Artificial surfactant and natural surfactant

Comparative study of the effects on premature rabbit lungs

COLIN MORLEY, BENGT ROBERTSON, BURKHARD LACHMANN, ROLAND NILSSON, ALEC BANGHAM, GERTIE GROSSMANN, AND NIGEL MILLER

Department of Paediatrics, University of Cambridge; Biophysics Unit, ARC Institute of Animal Physiology Babraham, Cambridge; Department of Paediatric Pathology, Karolinska Institute, Stockholm, Sweden; Research Institute for Lung Disease, Berlin-Buch, GDR

SUMMARY Premature newborn rabbits, delivered on day 27 of gestation, were treated with tracheal deposition of dry artificial surfactant containing dipalmitoyl phosphatidylcholine and unsaturated phosphatidylglycerol (7:3), or crude natural surfactant prepared by centrifugation of lung wash from adult rabbits. Before receiving surfactant, the animals were allowed to breathe for 7-27 min; they were then subjected to artificial ventilation under standardised conditions. In comparison with littermate controls, both groups of surfactant-treated animals showed statistically significant improvement in lung-compliance 30 and 60 min after onset of ventilation. However, necrosis of bronchiolar epithelium and hyaline membranes was present in nearly all animals, even in those treated with natural surfactant; this suggests that in order to prevent epithelial lesions, surfactant should be given as soon as possible and preferably at birth. Our findings confirm earlier observations that treatment with supplementary surfactant has a beneficial effect on lung mechanics in the premature neonate. The fact that this effect can be obtained not only with natural surfactant but also with dry artificial surfactant should increase the possibility of clinical application.

Respiratory distress syndrome (RDS) is a major cause of mortality and morbidity in premature babies.¹ It is characterised by failure of lung expansion at birth and the early development of atelectasis and hyaline membranes.² This is due to inadequate or unsatisfactory surfactant phospholipids in the immature lungs.³ At the moment the only treatment for this serious disease is symptomatic care with good nursing, and oxygen and respiratory support. Theoretically it should be possible to prevent RDS by placing surfactant substances within the lungs to maintain normal lung function until the babies are able to produce their own surfactant. This has been successfully demonstrated in surfactant-deficient premature animals by means of a surfactant washed from mature animal lungs and concentrated to a small volume by centrifugation. The results of these experiments have shown considerable improvement in lung expansion,⁴⁻⁵ static pressure volume curves,⁶⁻⁷ compliance,⁸ oxygenation,⁹ and survival,^{5 10} with prevention of bronchiolar epithelial lesions and hyaline membrane formation.^{8 11}

It is likely that a suspension of mature natural surfactant instilled into the lungs of premature

babies would have similar advantageous effects. Unfortunately lung wash fluid is difficult to obtain in large amounts and, because it contains foreign proteins, would be quite unsuitable for use in human babies. An alternative way to treat premature babies and prevent RDS would be with a nontoxic synthetic surfactant which could imitate all the physical properties of natural surfactant. Such an artificial surfactant has been developed¹² and this paper describes its effects on the lungs of ventilated premature rabbits and compares them with the effects produced by natural surfactant.

Material and methods

Preparation of natural surfactant. Crude natural surfactant was prepared as previously described by centrifugation of lung wash from mature rabbits.⁶ This produces a small amount of precipitate rich in surface active phospholipids (8 mg/ml, about 80% lecithin).¹³ It is mixed with an equal volume of supernatant to produce a fine particulate suspension and stored frozen until used.

Preparation of artificial surfactant. This was a fine

white powder, mean particle size 150 μm , consisting of pure dipalmitoyllecithin and phosphatidylglycerol. 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine was prepared by the method of Baer and Buchnea.¹⁴ Phosphatidylglycerol with a variety of fatty acid chains identical with that of the parent egg lecithin was produced by the methods of Confurius and Zwaal¹⁵ and Dawson.¹⁶ These substances were further purified on silicic acid columns and shown to be uncontaminated by other lipids or proteins. The lipids were dried together in a ratio of 7:3, thoroughly mixed with water, lyophilised, and then ground to a fine white dry powder which was stored at -20°C over silica gel, under nitrogen in gelatin capsules.

