# In Vivo Tests for Evaluation of Pulmonary Surfactant

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## Introduction

Before using surfactant preparations in animal experiments or in clinical trials, it is necessary that they fulfill specific physical properties [5, 9]. Some of these essential physical requirements for an effective lung surfactant have been reviewed by many workers [1, 4, 7, 27]. However, different clinical results concerning arterial oxygen tension have been reported. A marked improvement in arterial oxygen tension after surfactant replacement has been reported by Fujiwara et al. [6], whereas no effect on arterial oxygen tension was observed by Milner et al. [22], Morley et al. [23] and Wilkinson et al. [32]. All these workers used a surfactant which was highly effective in in vitro studies. This poses the question as to whether the physical characteristics of surfactant, obtained through in vitro studies, can be also be considered as a method of testing a preparation which has to be physiologically active in the lung.

In earlier investigations [12] no prognosis could be made from surface tension characteristics (Langmuir balance) of an exogenous surfactant on its effects on the improvement of thorax-lung compliance in immature rabbit fetuses. When testing pure artificial surfactant giving effective in vitro results, Obladen et al. [28] could not guarantee its in vivo effects.

In order to exclude the possibility that the lack of correlation between effects in vitro and in vivo are not specific to the premature rabbit fetus model, we made similar investigations in the lung lavage model and the viral pneumonia model [17]. These studies also showed that the shape of the surface area diagram of an exogenous surfactant, or any other parameter from the surface tension area diagram, did not correlate with the improvement of arterial oxygen tension in the lung lavage model or the improvement of thorax-lung compliance in mice infected with viral pneumonia. Our results are in agreement with other authors and lead us to conclude that the efficacy of various preparations of exogenous surfactants should mainly be evaluated in living animals with surfactant-deficient lungs [8, 17, 26, 30].

In recent years such experimental models of surfactant deficiency have been developed [20, 29], but conclusive criteria for the effectiveness of a surfactant have not yet definitely been defined. For this reason some standard levels for improvement of lung function, or threshold levels, must be established in standardized animal models with surfactant deficiency.

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## Immature Rabbit Fetuses (Day 27)

Using immature rabbit fetuses we developed a method which allows study of lung mechanics during spontaneous [15] as well as artificial ventilation in up to ten rabbit fetuses simultaneously [16]. In this model, especially under standardized conditions, compliance is a very sensitive parameter, and the difference between controls and animals treated with high-quality exogenous surfactant varied from 10- to 15-fold (Fig. 1).

Other criteria which can easily be analyzed in this model include the effect of instilled surfactant on compliance in relation to time and the decrease of the critical opening pressure under dynamic conditions. A very effective surfactant is, for example, characterized by maintaining the compliance constant for up to 1 h during pressure-controlled ventilation and decreasing the critical opening pressure to 15–20 cm $H_2O$ (Fig. 1; Table 1).

To prevent different results concerning the improvement of lung compliance when testing surfactant, the lungs of all rabbit fetuses should have the same degree of stiffness, or immaturity, before surfactant replacement. This avoids high standard deviations in thorax-lung compliance, especially when using a non-optimal surfactant (Fig. 2). Other parameters which should be kept constant when testing exogenous surfactant are the instilled volume and the concentration of total phospholipids. With a constant instilled volume, the lungs of animals treated with a surfactant of higher concentration were more

