

Application of a new ventilator-multi-plethysmograph system for testing efficacy of surfactant replacement in newborn rabbits

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Application of a new ventilator-multi-plethysmograph system for testing efficacy of surfactant replacement in newborn rabbits. B. Sun, T. Kobayashi, T. Curstedt, G. Grossmann, B. Robertson.

ABSTRACT: We applied a new ventilator-multi-plethysmograph system to evaluate the effect of surfactant replacement in newborn rabbits under well controlled, nearly physiological conditions characterized by normal ECG and adequate P_{CO_2} in right ventricular heart blood obtained at the end of the experiment. Up to 10 animals were ventilated in parallel with a pressure-constant common respirator system. Using a working pressure of 4.9 kPa (50 cmH₂O), we could adjust the pressure delivered to each animal within the range of 0.49–4.4 kPa (5–45 cmH₂O), by changing the length of an open high-resistance tube constituting the outflow limb of the connection between the common ventilator tube and the tracheal cannula. Immature newborn animals obtained after 27.5 days gestation and ventilated for 30 min with a tidal volume of 8–10 ml·kg⁻¹ had a mean±SD lung-thorax compliance of 4.2±1.1 ml·kPa⁻¹·kg⁻¹ (0.41±0.11 ml·cmH₂O⁻¹·kg⁻¹) and P_{CO_2} of 8.5±1.9 kPa. In littermates treated at birth with a large dose of natural surfactant (Curosurf, 200 mg·kg⁻¹, compliance increased to 6.0±1.0 ml·kPa⁻¹·kg⁻¹ (0.68±0.10 ml·cmH₂O⁻¹·kg⁻¹) ($p<0.01$) and P_{CO_2} decreased to 6.9±1.2 kPa ($p<0.01$). Near-term animals, obtained at 30 days gestation and ventilated under similar conditions had a compliance of 7.2±0.9 ml·kPa⁻¹·kg⁻¹ (0.71±0.09 ml·cmH₂O⁻¹·kg⁻¹) and P_{CO_2} of 6.4 (1.2) kPa. Administration of surfactant (same dose as above) to these mature animals at birth had no adverse effects.

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Surfactant deficiency is a main aetiological factor in the pathogenesis of the neonatal respiratory distress syndrome (RDS), as underscored by data from clinical trials showing efficacy of surfactant replacement therapy even in severe forms of this disease [1, 2]. The surfactants used in these and other [3, 4] clinical trials were first tested extensively for biophysical activity under various experimental conditions including instillation of the material into the airways of artificially-ventilated preterm newborn animals [5–8]. Rabbit foetuses, delivered prematurely at a gestational age of 27 days (term 31 days) provide a well-established experimental model for this purpose [9, 10]. With the body-plethysmograph system designed by LACHMANN *et al.* [11], multiple newborn rabbits can be subjected in parallel to pressure-constant ventilation, while the response to surfactant replacement therapy is evaluated by tidal volume measurements [12]. In the original version of this device, the insufflation pressure was not adjusted to the individual requirements and physiological conditions were, therefore, usually not attained. Although in a population of animals average tidal volumes might seem adequate at a certain ventilator setting, individual

animals were frequently either overventilated or underventilated. Using an intricate construction of valves and water-locks, IKEYAMA *et al.* [13] recently developed an alternative body-plethysmograph system for multiple animals, allowing individual adjustment of the pressure generated from a common ventilator source. In the present paper, we describe a more convenient solution to the same problem, originally designed by one of us (T. Kobayashi, unpublished). We have applied this innovation to studies on immature newborn rabbits in order to delineate new experimental criteria for a satisfactory therapeutic response to surfactant replacement therapy.

