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Research Article

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Duration and Characteristics of Treatment of Premature Lambs with Natural Surfactant

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ABSTRACT Premature lambs were treated with 50 mg/kg of natural surfactant lipid by tracheal instillation either at birth or shortly thereafter when respiratory failure was documented. All lambs were delivered by cesarean section and supported on infant ventilators with 100% oxygen under conditions to mimic the care of human infants with the respiratory distress syndrome. The natural surfactant used for therapy was recovered by lavage from sheep lung. Six 120-d gestational age lambs treated at birth had an initial mean oxygen pressure (pO_2) value of 270±35 mm Hg; this fell within 3 h to <100 mm Hg. By 8.3±0.3 h after birth the lambs were in severe respiratory failure with a mean pH < 7.1 and a mean pCO_2 > 70 mm Hg. Six untreated lambs had pH values below 7.0 within 40 min of life despite more intensive respiratory support than was given the treated animals. Treatment with natural surfactant of 17 lambs of 120 and 130 d gestational age after early respiratory failure resulted in a prompt increase in pO_2 values from about 35 mm Hg to values over 200 mm Hg and a fall in pCO_2 values to normal levels in the majority of animals. This response lasted only ~3 h, and a second treatment was less predictably effective.

INTRODUCTION

The respiratory distress syndrome in premature human infants results from inadequate surfactant to maintain the normal surface active properties of the lung (1, 2). Although many of the clinical characteristics of the syndrome result from complex pathophysiologic events and treatment methods, the high pulmonary surface tensions initiate the disease process. Effective replacement therapy with surfactant might reverse the disease process and permit the immature lung to develop adequate surfactant synthetic and secretory potential. Initial attempts to nebulize dipalmitoyl phosphatidylcholine into the airways of infants with the respiratory distress syndrome did not show large effects (3, 4). However, a surfactant suspension placed in the airways of prematurely delivered rabbits before the initiation of breathing increased survival and aeration of the lung (5). Nebulization of natural surfactant into a surfactant-depleted rat lung was ineffective, while instillation of a surfactant suspension resulted in normal pressure-volume measurements (6). The instillation of 50–170 mg total surfactant lipid per kg animal wt before the first breath into the tracheas of 120 d prematurely delivered lambs (term, 150 d) protected the animals from many of the early features of the respiratory distress syndrome for a study period of 2 h (7, 8). Fujiwara et al. (9) recently reported that a mixture of an acetone extract of surfactant from beef lung and synthetic phospholipids instilled in saline into the endotracheal tubes of 10 infants with the respiratory distress syndrome improved oxygenation and the clinical course of the disease. As these early positive results will stimulate more clinical trials, controlled animal experimentation is essential to carefully describe the characteristics of the response to surfactant replacement therapy.

METHODS

Preparation of lambs. Previously uninstrumented, datemated western mix breed ewes with twin pregnancies were prepared for cesarean section following sedation with 11 mg Rompun (Haver-Lockhart, Shawnee, Kan.) The animals received combined spinal-epidural anesthesia with 10 ml of 0.5% bupivacaine HCl plus 2% xylocaine, 1:1 (vol/vol), and the uterus was exposed by a midline incision. Each fetal head was mobilized, the uterus over the mid-fetal neck was opened, and the fetal trachea was exposed and opened. An uncuffed endotracheal tube (4.5 mm internal Diam for 120-d animals and 5.0 mm internal Diam for 130-d animals) was inserted and tied into the trachea, and the neck wound was closed. 5–10 ml of

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the fetal lung fluid was aspirated and the endotracheal tube was clamped. Cord blood was drawn for pH and blood gas measurements as each lamb was delivered. The lambs were weighed quickly. Animals randomized for treatment at birth then received the surfactant suspension, and all animals were ventilated by hand for 30 s to 1 min with an anesthesia bag delivering 100% oxygen at 25–30 cm H_2O pressure.