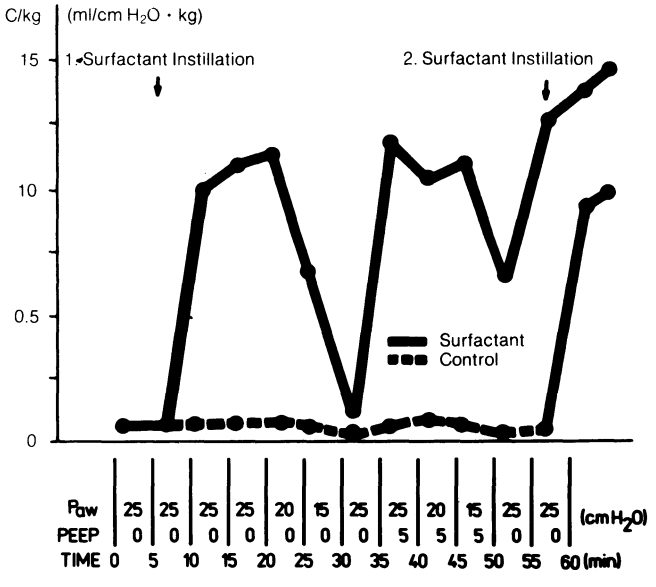


**Fig. 1.** Experimental protocol for testing exogenous surfactant in immature rabbit fetuses from gestational day 27. Course of the compliance shows the effects of a rather optimal exogenous surfactant. Note that tracheal instillation of surfactant leads, both in already treated animals and in controls, to an improvement in lung compliance after 55 min. *Paw,* peak airway pressure; *PEEP,* positive end-expiratory pressure. Mean values of eight treated animals and six controls

**Table 1.** Experimental conditions for testing exogenous surfactant and functional characteristics of effective lung surfactant in premature newborn rabbits (day 27)

| Experimental conditions | Functional improvements |
|---|---|
| Ventilator settings: pressure-controlled ventilation peak airway pressure 25 cm $H_2O$; 50 %–60 % inspiratory time; frequency 30–40/min | At 15 min after surfactant instillation compliance should be higher than 1.0 ml/cm $H_2O$ kg |
| Before surfactant instillation compliance has to be less than 0.1 ml/cm $H_2O$ kg in each animal | Compliance should be stable or |
| About 10 min after delivery 2–5 ml/kg surfactant should be given | Increase over an observation period of 1 h and never decreases when peak airway pressure of 25 cm $H_2O$ is used |
| | When peak airway pressure of 15 cm $H_2O$ leads to lung collapse and decrease in compliance below 0.2 ml/$H_2O$ kg additional PEEP of 4–5 cm $H_2O$ should improve compliance to more than 0.5 ml/cm $H_2O$ kg |

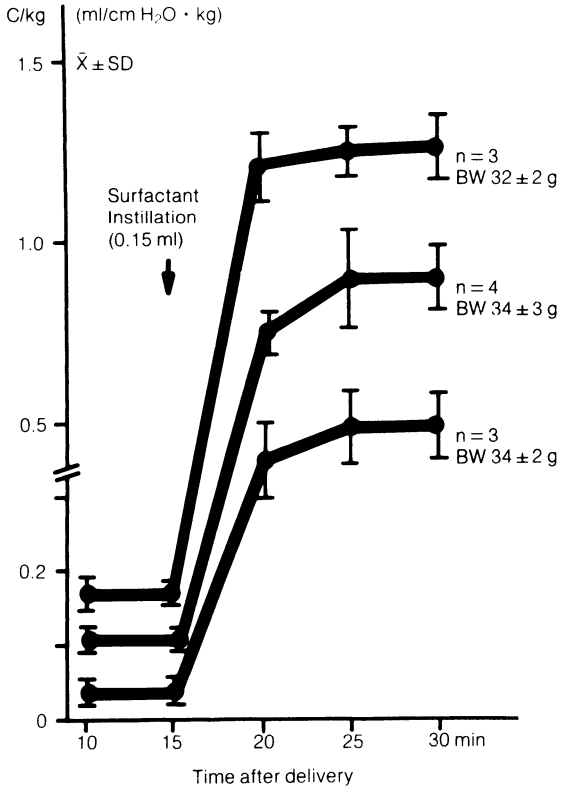


**Fig. 2.** Lung-thorax compliance in surfactant-treated immature newborn rabbits with different compliance immediately after delivery (gestational age 27 days) at various intervals after onset of pressure-controlled ventilation with standardized peak airway pressure of 25 cm $H_2O$. Note that the animals with the highest compliance after delivery show the greatest improvements