Animal experiments. The experiments were carried out on 39 newborn rabbits from 7 litters obtained on day 27 of gestation (term = 31 ± 1 day; day 0 = day of conception). Day 27 was chosen because at this age the animals are deficient in surfactant.¹⁷ At this stage they are also capable of responding to exogenous surfactant.^{5, 8} A survey of the material is given in Table 1.

The doe was killed by a rapid intravenous injection of 2 ml sodium pentobarbital (Mebumal vet., ACO, Sweden, 60 mg/ml) and 5 ml potassium chloride (150 mg/ml). The fetuses were immediately delivered by hysterotomy, placed in an incubator at 37°C , and stimulated to breathe by gentle manipulation and wiping with a damp gauze. The animals were weighed, placed under an infrared lamp, and tracheostomised through an incision in the larynx without anaesthesia. A metal cannula (external diameter 1.2 mm, internal diameter 0.8 mm) was tied in the trachea and then secured to the animal with a strip of adhesive plaster round the neck. The end of the cannula was joined to a 1 cm length of silicone tubing (internal diameter 1.2 mm).

Alternate animals from the same litter were used as controls with no extra treatment, while the others were treated with either 50 μl of natural surfactant (this dose corresponds to approximately 0.3 mg of lecithin or twice the number of molecules required to form a monolayer on the interior surface of the lung⁴) or 1.8 ± 0.5 mg (mean $\pm$ SD) of artificial surfactant powder. In some experiments both substances

were tested on the same litter. The tracheostomies were performed at intervals of 3–4 min so that the later animals had been delivered and breathing for about 20 min before the surfactant was deposited in the trachea. Since the first animal of the litter was always used as control, the mean interval between birth and tracheostomy was lower in control animals than in those receiving surfactant (Table 1).

The natural surfactant was instilled by injection from a fine polyvinyl chloride tube passed through the cannula into the trachea. The artificial surfactant powder was dropped into the cannula via a small funnel temporarily connected to the cannula tubing, while the animal's chest was squeezed so that the lung fluid filled the cannula and made contact with the powder. If no fluid appeared, about 1 drop of saline was added to facilitate delivery. When the animals gasped most of the powder was drawn into the trachea. However, an undetermined amount of powder was left in the funnel, and perhaps, in the upper part of the cannula (this is the reason why artificial surfactant was given in excess, compared with natural surfactant).

Ventilation. Immediately after the tracheostomy and the deposition of the surfactant substances each animal was sealed into a separate compartment of a multiple-chamber, constant-pressure, whole-body plethysmograph heated to 37 – 38°C (Fig. 1). At the same time a fluid-filled oesophageal catheter (Argyle umbilical artery catheter 3.5 Ch) was passed to the level of the xiphisternum. The tracheal cannula was connected to a metal tube of equal size, fixed into the side of the plethysmograph. The tracheal tube fitted at right-angles into the side of the ventilator tubing to minimise the dead space (<0.05 ml). Its resistance at a flow of 6 ml/second was $0.017 \text{ cmH}_2\text{O/ml} \times \text{second}$. The ventilator* was time cycled, pressure limited, delivering a decelerating gas flow. Each animal was initially ventilated for 1 min at a peak pressure of $35 \text{ cmH}_2\text{O}$, and end expiratory pressure of 1 – $2 \text{ cmH}_2\text{O}$, a rate of 60/min, an inspiration: expiration ratio of 1:1, and 100% oxygen. The rabbit was then moved to one of the other plethysmograph compartments. Each compartment was connected in parallel to a similar ventilator with identical settings, except for a lower peak pressure of $22 \text{ cmH}_2\text{O}$. Ventilation continued for one hour in each animal.

Recording of respiratory mechanics. The equipment used for ventilation and registration of lung mechanics is shown diagrammatically in Figs 1 and 2.

Lung mechanics were measured during artificial ventilation at 30 and 60 min. Inspiratory pressures

*Constructed by G Merker, medical engineer at Research Institute for Lung Diseases, Berlin-Buch GDR.

