Surfactant Metabolism during Hyperventilation of Newborn Lambs with Atrial Right to Left Shunts

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ABSTRACT. We studied the effect on surfactant metabolism of 8 h of mechanical ventilation at tidal volumes of 13 ± 0.3 ml/kg and very high tidal volumes of 28 ± 1.5 ml/kg, with and without added CO_2, in the presence of an atrial right to left shunt in 4- to 8-day-old lambs. Similarly aged, spontaneously breathing lambs were used as controls. Right to left atrial shunts were created by inflating a balloon in the right atrium after a Rashkind atrial septostomy, thus creating a stable, easily controlled atrial shunt. Radiolabeled surfactant phospholipid precursors were used to probe incorporation into and secretion of surfactant phosphatidylcholine, whereas intratracheally administered labeled natural surfactant was used to evaluate alveolar clearance. Protein leak from the vascular space to the lungs was measured using radioactive iodine-labeled albumins. At the end of the 8-h study period, tissue association of alveolar surfactant was significantly increased to 63% in the mechanically hyperventilated lambs as compared to 44% in those lambs mechanically ventilated but not hyperventilated ($p < 0.05$) and to 39% in the spontaneously breathing control animals ($p < 0.05$). No increased surfactant secretion or decreased compliance was detected with hyperventilation. However, the lambs had very large surfactant-saturated phosphatidylcholine pool sizes, and a large portion (50%) was already in the alveolar pool, even in the spontaneously breathing lambs. Precursor incorporation into saturated phosphatidylcholine was similar in all groups, and very low and comparable protein leaks were seen in the different groups of lambs. These experiments did not detect any adverse effects of 8 h of mechanical ventilation, large atrial shunts or mechanical hyperventilation on the surfactant system of newborn lambs. (*Pediatr Res* 25:83–88, 1989)

In neonates with persistent pulmonary hypertension, hyperventilation has been considered an essential part of the ventilatory support (1). Suprasystemic pulmonary arterial pressures cause right to left shunts at the patent foramen ovale and ductus arteriosus levels which result in progressive hypoxemia. The goal of hyperventilation is to attain a critically low $PaCO_2$ and alkaline pH which may promote reversal of the right to left shunt by decreasing pulmonary artery pressures to less than systemic levels (1). However, infants often become less responsive to the same

degree of hyperventilation after 48–72 h, requiring higher peak inspiratory pressures to maintain the same level of oxygenation. At this stage, ventilator-induced pulmonary parenchymal disease and oxygen toxicity are other major factors causing hypoxemia rather than the right to left shunting (2). It is also possible that this subsequent respiratory deterioration is in part due to damage to the surfactant system by pulmonary edema fluid (3), damage to type II cells, alterations in surfactant metabolism (4), or effects of mechanical ventilation on surfactant (5).

Previous studies in isolated rat and dog lungs showed decreased compliance and increased minimum surface tensions with increased duration of ventilation at large tidal volumes for periods of up to 4 h (6, 7). Oyarzun and Clements (8, 9) measured alveolar surfactant phospholipids in hyperventilated adult rabbits and found that alveolar surfactant increased during the first 2 h but substantially decreased after 4 h of increased tidal volume ventilation. They speculated that secretion was stimulated by hyperventilation, thereby increasing alveolar surfactant pools at the expense of tissue pools (8, 9). Similar conclusions were drawn by Wyszogrodski *et al.* from their study of hyperventilated adult cats (10). Morphometric analysis of hyperventilated and hypoperfused adult dog lungs showed decreased lamellar body volume densities in type II cells (11), and addition of CO_2 to the inspired gases was found to moderate this effect (12).

Because differences between adult and newborn surfactant physiology exist (13), perhaps developmental differences in the response to mechanical ventilation at different tidal volumes would also occur. Using newborn lambs, we asked if surfactant phospholipid metabolism is altered by either mechanical ventilation or mechanical hyperventilation in the presence of an atrial right to left shunt with and without normalization of arterial PCO_2 when compared to spontaneously breathing controls.

MATERIALS AND METHODS

Preparation of lambs. Healthy lambs 4 to 8 days of age were studied as four groups of five lambs each. Groups were ventilated as follows: group I: mechanically hyperventilated to $PaCO_2$ of 15–20 mm Hg, tidal volume 25–40 ml/kg; group II: mechanically hyperventilated to a similar level as group I except CO_2 was added to the inspired gases to maintain $PaCO_2$ at 35–45 mm Hg; and group III: mechanically ventilated to $PaCO_2$ of 35–45 mm Hg, tidal volume 10–14 ml/kg. Group IV lambs were allowed to breathe spontaneously without assisted ventilation.