Materials and methods

The ventilator-multi-plethysmograph system

The design of this modified ventilator-multi-plethysmograph system is illustrated in figure 1. From a common respirator unit (Servo Ventilator 900 B, Siemens- Elema, Solna, Sweden), a large gas flow

(5 l·min⁻¹) is delivered under constant pressure during the inspiratory phase of each ventilatory cycles. This gas (100% oxygen in the present experiments) flows through a wide tube (cross-sectional area: 1 cm²), to which the tracheal cannula of each animal is connected by means of a narrow, high-resistance silicon tube I.D. 1 mm) with an open outflow limb. The pressure drop from the large common ventilator tube to the end of each narrow tube is determined by the length of this latter tube, and the pressure delivered to the experimental animal can thus be reduced simply by cutting the outflow tube, and increased by adding pieces of tubing on the same side. During these manoeuvres, the inflow side of the tube is clamped to avoid sudden high peaks of inflation pressure due to accidental obstruction of the outflow tube. Pressure can also be modified by altering the length of the inflow limb. At a point opposite to the connection between the high-resistance silicon tube and the tracheal cannula, the pressure delivered to each animal was measured by means of a conventional pressure transducer (EMT 34, Siemens-Elema). The connections between the inflow and outflow portions of the narrow tube, the tracheal tube, and the exit to the pressure transducer were enclosed in a plastic block, attached directly to the plethysmograph wall to minimize deadspace.

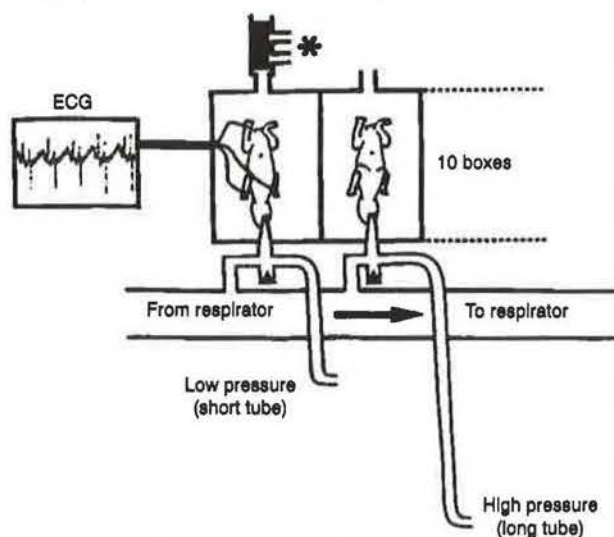


Fig. 1. — The design of the ventilator-multi-plethysmograph system applied in the present experiments. From the common ventilator tube, narrow high-resistance tubes lead to each tracheal cannula, continuing as open outflow limbs. Tidal volumes are measured with a "Fleisch-tube" connected to the opposite side of the plethysmograph box. The pressure delivered to each animal is kept at the level required to maintain the standardized tidal volume by adjusting the length of the open outflow limb of the high-resistance tube. *: flow and volume; ▲: pressure transducer.

With this arrangement and with the ventilator set at a working pressure of 4.9 kPa (50 cmH₂O) we could ventilate 10 animals in parallel, adjusting the individual insufflation pressure within the range of 0.49–4.4 kPa (5–45 cmH₂O) (fig. 2). Tidal volumes were measured by means of a specially designed "Fleisch-tube" [14] connected to the other side of the plethysmograph box, a differential pressure transducer (EMT 32,

Siemens-Elema), an amplifier (EMT 31, Siemens-Elema), an integrator unit (EMT 41, Siemens-Elema) and a recorder (Mingograf 81, Siemens-Elema).

Throughout the present experiments, the ventilator was set at a frequency of 40 cycles·min⁻¹ and 50% inspiration time. No positive end-expiratory pressure was applied. The ECG was recorded at regular intervals from subcutaneous needle electrodes connected to the animal inside the plethysmograph box.