Table 1 Survey of the material

Treatment	Body weight (g; $\bar{x} \pm \text{SD}$)	Time between birth and tracheostomy (min)	
		Range	$\bar{x} \pm \text{SD}$
Artificial surfactant (n=12)	29 ± 5	7–27	16 ± 7
Natural surfactant (n=10)	29 ± 5	11–27	19 ± 6
Controls (n=17)	30 ± 6	4–24	12 ± 7

MV_I and MV_E, inspiratory and expiratory magnetic valves

Fig. 1 Diagram of equipment for standardised ventilation of newborn rabbits. Flow ($\dot{V}$) and tidal volume (V_T) are measured with a Fleisch tube connected to each compartment of the plethysmograph.

Fig. 2 Diagram showing our method for recording lung mechanics during spontaneous ventilation. Oesophageal pressure (P_{oes}) is measured with a fluid-filled catheter, and flow ($\dot{V}$) and tidal volume (V_T) with a Fleisch tube connected to the tracheal cannula.

were recorded from the ventilatory tubing using a Siemens-Elma pressure transducer (EMT 34). Flow and volumes were measured by a specially made Fleisch tube connected to the plethysmograph, a differential pressure transducer (EMT 32 Siemens-Elma, Solna, Sweden) and an integrator unit (EMT 41). With this apparatus it is possible to measure entirely satisfactorily volumes of 0.005 ml and flow rates of 0.4 ml/second up to a frequency of 8 Hz.¹⁸ At 30 min and 60 min the animals were disconnected from the ventilator and encouraged to breathe spontaneously. Lung mechanics were then measured using the pressure transducer attached to the oesophageal catheter and the Fleisch tube attached to the tracheostomy tube. All parameters were recorded on a Siemens-Elma Mingograf 81. From this record lung compliance was computed, as earlier described.¹⁸

Histological-morphometric techniques. At the end of the period of ventilation the animals were killed with intraperitoneal sodium pentobarbital. The complete thorax was dissected out and the diaphragm was studied for evidence of pneumothorax. The whole thorax was then fixed in 10% neutral formalin. Conventional histological sections were prepared from the basal portions of the lungs and studied microscopically, with particular reference to alveolar air expansion, bronchiolar epithelial lesions, and hyaline membranes. The relative expansion of the alveolar compartment was determined morphometrically by point-counting and expressed as the alveolar expansion index.¹⁰ The degree of bronchiolar lesions and hyaline membranes was also assessed morphometrically, by the protocol of Nilsson *et al.*⁸ The evaluation of the histological sections was made without knowledge of the experimental conditions for each animal.

Statistical evaluation. The χ^2 and the Wilcoxon two sample 2-tailed tests were used for statistical evaluation of our results.

Results

Table 2 gives the number of animals showing spontaneous respiratory efforts at the end of the experimental period and the incidence of pneumothorax; these figures show no difference between the two groups of surfactant-treated animals and controls.

Compliance. Representative samples of the recordings used to calculate compliance are shown in Fig. 3. The calculated values are shown in Table 3.

Lung-thorax compliance during artificial ventilation was generally low in control animals both 30 and 60 min after onset of ventilation. The control animal

that showed the highest compliance at 30 min developed a pneumothorax and its lung-thorax compliance value was considerably reduced at 60 min.

Table 2 Incidence of pneumothorax and number of animals breathing spontaneously 30 and 60 min after onset of artificial ventilation

Treatment	No of animals		
	With pneumothorax	Breathing spontaneously	
		30 min	60 min
Artificial surfactant (n=12)	0	10	6
Natural surfactant (n=10)	4	8	6
Controls (n=17)	2	17	9

Differences between each group of surfactant-treated animals and controls are not statistically significant (χ^2 test, $P>0.1$).

Fig. 3 Recordings of insufflation pressure (P), volume (V), and flow ($\dot{V}$) in control and in animals treated with dry artificial surfactant (DSA) and natural surfactant (NSA). Recordings were obtained with the equipment shown in Fig. 1, after 1 hour of artificial ventilation. Figures for lung-thorax compliance are based on the mean amplitude of the volume tracing, omitting ventilatory cycles where V is augmented by spontaneous inspiratory efforts (arrows). Lung-thorax compliance in control 0.014, in DSA-treated animal 0.20 and in NSA-treated animal 0.37 ml/cmH₂O per kg.

