

## Sequential treatments of premature lambs with an artificial surfactant and natural surfactant.

M Ikegami, ... , H Jacobs, S J Jones

*J Clin Invest.* 1981;**68**(2):491-496. <https://doi.org/10.1172/JCI110280>.

### Research Article

To test an artificial surfactant in vivo, six 120-d gestational age lambs were treated at birth with a mixture of a 9:1 M ratio of [<sup>14</sup>C]dipalmitoyl phosphatidylcholine (DPC) and phosphatidylglycerol at a dose of 100 mg DPC/kg. Nine other lambs were not treated. The mean PO<sub>2</sub> values of the lambs treated with artificial surfactant were 65.7 +/- 11 mm Hg vs. 24.8 +/- 1.6 mm Hg for the untreated lambs (P less than 0.001). All lambs then were treated with 50 mg/natural surfactant lipid per kg, which promptly improved PO<sub>2</sub> in all lambs. The PO<sub>2</sub> values of those lambs previously treated with artificial surfactant remained greater than 100 mm Hg for 2.5 +/- 0.5 h vs. 0.9 +/- 0.3 h for lambs untreated with artificial surfactant (P less than 0.01). The pH and PCO<sub>2</sub> values were not strikingly different between the two groups of lambs. Airway samples taken from lambs treated with artificial surfactant before treatment with natural surfactant had minimal surface tensions of 32 +/- 2.9 dyn/cm, whereas the artificial surfactant reisolated from these samples by centrifugation had minimum surface tension of 0 dyn/cm. The minimum surface tension of artificial surfactant was inhibited by fetal lung fluid from the premature lambs, whereas the minimum surface tension of natural surfactant was much less sensitive to inhibition. Artificial surfactant did not [...]

Find the latest version:

<https://jci.me/110280/pdf>



# Sequential Treatments of Premature Lambs with an Artificial Surfactant and Natural Surfactant

MACHIKO IKEGAMI, ALAN JOBE, HARRIS JACOBS, and SALLY J. JONES, *Department of Pediatrics, Harbor-University of California at Los Angeles Medical Center, Torrance, California 90509*

**ABSTRACT** To test an artificial surfactant *in vivo*, six 120-d gestational age lambs were treated at birth with a mixture of a 9:1 M ratio of [ $^{14}\text{C}$ ]dipalmitoyl phosphatidylcholine (DPC) and phosphatidylglycerol at a dose of 100 mg DPC/kg. Nine other lambs were not treated. The mean  $\text{PO}_2$  values of the lambs treated with artificial surfactant were  $65.7 \pm 11$  mm Hg vs.  $24.8 \pm 1.6$  mm Hg for the untreated lambs ( $P < 0.001$ ). All lambs then were treated with 50 mg/natural surfactant lipid per kg, which promptly improved  $\text{PO}_2$  in all lambs. The  $\text{PO}_2$  values of those lambs previously treated with artificial surfactant remained  $>100$  mm Hg for  $2.5 \pm 0.5$  h vs.  $0.9 \pm 0.3$  h for lambs untreated with artificial surfactant ( $P < 0.01$ ). The pH and  $\text{PCO}_2$  values were not strikingly different between the two groups of lambs.

Airway samples taken from lambs treated with artificial surfactant before treatment with natural surfactant had minimal surface tensions of  $32 \pm 2.9$  dyn/cm, whereas the artificial surfactant reisolated from these samples by centrifugation had minimum surface tensions of 0 dyn/cm. The minimum surface tension of artificial surfactant was inhibited by fetal lung fluid from the premature lambs, whereas the minimum surface tension of natural surfactant was much less sensitive to inhibition. Artificial surfactant did not improve the pressure-volume characteristics of unventilated premature lung, whereas natural surfactant did. The change in specific activity of [ $^{14}\text{C}$ ]DPC following treatment with natural surfactant indicated that  $\sim 50\%$  of the DPC initially administered was no longer associated with the airways.