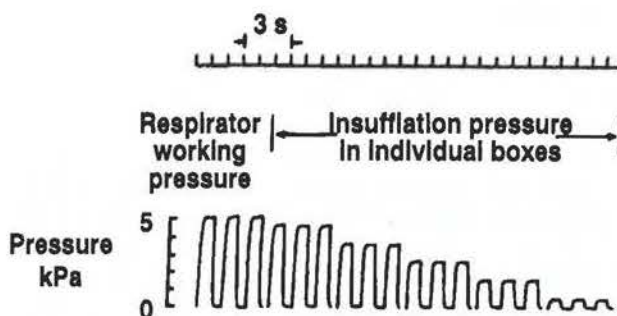


Fig. 2. — Recordings documenting individual variations in insufflation pressure, ranging from 0.49–4.4 kPa (5–45 cmH₂O) while the working pressure of the common ventilator system is set at 4.9 kPa (50 cmH₂O).

Preparation and characterization of surfactant

Surfactant ("Curosurf") was isolated from minced pig lungs by a combination of washing, centrifugation, extraction with chloroform:methanol and liquid-gel chromatography, as described previously [7, 15]. Containing approximately 99% polar lipids, mainly phospholipids, and 1% hydrophobic proteins (SP-B and SP-C) [15], this surfactant lowers the contractile force at an air-liquid interface to near 0 nM·m⁻¹ during surface compression [15]. The physiological activity of the batches used in the present experiments had been confirmed in earlier experiments on premature newborn rabbits ventilated with a standardized sequence of insufflation pressures (data not shown) as well as in clinical trials of surfactant replacement therapy for neonatal RDS [2]. Surfactant was administered at the same concentration as in the clinical trials, 80 mg·ml⁻¹.

Protocols for animal experiments

Newborn rabbits obtained by hysterotomy at a defined gestational age (see below) were anaesthetized at birth by intraperitoneal injection of 0.1 ml sodium pentobarbital (Mebumal vet. 6 mg·ml⁻¹, Nord Vacc, Skärholmen, Sweden) and tracheotomized. They were randomized either to receive surfactant suspension *via* the metal tracheal cannula or to serve as controls in which no material was instilled into the airways. After the tracheotomy and instillation procedures (average duration about 3 min), the animals were relaxed by intraperitoneal injection of 0.1 ml pancuronium

bromide (Pavulon, 0.2 mg·ml⁻¹, Organon, Oss, Holland) and kept at 37°C in the plethysmograph system, connected to the respirator unit. ECG electrodes were inserted before the box was closed, and the animal was not included in the study unless regular cardiac activity with normal QRS-complexes was recorded at the onset of ventilation. The tidal volumes were adjusted as described above, and a stable level could usually be attained within approximately 5 min after the onset of ventilation. For the rest of the experiments, two protocols were applied.

High vs low tidal volumes in immature animals (Protocol 1). These experiments were designed to investigate whether gas exchange and survival would differ between animals ventilated with small or large tidal volumes. A total of 50 immature newborn rabbits were obtained from 6 does at a gestational age of 27 days. These animals received either 0.1 ml surfactant [16] or served as controls, and in each of these groups the tidal volumes were adjusted to either 6–8 ml·kg⁻¹ or 10–12 ml·kg⁻¹. The animals were ventilated for 60 min, and simultaneous recordings of tidal volume, pressure, and ECG were obtained every 15 min. Compliance values for the respiratory system (lung-thorax compliance) were calculated from the quotient between tidal volume and peak insufflation pressure.

Final protocol, applied for testing multiple surfactant batches (Protocol 2). A total of 108 immature newborn rabbits were obtained from 16 does at a gestational age of 27.5 days. They were tracheotomized and received the same dose of surfactant as used in our clinical trials, 200 mg·kg⁻¹ body weight [2], or served as non-treated controls. Seven batches of surfactant were examined, 1–2 batches in each experiment. This implies that data for each batch were obtained from 2–6 litters (median = 3). The animals were ventilated for 30 min with an intermediate tidal volume (8–10 ml·kg⁻¹), adjusted as described above. (The reasons for these modifications of protocol 1 are given below).