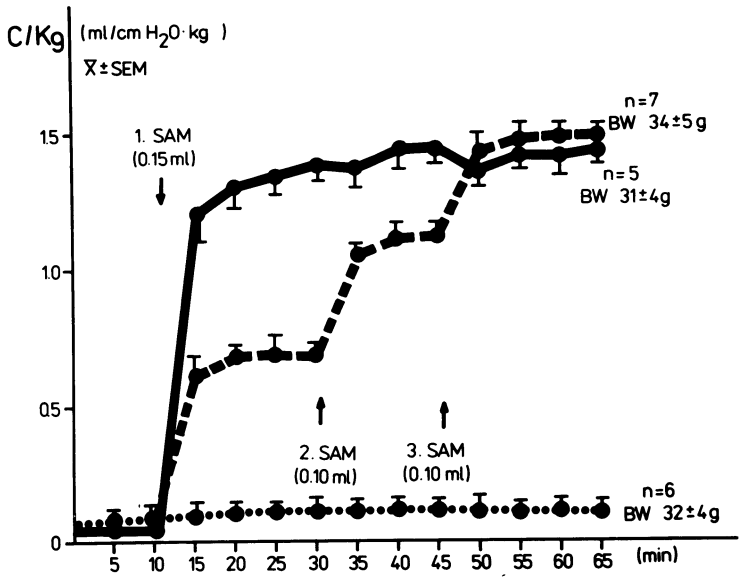


**Fig. 3.** Lung-thorax compliance in immature rabbit fetuses (gestational age 27 days) at various intervals after onset of artificial ventilation (peak airway pressure, 25 cm $H_2O$). Animals were treated with one preparation of exogenous surfactant, 80 mg/ml total phospholipids *(solid line)* and 30 mg/ml *(dashed line)*. Surfactant administration *(SAM)* is indicated with *arrows*. Note that compliance reached the same level when animals treated with less concentrated surfactant received the same amount of phospholipids as animals treated with the more concentrated surfactant

**Table 2.** Dependence of thorax-lung compliance on the volume instilled of a rather optimal (but not excellent) exogenous surfactant (total phospholipid concentration 60 mg/ml)

| | Min after delivery | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 |
| Total volume of surfactant | – | 50 | 100 | 150 | 200 | 250 |
| Group A compliance ml/cm $H_2O$ kg Mean ± SD | 0.06 ± 0.02 | 0.45 ±0.12 | 0.72 ±0.28 | 1.11 ±0.26 | 1.45 ±0.36 | 1.61 ±0.34 |
| Total volume of surfactant µl | – | 200 | – | – | – | – |
| Group B compliance ml/cm $H_2O$ kg Mean ± SD | 0.07 ±0.02 | 1.43 ±0.26 | 1.51 ±0.28 | 1.55 ±0.27 | 1.55 ±0.29 | 1.54 ±0.28 |

Group A (six animals, BW 38 ± 7 g) received five doses of surfactant (50 µl) at 10-min intervals. Group B (six animals, BW 33 ± 8 g) received one dose of 200 µl surfactant.

compliant than those treated with a lower concentration (Fig. 3, at 25 min). However, two additional surfactant instillations (lower concentration) finally led to the same degree of lung compliance compared with the higher concentration (Fig. 3, at 65 min). Additional instillations of surfactant (high concentration) in lungs already more compliant did not lead to significant changes in compliance during pressure-controlled ventilation with 25 cm water, but did lead to significant improvement in lung mechanics during ventilation with 20 cm water (mean ± SD after first surfactant instillation, 0.78 ± 0.19; second, 1.22 ± 0.24; third, 1.42 ± 0.17). Also, the stepwise instillation of surfactant (50 µl with a constant phospholipid concentration) finally led to the same improvement in lung mechanics compared with thorax-lung compliance when an initial large volume (200 µl) was used (Table 2).