## INTRODUCTION

The lungs of premature infants dying from the respiratory distress syndrome (RDS)<sup>1</sup> have abnormal surface

properties (1) and low levels of surfactant phospholipids (2). Early attempts to treat RDS in premature infants with nebulized dipalmitoyl phosphatidylcholine (DPC) did not appreciably improve pulmonary function (3). However, Shannon et al. (4) demonstrated that nebulized DPC restored the pressure volume relationships to surfactant-depleted excised adult dog lungs. Similarly, 9:1 mixture of sonicated DPC and phosphatidylglycerol (PG) was  $\sim 80\%$  as effective as natural surfactant in restoring the total lung capacity of surfactant-depleted adult rat lungs (5). A 7:3 mixture of "dry" DPC and PG improved the lung mechanics of artificially ventilated premature rabbits (6) and may have improved the pulmonary function of infants within the first 6 h of life (7). An acetone extract of beef lung surfactant with added DPC and PG, when instilled into the lungs of premature infants with RDS, rapidly reversed the respiratory failure (8). However, in all comparative studies natural surfactant was more effective than artificial surfactant preparations (5, 7, 9). Previously, we documented the duration and characteristics of the response of 120-d and 130-d gestational age premature lambs to natural surfactant therapy (10). We now report the sequential treatment of premature lambs with an artificial surfactant and natural surfactant.

## METHODS

**Animal preparation.** 15 lambs were delivered at 120 d gestational age by cesarean section of date-mated Western mixed breed ewes. General care and treatment of the lambs were as described (10). In summary, after tracheotomy to secure an endotracheal tube and sampling of fetal lung fluid and cord blood, the lambs were delivered and ventilated with 100% oxygen by hand for  $\sim 30$  s. Then the lambs were ventilated with Sechrist IV-100 pressure limited infant ventilators (Sechrist Industries, Inc., Anaheim, Calif.) with 100% humidified oxygen. Ventilator settings were a peak inspiratory pressure of 30 cm  $\text{H}_2\text{O}$ , a positive end expiratory pressure of 2 cm  $\text{H}_2\text{O}$ , a rate of 30 breaths/min and an inspiratory time of 1 s. These settings were not changed throughout the course of the experiment. A 5 Fr. catheter was placed in the distal aorta via an umbilical artery for continuous recording of blood pressure, heart rate, and for blood sampling for blood gas measurements.

Address reprint requests to Dr. Ikegami.

Received for publication 27 February 1981.

<sup>1</sup>Abbreviations used in this paper: DPC, dipalmitoyl phosphatidylcholine; PC, phosphatidylcholine; PG, phosphatidylglycerol; RDS, respiratory distress syndrome; SPC, saturated phosphatidylcholine.

All lambs were kept paralyzed with pancuronium bromide (Pavulon, Organon Teknika Corp., Aurora, Col.). Body temperature was maintained with radiant warmers and supplemental heat lamps. The lambs received a continuous infusion of 100 ml/kg per 24 h of 5% dextrose in water via the arterial catheter. Eight other lambs at 120 d gestational age were delivered but killed with pentobarbital without being permitted to breathe. The lungs of these lambs were used for pressure-volume measurements.

**Surfactants used for treatments.** The artificial surfactant was a molar ratio of 9:1 DPC and PG purchased from Calbiochem-Behring Corp. (San Diego, Calif.). The phospholipids were sonicated in an ice bath for 1.5 min at a concentration of 20 mg DPC/ml in 20 ml water with a Fisher Sonic Dismembrator, model 300, at 80% energy output using a regular sized probe (Fisher Scientific Co., Pittsburgh, Pa.). After sonication, the artificial surfactant was opalescent. For several artificial surfactant preparations, 0.13  $\mu$ Ci of [1-<sup>14</sup>C]DPC (55 Ci/mol) (Applied Science, State College, Pa.) was added per 100 mg of DPC before sonication.

Natural surfactant was isolated from the airway lavage fluid of sheep lung by a simple three-step centrifugation procedure (10).

**Treatment protocols.** After delivery and before the first breath, six lambs received by tracheal instillation 7 ml/kg of the artificial surfactant suspension containing 100 mg DPC/kg. The lambs were then ventilated by hand and placed on ventilators. Nine other lambs were not treated at birth. Previous studies showed no difference in the clinical course of untreated lambs or lambs treated at birth with either saline or water (11). Sequential blood gas measurements documented the respiratory status of the lambs for at least the first half-hour of life. When the  $PCO_2$  was  $>70$  mm Hg, all lambs subsequently were treated by tracheal instillation with 7 ml/kg of a suspension containing 50 mg natural surfactant lipid/kg (10).