Table 3 Lung-thorax compliance during artificial ventilation

Treatment	Compliance (ml/cmH ₂ O per kg; mean and range)			
	Time after onset of ventilation			
	30 min		60 min	
Artificial surfactant (n=11)	0.28**	0.05–1.14 (n=12)	0.27*	0.02–1.08
Natural surfactant (n=9)	0.49***	0.06–1.24 (n=10)	0.29**	0.05–1.33
Controls (n=17)	0.07	0.01–0.32 (n=17)	0.07	0.01–0.25

*P v. controls <0.05, **P v. controls <0.01, ***P v. controls <0.002.

In most animals treated with artificial or natural surfactant there was a clear increase in lung-thorax compliance during artificial ventilation (Fig. 3) and the difference v. controls was statistically significant for each of the two groups of surfactant-treated animals at both 30 and 60 min. In both these two groups however, some fetuses did not seem to respond to the treatment, as their lung-thorax compliance values remained in the same order as the controls. Three of the 4 animals that were treated with natural surfactant and developed a pneumothorax had clearly increased lung-thorax compliance at the 30-min interval. At 60 min, however, compliance figures for these 3 animals had returned to control levels.

Lung-thorax compliance, expressed per kg body weight, was unrelated to body size—that is, the largest fetuses did not have the most compliant lungs.

Data for lung compliance during spontaneous ventilation show the same pattern as the figures for lung-thorax compliance during artificial ventilation; however, there were fewer observations and a statistically significant improvement was found only for animals treated with natural surfactant, at the interval 30 min (Table 4). Values for tidal volume show no statistically significant differences between the groups (Table 5).

Histological-morphometric findings. Lung aeration was inadequate in all groups, even in surfactant-treated

Table 4 Lung compliance during spontaneous ventilation

Treatment	Compliance (ml/cmH ₂ O per kg; mean and range)			
	Time after onset of ventilation			
	30 min		60 min	
Artificial surfactant (n=10)	0.26	0.06–1.27 (n=6)	0.58	0.09–1.19
Natural surfactant (n=8)	0.42*	0.14–1.85 (n=6)	0.38	0.07–1.34
Controls (n=14)	0.11	0.03–0.27 (n=9)	0.10	0.02–0.15

P* v. controls <0.01.

animals, showing markedly increased compliance; and in many animals the terminal airspaces were distended by oedema or unresorbed fetal pulmonary fluid. The alveolar expansion index (which does not differentiate between aerated and fluid-filled airspaces) showed a wide range of variation in all

groups, without difference between surfactant-treated animals and controls (Table 6).

Bronchiolar lesions, characterised by necrosis, desquamation of epithelial cells, and hyaline membranes, were present in every animal except one. The single exception was an animal treated with natural surfactant; it had been tracheostomised 23 min after birth and had the highest compliance values in the material. In general, surfactant-treated animals with markedly improved compliance showed less than average epithelial lesions. There was no obvious correlation between the time interval from birth to tracheostomy, and the extent of bronchiolar epithelial lesions. Although the mean index for bronchiolar epithelial lesions was lower in animals treated with natural surfactant than in the other two groups, this difference was not statistically significant (Table 6). Fig. 4 shows the pattern of

Table 5 *Tidal volume during spontaneous ventilation*

Treatment	Tidal volume (ml/kg; mean and range)		Time after onset of ventilation	
	30 min		60 min	
Artificial surfactant	(n=10)	1.65 0.96-3.07	(n=6)	1.44 0.64-3.44
Natural surfactant	(n=8)	1.71 0.42-4.78	(n=6)	1.95 0.54-6.55
Controls	(n=14)	1.29 0.24-2.91	(n=9)	0.80 0.18-1.30

There are no statistically significant differences between surfactant-treated animals and controls.

Fig. 4 Lung section from an animal treated with dry artificial surfactant. This field has well aerated alveoli but there is necrosis and desquamation of the bronchiolar epithelium (arrow). Lung compliance at 60 min = 0.20 ml/cmH₂O per kg. H and E, $\times 160$.