To investigate whether the exogenous surfactant would have any adverse effects in mature animals, we also included 13 near-term rabbits obtained from 2 does at a gestational age of 30 days. These animals were ventilated under the same conditions as in the final protocol, with or without previous treatment with surfactant.

After the period of ventilation, the animals were killed by intracranial injection of 0.5 ml Lidocaine (Xylocaine, 20 mg·ml⁻¹, Astra, Södertälje, Sweden) (this leads to immediate cardiac arrest). The abdomen was opened and the diaphragm inspected for pneumothorax; thereafter the chest was opened in the anterior midline and blood was drawn from the right ventricle for determination of carbon dioxide tension (Pco₂).

In animals with evidence of pneumothorax, the moment of lung rupture could usually be identified retrospectively in our tracings from a sudden drop of tidal volume by more than 50%, occurring during ventilation with the same insufflation pressure [16].

Preparation and histological examination of lungs

The lungs were fixed by vascular perfusion with a mixture of 3.5% formaldehyde and 1% glutaraldehyde, while kept expanded at a deflation pressure of 0.98 kPa (10 cmH₂O) [10]. They were embedded in paraffin and transverse sections from the lower lobes (stained with haematoxylin and eosin) were examined with particular reference to alveolar expansion pattern and epithelial necrosis in conducting airways [17]. The volume density of the alveolar spaces was determined by point-counting, using the total parenchyma as a reference volume [10].

Statistical methods

Differences between the groups were evaluated with the Wilcoxon Mann-Whitney two-sample test (two-tailed) and with the Chi-squared test, using p=0.05 as the limit level of statistical significance.

Results

Physiological measurements from Protocol 1

The overall survival rate was 44% in this series of experiments, with no difference between surfactant-treated animals and controls or between animals ventilated with the lower or higher tidal volume range (table 1). Pneumothorax was a frequent complication, with a total incidence of no less than 50%. The majority of the pneumothorax episodes (17/25) occurred during the second 30 min of ventilation. The incidence of

Table 1. – Survey of experimental animals and controls, studied according to Protocol 1

Tidal volume ml·kg ⁻¹	Surfactant	n	Body weight g mean±SD	Abnormal ECG n	Pneumothorax n	Survival n (%)
6–8	-	11	26±6	0	5	6 (54)
6–8	+	13	27±5	2	6	5 (38)
10–12	-	13	28±5	0	7	6 (46)
10–12	+	13	28±5	2	6	5 (38)

All animals were delivered at a gestational age of 27 days and ventilated for 60 min.

Table 2. – Physiological data from animals studied according to Protocol 1

Group	n	Tidal volume ml·kg ⁻¹	Insufflation pressure kPa cmH ₂ O	Lung-thorax compliance ml·kPa ⁻¹ ·kg ⁻¹ ml·cmH ₂ O ⁻¹ ·kg ⁻¹	Pco ₂ kPa	Heart rate per min
Control	6	6.6±0.8	2.55±0.20 26±2	2.7±0.3 0.26±0.03	7.8±3.6	285±28
Surfactant	5	7.0±1.1	1.67±0.49** 17±5**	4.3±1.0** 0.42±0.10**	6.5±3.7	323±17
Control	6	10.7±0.5	2.36±0.59 24±6	4.9±1.4 0.48±0.14	6.8±1.1	317±12
Surfactant	5	11.5±0.3	1.86±0.49 19±5	6.6±1.5 0.65±0.15	5.6±2.1	306±9

The results are given as mean±SD. All animals were delivered at a gestational age of 27 days and ventilation for 60 min. **: p vs control group with same tidal volume range <0.01. Pco₂: carbon dioxide tension.