The lambs were ventilated with time-cycled, pressure-limited, Sechrist infant ventilators (Sechrist Instruments, Inc., Anaheim, Calif.) delivering warmed and humidified 100% oxygen, a positive end expiratory pressure of 2 cm H_2O and an initial peak inspiratory pressure of 25–30 cm H_2O. Levels of positive end expiratory pressure > 3 cm H_2O have been shown to compromise pulmonary function in premature lambs (10). The inspiratory time was held constant at 1.0 s and rate was varied from 20 to 40 breaths/min. Peak inspiratory pressures > 35 cm H_2O were not used because of the frequent occurrence of pneumothoraces in preliminary experiments. Serial arterial blood gas and pH measurements were made with a Radiometer blood gas instrument (Radiometer Co., Copenhagen, Denmark) on samples taken from the distal aorta via an umbilical artery catheter. Changes in ventilator rate and peak inspiratory pressure only were made based on the pH and blood gas values. All animals were paralyzed with 0.1 mg/kg pancuronium bromide (Pavulon, Organon Teknika Corp., Aurora, Col.) given at the initiation of ventilation and as needed.

Each lamb was dried superficially and placed under an Air Shields (Narco Air Shield, Hatboro, Pa.) infant radiant warmer and supplemental heat lamps. The core temperature was measured with a rectal temperature probe and, after a short period of initial hypothermia, the body temperature was maintained between 37 and 39°C.

The aortic catheter was used for continuous measurements of blood pressure and heart rate. The lambs received 100 ml/kg per 24 h of 5% dextrose in water as a continuous infusion via the aortic catheter. Several animals also had catheters placed into the pulmonary artery and the aortic arch via the neck vessels, procedures that did not change the status of the lambs. Transfusions were given for hypotension soon after delivery or for blood replacement with maternal blood that had been drawn into citrate-phosphate-dextrose transfusion packs. Several of the lambs received 2–6 meq/kg of sodium bicarbonate soon after delivery for metabolic acidosis. The hematocrits of the lambs were measured to exclude anemia.

Expiratory tidal volumes were measured intermittently with a 2/0 Fleisch pneumotachygraph using a Validyne pressure transducer and electronic integration (Validyne Engineering Corp., Northridge, Calif.). Total lung compliance per kilogram was calculated by dividing the tidal volume by the pressure change of the ventilator and the weight of the lamb.

Natural surfactant used for treatment. The surfactant was recovered by lung lavage of healthy adult ewes or 2–7-d old lambs being killed for other reasons. The airways were filled by gravity with physiologic saline until fully distended, and the saline-surfactant suspension was drained from the lungs. This lavage was repeated four times to yield a wash volume of ~8.5 liters per adult lung. The lavage fluid was centrifuged at 4°C at 8,000 *g* for 30 min. The resulting pellet was suspended in a small volume of saline and layered over 0.7 M sucrose in saline. The step gradient was centrifuged at 8,000 *g* for 30 min, and the surfactant at the interface was recovered. After dilution with saline and centrifugation at 4°C at 27,000 *g* for 20 min, the surfactant was resuspended in distilled water, and stored at −20°C under nitrogen gas.

Analyses of natural surfactant and fetal lung fluid. The quantity of surfactant lipid was estimated by weighing with a Cahn electrobalance (Cahn Instruments Div., Ventron Corp., Cerritos, Calif.) the dried chloroform:methanol extract (11) of an aliquot of the surfactant suspension. The phospholipid composition and phosphatidylcholine content of the surfactant and fetal lung fluid samples were measured by phosphate assay (12) following separation by two-dimensional thin layer chromatography (13). Protein was measured using bovine serum albumin as a standard (14). The surface active properties of the natural surfactant were assessed with a modified Wilhelmy balance at 20°C (8).

Treatment protocols. All lambs treated with surfactant were given a dose of 50 mg natural surfactant lipid/kg body wt. The dose was based on the wet weight of the animal at birth, which is ~10% higher than the weight measured after the fur has dried. The surfactant suspension was diluted in distilled water such that each animal received ~7 ml/kg of fluid. Immediately following birth the endotracheal tubes of six lambs were unclamped and the surfactant suspension was instilled into the lungs while the lambs were rotated. The lambs then were hand ventilated for 30 s to 1 min with 100% oxygen. The randomly selected twins received no treatment, as previous reports indicated that comparison animals receiving an equal volume of saline, water, or no instillation were not different (7, 8).