Ventilated groups. After premedication with intramuscular ketamine 10 mg/kg and acepromazine 0.1 mg/kg, lambs in groups I–III had a 5.0 Fr catheter placed in the distal aorta via the femoral artery using supplemental local anesthesia. This catheter was used for continuous blood pressure and heart rate monitoring and blood gas and pH determinations before the

onset of and during ventilation. Under local lidocaine anesthesia, the trachea was then exposed and a 5.0 mm internal diameter cuffed endotracheal tube was secured by tracheostomy. Pancuronium bromide 0.1 mg/kg was then administered intravenously to paralyze the animal. The lambs were subsequently ventilated with 100% humidified and warmed oxygen with Sechrist pressure limited ventilators (Sechrist Industries, Anaheim, CA), using initial settings of: flow, 15 liter/min; peak inspiratory pressure, 10 cm H_2O; positive end expiratory pressure, 1 cm H_2O; rate, 20 breaths/min; and inspiratory time, 0.7 s. A baseline arterial blood gas and pH were measured on these settings. A Rashkind balloon catheter was then introduced into a femoral vein and advanced with fluoroscopy guidance to the right atrium and then across the patent foramen ovale to the left atrium. The balloon was inflated in the left atrium and pulled across the foramen ovale to the right atrium, creating an atrial septal defect. The Rashkind catheter was then withdrawn from the femoral vein, and a 5.0 Fr catheter was inserted in its place and threaded 7–8 cm into the vessel.

To generate a right to left shunt at the atrial level, a catheter with a distensible distal balloon was threaded to the right atrium via the right external jugular vein in a manner similar to that described by Fahey and Lister (14). Balloon position was confirmed by fluoroscopy. The shunt was demonstrated clinically by inflating the balloon with 2–3 ml of contrast medium and documenting a substantial decrease in arterial PO_2 from > 300 mm Hg to 35–50 mm Hg in groups I, II, and III. The balloon was deflated in group III after the initial demonstration that a shunt could be created.

Each lamb then received ^{32}P-labeled natural rabbit surfactant (approximately 5 mg total lipid suspended in 10 ml 0.9% NaCl) intratracheally to label the alveolar pools. The time between initial placement on the ventilator and administration of ^{32}P natural surfactant averaged 48 ± 7 min. The lambs were rotated to four different positions during the intratracheal injection to try to distribute the surfactant suspension uniformly. After the tracheal injection, the ventilator rate was increased to 30 breaths/min, and peak inspiratory pressure increased to maintain ventilation. Only peak inspiratory pressure was subsequently changed to achieve the ventilatory goals for the animals in the different groups. The low rate, high tidal volume, low positive end expiratory pressure combination was selected for hyperventilation to avoid gas trapping. Group II lambs had CO_2 added to the inspired gases at the onset of hyperventilation to maintain $PaCO_2$ at 35–45 mm Hg.

A continuous infusion of 5% dextrose in 0.45% NaCl was given to each lamb at 100 ml/kg/24 h. Body temperature was monitored with a rectal probe during the study. The lambs were kept paralyzed with 0.1 mg/kg pancuronium bromide given intravenously every 4 h, and anesthesia was maintained with 10 mg/kg ketamine and 0.1 mg/kg acepromazine given by intramuscular injection every 4 h. Tidal volumes were measured hourly with a Fleisch pneumotachometer, and compliances were calculated as ml tidal vol/kg body weight divided by the difference between the peak inspiratory pressure and the positive end expiratory pressure (15).

Unventilated group. After premedication with the same dose of intramuscular ketamine and acepromazine as for the previous animals, the group IV lambs were intubated orally with a 4.5-mm internal diameter cuffed endotracheal tube, and the ^{32}P natural surfactant was given intratracheally. The lambs were then ventilated using 100% humidified and warmed oxygen with a flow of 15 liters/min; peak inspiratory pressure, 20 cm H_2O; positive end expiratory pressure, 1 cm H_2O; rate, 20 breaths/min for 5–10 min to distribute the surfactant in the airways. During this brief period of ventilation, a 3.5 Fr catheter was secured in a saphenous vein for subsequent vascular injections. The animals were extubated and returned to pens with their mothers for the duration of the experiment. They were subsequently disturbed for two brief periods (< 5 min) to inject radiolabeled precursors before the animals were killed. A blood sample for venous blood gas and pH measurements was obtained from the saphenous catheter just before the animals were killed.