Our observations are in agreement with those of other workers [2, 8, 24] who found that higher concentrations of surfactant improved the clinical and functional status to a greater extent than lower concentrations. Moreover, Metcalfe et al. [21] also showed that by increasing the instilled volume and amount of surface active lipids, this resulted in improved pressure-volume diagrams. From these results we concluded that there is no danger from the instilled volume in a range from 2–10 ml/kg, when experimental animals are artificially ventilated. Therefore, we believe that for reaching a threshold level of improvement in functional and clinical parameters, an excess of surface active material should be given. For a good intrapulmonal distribution the instilled volume must be larger than anatomical dead space.

## In Vivo Lung Lavage

We have used guinea pigs, rabbits, and dogs to develop a model of adult respiratory distress syndrome (ARDS) in which alveolar surfactant phospholipids are selectively removed by in vivo lung lavage [11, 13, 14]. Severe respiratory insufficiency was defined as a fall in $PaO_2$ below 60 mmHg during pressure-controlled ventilation with pure oxygen, positive end-expiratory pressure (PEEP) of 6–8 cm $H_2O$, peak airway pressure of 26–30 cm $H_2O$, and inspiratory time 33 %–50 % (Table 3).

We found the lung lavage model (particularly in small animals) useful for a variety of experimental purposes, especially for the testing of alternative surfactant preparations [10, 18, 19], as lung mechanics and arterial blood gases can be measured in up to eight guinea pigs simultaneously.

## Viral Pneumonia in Mice

Viral pneumonia results in a clinical situation equivalent to ARDS. Important functional changes in lungs infected by influenza virus are induced by surfactant deficiency secondary to destruction of type II cells [31]. Therefore, we used viral pneumonia as an additional surfactant-deficiency model for testing exogenous surfactant [20]. Mice infected with influenza virus, accord-

**Table 3.** Experimental conditions for testing exogenous surfactant and functional characteristics of effective lung surfactant in lung lavage model

| Experimental conditions | Functional improvements |
|---|---|
| Ventilator settings: pressure-controlled ventilation peak airway pressure 27–29 cm $H_2O$; PEEP 6–8 cm $H_2O$; inspiratory time 50 %; frequency 30/min; inspiratory $O_2$ concentration 100 % | $PaO_2$ should rise to more than 200 mmHg within 15 min after first surfactant instillation |
| At 10 min after lung lavage $PaO_2$ should be below 60 mmHg | After second surfactant instillation $PaO_2$ should be stable or increase within the following 30 min |
| At 10 and 40 min after lung lavage 3–4 ml/kg surfactant should be given | |

ing to Noack et al. [25], were used on day 6 after infection, when the compliance had diminished to 20 %–30 % of the initial value [3]. Compliance measurements were made according to Lachmann et al. [16] at a peak airway pressure of 25 cm $H_2O$ during pressure-controlled ventilation (Table 4).

## Conclusions

Although, to date, some workers have achieved excellent clinical results with their surfactant preparations (in contrast to others), we believe that before use of other exogenous surfactant preparations in clinical trials, some of the criteria discussed in this paper should be studied in different surfactant-deficiency models [30].

Our experience has shown that the respiratory distress syndrome (RDS) model of premature rabbit fetuses is the most sensitive model, while the model of viral pneumonia is the least sensitive when testing exogenous surfactant. With a less effective exogenous surfactant, a small improvement in lung function in the rabbit fetuses model can be observed (Fig. 4) while in the viral pneumonia model almost no improvement occurred.

**Table 4.** Experimental conditions and functional characteristics of exogenous surfactant in virus pneumonia model

| Experimental conditions | Functional improvements |
|---|---|
| Ventilator settings: pressure-controlled ventilation peak airway pressure 25 cm $H_2O$; inspiratory time 50 %; frequency 40/min | Compliance should improve more than 2.5-fold within 5 min after tracheal surfactant instillation |