Four of the lambs from the group of untreated lambs above and 13 other lambs were treated with surfactant after respiratory failure had been documented by at least three blood gas and pH measurements in spite of an FiO_2 of 1.0 and peak inspiratory pressures of at least 30 cm H_2O. Respiratory failure was defined as a carbon dioxide pressure (pCO_2) > 70 mm Hg. Each lamb then was treated with the surfactant suspension and then was ventilated with no changes in ventilator settings for at least 1 h after treatment. The lambs were killed with 2 ml of 360 mg/ml sodium pentobarbitol.

All values are given as means±SE. Significance has been tested by a two-tailed *t* test.

RESULTS

Natural surfactant used for therapy. A simple three-step centrifugation procedure was used to isolate the large amounts of natural surfactant required for these treatment protocols. The mean amount of surfactant lipid recovered from an adult ewe was 396±140 mg ($n = 8$). The phospholipid compositions of the surfactant isolated from young lambs and adult sheep were similar except for the higher phosphatidylglycerol content of the material recovered from the adults (Table I). The phosphatidylcholine to protein ratios indicated that very little protein was present. When tested on a surface balance with a minimal surface area of 12.8 cm^2, surfactant containing 19±4 pmol ($n = 4$) phosphatidylcholine decreased the surface tension to <10 dyn/cm.

Analysis of fetal lung fluid. The fetal lung fluid from both 120 and 130 d gestational age animals contained similar and very small amounts of phosphatidylcholine (Table I). While phosphatidylcholine was the dominant species identified, no phosphatidylglycerol was found. Sphingomyelin and phosphatidylethanol-

TABLE I
Composition of Phospholipids of Natural Surfactant and Fetal Lung Fluid

	Natural surfactant		Fetal lung fluid	
	Adult ewes	Lambs	120 d GA*	130 d GA
	$n = 9$	$n = 5$	$n = 8$	$n = 7$
Phospholipids (percent composition)				
Phosphatidylcholine	79.3±0.7	83.9±1.2	45.7±1.7	53.7±3.1
Phosphatidylethanolamine	5.1±0.3	4.3±0.2	18.8±1.9	19.8±2.9
Sphingomyelin	1.2±0.1	0.8±0.1	28.3±1.3	17.6±2.8
Phosphatidylinositol	0.7±0.3	2.3±0.5	7.2±1.5	8.9±2.3
Phosphatidylglycerol	11.2±0.7	6.3±0.9	—	—
Lyso-*bis*-phosphatidic acid	1.9±0.9	1.7±0.1	—	—
Lyso-phosphatidylcholine	0.7±0.2	0.4±0.1	—	—
μM Phosphatidylcholine/mg protein	5.7±0.6	5.4±0.8	—	—
pM Phosphatidylcholine/ml fetal lung fluid	—	—	6.4±2.4	4.9±1.8

* GA, gestational age.

amine were present in high amounts relative to the phospholipid distribution of the surfactant.

Treatment at birth. No differences were noted between the six lambs treated at birth and the six initially untreated twins (Table II). Three of the treated lambs were first-born and three were second-born. The mean pH, blood gas values, peak inspiratory pressures, and compliance measurements for the treated and untreated lambs are shown in Fig. 1. The initial high pO_2 values fell to <100 mm Hg before the pCO_2 values rose. Mean blood pressure and heart rate values did not change during the study period. A treatment failure was arbitrarily defined as the pH falling below 7.10 as a result of an elevated pCO_2. The mean time to treatment failure was 8.3±0.3 h. In contrast, severe respiratory failure developed in the untreated lambs in spite of higher peak inspiratory pressures. The untreated lambs had mean pH values of 6.98 and pCO_2 values of 90 mm Hg by 40 min.