Preparation of radioactive natural surfactant. Radioactive large aggregate natural rabbit surfactant was isolated as previously described by centrifugation of alveolar washes from lungs of 3-day-old rabbits that were each given 1 mCi of $[^{32}P]$orthophosphate by tracheal injection 24 h before the animals were killed (16). This radiolabeled surfactant was then suspended in 0.9% NaCl with unlabeled natural sheep surfactant to a concentration of approximately 5 mg total surfactant lipid and 2 μCi ^{32}P radioactivity/10 ml. Although the labeled tracer surfactant was from rabbits rather than sheep, we found few differences in rates of clearance or tissue association patterns for species heterogenous surfactants (17).

Radioactive label administration. All lambs received two different radiolabeled precursors of surfactant phosphatidylcholine intravenously at different times as well as the intratracheal labeled surfactant. Surfactant pools in all animals were labeled with $[^3H]$palmitic acid (0.67 mCi/kg) given by intravascular injection about 22 h before the animals were killed, primarily to study surfactant secretion. At 1 h before the animals were killed, $[^{14}C]$palmitic acid (25 μCi/kg) was given by intravascular injection to evaluate precursor incorporation into newly synthesized saturated phosphatidylcholine. Protein leak into the lungs was studied using $[^{125}I]$albumin given by intravascular injection 2 h before death. $[^{131}I]$albumin was given by intravascular injection 2 min before the animals were killed to estimate the blood volume retained within the lung tissue (15). Preparation of iodinated albumin using monomer standard bovine albumin and chloramine T was as previously described (15). Trichloroacetic acid precipitation of the iodinated albumin product yielded > 97% trichloroacetic acid-precipitable radiolabeled protein complex.

Cardiac outputs, left to right ductal shunts, and atrial right to left shunts were measured in the mechanically ventilated lambs about 3 h before the animals were killed using radiolabeled 15-μm diameter microspheres. ^{57}Co microspheres were injected over 30 s into a 5.0 Fr catheter inserted into the left ventricle via a right carotid artery cutdown while a reference sample was simultaneously being drawn from the aortic catheter into a heparinized glass syringe at a rate of 6 ml/min for 2 min using a Harvard pump (18). The left ventricular catheter was then removed. ^{46}Sc microspheres mixed with 8 ml maternal blood were divided into two 4-ml aliquots. One 4-ml aliquot was injected through the femoral vein catheter while the other 4-ml aliquot was simultaneously injected into the left external jugular vein. After the animals were killed, the lungs were removed, and the rest of the body was carbonized and counted for microsphere content. Aliquots of lung homogenate also were taken in triplicate for microsphere counts. Cardiac outputs were measured using the reference sample drawn from the aorta during the left ventricular injections and using total radioactivity as ^{57}Co counts in the body plus the lungs and the reference sample (18). The percentage of recovery in the lungs of the ^{57}Co microspheres injected into the left ventricle was a measure of left to right ductal shunting. Atrial right to left shunts were determined using the following formula: $[1-(^{46}Sc$ lungs/^{46}Sc lungs + carcass$)] \times 100\% = \%$ right to left shunt. Microsphere-associated radioactivity was counted using a 5-channel counter because of the presence of ^{125}I and ^{131}I. Appropriate isotope standards were used for computerized cross channel and background corrections.

Processing of lungs. The lambs were killed with intravenous pentobarbital followed by exsanguination 8 h after receiving the intratracheal natural surfactant labeled with ^{32}P. Groups I–III lambs had their endotracheal tubes cross-clamped, whereas lambs in group IV had endotracheal tubes secured by tracheostomy and cross-clamped. The chest of each lamb was then rapidly opened to expose the heart, and blood for the determination of labeled iodine content was aspirated from the ventricle into a heparinized syringe. In the mechanically ventilated lambs, the position of the intraatrial balloon was confirmed. The lungs of

all lambs were carefully removed from the chest, inspected, and weighed. The hearts and major vessels were examined.

