

Prevention of Hyaline Membrane Disease in Premature Lambs by Apneic Oxygenation and Extracorporeal Carbon Dioxide Removal

A. Pesenti^{1*}, T. Kolobow¹, D. K. Buckhold², J. E. Pierce², H. Huang¹ and V. Chen¹

¹Laboratory of Technical Development and ²Laboratory of Animal Medicine and Surgery Section, National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA

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Abstract. Hyaline membrane disease is found only in lungs where pulmonary ventilation has been established, i.e. after birth. We delivered eleven fetal lambs of a gestational age of 128–130 days but instead kept their lungs in total apnea and inflated to constant pressure, while removing all metabolically produced carbon dioxide with an extracorporeal membrane lung. Oxygen was provided by the membrane lung, and by apneic oxygenation through the natural lungs. Hence, arterial blood gases remained always normal, without any pulmonary ventilation. After 6–66 h the lungs had sufficiently cleared to allow normal mechanical pulmonary ventilation in 10 out of 11 lambs so treated. In a control group treated with mechanical ventilation alone, five of seven lambs died within the first 24 h of severe hyaline membrane disease.

Key words: Hyaline membrane disease – Lambs – Apneic oxygenation – Extracorporeal CO₂ removal

Introduction

The pathology seen in HMD is never found while in utero, but only following birth *and* pulmonary ventilation. Newborns who go on to develop HMD usually lack severe pulmonary disorder at the time of birth. Following a lag of several hours, the disease leads to a fullblown syndrome with a poor prognosis.

The fetal lungs have sufficient capillarity and anatomical vascularity and potential air spaces to sustain potential effective pulmonary gas exchange well before the fetus is now considered viable [3]. The lungs of those fetuses can already be inflated with air, and the lungs will remain inflated as long as the intrapulmonary pressure (IP) is maintained. This suggests that pulmonary oxygen transfer may already be feasible while normal pulmonary ventilation for CO₂ removal may not yet be fully possible.

It has been previously shown in sedated and paralyzed newborn lambs [12] and in patients with adult respiratory distress syndrome (ARDS) [8] that total metabolic oxygen needs can be transported through the natural lungs during apnea, the arterial blood PO₂ remaining normal; when all metabolically produced CO₂ is then removed by the extracorporeal membrane lung, the blood PCO₂ will also remain normal. Such a technique, termed apneic oxygenation with extracorporeal removal of CO₂ (ECCO₂R), does not require pulmonary ventilation, and is accomplished with lungs completely at rest and yet inflated to a constant IP. We felt this procedure ideally suited to a population of immature, previable newborns whose lungs cannot yet sustain ventilation without high risk of developing HMD, to tide the newborn over the critical first few hours or days of life when clinical HMD has its highest incidence.

To evaluate our hypothesis we performed a controlled study using the fetal lamb of about 130 days gestation as the animal model (term: 145–147 days). MV was instituted immediately after birth in a control group and after termination of apneic oxygenation ECCO₂R in a treated group. We then compared respiratory function during MV and survival between the two groups.

* Present address: Istituto di Anestesiologia e Rianimazione, University of Milan, Italy

Materials and Methods

Eighteen sheep fetuses of 128–130 days gestational age of Hampshire or Corriedale breeds were assigned either to a control group (7 lambs) or to a treated group (11 lambs). The average weight of control and treated groups were 3.3 ± 0.2 SEM kg (range 2.7–4.2 kg) and 3.2 ± 0.1 SEM kg (range 2.4–3.7 kg) respectively. Gestational age was accurately known through a single three-day exposure of the ewe to a ram.

The ewes were anesthetized with inhalation anesthesia using a mixture of nitrous oxide, oxygen and fluothane. A midline incision was performed in the abdominal wall, and the uterus was exposed for hysterotomy.