Treatment after early respiratory failure. The 130-d gestational age lambs were ~1 kg larger than the 120-d lambs, and the mean pH at the time of treatment was slightly higher in the 130-d lambs; other values were comparable (Table III). All animals were in severe respiratory failure at the time of treatment; however, they were not hypertensive or tachycardic. The responses to surfactant treatment were graded by the composite changes in pH, pO_2, and pCO_2 values. A 3+ response was an increase in $pO_2 > 100$ mm Hg combined with a fall in pCO_2 of at least 40 mm Hg and a rise in pH > 0.25. A 2+ response was a change in pO_2 of >50 mm Hg combined with a fall in pCO_2 of >20 mm Hg, and an increase in pH > 0.10. A 1+ response was an improvement in oxygenation and no improvement in pH and pCO_2 values. The responses of the 17 animals divided by gestational age are shown in Table IV.

Sequential blood gas values for a 120-d gestational age lamb who had a 3+ response to surfactant demonstrate rapid deterioration of the pH and pCO_2 values measured in the cord blood (Fig. 2). At 30 min of life and 2 min after surfactant treatment, the pO_2 rose from 30 to 205 mm Hg. The pCO_2 decreased from levels > 100 mm Hg to the normal range within 30 min as the total compliance improved. The pH values reflected the fall in pCO_2.

The mean values for the seven lambs with 3+ responses (Fig. 3) demonstrate the consistent observation that the pO_2 values are maximal within ~3 min of therapy, at a time when little change in compliance, pH, or pCO_2 values are noted. The pO_2 values reach low levels before the pCO_2 begins to rise. The curves of the mean values of the sequential measurements for the seven lambs having 2+ responses to surfactant treatment are qualitatively similar to those for the 3+ response (Fig. 4). Blood pressure and heart rates changed little following the surfactant treatments. The duration of the 3+ and 2+ responses was defined as the time from treatment for the pH to again fall below 7.10 as a result of respiratory acidosis. The mean duration of effect was 3.6±0.4 h in seven 120-d gestational age

TABLE II
Lambs Treated at Birth

	Treated twin (6)*	Untreated twin (6)*
Gestational age, d	120.3±0.4	120.3±0.4
Weight, *kg*	2.1±0.2	2.0±0.1
Cord blood values		
pH	7.31±0.02	7.29±0.02
pO_2, *mm Hg*	20±2	19±2
pCO_2, *mm Hg*	34±3	42±2

* No. of lambs.

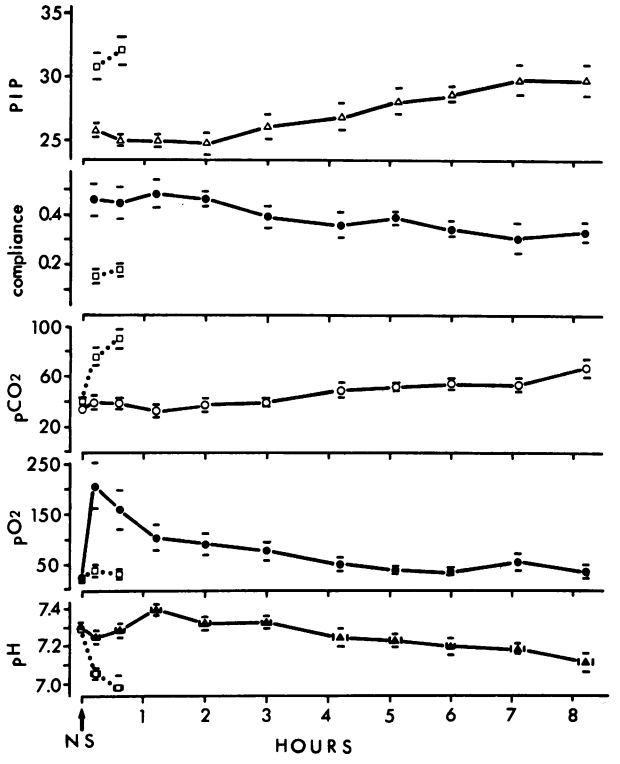

FIGURE 1 Sequential measurements for six lambs treated at birth and for six untreated lambs. The graph shows the mean changes ±SE in pH, pO_2 in mm Hg, pCO_2 in mm Hg, compliance and peak inspiratory pressure (PIP) in centimeters H_2O vs. time from birth in hours. Natural surfactant (NS) was given at birth. The initial pH, pO_2, and pCO_2 values were from cord blood. The error bars for the time interval are given on the pH curve only but apply to the other measurements. The data points connected by solid lines are for the treated animals, the interrupted lines represent the untreated animals.