Sufficient saline to distend the lungs fully was washed in and out of the lungs three times and recovered. This wash procedure was repeated with four more aliquots of saline, and the five alveolar washes were pooled and the volume recorded (19). The lungs were then weighed, minced with scissors, and degassed in a vacuum chamber. On average, 7% of the saline volume was retained in the lung tissue, and this amount was similar in the four groups, supporting the consistency of the alveolar wash procedure. The tissue was then gently homogenized with an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH) in 350–450 ml 0.25 M sucrose/Tris buffer at pH 7.4 (20). The final volume of the homogenized lung tissue was recorded. Duplicate aliquots from each lung homogenate were processed for lamellar bodies and microsomes using sucrose step gradients (20). The lamellar bodies and microsomes resuspended in saline as well as aliquots of alveolar washes and lung homogenates in triplicate were extracted with chloroform/methanol (21). Saturated phosphatidylcholine was recovered by column chromatography after osmium tetroxide oxidation (22). Phosphorous content was measured on an aliquot of the saturated phosphatidylcholine solution according to Bartlett (23); the remainder was used to measure radioactivity in Aquasol scintillation fluid (New England Nuclear, Boston, MA). Specific activities of ^{3}H and ^{14}C were determined for each sample as cpm/μmol saturated phosphatidylcholine. Recovery of ^{32}P-saturated phosphatidylcholine was determined in extracted alveolar washes and lung homogenates and expressed as a percentage of the total amount given to the lambs. Saturated phosphatidylcholine pool sizes were calculated for alveolar washes and lung homogenates of each animal and expressed as μmol saturated phosphatidylcholine/kg body weight.

Radiolabeled albumin associated with alveolar washes, lung tissue and blood were measured. The ^{131}I radioactivity associated with the lung homogenate was assumed to be from intravascular blood and was used to estimate the amount of blood retained in the lung tissue (15). The blood-associated ^{125}I radioactivity was then subtracted from that measured for each lung. The remaining lung-associated ^{125}I radioactivity was expressed as a percentage of total injected dose. Protein in alveolar wash was measured according to Lowry (24) using a BSA standard.

Data analysis. Data are expressed as means $\pm$ SEM. Statistical comparisons were performed by ANOVA followed by the Student Newman-Keuls multiple comparison procedure. Differences between groups were considered significant at $p < 0.05$.

RESULTS

Description of animals. The four groups of lambs had similar ages, body weight, and sex distributions (Table 1). The mean heart rate in group I lambs (hyperventilated) was slightly higher than those of the other two mechanically ventilated groups (II,

hyperventilated with added CO_2 and III, ventilated with the lower tidal volumes); however, cardiac outputs and mean blood pressures were similar in the three groups. Atrial right to left shunt was measured at 5 ± 1 h after onset of ventilation. Shunt values, obtained by calculation of the fraction of injected ^{46}Sc microsphere counts found in the carcass, were plotted on a semilog scale against the corresponding PaO_2 at the time of injection, and a linear regression curve was generated ($r = 0.98$). Average shunt during the 8-h study period was calculated from the mean PaO_2 values in each group using this curve (Table 1). The hyperventilated groups (I and II) of lambs had similar shunts that were significantly different from the lambs ventilated at the lower tidal volumes (group III, $p < 0.01$). Recovery of microspheres injected into the left ventricle from the lungs of the three groups of ventilated lambs was similar at 4 to 6%. This low value indicated no significant left to right shunt at the level of the ductus arteriosus.

Tidal volumes were about 28 ml/kg and similar in the hyperventilated groups, whereas group III lambs were ventilated with significantly lower mean tidal volumes of 13 ml/kg (Fig. 1A). The tidal volumes in groups I and II were achieved with mean peak inspiratory pressures of 23 cm H_2O, whereas 16 cm H_2O peak inspiratory pressure was used to support the group III lambs. Lung compliances, which were normal in all lambs at the onset of ventilation, increased after the onset of hyperventilation in groups I and II and persisted at those increased levels with time, whereas lung compliances in group III remained unchanged throughout the experiment (Fig. 1B). Arterial PCO_2 values were successfully normalized to an average value of 38 mm Hg in group II with additional inspired CO_2, and this was similar to the mean value of 40 mm Hg for group III, but significantly higher than the mean value of 18.7 mm Hg for group I ($p < 0.01$, Fig. 1C). Mean PaO_2 values in group III lambs were 330 mm Hg and different from groups I and II, which had comparable low mean PaO_2 values of about 42 mm Hg ($p < 0.01$, Fig. 1D). The pH values reflected the PCO_2 values in the different groups of lambs (Table 1). Venous PCO_2 and pH in group IV lambs measured just before the animals were killed indicated normal gas exchange.