**Analysis of airway samples and alveolar wash.** Airway samples were recovered by suction of the airways with 8 Fr. catheters ~ every 40 min after the initiation of ventilation. Of the ~0.5-ml samples recovered, 0.2 ml was stored at  $-20^\circ\text{C}$  for surface activity measurements and the rest was extracted with chloroform/methanol for phospholipid analysis. After death, the lungs were removed intact and filled with physiologic saline by syringe until fully distended; the saline was withdrawn and reinfused three times, and recovered as the alveolar wash sample. The alveolar wash was stored at  $-20^\circ\text{C}$  for later analysis.

Phospholipid compositions and the phosphatidylcholine (PC) contents of the airway samples were measured by phosphate assay (12) following separation of the phospholipids by one- or two-dimensional thin-layer chromatography (13). Saturated phosphatidylcholine (SPC) was recovered from lipid extracts following treatment with osmium tetroxide according to Mason et al. (14). Each sample of SPC was divided for measurement of phosphate and for determination of radioactivity. Specific activities were expressed as counts per minute per micromole SPC. The majority of the SPC recovered from lung and surfactant samples is DPC (14).

**Pressure-volume characteristics.** Pressure-volume curves were measured using the left lungs of unventilated premature lambs as before (11). After the removal of any airway gas in a vacuum jar, the lung was placed in a saline bath at  $37^\circ\text{C}$ , 50 mg natural surfactant lipid or artificial surfactant containing 100 mg DPC in 7 ml water was instilled, and the lung was ventilated by hand with 100% oxygen at pressures of 25/2 cm  $H_2O$  for 5 min. After gas was removed again from the lung, a second pressure-volume curve was measured.

**Surface tension measurements.** The surface tensions of the alveolar wash samples taken at death were measured with

a Wilhelmy balance (15). The dynamic alveolar model (16) was used to measure the minimum surface tensions of the airway samples. This method requires only a 2- $\mu$ l sample to measure surface tension by recording the pressure-volume relationships of a micro bubble oscillating 16 times per minute. The PC concentration of each sample measured by the dynamic alveolar model was 1  $\mu$ mol PC/ml.

Surfactant was recovered from the airway samples taken before treatment with natural surfactant. The airway samples were layered over 0.7 M sucrose in 0.9% saline and centrifuged at 8,000 g for 30 min. The white pellet over the sucrose was recovered, diluted with saline, and again centrifuged for 15 min at 27,000 g. The pellet was suspended in distilled water to a concentration of 1  $\mu$ mol PC/ml. This method is equivalent to the procedure for the isolation of natural surfactant from lung lavage (10). The surface tension of the reisolated material was measured with the dynamic alveolar model.

Natural and artificial surfactants each were diluted to 1  $\mu$ mol PC/ml with saline and varying amounts of fetal lung fluid that had been collected at delivery from 120-gestational d lambs. Surface tensions then were measured with the dynamic alveolar model. All values are expressed as mean  $\pm$  SE. Significance was tested by the Student two-tailed *t* test.

## RESULTS

**Artificial and natural surfactant.** After sonication of the weighed DPC and PG and separation of these lipids by two-dimensional thin-layer chromatography, the molar ratio of the artificial surfactant determined by phosphate measurement was 9.1 parts DPC to 1 part PG. The phospholipid composition of the natural surfactant has been published (10). The minimum surface tension of the artificial surfactant measured with the dynamic alveolar model at a concentration of 1  $\mu$ mol PC/ml was  $3.2 \pm 1.5$  dyn/cm ( $n = 6$ ). When artificial surfactant containing 0.4  $\mu$ mol DPC was applied to saline in the trough of a Wilhelmy balance, the minimal surface tension stabilized at 6.9 dyn/cm after 20 3-min cycles. In contrast, natural surfactant containing 1  $\mu$ mol PC/ml lowered surface tension by the dynamic alveolar model to 0 dyn/cm. Natural surfactant containing 0.1  $\mu$ mol PC lowered surface tension on the Wilhelmy balance to  $<10$  dyn/cm by the fifth cycle.

**Clinical responses.** The gestational ages, body weights, cord blood gases, and times from birth to treatment with natural surfactant of the artificial surfactant treated and untreated lambs were not different (Table I). The pH values of both groups of lambs were similar throughout the experimental period (Fig. 1). The lambs that were treated with artificial surfactant at birth had higher mean  $PO_2$  values than the untreated lambs ( $65.7 \pm 11$  mm Hg vs.  $24.8 \pm 1.6$  mm Hg) before subsequent treatment with natural surfactant ( $P < 0.001$ ). Mean  $PCO_2$  values for the entire prenatal surfactant period were  $106 \pm 6$  for the artificial surfactant treated group and  $90 \pm 5$  for the control group ( $0.05 > P < 0.01$ ).