Control Group

In two twin pregnancies, the first fetus was assigned to the control group and underwent MV. The other five control lambs were treated in the same manner. All lambs were first intubated with a 4 mm ID cuffed endotracheal tube and all free lung fluid was aspirated and saved for minimum surface tension measurement. The lungs were then manually bag ventilated with 40% oxygen at a maximal inspiratory pressure of 25 cm H₂O and expiration pressure of 5 cm H₂O. One umbilical artery was then cannulated with a 1.8-mm ID spring wire reinforced polyurethane catheter [9] advanced to the level of the renal arteries for blood pressure measurement, and for sampling of arterial blood. The cord was then ligated, and the lambs delivered and placed in a small box with an electric pad heater; they were rapidly dried with a hair dryer and an infra-red heat lamp. The body temperature was kept at 38°C–39°C. The lambs were immediately placed on MV with a Siemens Servo-Ventilator 900B (Elema-Shönander, Sweden), at a respiratory rate ranging from 50–70 breaths per minute, and a tidal volume (TV) of 6–10 ml kg⁻¹. The inspiratory time was 25% of the respiratory cycle with an inspiratory pause of 10%. Expiratory volume (V_E) was checked with a spirometer (Collins Inc., Braintree, MA). The peak inspiratory pressure limit was set at 25 cm H₂O. When necessary to maintain adequate alveolar ventilation, the peak limit pressure was raised to 35 cm H₂O. Positive end expiratory pressure (PEEP) ranged between 3 and 5 cm H₂O for best PaO₂ consistent with adequate alveolar ventilation. The FIO₂ was kept between 0.40 and 0.80 so as to maintain a PaO₂ over 40 mmHg. The blood gases were taken soon after MV began, and after 1, 3, and 6 h, and then at intervals of 6 h. Blood gases were measured with an IL 213 Blood Gas Analyzer (Instru-

mentation Laboratories, Lexington, MA) within 5 min of the sampling. Effective lung compliance (ELC) was computed from TV, plateau pressure and PEEP, at same time intervals. Chest X-ray pictures were taken at variable intervals. Pneumothorax, if it developed, was treated with chest tube drainage with a water valve control. Every effort was made to maintain optimal pulmonary care.

All animals received a parenteral nutrition solution composed of as follows: 100 ml glucose 50% (w/v), 150 ml Aminosol® 5% amino-acids (Abbott Laboratories, North Chicago, IL), 5 ml calcium gluconate 10% solution (Abbott), 1 ml magnesium sulfate 50% solution (Eli Lilly & Co., Indianapolis), 5 million U Penicillin G Potassium (Eli Lilly & Co.), 8 U Iletin® insulin (Eli Lilly & Co.). Total fluid intake was kept at 8 ml h⁻¹. Blood volume removed for sampling was replaced with Lactated Ringer's. Bicarbonate (7.5%) was given when necessary to counteract metabolic acidosis (Base Excess –5 mEq l⁻¹ or less).

At autopsy, the right lung was washed with a total of 150 ml of saline in four aliquots for minimum surface tension measurements. Surface tension measurements were done on a modified Wilhelmy surface balance [5]. Samples were taken from the right and left upper and lower lobes for histologic examination, fixed in buffered formalin, and 5 µm sections were obtained from paraffin blocks.

Treated Group

The two second twins, as well as the other nine single pregnancy fetuses were assigned to the treated group and were placed on apneic oxygenation, with ECCO₂R [12].

This group of animals was similarly intubated as in the control animals. A venous drainage catheter 3.2 mm ID made of 0.25 mm wall stainless steel spring wire reinforced polyurethane was advanced through the right external jugular vein to the right atrium. The right common carotid artery was cannulated for blood gas and blood pressure monitoring. Manual bag ventilation was then instituted as in the control group. Immediately thereafter, one umbilical vein was cannulated with a 3.2-mm ID stainless steel spring wire reinforced polyurethane catheter which was advanced 2–4 cm beyond the abdominal wall. The remaining umbilical vessels were tied, and the fetus was placed into a thermostatically controlled box for apneic oxygenation, and the venous blood lines were connected to the extracorporeal blood circuit for veno-venous (jugular to umbilical vein) bypass.

The Extracorporeal Perfusion Circuit

The extracorporeal perfusion circuit was made of 4.5 mm ID silicone rubber tubing with appropriate blood sampling ports. Venous blood drained from the right external jugular vein into a collapsible silicone rubber blood reservoir (15 ml in volume) connected to a microswitch to sense venous return, and to stop the blood pump if venous return slowed down. Blood then flowed through a roller pump, set occlusively, using 9.2 mm ID polyurethane pump chambers, through a 0.8–1.0 m² spiral coiled carbon dioxide membrane lung (CDML) [11] and back into the umbilical venous catheter. Blood connectors were of stainless steel, internally coated with silicone rubber. Extracorporeal blood flow was measured with a Biotronex Model 615 (Biotronex Laboratories, Inc., Silver Spring, MD) extracorporeal blood flow meter. Venous blood oxygen saturation was measured with an on line oximeter [15]. The entire perfusion circuit, including the membrane lung and the blood pump, were placed in a thermostated chamber kept at 41 °C.