Surfactant measurements. Surfactant pool sizes in both the alveolar and tissue compartments, estimated by saturated phosphatidylcholine content, were comparable in all groups (Table 2). Intratracheal administration of trace amounts of ^{32}P natural surfactant permitted us to label alveolar surfactant pools without significantly altering the sizes of the existing pools. Significantly less of the saturated phosphatidylcholine from the ^{32}P natural surfactant was recovered by alveolar wash from the two mechanically hyperventilated groups as compared to the lambs mechanically ventilated at the lower tidal volumes and the spontaneously breathing controls that had similar values, indicating a higher extent of tissue association with the increased ventilation (Fig. 2). Recovery of label in the total lung (the sum of the alveolar

Table 1. *Characteristics of lambs studied**

	Group I: hyperventilated without added CO_2	Group II: hyperventilated with added CO_2	Group III: ventilated to normal CO_2	Group IV: spontaneously breathing	$p < 0.05$
No. of animals	5	5	5	5	
Age (days)	6.4 ± 0.4	4.8 ± 0.5	5.4 ± 0.8	4.4 ± 0.2	
Body wt (kg)	3.6 ± 0.1	3.2 ± 0.2	3.3 ± 0.3	3.5 ± 0.3	
Male:female	1:4	3:2	4:1	1:4	
Heart rate (beats/min)	246 ± 6	228 ± 6	218 ± 6		II, III < I
Mean arterial blood pressure (mm Hg)	81 ± 1	84 ± 4	86 ± 3		
Cardiac output (ml/kg/min)	221 ± 45	282 ± 10	274 ± 14		
% right to left shunt	56.5 ± 1.4	57.9 ± 1.3	4.7 ± 1.3		III < I, II
Mean pH when killed	7.43 ± 0.01	7.34 ± 0.02	7.37 ± 0.01	7.38 ± 0.01	II, III, IV < I

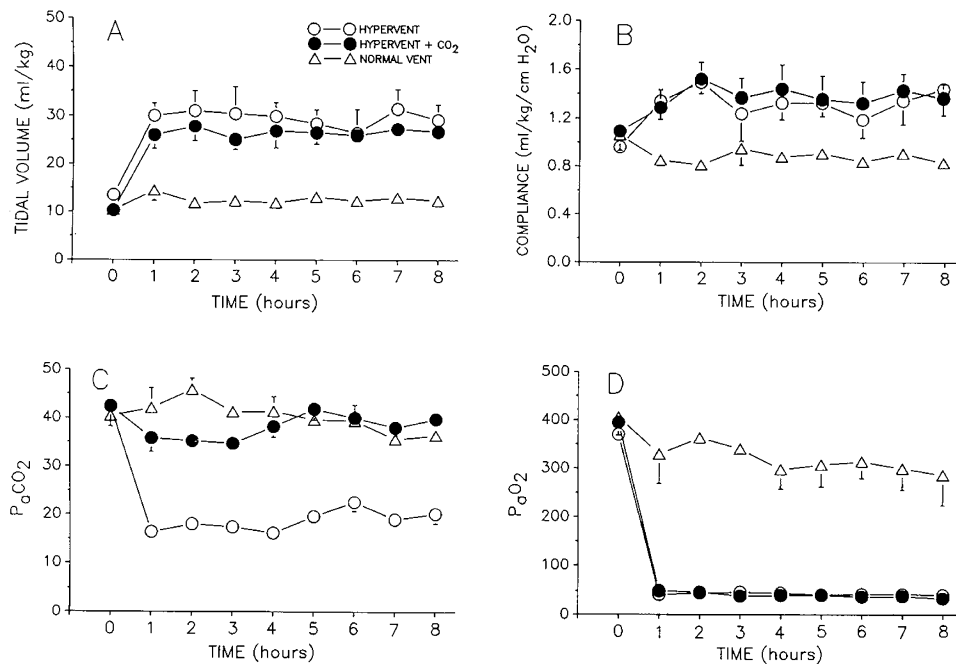* Values are given as means $\pm$ SEM.

Fig. 1. Comparison of mechanically ventilated lambs from onset of mechanical ventilation to time animals were killed. Symbols for each group of lambs are identified on the figure. Time 0 represents measurements just before administration of intratracheal tracer surfactant labeled with ^{32}P. Values are means ± SEM. Representative error bars not shown fall within the data point.