and 2.8±0.3 in six 130-d gestational age animals with 3+ and 2+ responses. One 120-d old lamb with a 2+ response never had a pH > 7.10 and thus was not included in the estimation of duration. The 3+ responses lasted 3.9±0.2 h while the 2+ responses lasted only 2.4±0.1 h. The two lambs with 1+ oxygenation responses had increases in pO_2 of 79 and 118 mm Hg. Of the three lambs with 1+ or no responses, two had pneumothoraces and died concurrently with or shortly after the surfactant therapy.

Retreatment with natural surfactant. Eight 120-d gestational age lambs treated either before the first breath ($n = 5$) or rescued with a 3+ response ($n = 3$) were retreated with the same dose of surfactant. Two 130-d gestational age lambs with 3+ initial responses similarly were retreated. The mean values of these 10 lambs just before retreatment were: pH, 7.05±0.03; pO_2, 29±4 mm Hg; pCO_2, 79±4 mm Hg, peak inspiratory pressure, 31±1 cm H_2O. Only 4 of 10 animals so treated had 3+ or 2+ responses (Table IV).

TABLE III
Lambs Rescued from Respiratory Failure

	Gestational age		
	120±0.3	130±0.2	*P* value
Number of animals	9	8	
Birth weight, *kg*	2.13±0.20	3.12±0.22	<0.01
Cord blood values			
pH	7.29±0.01	7.30±0.02	NS*
pO_2, *mm Hg*	21±2	26±3	NS
pCO_2, *mm Hg*	43±2	40±3	NS
Age at treatment, *h*	1.4±0.5	3.1±0.7	NS
Pretreatment values			
pH	6.89±0.03	7.03±0.02	<0.01
pO_2, *mm Hg*	30.8±5.2	37.3±6.5	NS
pCO_2, *mm Hg*	110±8	90±5	NS
Mean blood pressure, *mm Hg*	63±3	55±5	NS
Heart rate	175±9	159±11	NS
Pmax of ventilator at treatment, *cm* H_2O	32.0±0.5	31.9±0.5	NS

* NS indicates $P > 0.05$.

DISCUSSION

Stahlman et al. (15) demonstrated that prematurely delivered lambs with respiratory distress have a clinical and pathological course similar to that described for the premature human with the respiratory distress syndrome. The lungs of prematurely delivered lambs are mechanically unstable even at 140 d of the ~150-d full gestation (16). Our experience and that of others (15) indicates that the degree of lung maturity is quite variable in lambs delivered after 130 d gestational age. Therefore, we used lambs delivered at 120 and 130 d gestational age to have homogenous groups of animals with severe lung immaturity. The phospholipid profiles and phosphatidylcholine contents of the fetal lung

TABLE IV
Response to Natural Surfactant Treatment after Early Respiratory Failure

	Response to initial treatment				Response to second treatment			
Gestational age	3+	2+	1+	—	3+	2+	1+	—
d								
120	5	3	1	0	1	2	2	3
130	2	4	1	1		1	1	

3+, ↑ pO_2 > 100 mm Hg; ↓ pCO_2 > 40 mm Hg; ↑ pH > 0.25.
2+, ↑ pO_2 > 50 mm Hg; ↓ pCO_2 > 20 mm Hg; ↑ pH > 0.10.
1+, oxygenation response only.
—, no response.