To prime the system, the CDML was first flushed with carbon dioxide [10] and was then primed with 250 ml heparinized (8 U ml⁻¹) Lactated Ringer's solution to displace all CO₂ from the blood phase. After priming was completed, room air was passed through the CDML gas compartment for 5 min to flush out all CO₂. The prime was exchanged 2–5 min before bypass with fresh heparinized sheep blood to avoid hemodilution. One minute before the pump was started, the gas flow to the CDML was opened to lower PCO₂ of the blood primed in the CDML.

The CDML was ventilated with a mixture of oxygen and room air humidified to 40 °C at a flow rate of 0.3 to 3 l min⁻¹. The pressure in the CDML gas compartment was kept 200 mmHg below atmospheric pressure by connecting effluent gas to a source of controlled suction (so as to prevent any accidental gas leak into the blood compartment). The extracorporeal blood flow was kept in the range of 80–120 ml kg⁻¹ min⁻¹.

For adequate monitoring of the perfusion, pressures were measured before and after the membrane lung with Bentley Trantec Model 800 (Bentley Laboratories, Irvine, CA) pressure transducers. All data were recorded on an ink recorder (Gould, Inc., Cleveland, OH).

Apneic Oxygenation with Extracorporeal Removal of Carbon Dioxide [12]

Immediately on placing the cannulated animals into the thermostatically controlled box the lambs were connected to apneic oxygenation [7] with ECCO₂R (Fig. 1).

The tracheal tube was connected to a 4 l rubber balloon connected to a water valve as a pressure limit, set initially at 15 cm H₂O. This allowed the animals to make voluntary respiratory motions particularly in the initial moments of bypass when the PCO₂ was still high. Following normalization of PaCO₂ the animals ceased virtually all voluntary respiratory effort for the

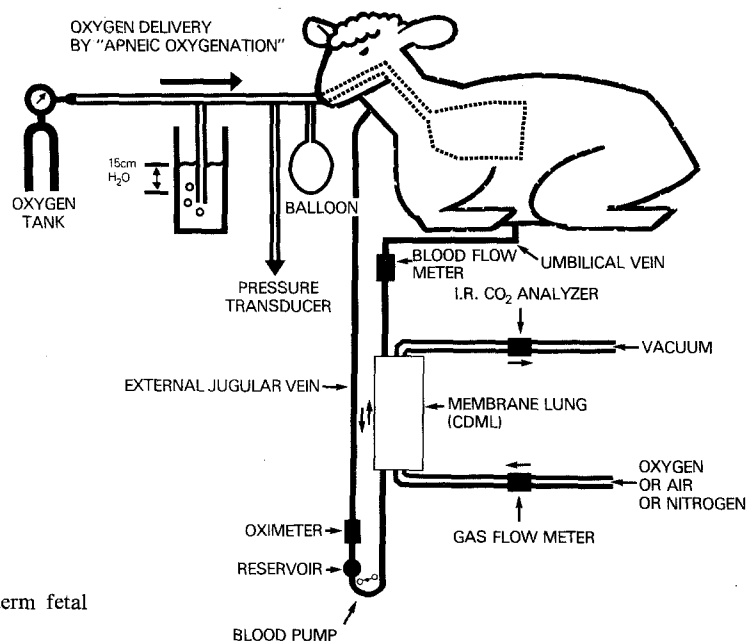


Fig. 1. Apneic oxygenation with ECCO₂R in the pre-term fetal lamb

duration of ECCO₂R [12]. A small Teflon catheter was placed through a side-arm of the airway tube and advanced to the level of the carina through which 100% oxygen flowed at 40–50 ml min⁻¹. Excess oxygen not taken up by the natural lungs was vented through the water valve. When oxygen flow to the capillary was reduced so that no gas escaped through the water valve, all oxygen so supplied was hence taken up by the natural lungs; this provided an easy means to estimate total pulmonary oxygen uptake ($\dot{V}O_2$) as no CO₂ was removed through the natural lungs. As the CDML was being ventilated and not the natural apneic lungs, the CDML alone governed the PN₂ in the alveolar gases; hence, although 100% oxygen was applied to the level of the carina (at a rate slightly in excess of pulmonary oxygen consumption), the alveolar PO₂ did not reflect 100% oxygen, and pulmonary oxygen toxicity was avoided.

