparable in both groups (Table 1). The post-lavage P/V curves had an inflection point at a mean pressure of 11.7 ± 0.9 mmHg in the low PEEP group and 10.8 ± 1.9 mmHg in the high PEEP group, the compliance below this point being significantly less than pre-lavage values. There was some retention by the lungs of the instilled saline, mostly from the initial lavage. Approximately 20% was retained from this first lavage, then only very small volumes from subsequent lavages.

After stabilization on the assigned method of ventilation, there was a significant difference in PaO_2 levels between the two groups, the PaO_2 being lower in the low PEEP animals throughout the study period (Table 1, Fig. 7). In order to keep the $PaCO_2$ within normal limits it was necessary to increase the

Table 1. Mean ± SD of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) tensions, arterial pH, base excess (BE) and the arterio-venous oxygen content differences [$(a-\bar{v})dO_2$] during the stages of the experiment

Time	Group	PaO_2 (kPa)	$PaCO_2$ (kPa)	pH	BE (mmol l^{-1})	$C_aO_2-C_{\bar{v}}O_2$ (vol%)
Pre-lavage	I	63.7± 4.5	4.3±0.5	7.45±0.07	−3.1 ±2.2	7.3±1.8
	II	62.6± 7.6	4.1±0.7	7.45±0.07 $n=7$	−0.36±3.5	6.3±1.6 $n=6$
Post-lavage	I	7.7± 1.2	5.6±0.9	7.35±0.04	−3.1 ±2.1	–
	II	8.1± 1.0	5.3±0.7 $n=7$	7.30±0.09	−2.0 ±2.9 $n=6$	–
1 h	I	11.7± 5.8[b]	4.3±0.6	7.36±0.06	−6.3±4.0	6.9±1.0 $n=6$
	II	37.9±18.8	4.6±1.1	7.37±0.09	−4.0 ±5.5	6.3±2.2 $n=6$
2 h	I	15.2± 8.3[a]	4.1±0.8[a]	7.35±0.1	−6.8 ±4.5	8.3±1.9 $n=6$
	II	39.6±21.8	5.1±1.0	7.34±0.03	−4.8±2.1	7.9±2.7 $n=6$
3 h	I	19.3±18.4[a] $n=7$	3.9±1.3[a] $n=7$	7.37±0.07 $n=7$	−4.9 ±4.4 $n=7$	8.4±1.6 $n=6$
	II	41.1±20.2	5.0±1.0	7.32±0.07	−7.1 ±3.6	8.4±2.2 $n=6$
4 h	I	19.2±12.3 $n=6$	4.0±1.4[a] $n=6$	7.30±0.1 $n=6$	−10.6 ±4.3 $n=6$	7.8±1.3 $n=4$
	II	33.6±21.8 $n=6$	5.5±0.7 $n=6$	7.31±0.1 $n=6$	−5.0 ±4.8 $n=6$	7.5±2.1 $n=4$
5 h	I	13.5± 6.2 $n=5$	4.5±0.9 $n=5$	7.28±0.05 $n=5$	−11.0 ±2.7 $n=5$	8.6±0.9 $n=3$
	II	29.3±18.4 $n=6$	5.0±1.2 $n=6$	7.29±0.12 $n=6$	−8.1±3.6 $n=6$	7.3±0.8 $n=2$

Group I = low PEEP; group II = high PEEP. Statistical significance between the two groups: [a] $p \leqslant 0.05$, [b] $p \leqslant 0.001$

respiratory rate to a greater extent in the high PEEP group. All the animals tended to develop a non-respiratory acidosis and bicarbonate was given each hour to restore the pH to normal. The mean total quantities of sodium given to the animals by continuous infusion and as 8.4% $NaHCO_3$ were 18.3±7.2 mmol in the low PEEP group and 18.1±7.4 m mol in the high PEEP group. In addition 5 ml aliquots of Haemaccel were given to maintain arterial pressure when the mean airway pressure was increased to 15 mmHg.

The mean ($CaO_2-C\bar{v}O_2$) values in the post-lavage period were similar in both groups throughout the study (Table 1). Mean airway pressures were the same in both groups but the peak pressures remained higher in the high PEEP group ($p=0.04$) throughout the study (Table 2), although still in the range 24±3 mmHg. In the high PEEP group, 6 of the 8 animals completed the protocol and the mean survival time was 272 min. In the low PEEP group 4 animals reached the end of the protocol, with a mean survival time of 254 min. In both groups of animals death was associated with acidosis, combined with hypoxia in the low PEEP group. There was considerable variation in the amount of sodium bicarbonate needed to correct acidaemia in the animals. The low PEEP animals were given a mean dose of 7.7±4.1 mmol kg^{-1} of 8.4% $NaHCO_3$ during the course of the experiment (range 2.0−15.6 mmol kg^{-1}). The corresponding mean dose for the high PEEP animals was 7.1±3.8 mmol kg^{-1} (range 2.3−12.7). There would therefore have been no difference in CO_2 production due to the bicarbonate administration.