All lambs showed a dramatic initial response to natural surfactant therapy. However, the  $PO_2$  values of the

TABLE I  
Comparison of Artificial Surfactant Treated  
and Untreated Lambs

	Artificial surfactant at birth	No treatment at birth
Number of lambs	6	9
Gestational age, <i>d</i>	120.5±0.2	120.2±0.2
Weight, <i>kg</i>	2.1±0.1	2.1±0.1
Cord blood values		
pH	7.30±0.01	7.30±0.02
PCO <sub>2</sub>	46±2	43±3
PO <sub>2</sub>	30±5	28±2
Time of treatment with natural surfactant, <i>h</i>	1.09±0.63	0.40±0.08

lambs not treated initially with artificial surfactant fell to >100 mm Hg within 0.9±0.3 h of treatment with natural surfactant. The lambs pretreated with artificial surfactant maintained PO<sub>2</sub> values >100 mm Hg for 2.5±0.5 h after natural surfactant therapy (*P* < 0.01).

**Labeling studies.** <sup>14</sup>C-labeled artificial surfactant was used so that the quantities of SPC in the airways could be assessed by the changes in specific activities of SPC recovered in airway samples and alveolar wash samples. After treatment with artificial surfactant containing 100 mg DPC/kg, the mean specific activity fell from a normalized value of 1 to a mean value of 0.87 (Fig. 2). Assuming all the SPC remained in the airways, this fall in specific activity indicated that the treatment dose mixed with ~15 mg/kg airway-associated SPC from endogenous sources. After treatment with 50 mg natural surfactant lipid/kg, the specific activity of the [<sup>14</sup>C]SPC from the airway samples fell to a mean value of 0.59. This second fall in specific activity reflected the combined amounts of SPC from endogenous sources and the artificial surfactant that mixed in the airways with the natural surfactant. 50 mg of natural surfactant lipid/kg contains ~25 mg SPC/kg. Thus, only ~52 mg SPC/kg mixed with the SPC of the natural surfactant, indicating that ~50% of the DPC given at birth as artificial surfactant was no longer in the airways. The similarity of the specific activities of SPC in alveolar wash and airway samples indicated that the airway samples were representative of the airway-associated lipids.

**Surface tension measurements.** The mean minimum surface tension of the alveolar washes from lambs receiving artificial surfactant was 11.4±3.5 dyn/cm (*n* = 6), whereas the alveolar washes from the control lambs had a minimum surface tension of 26.6±3.6 dyn/cm (*n* = 5) (*P* < 0.01). Thus, artificial surfactant treatment seems to affect the surface tensions of alveolar washes recovered at death from lambs subsequently treated with natural surfactant. However, the minimal

surface tension of airway samples recovered 18.6±3.5 min after birth from lambs treated with artificial surfactant was 32.9±2.3 dyn/cm (*n* = 6). When the artificial surfactant was reisolated from these airway samples by centrifugation, the minimal surface tension was 0 dyn/cm. Phospholipid analysis of the reisolated artificial surfactant showed only PC and PG in 9.1:1 M ratio. The artificial surfactant was present, but its surface tension lowering properties were inhibited.

To test whether fetal lung fluid from premature lambs would differentially inhibit surfactant function, the surface tensions resulting from mixtures of artificial or natural surfactant with fetal lung fluid were measured. Natural surfactant suspensions containing 0.5 μmol PC/ml lowered the minimum surface tension to 0 dyn/cm on the dynamic alveolar model. Natural surfactant concentration was held constant at 1 μmol PC/ml and increasing amounts of lung fluid from four 120-d gestational age lambs were added. The minimal surface tension of the natural surfactant mixture was 0 dyn/cm until >50% of the suspending fluid was fetal lung fluid (Fig. 3). The surface activity of the artificial surfactant tested at a concentration of 1 μmol DPC/ml was inhibited by small amounts of fetal lung fluid.