The extracorporeal CDML allowed in addition to total CO₂ removal also some supplemental transfer of oxygen. When needed, to keep PaO₂ over 40 mmHg, the oxygen concentration to the CDML could be raised to provide supplemental oxygen transport to blood, in addition to oxygen uptake by the natural lungs. Initially, 50% oxygen was used to ventilate the CDML but slowly it was reduced to 21%–30% oxygen. CDML gas flow was adjusted to keep PaCO₂ in the normal range.

Frequent measurements of blood gases, in addition to continuous readout in mixed venous blood oxygen saturation, provided a ready indication for changing the blood flow or the gas flow through the CDML. Intravenous alimentation was as in the control group except that heparin at 25 U kg⁻¹ h⁻¹ was also administered through the infusion to avoid thrombus formation in the extracorporeal circuit. No tests to determine the clotting status was performed.

We weaned the animals from bypass when chest X-ray films showed aerated lung fields with at least one clear cardiac border, and when blood gases and ELC during a short trial period of MV confirmed the feasibility of pulmonary ventilation. The gas flow through the CDML was then stopped, but blood flow was maintained for a few more hours.

Measurements were made at 0, 1, 3, 6, 12, 18, and 24 h for blood gases and ELC; chest X-ray films were taken as needed. After 4–6 h on MV with all respiratory parameters steady and improving, the perfusion was stopped, the blood catheters were removed and the veins ligated.

To relate pulmonary improvement during MV at different F_{IO₂}, we computed the ratio of P_{aO₂}/P_{AO₂} =

$$\frac{P_{aO_2}}{(P_B - P_{H_2O}) \times F_{IO_2} - P_{aCO_2}}$$

The PA chest X-ray films were scored by four independent observers according to Fletcher [6] and modified as follows:

- 0 – no aeration, no air bronchogram
- 1 – aeration of trachea and major bronchi
- 2 – partial parenchymal aeration associated with ill defined cardiac borders
- 3 – moderate parenchymal aeration associated with well defined cardiac borders
- 4 – complete clearing of the lungs except for minimal opacity at lung bases
- 5 – complete aeration of lungs

Half score points were allowed, and the mean score was computed. In no case did scores vary by more than 0.5 among the observers.

After 24 h of MV, the animals were electively sacrificed. Lung section for pulmonary histology, and saline lung wash for minimum surface tension measurements were as in the control group.

Results

Blood gases and pH immediately after delivery and bagging did not differ significantly among the control and the treated groups (pH 7.14 ± 0.06 SE versus 7.17 ± 0.04 SE; PaCO₂ = 76 ± 14 SE mmHg versus 59 ± 6.4 SE mmHg; PaO₂ = 107 ± 39 SE mmHg versus 131 ± 12 SE mmHg, respectively). Mean chest X-ray score within 3 h from delivery was 2.80 ± 0.30 SEM in the control group and 2.81 ± 0.39 SEM in the treated group, the difference being not significant. Five of seven control lambs died after 20 ± 2.6 SEM h of MV of HMD, and two of the dead had a pneumothorax that required venting.

None of the 11 treated lambs died while on bypass with ECCO₂R. There was no bleeding from the cut-down in the neck. Platelet counts by the end of perfusion, and just prior to disconnecting from the bypass were around 100,000 mm⁻³. One animal in the treated group died of accidental massive air embolism while on MV but still connected to the perfusion circuit, all parameters of lung status at that time suggested normally improving lungs. A second fetus was severely jaundiced at birth and pulmonary function was never sufficient to remove all carbon dioxide without partial assistance from the extracorporeal CDML; this animal became hypotensive and was sacrificed. The overall mortality of the control group was 71% while the mortality of the treated group (excluding the one sheep with accidental air embolism) was 10% (*p* < 0.01).

Performance During Bypass

The extracorporeal bypass lasted 31 ± 6.2 SEM h (range 6–66 h). P_{aCO_2} during bypass averaged 35.4 ± 0.9 SE mmHg ($n = 81$). In three animals, we had an early mild metabolic acidosis which was corrected by giving bicarbonate.