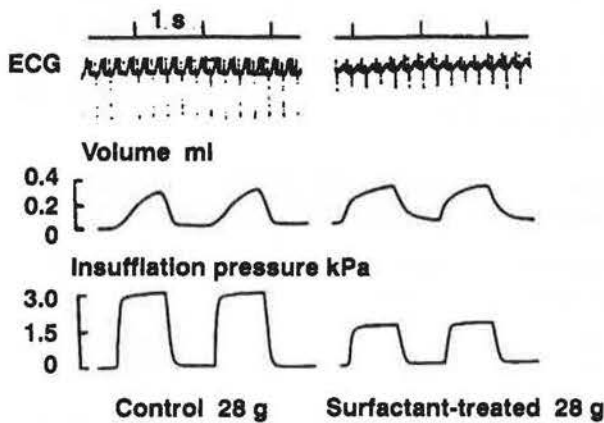


Fig. 3. – Simultaneous recordings of ECG, tidal volumes and insufflation pressures in a surfactant-treated immature animal and a control (gestational age 27 days, Protocol 1). The pressure required to maintain the standardized tidal volume (10–12 ml·kg⁻¹) is significantly lower in the animal receiving surfactant. There is also a difference in the shape of the volume tracing: the lungs of the control animal open more slowly during inflation and eject the expiratory tidal volume much more rapidly than do the lungs of the surfactant-treated animal. Conversion factor: 1 kPa=10.2 cmH₂O.

pneumothorax did not differ significantly between surfactant-treated animals and controls or between animals ventilated with lower or higher tidal volumes. Among surfactant-treated animals without pneumothorax, two in each group were excluded because of ECG abnormalities appearing during the period of ventilation.

Data on tidal volumes, insufflation pressure, compliance, heart rate and blood gases are shown in table 2. The tidal volume measurements document that all four groups were well adjusted to the experimental protocol, and the pressure measurement for each animal was usually established within 10–15 min of ventilation (data not shown). The pressure levels required to maintain the standardized tidal volume were significantly lower in animals receiving surfactant than in controls (fig. 3), and the corresponding improvement in compliance amounted to 62% in animals ventilated with the lower

(6–8 ml·kg⁻¹) and 35% in those ventilated with the higher (10–12 ml·kg⁻¹) tidal volume range. Average values for Pco₂ were acceptable, without significant differences between the groups (table 2).

Physiological measurements from Protocol 2

The overall survival rate in these experiments was 86%, with no difference between surfactant-treated animals and controls (table 3). Pneumothorax occurred in 2 of 66 surfactant-treated animals and in 2 of 42 controls. Twelve immature animals without pneumothorax were excluded because of ECG abnormalities developing during the period of ventilation. Physiological data from experiments testing 7 batches of surfactant are shown in figure 4. Average tidal volumes were generally close to the upper limit of the standardized range, and in one group of surfactant-treated animals were slightly above 10 ml·kg⁻¹. The mean compliance improvement in surfactant-treated animals, reflected by the significantly lower pressure requirements, amounted to 6.9%: 6.9 vs 4.2 ml·kPa⁻¹·kg⁻¹ (0.68 vs 0.41 ml·cmH₂O⁻¹·kg⁻¹) (p<0.001). Average Pco₂ was significantly lower in surfactant-treated animals than in controls: 6.9 vs 8.5 kPa (p<0.001).

Corresponding physiological data from near-term newborn rabbits are included in table 3 and figure 4. There were not significant differences in insufflation pressure, compliance or Pco₂ between surfactant-treated and non-treated mature animals. The values for compliance and Pco₂ in these animals were 7.2±0.9 ml·kPa⁻¹·kg⁻¹ (0.71±0.09 ml·cmH₂O⁻¹·kg⁻¹), and 6.4±1.2 kPa, respectively.

Histological and morphometric observations

The lungs of surfactant-treated immature animals (both protocols) had a nearly uniform alveolar expansion pattern with well aerated terminal airspaces and usually well preserved epithelium in conducting airways. In littermate

Table 3. – Survey of experimental animals and controls, studied according to Protocol 2

Gestational age day	Surfactant	n	Body weight g mean±SD	Abnormal ECG n	Pneumothorax n	Survival n (%)
27.5	-	42	31±5	3	2	37 (88)
27.5	+	66	32±5	9	2	55 (83)
30	-	6	42±9	0	0	6 (100)
30	+	7	44±7	0	0	7 (100)

The animals were delivered at a gestational age of 27.5 or 30 days and ventilated for 30 min.