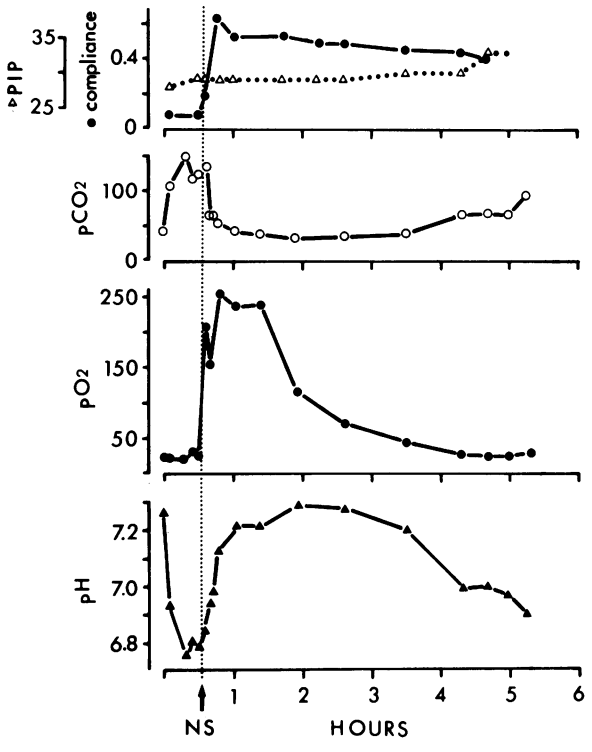

FIGURE 2 Treatment of a lamb with natural surfactant after early respiratory failure. This 120-d, 1.9-kg lamb was supported on an FiO_2 of 1.0 on ventilator pressures of 30/2 cm H_2O at a rate of 30 breaths/min when treatment with natural surfactant (NS) was given at 30 min of age. The initial pH, pO_2 and pCO_2 values are from the cord blood.

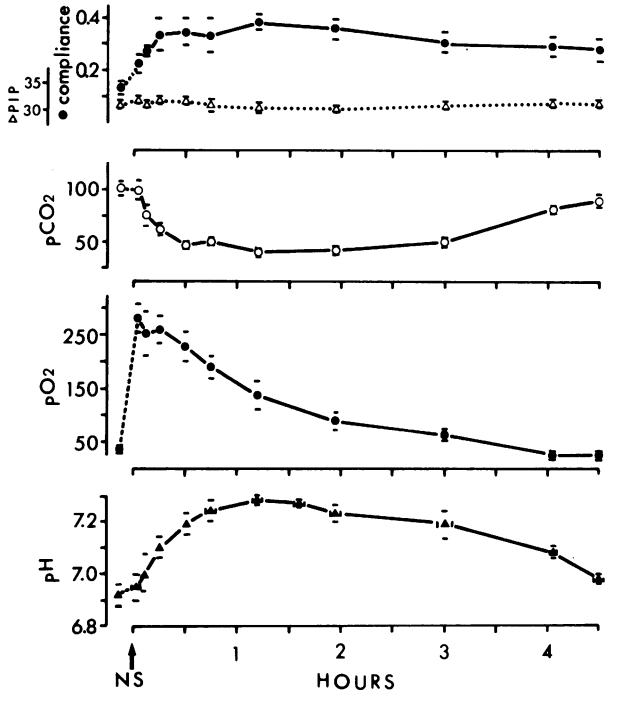

FIGURE 3 Mean values for the seven lambs with 3+ responses to treatment after early respiratory failure. The initial points are the mean values immediately pretreatment. Natural surfactant (NS) was instilled and the sequential changes following therapy are shown vs. time.

fluids support the concept that surfactant deficiency is a component of the lung immaturity in these premature lambs.

The phospholipid composition and surface tension characteristics of the natural surfactant isolated from lambs or ewes and used for the treatments were similar. The method of isolation was somewhat different from that reported by Adams et al. (7) who used only lambs for the isolation. The 6.3% phosphatidylglycerol that we found in the surfactant isolated from lambs is similar to the 4.4% phosphatidylglycerol in the preparation used earlier by Adams et al. (7). The surface activity of the natural surfactant is comparable to that used previously in the lamb studies (8) and to that of highly purified surfactant from dog lung (17).