He always achieved adequate oxygenation of arterial blood in the treated animals, as oxygen was supplied both through the natural lungs as well as through the CDML. P_{aO_2} during bypass averaged 63.8 ± 4.4 SE mmHg ($n = 81$). As the pulmonary function improved we reduced oxygen concentration to the CDML to decrease natural lung F_{AO_2} . By the third hour of apneic oxygenation the oxygen transport through the natural lungs reached over $10 \text{ ml kg}^{-1} \text{ h}^{-1}$, and continued to rise.

The IP was set at 15 cm H_2O and was then gradually reduced at the end of bypass to a mean of 10 cm H_2O . Lowering of the IP was done slowly over a period of 6–12 h.

Performance During Mechanical Ventilation

Adequate ventilation was obtained in 10 out of 11 treated lambs as shown by normal P_{aCO_2} . The one exception was the severely jaundiced lamb at delivery which was sacrificed during MV while still having some CO_2 removed by the CDML.

Adequate ventilation of natural lungs became impossible in 5 out of 7 control lambs; these animals all died not of hypoxia, but with progressive hypercapnea and combined respiratory and metabolic acidosis (Fig. 2).

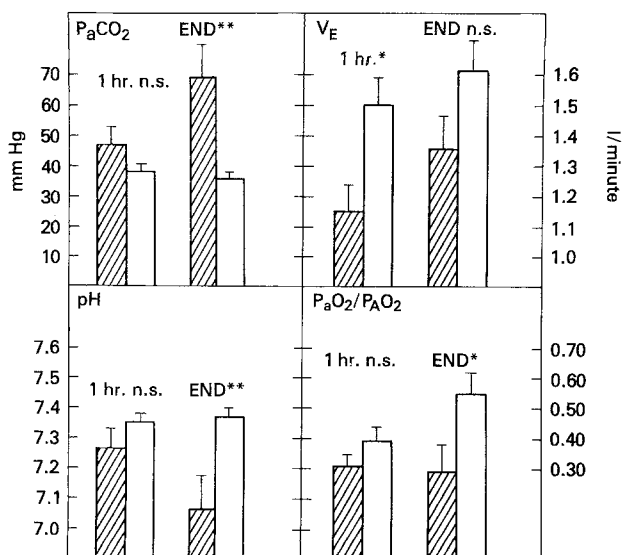


Fig. 2. Respiratory parameters in control (shaded area) and treated fetuses 1 h after beginning of mechanical ventilation, and at 24 h (or death). Mean values \pm SEM. Controls $n = 7$; treated $n = 10$. * Significant at $p < 0.05$; ** significant at $p < 0.01$

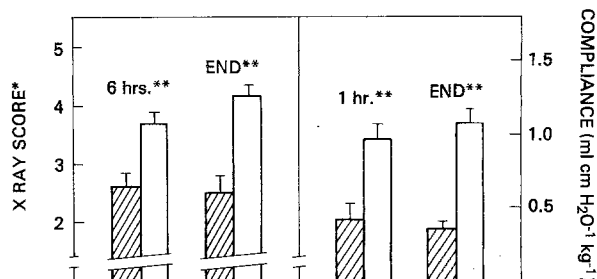


Fig. 3. (Modified after [3]). **Left** chest X-ray score in control (shaded area) and treated groups, after 6 h after beginning mechanical ventilation, and at the end of 24 h (or death). **Right** effective lung compliance in control (shaded area) and treated groups, 1 h after beginning of mechanical ventilation and at the end of 24 h (or death). Mean \pm SEM. Controls $n = 7$; treated $n = 10$. ** significant at $p < 0.01$

The PaO_2/PAO_2 ratio was not significantly different in both groups at the beginning of MV. This ratio did not change among the animals in the entire control group while it rose significantly at the end of MV in the treated group (Fig. 2). However, hypoxia was not a significant factor in the five lambs in the control group which died, since the PaO_2 was always high enough with supplemental oxygen to sustain life (68 ± 10 SEM mmHg at first hour, and 91 ± 41 SEM mmHg just before death). Rather, death occurred from combined respiratory and metabolic acidosis.

The FIO_2 at the end of MV in the treated group was 0.27 ± 0.02 SEM, with a PaO_2 of 82 ± 9 SEM mmHg. The FIO_2 in the control group at just prior to death, and including the two survivors just prior to sacrifice, was 0.65 ± 0.13 SEM with a PaO_2 of 90 ± 30 SEM mmHg.