Table 6 Morphometric findings, including data for animals with pneumothorax

Treatment	Alveolar expansion index ($\bar{x} \pm SD$)	Index of bronchiolar epithelial lesions ($\bar{x} \pm SD$)
Artificial surfactant (n=12)	0.70 $\pm$ 0.24	0.29 $\pm$ 0.19
Natural surfactant (n=10)	0.65 $\pm$ 0.17	0.20 $\pm$ 0.14
Controls (n=17)	0.70 $\pm$ 0.27	0.28 $\pm$ 0.15

Differences between surfactant-treated animals and controls are not statistically significant.

expansion and epithelial lesions in one animal treated with dry artificial surfactant.

Focal intra-alveolar haemorrhage was found in some animals in each group, again without difference between surfactant-treated animals and controls.

Discussion

Two main problems have delayed, until recently, the discovery of an artificial surfactant which will mimic the physical properties of natural surfactant.

Firstly, the main component of natural surfactant, dipalmitoyllecithin, has a crystal to liquid transition temperature of about 41°C¹⁹ and is therefore not surface active at 37°C; this means that this phospholipid cannot be used alone as substitute for natural surfactant. However, when combined with phosphatidylglycerol in the proportions used in the present study, the melting point of the phospholipid system is sufficiently reduced to make the artificial surfactant surface active at body temperature.¹²

Secondly, in previous attempts to produce an artificial surfactant the phospholipids have generally been thoroughly mixed with water or nebulised as a mist. This process traps the lipids into liposomes from which only few molecules can escape to form a surface active layer.²⁰ This problem has now been overcome either by keeping the surfactant dry²¹ or by dispersing the lipids in a nonaqueous carrier.²²

An alternative technique for preparation of artificial surfactant was recently suggested by Fujiwara *et al.*²³ In this procedure synthetic dipalmitoyllecithin and phosphatidylglycerol are added to a Folch extract of minced lung tissue. Unfortunately, this 'artificial surfactant' contains all sorts of lipids and 2% protein.²³ Fujiwara *et al.*²⁴ reported that their surfactant preparation is effective at improving lung-thorax compliance in premature rabbit fetuses, and they emphasised that it is acting as a water-in-oil system rather like dry surfactant.

The artificial surfactant used in the present experiments has the following important properties.^{12 21}

(1) When placed on an aqueous surface at 37°C, it spreads a monolayer very rapidly to an equilibrium surface pressure of 42–47 mN/m. This allows the powder to be placed on the surface of the bronchi, from where it will spread spontaneously along the air-liquid interface, towards the periphery of the lung. (2) When part of the monolayer is removed it is immediately replenished from particles still present on the surface. (3) On rapid compression by about 50% of the surface area the surface 'solidified' and the surface pressure rose immediately to 72 mN/m. This was repeatable all the time particles were still present on the surface. (4) It is sterile and has no proteinaceous contamination. It therefore mimics the physical properties of natural surfactant and is most unlikely to harm living lung tissue.

This study was the first attempt to test the effect of this dry surfactant in living experimental animals. The method used was based on the principles for registration of lung mechanics in small animals that were originally developed by Lachmann *et al.*²⁵ in experiments on guinea-pigs. When applied to premature newborn rabbits, during artificial or spontaneous ventilation, this technique is particularly useful for testing various forms of surfactant preparations and other therapeutic regimens that might facilitate neonatal lung adaption in the premature neonate.¹³

The setting of the ventilator in our experiments was arrived at by experience and may not be optimal. The pressure levels are in the same order as those registered in a previous experimental series in which animals treated with natural surfactant were ventilated for one hour with a standardised tidal volume of 10 ml/kg.⁸ In our study all animals were ventilated with the same pressure, so there was a risk that animals with highly compliant lungs would become greatly overventilated and run the risk of pneumothorax. This complication obviously occurred in some animals, especially among those treated with natural surfactant. Obviously a tension pneumothorax would alter the apparent compliance of the lung-thorax system. However, since the duration and exact influence of pneumothorax could not be assessed, all compliance values were recorded as they were measured.