The measurements of EELV showed a significant difference ($p<0.001$) between the two groups (Table 3). In the post-lavage period, the high PEEP group had EELV values of 32−37 ml, whereas the low PEEP group had a range of 2.7−3.8 ml.

The histological findings in the two groups were markedly different and are summarized in Table 4. The scores for the two lungs were combined since there was no significant difference between them. The mean scores for each lung were 2.43±1.1 in group I and 0.6±0.8 in group II. The high PEEP group had significantly less hyaline membrane formation ($p=0.001$). The scores for neutrophil infiltration were not significantly different. One-way analysis of variance revealed no significant effect of time in either group.

Discussion

It is now generally agreed that the deficiency of surfactant in NRDS leads to an increase in surface tension and a loss of alveolar stability, so that alveolar collapse tends to occur at the end of expiration [2]. The increase in surface tension also alters the transmural capillary pressure gradient and so increases fluid filtration. When the lung is ventilated, the regional differences in compliance probably lead to intraregional stresses and alveolar-capillary leak which

Table 2. Mean ± SD of the peak airway (P_{PAW}), mean airway ($P_{\overline{AW}}$) and positive end-expiratory (PEEP) pressures and respiratory rate (RR) throughout the experiment

Time	Group	P_{PAW} (mmHg)	$P_{\overline{AW}}$ (mmHg)	PEEP (mmHg)	RR (min^{-1})
Pre-lavage	I	10.6 ± 1.1	5.2 ± 0.7	0.25 ± 0.5	24.5 ± 3.2
	II	9.9 ± 1.9	4.9 ± 0.8	0.25 ± 0.5	24.3 ± 4.0
Post-lavage	I	17.4 ± 2.7	10.9 ± 3.4	1.9 ± 0.4	30.8 ± 6.8
	II	17.2 ± 3.2	9.2 ± 2.1	1.8 ± 0.7	26.6 ± 7.6
1 h	I	21.0 ± 2.1[a]	15.2 ± 1.6	1.6 ± 0.7	32.9 ± 6.3
	II	23.1 ± 2.0	14.5 ± 1.1	10.3 ± 1.2	36.8 ± 15.2
2 h	I	21.4 ± 2.4	15.2 ± 1.6	2.0 ± 1.1	32.9 ± 5.7
	II	25.4 ± 4.6	14.7 ± 1.4	10.3 ± 1.3	39.0 ± 13.6
3 h	I	21.4 ± 2.6[a] $n = 7$	15.2 ± 1.4 $n = 7$	2.1 ± 1.2 $n = 7$	29.6 ± 3.8[a] $n = 7$
	II	26.9 ± 3.4	14.8 ± 1.4	10.5 ± 1.1	40.1 ± 12.7
4 h	I	21.3 ± 2.6[a] $n = 6$	15.0 ± 1.3 $n = 6$	2.2 ± 0.7 $n = 6$	31.2 ± 5.3[a] $n = 6$
	II	25.3 ± 3.1 $n = 6$	14.5 ± 0.5 $n = 6$	10.9 ± 0.9 $n = 6$	43.5 ± 13.6 $n = 6$
5 h	I	23.0 ± 2.9 $n = 5$	15.5 ± 1.1 $n = 5$	2.2 ± 1.1 $n = 5$	29.6 ± 5.7[b] $n = 5$
	II	25.5 ± 0.5 $n = 6$	14.6 ± 0.5 $n = 6$	10.9 ± 0.8 $n = 6$	45.2 ± 14.4 $n = 6$

Group I = low PEEP; Group II = high PEEP. Statistical significance between the two groups: [a] $p \leqslant 0.05$, [b] $p \leqslant 0.001$

Table 3. Mean ± SD of the static lung compliances measured below and above the inflection point on the pressure volume curve of the lung and of the end-expiratory lung volume (EELV)