Mean values of ELC one hour after onset of MV were significantly higher in the treated group (0.93 ± 0.10 SEM ml (cm H_2O) $^{-1}$ kg^{-1}) than in the control group (0.48 ± 0.10 SEM ml (cm H_2O) $^{-1}$ kg^{-1}) (Fig. 3).

ELC remained steady during MV in the control group, while it continued to rise during MV in the treated group, although the rise was not statistically significant.

The chest X-ray score during the first 6 h, and at the end of MV, showed a significantly higher score in the treated group (Fig. 3); there was substantial improvement with continuing MV. The high score at the end of MV in the treated group (4.15 ± 0.15 SEM) indicated almost complete clearing of both lung fields (Fig. 4). The control group, however, showed no improvement in mean chest X-ray score in all seven animals during the course of MV. Eight saline lung washes were available from the treated group, and four from the control group (including one survivor). The minimal surface tension did not differ among the two groups, being 17.6 ± 0.9 SEM dynes

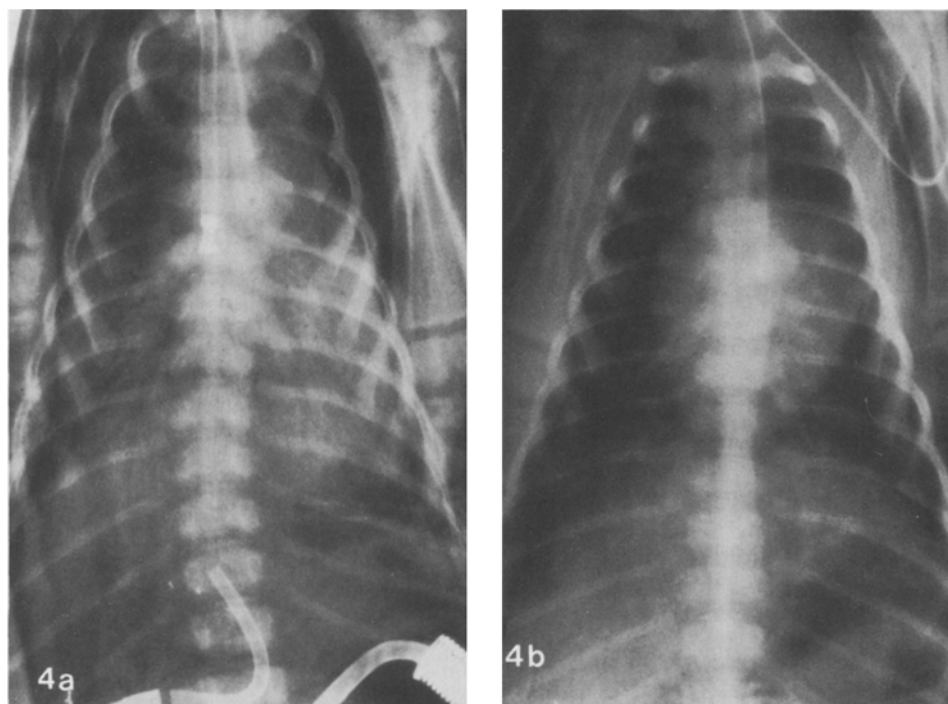


Fig. 4 a–b. Chest X-ray films in a treated animal. **a** 1 h after beginning apneic oxygenation + ECCO₂R (Score 2.13). **b** after 24 h mechanical ventilation (Score 4.0)

cm⁻¹ in the treated lambs and 17.1 ± 2.1 SEM dynes cm⁻¹ in the control group. Gross examination of lungs in the control group of animals which died showed extensive patchy atelectasis with a liver like appearance. These lungs after inflation promptly collapsed. On histologic examination there was extensive atelectasis with hyaline membrane formation, nuclear fragmentation and cell debris in the alveolar spaces, swelling of the alveolar septae, and intraalveolar red blood cell accumulation.

The lungs of the treated animals grossly appeared healthy, and stayed well aerated. On histologic examination there was good alveolar expansion with some subpleural hyperexpansion, but no alveolar cell damage.

Discussion

The fetal lamb is widely used as a model of HMD in pediatric research. At a gestational age of 128–130 days the fetal lamb has a very high mortality [14], thought to be related to surfactant deficiency, leading to collapse of distal airways during expiration [1].