**Pressure-volume measurements.** The mean enclosed volumes, volumes at 7 cm H<sub>2</sub>O and residual volumes that characterized the pressure-volume curves of the left lungs of 120-d gestational age lambs killed

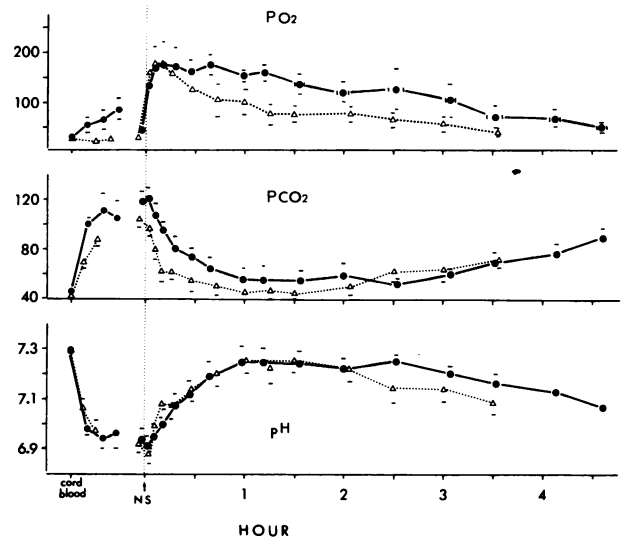


FIGURE 1 Sequential PO<sub>2</sub> (millimeters of mercury), PCO<sub>2</sub> (millimeters of mercury), and pH measurements of lambs. The mean values±SE for lambs treated at birth with artificial surfactant containing 100 mg/kg DPC are shown as —●. The mean values for nine lambs not initially treated with surfactant are indicated by ····Δ. The standard errors of the time intervals were ≤10 min. The initial values are from cord blood, followed by measurements made until treatment of all lambs with 50 mg natural surfactant lipid/kg (NS) at zero time.

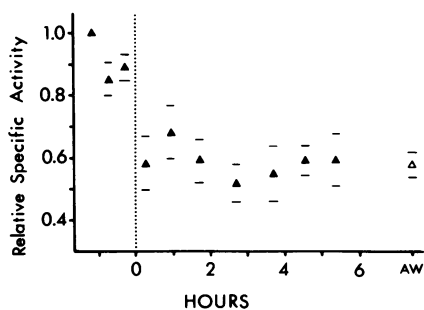


FIGURE 2 Changes in specific activity of SPC associated with artificial surfactant. Five of the lambs received [ $^{14}$ C]DPC labeled artificial surfactant at birth. The mean  $\pm$  SE specific activities (counts per minute per micromole SPC) of airway samples taken both before and after treatment with 50 mg/kg natural surfactant lipid at zero time (dotted line) are shown vs. time. All specific activities were normalized such that the artificial surfactant used for treatment had a sp act of 1. The mean specific activity in the alveolar washes (AW) at death is shown as an open triangle.

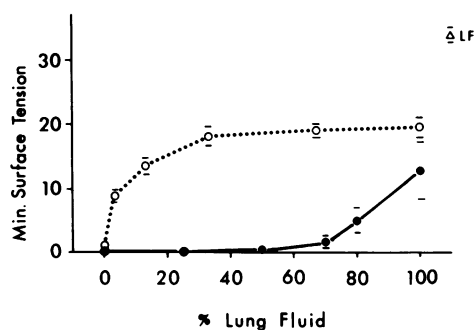


FIGURE 3 Minimum (min.) surface tensions of artificial and natural surfactant mixed with fetal lung fluid. Four samples of fetal lung fluid (LF) were mixed with saline to give varying percents of lung fluid in saline. Sufficient artificial or natural surfactant was added to each mixture such that the final concentrations were constant at 1  $\mu$ mol PC/ml or DPC/ml, respectively, and minimal surface tensions were measured. The mean minimal surface tension of the LF is indicated by an open triangle.

at birth are listed in Table II. Lungs treated with natural surfactant contained more air than the untreated lungs, whereas lungs treated with artificial surfactant contained less air than untreated lungs. Artificial surfactant did not improve pressure-volume characteristics of lungs from unventilated premature lambs.

## DISCUSSION

These experiments were designed to test an artificial surfactant in an in vivo premature lamb preparation known to respond predictably to natural surfactant therapy given either at birth or after the onset of respiratory failure (10). A simple mixture of DPC and PG was selected as the artificial surfactant. DPC is the major surface active component of natural surfactant (17), and PG has been proposed as an important modifier of the surface activity properties of DPC (18). Although DPC/PG mixtures and natural surfactant have somewhat different surface properties, DPC/PG mixtures will restore the pressure volume

characteristics to surfactant-depleted adult rat lungs (5) and improve the lung mechanics of ventilated premature rabbits (6). The sonication procedure used to prepare the 9:1 M ratio of DPC to PG generated a surfactant with low surface tension properties at low surface concentrations. However, the number of cycles required for the Wilhelmy balance to reach the minimal surface tensions was more than required for the natural surfactant. Fujiwara and Adams (19) have recently reviewed some of the properties of surfactants that may be important for function within the lung.