Table 2. *Measurements of surfactant phosphatidylcholine* *

	Group I: hyperventilated without added CO_2	Group II: hyperventilated with added CO_2	Group III: ventilated to normal CO_2	Group IV: spontaneously breathing	$p < 0.05$

Saturated phosphatidyl-
  choline pool size
  (μmol/kg)					

AW	122 ± 14	120 ± 15	144 ± 12	137 ± 15	
AW + L	249 ± 24	258 ± 28	255 ± 14	247 ± 16	

Ratio of ^{32}P specific activ-
  ity microsome/lamel-
  lar bodies
	0.27 ± 0.04	0.33 ± 0.06	0.31 ± 0.09	0.60 ± 0.06	I, II, III < IV

^3H specific activity					
AW	7585 ± 961	11763 ± 492	12779 ± 2245	11159 ± 1184	
L	19264 ± 2932	21528 ± 2583	29153 ± 5230	32027 ± 3743	
LB	20426 ± 2744	23910 ± 3381	28144 ± 6764	18391 ± 1521	
M	22677 ± 2394	22881 ± 1428	30327 ± 4521	26430 ± 3893	
% ^3H label in AW	27.7 ± 2.1	32.9 ± 2.9	37.8 ± 3.4	28.3 ± 2.5	
^{14}C specific activity					
L	1260 ± 307	1115 ± 524	2073 ± 427	2152 ± 249	
M	2229 ± 599	2285 ± 890	3288 ± 816	2518 ± 489	

* Values are given as means ± SEM. Abbreviations: AW = alveolar wash; L = lung homogenate after alveolar wash; LB = lamellar body fraction; M = microsome fraction. Specific activity denotes cpm/μmol saturated phosphatidylcholine.

wash plus lung homogenate) was similar in groups II, III, and IV but was decreased in group I when compared to group IV only. The ratio of ^{32}P specific activities in the microsomes compared to the lamellar bodies was significantly lower in the mechanically ventilated groups as compared to the spontaneously breathing groups of lambs, suggesting that more of the labeled tracer surfactant was maintained in the resecretion pathway with ventilation (Table 2).

The specific activities of [^3H]palmitic acid-labeled saturated phosphatidylcholine in the lamellar body, microsome, lung tissue and alveolar compartments were comparable for the four groups 22 h after the labeled precursor was given. About 30% of the total lung [^3H]palmitic acid-labeled saturated phosphatidylcho-

line was recovered by alveolar wash in all groups. Specific activities of the [^{14}C]palmitic acid-labeled saturated phosphatidylcholine in the various fractions were not significantly different between the groups of animals. Very little [^{14}C]pamitic acid-labeled saturated phosphatidylcholine was recovered in the alveolar washes or lamellar bodies. This indicates that synthesis, but not storage and secretion, had occurred within the hour between isotope injection and the time the animals were killed.

Protein leak measurements. No differences in protein leak, measured by recovery of intravascularly administered [^{125}I]albumin from the airspaces and lung tissue, were found between the groups (Table 3). Similarly, total protein content in the alveolar wash was not different in the four groups.

DISCUSSION

Effects of hyperventilation on cardiac outputs, organ blood flows, and cellular metabolism have been reported in newborn animal models (25), but there are no studies of effects of mechanical hyperventilation on the surfactant system in the newborn. Similarly, effects of mechanical ventilation on the surfactant system of the newborn are not well described. We developed a newborn lamb model where mechanical hyperventilation was performed after creation of an atrial right to left shunt. The shunt was created to better mimic the clinical situation of decreased pulmonary blood flow and low systemic PaO_2 values that is managed clinically with hyperventilation. We recognize that these animals do not have the associated problems of pulmonary hypertension and any underlying lung disease. However, the model has the advantages that a thoracotomy was not required, and the magnitude of the atrial shunt could easily be adjusted by changing the volume of the atrial balloon. We did not see a fall in left ventricular output, the predictable result of balloon inflation in the right atrium in the absence of an atrial septal defect (14). The animals were quite stable for the 8-h study period. This model should be useful to others who want to change arterial PO_2 values without changing alveolar PO_2 values or cardiac output.