Time	Group	Compliance (ml $mmHg^{-1}$)		EELV (ml)
		Below	Above	
Pre-lavage	I	–	4.3 ± 1.6	–
	II	–	4.9 ± 1.0	–
Post-lavage	I	0.4 ± 0.1[a]	3.6 ± 1.0	–
	II	0.6 ± 0.2	4.2 ± 2.0	–
1 h	I	1.5 ± 0.1	4.0 ± 0.8	3.3 ± 1.4[b]
	II	0.7 ± 0.3	4.4 ± 2.4	33.3 ± 13.0
2 h	I	0.4 ± 0.1	4.0 ± 0.8	3.0 ± 1.5[b]
	II	0.6 ± 0.2	3.0 ± 1.0	34.9 ± 12.2
				$n = 7$
3 h	I	0.4 ± 0.2	3.6 ± 1.2	2.7 ± 1.4[b]
	II	0.5 ± 0.2	3.9 ± 1.2	32.7 ± 9.1
		$n = 5$	$n = 5$	$n = 6$
4 h	I	0.4 ± 0.1[a]	3.2 ± 0.8	3.8 ± 3.0[b]
		$n = 6$	$n = 6$	$n = 6$
	II	0.6 ± 0.3	4.2 ± 1.2	36.6 ± 15.9
		$n = 5$	$n = 5$	$n = 5$
5 h	I	0.4 ± 0.1	3.3 ± 1.1	2.8 ± 1.7[b]
		$n = 5$	$n = 6$	$n = 6$
	II	0.5 ± 0.1	3.7 ± 1.2	31.7 ± 19.8

Group I = low PEEP; Group II = high PEEP
[a] $p \leqslant 0.05$, [b] $p \leqslant 0.001$

Table 4. Survival time and histological scores (range 0–4) of hyaline membrane formation in the individual animals of both groups. Animals surviving 5 h were sacrificed. Hyaline membrane scores in high PEEP group (II) were significantly less than those in low PEEP group (I)

	Survival time	Hyaline membrane scores (0 to 4)	
		Right lung	Left lung
Low PEEP			
1	240	3	3
2	160	0	1
3	300	1	3
4	200	2	2
5	300	3	3
6	300	3	3
7	300	4	4
8	300	2	2
Mean score (for each lung) 2.43 ± 1.1			
High PEEP			
1	300	0	0
2	300	0	1
3	300	0	0
4	300	2	2
5	180	0	0
6	300	1	1
7	300	0	2
8	195	0	0
Mean score (for each lung) 0.6 ± 0.8			

($p = 0.001$)

results in the formation of hyaline membranes due to the condensation of blood proteins with necrotic epithelium [10, 24]. It has been suggested that this may be exacerbated by high peak airway pressures [24]. Hyaline membrane formation may also occur in adults with respiratory failure due to various causes during the acute phase of the disease [3]. The model of NRDS used in this study has the virtues of stability and reproducibility. It imitates the deficiency of surfactant that is the primary pathogenetic feature of the disease without causing severe damage to the alveolar structures [8, 9] and it produces histological changes which are very similar to those seen in NRDS. However, since the rabbits are mature, it is probable that the capacity for surfactant regeneration is retained so that the time course of recovery is shortened.

The number of lavages needed to produce a PaO_2 of less than 10 kPa, in this study, ranged from three to six. After the lavages had been completed a definite inflection point appeared in the inflation limb of the P/V curve. Below the inflection point pressure the lung compliance was markedly reduced, but above this the slope of the P/V curve was comparable with pre-lavage values (Table 3). The inflection point was situated between airway pressures of 9.5 to 14 mmHg.

Similar changes in the P/V curve have been reported in adults during the early stages of the respiratory distress syndrome [11, 17]. The inflection point pressure is thought to represent the pressure at which collapsed small airways and alveoli re-open. Thus, in the group of animals ventilated with low levels of PEEP we believe that the alveoli were collapsed and re-expanded during each respiratory cycle, whereas, in the animals in which the PEEP level was set above the measured inflection point, most of the alveoli should have remained inflated at all times. Although collapse would have tended to occur when the rabbits were disconnected to take readings, this would have been reversed by the sustained inflation generated by the recording of the P/V curve [4, 5, 7].

The use of 100% oxygen as the inspired gas is open to criticism since it is known that this may lead to absorption collapse. However, it seems unlikely that this could have had a major influence on the results since there was little change in PaO_2 with time in either group. Furthermore, any collapse from this source should have been reversed by the hyperinflation manoeuvre associated with the recording of the P/V curve.

The choice of ventilatory pattern in these studies was dictated by the need to maintain blood gases within the normal range and to have similar mean and peak airway pressures in each group. In a previous study using levels of PEEP equal to the inflection point pressure and 5 mmHg below the inflection point pressure we found that there was less hyaline membrane formation in the high PEEP group but noted that the arterio-venous O_2 content differences were also higher in this group, presumably because of the higher $\overline{P_{AW}}$ associated with the higher levels of PEEP. Since differences in pulmonary blood flow could have affected hyaline membrane formation we eliminated this factor by keeping $\overline{P_{AW}}$ close to 15 mmHg in both groups in the present study. This resulted in similar arterio-venous O_2 content differences in each group, though the values were greater than normal, thus suggesting that the cardiac outputs were reduced despite the augmentation of blood volume by the regular addition of a plasma substitute. High peak airway pressures are also known to be associated with hyaline membrane formation, so these were limited to 25 mmHg in both groups.