The six 120-d gestational age lambs treated with natural surfactant at birth had similar responses that lasted ~8 h. The high initial pO_2 values indicate that even at 120 d gestational age the premature lamb lung can support excellent oxygenation. Although the initial high pO_2 values rapidly fell, the lambs were otherwise quite stable until the pCO_2 began to rise late in the experimental period. These animals, however, were not "cured" of respiratory distress. In spite of surfactant therapy and 100% oxygen, the ventilatory requirements continued to increase. None of the animals treated at birth had a pneumothorax. However, at death, the lungs were airless and the lung wash was hemorrhagic. The surfactant therapy predictably stabilized the clinical course of the lambs such that the preparation could be used to study other aspects of neonatal adaption in premature lambs.

If the lambs at either 120 or 130 d gestational age were in severe respiratory failure despite ventilatory support, treatment resulted in less predictable responses. Some lambs had very rapid and dramatic rises in the pO_2 to >300 mm Hg with later falls in pCO_2 from >100 mm Hg to the normal range with correction of the pH from <7.0 to the normal range. Others had smaller changes in the blood gas and pH measurements. The highest mean pO_2 values were measured within 3 min of treatment demonstrating that the premature lung was capable of supporting excellent oxygenation even while severe acidosis persisted. The pO_2 values fell as the acidosis cleared. Whereas surfactant therapy resulted in a prompt increase of the total compliance, the compliance values did not fall to pretreatment levels despite the recurrence of respiratory failure.

The lambs treated with respiratory failure had occurred required a higher maximal ventilator pressure than the lambs treated at birth (30 cm H_2O vs. 25 cm

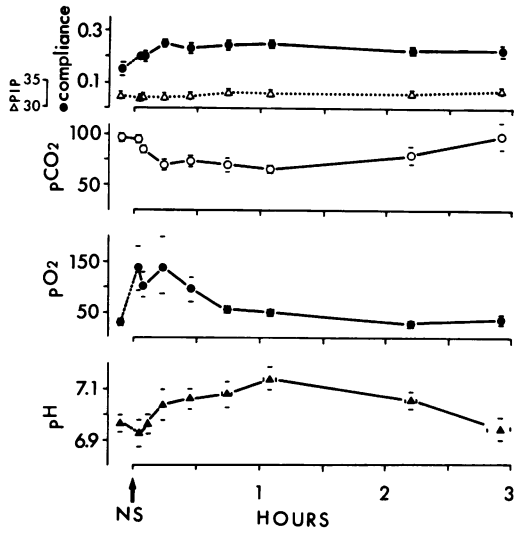

FIGURE 4 Mean values for the seven lambs with 2+ responses to treatment after early respiratory failure. The initial points are the mean values immediately pretreatment. Following instillation of natural surfactant (NS), the responses to treatment vs. time are shown.

H_2O). The response to treatment was of shorter duration (~3 vs. 8 h), and 3 of 17 lambs showed little or no response to therapy. Apparently the intercurrent episode of severe hypoxia, hypercarbia, and acidosis effected the response to treatment. A second treatment given to lambs who initially had good responses resulted in 3+ or 2+ responses in only 4 of 10 trials, again suggesting that the preceding course will effect the subsequent response to natural surfactant therapy. Possibly much of the variability in the responses noted may relate to the distribution of the surfactant suspension in the lungs. Adams et al. (7) noted homogeneous inflation of the lungs of lambs given surfactant at birth. Surfactant reached the alveolar surface of premature rabbits treated before the first breath (18). However, chest films taken of a number of the lambs who were treated following respiratory failure showed nonuniform aeration.

These studies in premature lambs with immature lungs define a very stringent test system for evaluating the effects of natural surfactant therapy or for testing artificial surfactant mixtures. Many questions relating to the efficacy and safety of surfactant replacement therapy remain to be answered. For example, the effects of surfactant therapy on cardiovascular dynamics are unknown. The asynchrony of the oxygenation response and decrease in pCO_2 values in these lambs is unexplained as are the reasons for the reversal of the effects of replacement therapy within hours.

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