Our recordings of lung-thorax compliance during artificial ventilation indicate that the standardised insufflation pressure was probably not high enough to expand the lungs of control animals. Our observations further show that artificial surfactant improved the neonatal lung expansion in most animals but that this effect was less consistent and somewhat less prominent than that obtained with natural surfactant. However, in some of the animals treated with either type of surfactant, lung-thorax compliance improved

to a level even exceeding that of normal term rabbit neonates.²⁶ Why the artificial surfactant failed to influence lung expansion in some animals is not known. It may have been due to ineffective delivery of the powder; whereas natural surfactant was easily instilled by injection, the dry surfactant was delivered with some difficulty and the precise amount of powder entering the lungs was not known. It is possible that the administration procedure was unsuccessful in some animals or that the powder (which is highly hygroscopic and swells on contact with water) might occasionally have blocked the tracheal tube or the airways. This complication was observed in some of our preliminary experiments but was avoided as far as possible in the present study, in which only fine grain powder was administered and care was taken to flush the cannula with small amounts of saline when the artificial surfactant was not clearly aspirated with the fetal pulmonary fluid.

An unexpected finding in the present study was that bronchiolar epithelial lesions developed in both groups of surfactant-treated animals. This finding is contrary to our earlier observation that the development of these lesions can be prevented in the premature animals by treatment with supplementary surfactant.⁸ However, in those earlier experiments surfactant was administered at birth, whereas in this study the time interval between birth and surfactant treatment ranged between 7 and 27 min.

Although no clear correlation was found between the duration of the interval and the extent of epithelial lesions, our data suggest that in order to prevent these lesions in the premature lung, supplementary surfactant should be given as soon as possible and preferably at birth. Presumably bronchiolar lesions analogous to those observed in the present study can develop during spontaneous ventilation too. Similar lesions have been observed in premature newborn infants dying shortly after birth, even in infants who have only been gasping and in whom no artificial ventilation has been applied.^{2, 27}

In conclusion, our findings confirm that the compliance of the premature neonatal lung can indeed be improved by treatment with supplementary surfactant, even if surfactant is administered after a period of spontaneous breathing. The fact that this can be achieved not only with natural surfactant but also with a protein-free preparation of synthetic phospholipids should increase the possibility of clinical application.

The experiments were performed in the laboratory of Dr Bengt Robertson, St Görans Sjukhus, Stockholm, Sweden.

C M was supported by a Wellcome Foundation travelling fellowship, the Medical Research Council, and Birthright; B R and G G were supported by the Swedish Medical Research Council (Project No. 3351); R N was supported by the Swedish National Association against Heart and Chest Diseases.

References

- Farrell P M, Wood R E. Epidemiology of hyaline membrane disease in the United States. Analysis of national mortality statistics. *Pediatrics* 1976; **58**: 167-76.
- Gandy G, Jacobson W, Gairdner D. Hyaline membrane disease. I. Cellular changes. *Arch Dis Child* 1970; **45**: 289-310.
- Gluck L, Kulovich M V, Eidelman A L, Cordero L, Khazin A F. Biochemical development of surface activity in mammalian lung. IV. Pulmonary lecithin synthesis in the human fetus and newborn and etiology of the respiratory distress syndrome. *Pediatr Res* 1972; **6**: 81-99.
- Enhörning G, Grossmann G, Robertson B. Morphometric analysis of lung expansion in the premature rabbit fetus after pharyngeal deposition of surfactant (abstract). *Acta Pathol Microbiol Scand (A)* 1972; **80**: 694.
- Enhörning G, Robertson B, Milne E, Wagner R. Radiologic evaluation of the premature newborn rabbit after pharyngeal deposition of surfactant. *Am J Obstet Gynecol* 1975; **121**: 475-80.
- Enhörning G, Robertson B. Lung expansion in the premature rabbit fetus after tracheal deposition of surfactant. *Pediatrics* 1972; **50**: 58-66.
- Grossmann G. Expansion pattern of terminal air spaces in the premature rabbit lung after tracheal deposition of surfactant. *Pfluegers Arch* 1977; **367**: 205-9.
- Nilsson R, Grossmann G, Robertson B. Lung surfactant and the pathogenesis of neonatal bronchiolar lesions induced by artificial ventilation. *Pediatr Res* 1978; **12**: 249-55.
- Enhörning G, Hill D, Sherwood G, Cutz E, Robertson B, Bryan C. Improved ventilation of prematurely-delivered primates following tracheal deposition of surfactant. *Am J Obstet Gynecol* 1978; **132**: 529-36.
- Enhörning G, Grossmann G, Robertson B. Tracheal deposition of surfactant before the first breath. *Am Rev Respir Dis* 1973; **107**: 921-7.
- Cutz E, Enhörning G, Robertson B, Sherwood W G, Hill D E. Hyaline membrane disease. Effect of surfactant prophylaxis on lung morphology in premature primates. *Am J Pathol* 1978; **92**: 581-94.
- Bangham A D, Morley C J, Phillips M C. The physical properties of an effective lung surfactant. *Biochim Biophys Acta* 1979; **573**: 552-6.
- Robertson B. Neonatal pulmonary mechanics and morphology following experimental therapeutic regimens. In: Scarpelli E M, Cosmi E V, eds. *Reviews in perinatal medicine*. Vol. 4. New York: Raven Press, 1980. In press.
- Baer E, Buchnea D. Synthesis of saturated and unsaturated L- α -lecithins. Acylation of the cadmium chloride compound of L- α -glycerylphosphorylcholine. *Can J Biochem Physiol* 1959; **37**: 953-9.
- Confurius P, Zwaal R F A. The enzymatic synthesis of phosphatidyl serine and purification by CM cellulose column chromatography. *Biochim Biophys Acta* 1977; **488**: 36-42.
- Dawson R M C. The formation of phosphatidylglycerol and other phospholipids by the transferase activity of phospholipase D. *Biochem J* 1967; **102**: 205-10.