To achieve these aims it was necessary to increase the inspiratory : expiratory time ratio to a mean of 2.7 : 1 in the low PEEP group at 1 h after lavage with an end-inspiratory pause of 24% of the inspiratory time. It was also necessary to increase the fresh gas flow and respiratory frequency in the high PEEP group since the tidal volume was restricted by the need to keep the peak airway pressures below 25 mmHg. These differences could have caused a difference in hyaline membrane formation. However, the differences in frequency were small and in studies with this model Lachmann et al. [9] found that increasing the inspiratory : expiratory time ratio decreased hyaline membrane formation. Since the differences in EELV between the high and low PEEP groups confirmed the position of the lungs on the P/V curve it seems reasonable to conclude that the higher incidence of hyaline membrane formation in the low PEEP group was associated with the intra-regional stresses resulting from the repeated closing and re-opening of alveolar units.

A number of other authors have postulated that hyaline membrane formation is associated with mechanical damage to the lung [5, 9, 20, 24] but there is still debate concerning the mechanisms by which the prevention of lung deflation may prevent hyaline membrane formation. The improvement in the distribution of ventilation that occurs will minimize regional lung hypoxia, and since this has been shown to play an important role in the inhibition of the metabolic activity of lung cells [23], it is possible that the reduction in lung damage is an indirect biochemical effect. Another factor which might be involved in the limitation of lung damage is the decrease of fluid filtration into alveoli that are maintained above the critical radius [20]. The maintenance of lung volume may also influence the activation of polymorphonuclear leucocytes (PMN). Although there was no statistically significant difference between the neutrophil counts of

the two groups studied, previous work has shown that PMN's may play an important role in determining the severity of changes seen in lung disease requiring ventilation. PMN's are a source of oxygen-derived free radicals, particularly in the presence of hyperoxia [24], and may thus promote lung injury. Leucocyte depletion has been shown to reduce lung damage due to hyperoxia [22] and granulocyte depleted rabbits mechanically ventilated after lung lavage have been found not to form hyaline membranes [6]. Finally, the beneficial effects of the maintenance of lung volume observed in this experimant may be due to an increase in surfactant availability. It has been shown by other workers that PEEP causes an increase in surfactent activity in hyperventilated cat and dog lungs, either by stimulating surfactant production and/or inhibiting its inactivation [27], or by hindering its movement into the airways [4]. McClenahan and Urtnowski [12] concluded that recovery of surfactant activity following its reduction during mechanical ventilation is an active cellular metabolic process. Thus, the beneficial effect of PEEP in this experiment may be mediated via a biochemical effect upon surfactant production.

Previous studies have compared CMV with techniques which decreased tidal volume but maintained end-expiratory lung volume [5, 16]. This raises the question whether the formation of hyaline membranes is accentuated by high peak pressures or large airway pressure changes, or whether repeated opening and closing of alveolar units is more important. The present experiments confirm our previous studies [1] and suggest that maintenance of an EELV above the inflection point is the major factor in the prevention of hyaline membrane formation.

Although the study by Hamilton et al. [5] suggests that HFO may offer some advantages in NRDS, other workers have failed to confirm this. Truog et al. [26] compared the effects of HFO with those of CMV in premature primates. The only difference they detected was a lower level of $PaCO_2$ in the HFO group. They found no improvement in PaO_2 with HFO, but they do not state what bias flow of oxygen was used. If this was 1 $l\,min^{-1}$ as in their previous study [25], then a higher flow rate might have improved oxygenation and reduced lung damage. Also, it may be inappropriate to compare the findings in two such different models of NRDS. Quan et al. [18] also failed to show any improvement in hyaline membrane formation with high-frequency jet ventilation (HFJV). Again there were differences between their model and that of Hamilton which may account for this. The difference in ventilation rate may be important and the lower mean airway pressure during HFJV (8–12 cm H_2O) may have contributed to the lack of improvement in oxygenation in that group.

In conclusion, it would appear that the maintenance of an adequate lung volume throughout the ventilatory cycle is of prime importance in providing good oxygenation in NRDS. The mean airway pressure is not in itself the determining factor in this condition, as has been previously suggested [17]. High I:E ratios were used in this study in order to match $\overline{P_{AW}}$ and these did not confer any beneficial effect upon the histological changes which developed in the low PEEP group. This is contrary to the findings of Lachmann et al. [9]. Lung inflation above the critical volume at which airway and alveolar collapse occurs can be achieved by various means. These include the use of high levels of PEEP and HFO, which would both be expected to maintain high EELV's. It would be interesting to compare the relative merits of these methods of ventilation in the presence of NRDS.

Acknowledgments. The authors thank Mr. Richard Madgwick for his expert technical assistance, Dr. David Holland for his statistical advice, and Miss Joan Rawlings for secretarial help.

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