Previous studies reported effects of hyperventilation on lung function using adult animal lung models. Decreased compliance after periods up to 4 h of increased ventilation were attributed to altered surfactant function because extracts from these lungs exhibited increased minimal surface tensions (6–10). Increased alveolar surfactant phospholipids were found by Oyarzun and Clements (8, 9) after hyperventilation of adult rabbits for periods up to 4 h. They postulated that increased secretion of tissue pools occurred followed by inactivation. High peak inspiratory pressure hyperventilation of adult sheep for periods up to 48 h similarly resulted in development of decreased lung compliance, acute respiratory failure, and increased alveolar wash fluid minimal

surface tension values as early as 6 to 12 h after onset of hyperventilation (26). Morphometric analysis of adult dog lungs hyperventilated for 4 h in the presence of relative hypoperfusion induced by pulmonary artery occlusion documented decreased lamellar body volume densities in type II cells, an effect that was moderated by the addition of CO_2 to the inspired gases (11). In subsequent studies using excised adult dog lungs subjected to high tidal volume ventilation with and without varying amounts of added CO_2, alveolar hypocapnia rather than the level of ventilation appeared to be the primary mediator of lamellar body depletion in type II cells (12).

Alveolar wash-saturated phosphatidylcholine pool sizes were not different in the four groups that we studied. The alveolar pool sizes were very large in these approximately 1-wk-old lambs. For comparison, adult rabbits, rats, and sheep would have pool sizes of saturated phosphatidylcholine of about 4 to 10 μmol/kg body weight, while 1-wk-old rabbits have alveolar pool sizes of about 40 μmol/kg body weight (13). The alveolar surfactant-saturated phosphatidylcholine pool was about 50% of the total lung surfactant-saturated phosphatidylcholine, a value very much higher than the approximately 15% of total lung surfactant-saturated phosphatidylcholine found in alveolar washes of adult rats and rabbits (27). Mechanical ventilation at the lower tidal volumes did not change alveolar wash-saturated phosphatidylcholine pool sizes or total lung-saturated phosphatidylcholine as compared to spontaneously breathing controls. Ennema et al. (4) similarly found no differences in the alveolar wash saturated phosphatidylcholine pool sizes in mechanically ventilated and spontaneously breathing adult rabbits. However, in contrast to what others have reported in adult animals (8–10), we found no increase in alveolar surfactant pool size after mechanical hyperventilation of these term newborn lambs. It is conceivable that so much of the available lung-saturated phosphatidylcholine was already in the alveoli that stimulated secretion either did not occur or was not detectable. Perhaps mean PCO_2 values of about 20 mm Hg and/or the increased tidal volumes were not sufficient to increase surfactant secretion. That the spontaneously breathing control lambs had similarly large alveolar surfactant pool sizes suggested that the secretory mechanisms in these 1-wk-old lambs were functioning at much higher levels than previously reported in adult or preterm animals (27–29).

Using two radiolabeled precursors administered at specified times, we tried to probe surfactant synthesis and secretion. In support of lack of a large effect of mechanical ventilation on surfactant secretion was the equivalent recovery in all groups of the [³H]palmitic acid-labeled saturated phosphatidylcholine in the tissue and alveolar pools 22 h after isotope injection. Peak alveolar recovery of labeled saturated phosphatidylcholine was shown to occur about 40 h after precursor injection in newborn lambs (20). Therefore, we anticipated that if increased secretion occurred, we would see increased amounts of labeled saturated phosphatidylcholine in the alveolar wash. However, all four groups of animals had secreted about 30% of the total lung [³H] palmitic acid labeled saturated phosphatidylcholine by 22 h, indicating that these somewhat older lambs had already secreted a higher percentage of labeled saturated phosphatidylcholine than
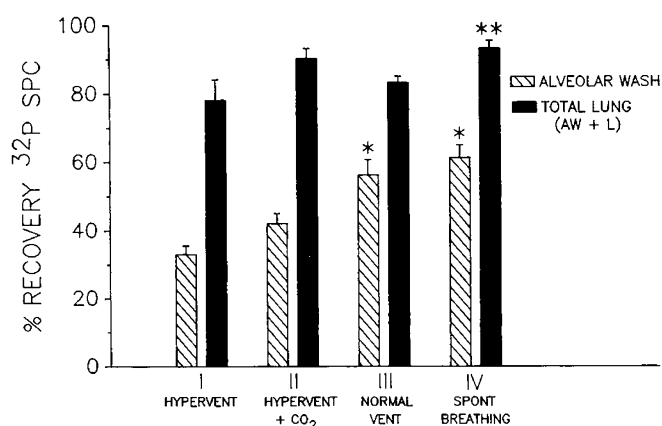

Fig. 2. Percentage of recovery of ^{32}P-labeled saturated phosphatidylcholine in alveolar wash (AW) and total lung ($AW+L$ = AW+lung homogenate after alveolar wash). Values are shown as means ± SEM. *p < 0.05 I, II < III, IV; **p < 0.05 I < IV.