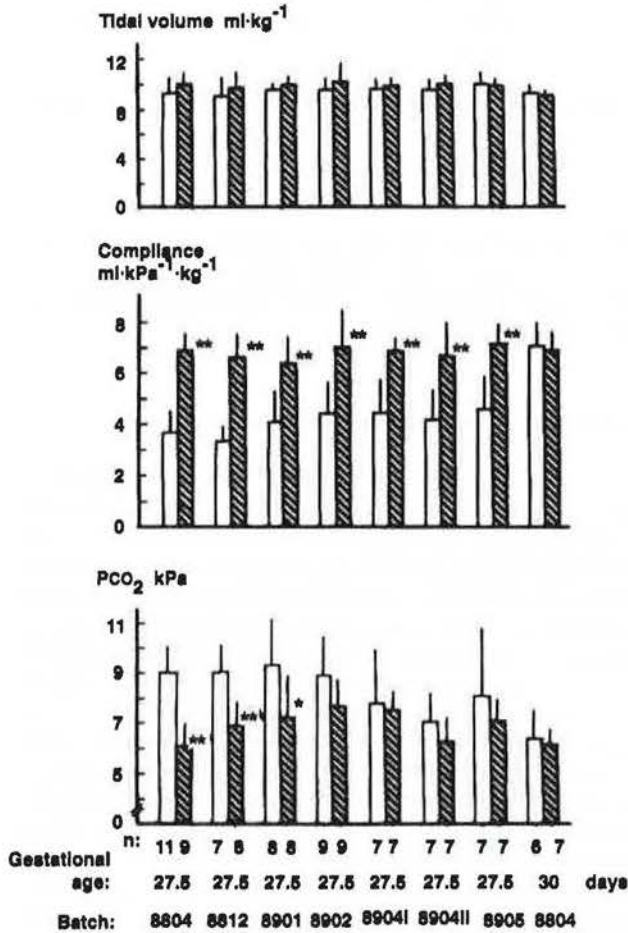


Fig. 4. – Diagrams showing physiological data (mean±SD) from experiments testing different batches of surfactant in immature newborn rabbits (Protocol 2) and from experiment in near-term animals (30 days). The values were obtained after 30 min of artificial ventilation. Conversion factor: 1 kPa= 10.2 cmH₂O. *: p vs littermate controls <0.05; **: p vs littermate controls <0.01. □: controls; ▨: surfactant; Pco₂: carbon dioxide tension.

controls, on the other hand, alveolar air expansion was characteristically incomplete and irregular, and necrosis of airway epithelium was a common finding. The difference between surfactant-treated animals and controls was further corroborated by our morphometric findings (tables 4 and 5): the alveolar volume density was, on average, increased by 61% in immature animals receiving surfactant (table 5). Treatment with surfactant had no effect on the alveolar expansion pattern of near-term animals (table 5).

Table 4. – Alveolar volume density in immature animals studied according to Protocol 1

Tidal volume ml·kg ⁻¹	Surfactant	n	Volume density ml·kg ⁻¹
6–8	-	6	0.28±0.09
6–8	+	5	0.64±0.08**
10–12	-	6	0.45±0.19
10–12	+	5	0.64±0.06*

The values are given as mean±SD. *: p vs control group with same tidal volume range <0.05; **: p vs control group with same tidal volume range <0.01.