Using a similar treatment protocol, premature lambs given 50 mg surfactant lipid/kg at birth maintained pH values  $> 7.1$  for  $> 8$  h (10). The six lambs treated with artificial surfactant containing 100 mg DPC developed severe respiratory failure in spite of improved oxygenation relative to the untreated controls. After subsequent treatment with 50 mg natural surfactant lipid/kg, the lambs pretreated with artificial surfactant had a more prolonged improvement in  $PO_2$  values than the lambs receiving only the natural surfactant. The

TABLE II  
Characteristics of Pressure-Volume Curves of Premature Lamb Lung

	Enclosed volume	Volume at 7 cm H <sub>2</sub> O	Residual volume
	ml/g lung	ml/g lung	ml/g lung
Untreated (n = 8)	1.57 $\pm$ 0.34	0.12 $\pm$ 0.02	0.03 $\pm$ 0.01
Natural surfactant (n = 4)	8.03 $\pm$ 1.80*	0.31 $\pm$ 0.07*	0.26 $\pm$ 0.07*
Artificial surfactant (n = 4)	0.34 $\pm$ 0.32†	0.03 $\pm$ 0.01†	0.02 $\pm$ 0.01

\*  $P < 0.05$  vs. untreated;

†  $P < 0.01$  vs. untreated.

responses to treatment with natural surfactant were otherwise similar to those reported previously (10). Artificial surfactant alone improved oxygenation and seemed to interact with natural surfactant to cause a prolonged oxygenation response. However, this artificial surfactant was ineffective at reversing PCO<sub>2</sub> elevations characteristic of the severe lung immaturity in these premature lambs.

We verified that the sonicated mixture of DPC/PG was functionally equivalent to that reported (5). The artificial surfactant restored the pressure-volume characteristics to surfactant-depleted adult rat lungs (data not shown). However, the artificial surfactant did not improve the pressure-volume characteristics of previously unventilated premature lamb lung, whereas the natural surfactant did (Table II). Two experiments suggest a possible mechanism for the failure of artificial surfactant to affect the pressure-volume curves and for the poor clinical response to artificial surfactant. The minimal surface tension of the artificial surfactant used for treatment was 3.2 dyn/cm, which became 32.9 dyn/cm in the airway samples from the lambs. However, highly surface-active material was recovered by centrifugation from these same airway samples. The artificial surfactant was present, but the surface tension properties were reversibly inhibited. Fetal lung fluid from these same premature lambs inhibited the surface active properties of artificial surfactant to a much greater degree than natural surfactant. Alveolar washes from premature lambs treated with natural surfactant contained inhibitors of the surface activity of natural surfactant (15). Normal adult lung may not inactivate surfactant; thus a mixture of DPC/PG will be effective in a surfactant depleted adult lung, but not in the immature lung. Residual surfactant remaining in the adult lung also may interact with an artificial surfactant to help restore the surface tension properties to the lung.

The change in specific activities of the radiolabeled artificial surfactant resulting from subsequent treatment with natural surfactant suggested that ~50% of the DPC given as artificial surfactant at birth was no longer associated with the airways. The artificial surfactant may be rapidly absorbed into the lungs and/or cleared from the lungs, further compromising function. Mixtures of DPC and PG when delivered as liposomes to the airways of adult rabbits were cleared from the airways at a rate of ~8% per h (20). The immature lung may clear artificial surfactant from the airway more rapidly than the adult lung.

These experiments demonstrate that a 9:1 mixture of DPC and PG was not nearly as effective as natural surfactant for the treatment of severe respiratory failure in premature lambs. The reasons for the poor response may be unique to the immature lung and

important for the design of other artificial surfactants. Premature humans with RDS responded to a mixture of artificial and natural surfactant lipids that contained some surfactant proteins (8). These infants had much less severe respiratory failure at the time of treatment at ~12 h of age (PCO<sub>2</sub> = 50 mm Hg and pH = 7.13) than the respiratory failure characteristic of premature lambs. A more mature lung may respond more favorably to an artificial surfactant than does the very immature lung tested by this experimental protocol.