Table 3. *Measurements of protein leak**

	Group I: hyperventilated without added CO_2	Group II: hyperventilated with added CO_2	Group III: ventilated to normal CO_2	Group IV: spontaneously breathing	$p < 0.05$
% recovery of ^{125}I-albumin					
AW	0.56 ± 0.11	0.50 ± 0.07	0.54 ± 0.07	0.56 ± 0.08	NS
L	0.98 ± 0.18	0.86 ± 0.14	0.82 ± 0.09	0.53 ± 0.08	NS
AW protein (mg/kg)	104 ± 17	93 ± 11	97 ± 3	100 ± 17	NS

* Values are expressed as means ± SEM. Abbreviations: AW = alveolar wash; L = lung homogenate after alveolar wash; NS = no significant difference.

would be anticipated for either newborn or adult animals (29). However, lamellar body specific activities were about twice the specific activities in the alveolar washes, suggesting that more labeled saturated phosphatidylcholine was available for secretion (29). Mechanical hyperventilation did not alter lamellar body to alveolar wash specific activity ratios which should be relatively sensitive indicators of surfactant metabolism.

The [^{14}C]palmitic acid was given 1 h before sacrifice to see if the amount of incorporation into saturated phosphatidylcholine would change. Palmitate incorporation into lung-saturated phosphatidylcholine previously was found to be very rapid and should be completed in less than 1 h with no secretion of labeled saturated phosphatidylcholine (20). A decrease in incorporation would be consistent with decreased synthesis, assuming that palmitate precursor pools did not change. Because no significant change occurred with the mechanical hyperventilation, we assume as a first approximation that there was no effect on synthesis. However, the values for the hyperventilated groups tended to be lower, and values for isotope incorporation following bolus intravascular injection in whole animals vary considerably, making the conclusion that synthesis was not affected quite tentative.

Intratracheal administration of trace amounts of ^{32}P natural surfactant allowed us to label the alveolar surfactant pools in these lambs. More of the ^{32}P surfactant was lung tissue associated in the hyperventilated groups compared to the other two groups. Added CO_2 had no modifying effect on these findings. In general, there was more variability in label recovery in lambs mechanically hyperventilated without added CO_2. This may have been a factor in the small but apparently significantly increased total lung clearance of saturated phosphatidylcholine in these lambs compared to the spontaneously breathing group. The increased tissue association could be either a nonspecific phenomenon or an indication of increased recycling (13). In support of the latter was the increased ^{32}P specific activity in lamellar bodies relative to the microsomes in the ventilated groups compared to the unventilated group. Assuming that the exogenously administered labeled surfactant followed the same pathways as the existing alveolar surfactant pools in these lambs, it is possible that increased secretion of tissue surfactant pools occurred but was masked both by very large alveolar pools and rapid recycling into the tissue compartment because intraalveolar surfactant pools were similar in all groups.

Lung injury is known to occur with prolonged hyperoxia and mechanical ventilation. Hyperoxia causes pulmonary edema and atelectasis (2), neither of which were apparent in these lambs. Artificial ventilation of surfactant-deficient lungs has been shown to increase protein leak, with the magnitude of leak increasing with increased peak inspiratory pressure used to ventilate the lung and with decreasing gestational age (15). In contrast, the lungs of mature lambs with adequate surfactant pools show little protein leak despite hyperventilation and higher peak inspiratory pressures (15). The lambs in this study had minimal protein leaks that were similar for all groups. Although no microscopic studies were performed, no significant ventilator or oxygen-induced lung injury was detected in these lambs, as indicated by the absence of increased protein leaks, gross atelectasis or hemorrhage, and unaltered compliances during the course of the study.

In summary, these experiments showed no large effects of mechanical ventilation, even at very high tidal volumes on the surfactant system, a result that was different from those reported in the literature, at least for hyperventilated adult animals. The use of a newborn model where larger surfactant pool sizes were present may account for the preservation of dynamic compliances in these lambs as compared with hyperventilated adult lung models. Although no surface tension measurements were performed, altered surfactant function was unlikely in view of compliance measurements which were normal and actually increased with hyperventilation during the course of the study. The

longer period of hyperventilation than previously studied in experimental animals may additionally have allowed for a new equilibrium to be reached. Finally, the lack of an obvious effect on surfactant secretion may have been masked by an increased rate of recycling. More elaborate studies, perhaps with a greater emphasis on quantifying the recycling pathway, would be needed to detect any more subtle effects on surfactant metabolism.

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