Table 5. – Alveolar volume density in immature and mature animals ventilated according to Protocol 2

Gestational age days	Surfactant batch	n	Volume density
27.5	-	11	0.30±0.10
27.5	8804	9	0.52±0.07***
27.5	-	7	0.28±0.10
27.5	8812	8	0.52±0.07***
27.5	-	8	0.37±0.09
27.5	8901	8	0.49±0.05**
27.5	-	9	0.36±0.08
27.5	8902	9	0.53±0.07***
27.5	-	7	0.38±0.07
27.5	8904I	7	0.60±0.10***
27.5	-	7	0.29±0.08
27.5	8904II	7	0.52±0.06***
27.5	-	7	0.33±0.10
27.5	8905	7	0.51±0.04***
30	-	6	0.49±0.03
30	8804	7	0.49±0.02

Some immature animals served as controls for more than one batch of surfactant. The values are given as mean±SD. The values are given as mean±SD. **: p vs littermate controls <0.01; ***: p vs littermate controls <0.001

Discussion

The data from the present experiments provide some new guidelines for experimental evaluation of surfactant substitutes in artificially ventilated immature newborn rabbits. Contrary to earlier experiments [18] involving tidal volume measurements in animals ventilated with a standardized sequence of different insufflation pressures, our present methods were designed to provide adequate lung perfusion and gas exchange during the period of ventilation, as documented by normal ECG and

acceptable final values of P_{CO_2} in heart blood. In our opinion, the equipment described in this paper is more convenient to use than the elaborate system of valves and adjustable water-locks designed for the same purpose by IKEGAMI *et al.* [13]. We also believe that our modified ventilator-multi-plethysmograph system may be applicable to other experimental models of neonatal lung disease, based on long-term ventilation of more mature animals.

In our experiments on animals delivered according to Protocol 1 (tables 1 and 2), pneumothorax was an unacceptably frequent complication irrespective of whether the animals were ventilated with a low (6–8 ml·kg⁻¹) or a high (10–12 ml·kg⁻¹) tidal volume. The tendency to lung rupture was probably related to structural factors including the low content of elastin and collagen in very immature lungs [19]. Although the rupture was no doubt triggered by the ventilatory pressure waves, the incidence of pneumothorax was not directly related to the pressure required to maintain the standardized tidal volume. Pneumothorax was as common in surfactant-treated animals as among controls, although the former animals could be ventilated with a significantly lower pressure. Our “retrospective” analysis of the tidal volume tracings from animals delivered after 27 days of gestation furthermore revealed that the episodes of pneumothorax mainly occurred during the second 30 min of ventilation.

These observations prompted us to modify our first protocol by delivering the animals approximately 12 h later (corresponding to 27.5 days of gestation) and shortening the period of ventilation to 30 min. We also chose to ventilate the animals with an intermediate tidal volume (8–10 ml·kg⁻¹) similar to that needed for healthy preterm newborn babies [20]. With this new protocol, pneumothorax was a much less frequent complication (table 3), and the animals could usually be maintained in stable conditions during the scheduled period of ventilation, with acceptable P_{CO_2} at the end of the experiment.

In principle, the response to surfactant replacement therapy varies with the degree of lung maturity in the treated animals [10, 21]. In an extremely immature foetal rabbit lung without fully developed terminal air sacs (<26 days of gestation), compliance levels close to those of a full-term lung cannot be reached simply by upgrading the intra-alveolar pool of surfactant. In contrast, surfactant-deficient lungs which have just entered the terminal-sac stage of development respond very well to replacement therapy. On the other hand, nearly mature animals receiving surfactant at birth show no further increase in compliance (fig. 4). The compliance values observed after 27.5 days gestation in surfactant-treated animals amount to 95% of the “ceiling” compliance level of nearly full-term animals ventilated under similar conditions.

We found that a surfactant preparation (“Curosurf”) with well documented clinical efficacy [2] increases lung compliance of immature newborn rabbits by 64%, from a mean control level of 4.2 ml·kPa⁻¹·kg⁻¹ (0.41 ml·cmH₂O⁻¹·kg⁻¹). This effect was associated with a 61%

increase in the volume density of the alveolar compartment, assessed at a critical point of the pressure-volume loop, confirming that these lungs were indeed well stabilized by the exogenous surfactant material. Treatment with surfactant also largely prevented the development of airway epithelial necrosis during artificial ventilation, in accordance with earlier observations [18, 22].