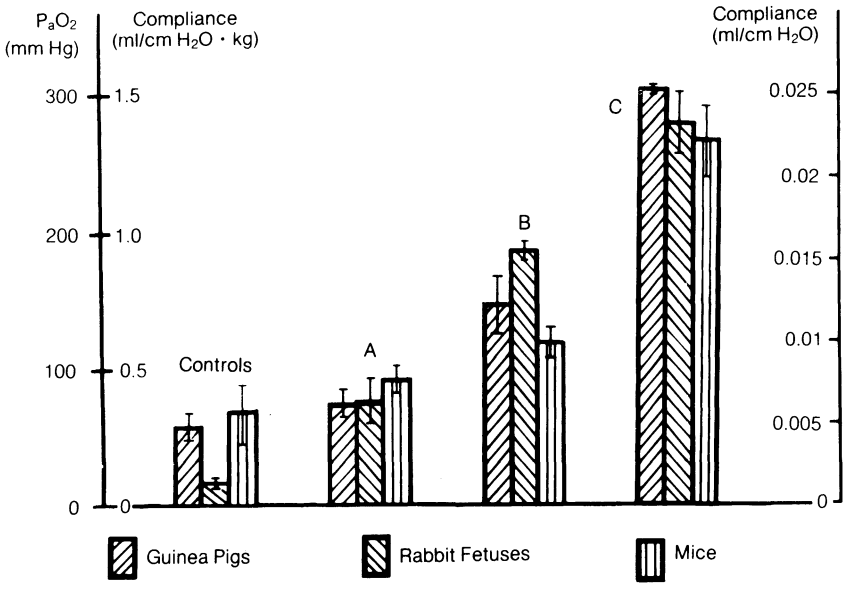


**Fig. 4.** Changes in $PaO_2$ and compliance in guinea pigs with ARDS, immature rabbit fetuses (gestational age 27 days), and mice with viral pneumonia after tracheal instillation of different effective exogenous surfactants *(A, B, C)*. Compliance is standardized by body weight in rabbit fetuses but not in mice. Number of animals in each group varied between six and eight

A suboptimal surfactant led to slight improvements in the three animal models and only a functionally effective surfactant fulfilled all our criteria for functional improvements in lung function.

## References

1. Bangham AD, Morley CJ, Phillips MC (1979) The properties of an effective lung surfactant. Biochim. Biophys Acta 573:552–556
2. Berggren P, Curstedt T, Grossman G, Nilsson R, Robertson B (1985) Physiological activity of pulmonary surfactant with low protein content; effect of enrichment with synthetic phospholipids. Exp Lung Res 8:29–51
3. Bergmann K CH, Lachmann B, Noack K (1984) Lung mechanics in orally immunized mice after aerolized exposure to influenza virus. Respiration 46:218–221
4. Clements JA (1977) Functions of the alveolar lining. Am Rev Respir Dis 115:67–71
5. Fujiwara T, Tanaka Y, Takei Y (1979) Surface properties of artificial surfactant in comparison with natural and synthetic surfactant lipids. IRCS Med Sci 7:311
6. Fujiwara T, Chida D, Watabe Y, Maeta H, Morita T, Abe T (1980) Artificial surfactant therapy in hyaline-membrane disease. Lancet 1:55–59
7. Goerke J (1974) Lung surfactant. Biochim Biophys Acta 344:241–261
8. Ikegami M, Adams FH, Towers B, Osher AB (1980) The quantity of natural surfactant necessary to prevent the respiratory distress syndrome in premature lambs. Pediatr Res 14:1082–1085
9. King RJ and Clements JA (1972) Surface active materials from dog lung. II. Composition and physiological correlations. Am J Physiol 223:715–726

10. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H (1981) Improved gas exchange after tracheal instillation of surfactant in the experimental adult respiratory distress syndrome (abstract) Crit Care Med 9:158
11. Lachmann B, Robertson B, Vogel J (1980) In vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesth Scand 24:231–236
12. Lachmann B (1982) Animal models for the in vivo testing of exogenous surfactant (abstract). Mackinac workshop on exogenous surfactant, October 6–9, 1982
13. Lachmann B, Jonson B, Lindroth M, Robertson B (1982) Modes of artificial ventilation in severe respiratory distress syndrome. Lung function and morphology in rabbits after wash-out of alveolar surfactant. Crit Care Med 10:724–732
14. Lachmann B, Danzmann E, Haendly B, Jonson B (1982) Ventilator settings and gas exchange in respiratory distress syndrome. In: Prakash O (ed) Applied physiology in clinical respiratory care. Nijhoff, The Hague, pp 141–176
15. Lachmann B, Grossman G, Nilsson R, Robertson B (1981) Effect of supplementary surfactant on in vivo lung mechanics in the premature rabbit neonate. Eur J Pediatr 136:173–179
16. Lachmann B, Grossman G, Freyse J, Robertson B (1981) Lung-thorax compliance in the artificially ventilated premature rabbit neonate in relation to variations in inspiration:expiration ratio. Pediatr Res 15:833–838
17. Lachmann B (1986) New aspects in pathophysiology and therapy of respiratory distress syndrome: criteria for characterisation of exogenous surfactant in surfactant deficient animal models. In: Cosmi EV, Di Renzo GC (eds) Selected topics in perinatal medicine. Rome, CIC, pp 177–198
18. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H (1983) Surfactant replacement therapy in the experimental adult respiratory distress syndrome (ARDS). In: Cosmi, Scarpelli (eds) Pulmonary surfactant system. Elsevier, Amsterdam, pp 231–235
19. Lachmann B, Berggren P, Curstedt T, Grossman G, Robertson B (1984) Surfactant replacement in experimental respiratory distress syndrome induced by lung lavage. Prog Resp Res 28:251–256 In: Robertson B, Van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant.
20. Lachmann B, Danzmann E (1984) Adult respiratory distress syndrome. Elsevier, Amsterdam, pp 505–548
21. Metcalfe IL, Burgoyne R, Enhorning G (1982) Surfactant supplementation in the preterm rabbit: effects of applied volume on compliance and survival. Pediatr Res 16:834–839
22. Milner AD; Vyas H, Hopkin IE (1983) Effects of artificial surfactant on lung function and blood gases in idiopathic respiratory syndrome. Arch Dis Child 58:458–460
23. Morley CJ, Miller N, Bangham AD, Davis JA (1981) Dry artificial lung surfactant and its effect on very premature babies. Lancet 1:64–68
24. Nilsson R, Grossman G, Berggren P, Robertson B (1981) Surfactant treatment in experimental hyaline membrane disease. Eur J Respir Dis 62:441–449
25. Noack K, Bergmann K CH, Eckert H, Luther P, Tischner H, Pohl WD (1982) Tierexperimentelles Modell einer aerogenen Influenza-Infektion. Z Erkrank Atm-Org 159:155–165
26. Nohara K, Berggren P, Curstedt T, Grossman G, Nilsson R, Robertson B (1986) Correlation between physical and physiological properties of various preparations of lung surfactant. Eur J Respir Dis 69:321–335
27. Notter RH, Taubold R, Mavis RD (1982) Hysteresis in saturated phospholipid films and its potential relevance for lung surfactant function in vivo. Exp Lung Res 3:109–127
28. Obladen M, Kampmann W, Zimmerman I, Lachmann B (1984) Artificial surfactant in preterm rabbits with and without RDS: difference of in vitro and in vivo activity. Eur J Pediatr 144:195–199
29. Robertson B (1980) Surfactant substitution; experimental models and clinical applications. Lung 158:57–68
30. Robertson B, Lachmann B (1988) Experimental evaluation of surfactants for replacement therapy. Exp Lung Res 14:279–310

31. Stinson SF, Ryan DP, Hertweck MS, Hardy JD, Hwang-Kow SY, Loosli CG (1976) Epithelial and surfactant changes in influenzal pulmonary lesions. Arch Pathol Lab Med 100:147–153
32. Wilkinson AR, Jenkins PA, Jeffrey JA (1985) Two controlled trials of dry artificial surfactant: Early effects and later outcome in babies with surfactant deficiency. Lancet 2:287–291