#### ACKNOWLEDGMENTS

This research was supported by Health and Human Development Grant HD-12714, by a grant from the March of Dimes Birth Defects Foundation, and by a Research Career Development Award HD-HL 00252 to Dr. Jobe.

#### REFERENCES

1. Avery, M. E., and J. Mead. 1959. Surface properties in relation to atelectasis and hyaline membrane disease. *Am. J. Dis. Child.* **97**: 517-523.
2. Adams, F. H., T. Fujiwara, G. C. Emmanouilides, and A. Scudder. 1965. Surface properties and lipids from lungs of infants with hyaline membrane disease. *J. Pediatr.* **66**: 357-364.
3. Chu, J., J. A. Clements, E. K. Cotton, M. H. Klaus, A. Y. Sweet, and W. H. Tooley. 1967. Neonatal pulmonary ischemia. *Pediatrics.* **40**: 709-782.
4. Shannon, D. C., H. Kazumi, E. W. Merrill, K. A. Smith, and P. S. L. Wong. 1970. Restoration of volume-pressure curves with lecithin fog. *J. Appl. Physiol.* **28**: 470-473.
5. Ikegami, M., J. Silverman, and F. H. Adams. 1979. Restoration of lung pressure-volume characteristics with various phospholipids. *Pediatr. Res.* **13**: 777-780.
6. Morley, C. J., B. Robertson, B. Lachmann, R. Nilsson, A. Bangham, G. Grossman, and N. Miller. 1980. Artificial surfactant and natural surfactant: comparative study of the effects on premature rabbit lungs. *Arch. Dis. Child.* **55**: 758-765.
7. Morley, C. J., A. D. Bangham, N. Miller, and J. A. Davis. 1981. Dry artificial lung surfactant and its effect on very premature babies. *Lancet.* **I**: 64-68.
8. Fujiwara, T., H. Maeta, S. Chida, T. Morita, Y. Watabe, and T. Abe. 1980. Artificial surfactant therapy in hyaline membrane disease. *Lancet.* **I**: 55-59.
9. Ikegami, M., T. Hesterberg, M. Nozaki, and F. H. Adams. 1977. Restoration of lung pressure-volume characteristics with surfactant: comparison of nebulization versus instillation and natural versus synthetic surfactant. *Pediatr. Res.* **11**: 178-182.
10. Jobe, A., M. Ikegami, T. Glatz, Y. Yoshida, E. Diakomanolis, and J. Padbury. 1981. The duration and characteristics of treatment of premature lambs with natural surfactant. *J. Clin. Invest.* **67**: 370-375.
11. Adams, F. H., B. Towers, A. B. Osher, M. Ikegami, T. Fujiwara, and M. Nozaki. 1978. Effects of tracheal instillation of natural surfactant in premature lambs. I. Clinical and autopsy findings. *Pediatr. Res.* **12**: 841-848.

12. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466-468.
13. Jobe, A., E. Kirkpatrick, and L. Gluck. 1978. Labeling of phospholipids in the surfactant and subcellular fractions of rabbit lung. *J. Biol. Chem.* **253**: 3810-3816.
14. Mason, R. J., J. Nellenbogen, and J. A. Clements. 1976. Isolation of disaturated phosphatidylcholine with osmium tetroxide. *J. Lipid Res.* **17**: 281-284.
15. Ikegami, M., A. Jobe, T. Glatz. 1981. Surface activity following natural surfactant treatment of premature lambs. *J. Appl. Physiol.* In press.
16. Nozaki, M. 1970. Pressure-volume relationship of a model alveolus. *Tohoku J. Exp. Med.* **101**: 271-279.
17. Van Golde, L. M. G. 1976. Metabolism of phospholipids in the lung. *Am. Rev. Respir. Dis.* **114**: 977-1000.
18. Hallman, M., and L. Gluck. 1976. Phosphatidylglycerol in lung surfactant, possible modifier of surfactant function. *J. Lipid Res.* **17**: 257-262.
19. Fujiwara, T., and F. H. Adams. 1980. Surfactant for hyaline membrane disease. *Pediatrics.* **66**: 795-798.
20. Oyarzun, M. J., J. A. Clements, and A. Baritussio. 1980. Ventilation enhances pulmonary alveolar clearance of radioactive dipalmitoyl phosphatidylcholine in liposomes. *Am. Rev. Respir. Dis.* **121**: 709-721.