Although absolute criteria for acceptance of new batches of surfactant for replacement therapy cannot be derived from this study (no “gold standard” is available), tentative guidelines have been outlined above. In our opinion, potential surfactant substitutes should be evaluated *in vivo* under conditions allowing effective ventilation and perfusion of the lungs. With the equipment described in this paper, such conditions can be maintained in newborn rabbits ventilated in parallel, a type of experimental design which easily includes littermate controls and permits a rapid screening of multiple samples. We postulate that the experimental evaluation should include functional as well as morphological parameters [10, 17, 18]. This is to identify suboptimal surfactant preparations with a detergent-like effect on the immature lung [23, 24], perhaps improving gas exchange and compliance without providing adequate stabilization of alveoli and protection of airway epithelium. We also propose that a surfactant suitable for replacement therapy should not cause a deterioration of lung compliance when instilled into the airways of mature newborn animals.

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- Application d'un nouveau système de ventilateur multipléthysmographe à ventilateur pour tester l'efficacité du remplacement de surfactant chez les lapins nouveau-nés. B. Sun, T. Kobayashi, T. Curstedt, G. Grossmann, B. Robertson.*
 RÉSUMÉ: Nous avons appliqué un nouveau système de ventilateur multipléthysmographe a pour évaluer l'effet du remplacement de surfactant chez des lapins nouveau-nés, dans des conditions bien contrôlées et presque physiologiques, caractérisées par un électrocardiogramme normal et une P_{aCO_2} adéquate dans le sang ventriculaire droit obtenu à la fin de l'expérimentation. Jusqu'à 10 animaux ont été ventilés en parallèle avec un système de respirateur commun à pression constante. En utilisant une pression de travail de 4.9 kPa (50 cmH_2O), nous avons pu ajuster la pression administrée à chaque animal dans une marge de 0.49-4.4 kPa (5-45 cmH_2O), en modifiant la longueur d'un tube ouvert à forte résistance constituant le bras de sortie de la connexion entre le tube du ventilateur commun et la canule trachéale. Des animaux nouveau-nés immatures, obtenus après 27.5 jours de gestation et ventilés après 30 minutes avec un volume courant de 8 à 10 $ml \cdot kg^{-1}$, avaient une compliance poumon-thorax moyenne de $4.2 \pm 1.1 ml \cdot kPa^{-1} \cdot kg^{-1}$ ($0.41 \pm 0.11 ml \cdot cmH_2O^{-1} \cdot kg^{-1}$) et une P_{aCO_2} de $8.5 \pm 1.9 kPa$. Chez les animaux d'une même portée, traités à la naissance avec une forte dose de surfactant naturel (Curosurf, 200 $mg \cdot kg^{-1}$), la compliance a augmenté à $6.9 \pm 1.0 ml \cdot kPa^{-1} \cdot kg^{-1}$ ($0.68 \pm 0.10 ml \cdot cmH_2O^{-1} \cdot kg^{-1}$) ($p < 0.01$), et la P_{aCO_2} a diminué à $6.9 \pm 1.2 kPa$ ($p < 0.01$). Chez des animaux presque à terme, obtenus après 30 jours de gestation et ventilés dans des conditions similaires, la compliance est de $7.0 \pm 0.09 ml \cdot kPa^{-1} \cdot kg^{-1}$, ($0.71 \pm 0.09 ml \cdot cmH_2O^{-1} \cdot kg^{-1}$), et la P_{aCO_2} de 6.4 (1.2) kPa. L'administration de surfactant (à la même dose que plus haut) à des animaux arrivés à maturité au moment de la naissance, n'a eu aucun effet défavorable.
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