- ¹⁷ Rooney S A, Wai-Lee T S, Gobran L, Motoyama E K. Phospholipid content, composition, and biosynthesis during fetal lung development in the rabbit. *Biochim Biophys Acta* 1976; **431**: 447–58.
- ¹⁸ Lachmann B, Grossmann G, Nilsson R, Robertson B. Lung mechanics during spontaneous ventilation in premature and full term rabbit neonates. *Respir Physiol* 1979; **38**: 283–302.
- ¹⁹ Albon N, Sturtevant J M. Nature of the gel to liquid crystal transition of synthetic phosphatidylcholines. *Proc Natl Acad Sci USA* 1978; **75**: 2258–60.
- ²⁰ Ikegami M, Hesterberg T, Nozaki M, Adams F H. Restoration of lung pressure-volume characteristics with surfactant. Comparison of nebulization versus instillation and natural versus synthetic surfactant. *Pediatr Res* 1977; **11**: 178–82.
- ²¹ Morley C J, Bangham A D, Johnson P, Thorburn G D, Jenkin G. Physical and physiological properties of dry lung surfactant. *Nature* 1978; **271**: 162–3.
- ²² Grossmann G, Larsson I. Surface properties of natural surfactant in comparison with various synthetic lipids. *IRCS J Med Sci* 1978; **6**: 478.
- ²³ Fujiwara T, Tanaka Y, Takei T. Surface properties of artificial surfactant in comparison with natural and synthetic surfactant lipids. *IRCS J Med Sci* 1979; **7**: 311.
- ²⁴ Fujiwara T, Maeta H, Chida S, Morita T. Improved lung-thorax compliance and prevention of neonatal pulmonary lesion in prematurely delivered rabbit neonates subjected to IPPV after tracheal instillation of artificial surfactant. *IRCS J Med Sci* 1979; **7**: 313.
- ²⁵ Lachmann B, Bergmann K C, Winsel K, Muller E, Petro W, Schafer C, Vogel J. Experimentelles Atemnotsyndrom nach Injektion von Anti-Lungen-Serum III Mitteilung: Atemmechanische, blutgasanalytische, physikochemische, und biochemische Befunde im Chronischen Versuch; Therapieversuche. *Paediatr Grenzgeb* 1975; **14**: 211–33.
- ²⁶ Nilsson R. Lung compliance and lung morphology following artificial ventilation in the premature and full term rabbit neonate. *Scand J Respir Dis* 1979; **60**: 206–14.
- ²⁷ Finlay-Jones J M, Papadimitriou J M, Barter R A. Pulmonary hyaline membrane: light and electron microscopic study of the early stage. *J Pathol* 1974; **112**: 117–24.

Correspondence to Dr C J Morley, Department of Paediatrics, Level E8, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

Received 15 January 1980