Our choice of the 128–130 days gestation lamb was also based on not only the high mortality ascribed to this age group, but in part also because some surfactant is presumed to be released beginning at about this stage of development [13] allowing some ventilation to take place at birth.

HMD is not a disease that develops in utero, but one that requires that breathing be established. We reasoned that by keeping the lungs motionless and in-

flated we will avoid the sequence that in this age group of animals almost always results in respiratory insufficiency and death. No breathing, and no disease. We aimed at “buying time”, for the immature lung to mature. The immature lungs, through apneic oxygenation and ECCO₂R were spared the effects of ventilation, barotrauma, and V_A/Q mismatching.

All lambs so treated (excluding the one only jaundiced lamb where this treatment did not succeed) had a progressive clearing of the lung fields, and an ELC almost double the ELC of the control group; they subsequently tolerated MV for 24 h with further improvement in the chest X-ray films, and in pulmonary function. Clearly, the lungs kept improving and we assume after some further care the animals could have been extubated and breathed on their own. Moreover, we found that in this age group it was possible to gain maximum effect of this treatment after as little as 6 h of bypass. As experience was gained, we gradually reduced the total time on bypass from 66 h to 6–12 h during the latter part of the study.

The brevity of this treatment poses interesting questions as to the underlying mechanism to pulmonary improvement. Minimum surface tension measurement of the saline lung washes did not differ from each other. They were abnormally high. In a single study not reported here, we sacrificed a lamb of 128–130 days gestational age treated as in this protocol with apneic oxygenation and ECCO₂R, prior to placing him on MV; minimum surface tension measurement of lung wash was 17.7 dynes cm⁻¹, in line with the mean values obtained in the present

series of animals. It was not a massive outpouring of surfactant recoverable by saline lung wash, as inferred from minimum surface tension measurements, which cleared the lungs, and which improved on the ELC. Besides, during apnea, there is no breathing, and it is not clear at all that surfactant during apnea can play any role in 1) clearing of the lungs or 2) improving on the lung compliance. We have no information at this time whether there is any qualitative difference in surface active material among the lung wash in the control and study group of animals, or in surfactant not recovered during saline lung wash.

The control animals (although two out of seven survived) did not show any improvement in mean ELC or in the mean X-ray score. Apneic oxygenation with ECCO₂R apparently allows us to buy time for the lungs to reach a state compatible with adequate pulmonary ventilation. The exact mechanism remains to be elucidated.

Our choice of 10–15 cm H₂O optimal constant distending pressure to keep the lungs inflated during apneic oxygenation was based on pilot experiments; this value is in good agreement with the mean airways pressure suggested as optimal in lambs of similar gestational age [4].

Similar mean airways pressures were achieved in the control group (range 9–13 cm H₂O): the need to limit peak pressure and to maintain a reasonable ventilation in highly uncompliant lungs limited in the control group the use of PEEP to no more than 5 cm H₂O.

Our choice of pre-pulmonary bypass [16] rather than veno-arterial bypass [2] was based on simplicity of this approach as it required only one venous cut-down, the umbilical vein being still accessible to direct cannulation. Moreover the veno-venous bypass intrinsically causes minimal hemodynamic derangement, allowing the lungs to be perfused with oxygenated blood at normal PCO₂. This results in a decrease in pulmonary vascular resistances, and a rise in pulmonary blood flow [17]. Hence apneic oxygenation can be expected to result in a high PaO₂ without the need of pulmonary ventilation.

We feel that our findings have relevance to clinical neonatology. We are at this time impressed by the lack of bleeding in any of our bypasses, and by the simplicity of the bypass. Some positive clinical experience in the treatment of HMD with extracorporeal blood oxygenation is already available [2].

Our experience suggests that management of the lungs with apnea and ECCO₂R may be a reasonable way to achieve normal pulmonary ventilation, and hence the prevention of HMD in a susceptible patient population. We can also speculate that a therapy which was shown to prevent HMD may also have a place in the treatment of HMD. From our preliminary

unreported studies in lambs, such cure can be expected within 24 h of instituting this treatment even in the most severe cases of HMD.

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Theodor Kolobow, M.D.
National Heart, Lung and Blood Institute
Laboratory of Technical Development
Building 10, Room 5D 20
Bethesda, MD 20205, USA