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Changes in the Pulmonary Alveolar Subphase at Birth in Term and Premature Lambs

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ABSTRACT. To study the changes in alveolar fluid at birth, we measured alveolar Cl^- as soon as possible after onset of ventilation in 11 anesthetized lambs. The lambs ranged from 129 to 144 days gestation and were delivered by cesarean section. Subpleural alveoli were punctured with Cl^- selective microelectrodes as soon as 5 min and as late as 135 min after the onset of mechanical ventilation. The $[Cl^-]$ was 151 ± 7 mEq/liter (mean ± SD, $n = 11$) in fetal lung fluid collected before ventilation. After about 40 min of ventilation, alveolar Cl^- was not different from that in term lambs 24 to 72 h old (94 ± 6 mEq/liter, $n = 21$). Assuming first order kinetics the mean $t_{½}$ was 10.4 min. There was no difference as a function of gestation. Thus, alveolar chloride decreases rapidly and alveolar fluid assumes a mature character very soon after the start of breathing in term and premature lambs. The onset of ventilation appeared to stimulate these rapid changes. (*Pediatr Res* 23: 418–422, 1988)

Abbreviations

PD, potential difference
$t_{½}$, time to complete one-half the change in a kinetic process
k, rate constant for a kinetic process
$[Cl^-]$, chloride (or other) ion concentration

Fetal lungs secrete fluid at a rate of about 5 ml/kg body weight/h (1). This secretion is vital to normal lung development *in utero* (2, 3) and contributes to the accumulation of 25 to 30 ml/kg body weight of this fluid in the fetal lung (4, 5). Active transport of ions across the alveolar and airway epithelia drive this secretion (5, 6), and the resulting fluid is rich in Cl^-, and H^+, low in Ca^{++}, HCO_3^- and protein (1, 7). Secretion is driven by an ATPase-dependent Na^+/K^+ pump and depends on a Na-Cl cotransporter on the basolateral surface of the respiratory epithelium (8).

The transition from fetal to air-breathing life is critical and complicated by the large volume of fluid that fills the fetal lungs. This fluid must clear out of the lung in order for normal gas exchange to occur. Studies have shown that near term the secretion of fetal lung fluid slows and might even reverse (9–11). Because of this change there may be as little as 6 ml/kg body weight of fluid remaining in the lung at the onset of air breathing (12). This change is affected by β-mimetics and other agents (9–13) that might naturally increase at term and with labor. However, the remaining fluid constitutes a considerable barrier to normal gas exchange that must clear from the lung with the first few breaths. This remaining fluid apparently moves rapidly from the alveoli to the interstitial and intravascular spaces (13).

In previous studies using micropuncture techniques (14, 15), we found that the electrolyte composition of the alveolar subphase in mature rabbits is quite different from that of fetal lung fluid in lambs. In particular, the concentration of Cl^- is about 50 mEq/liter lower in the mature subphase than it is the fetal fluid (7, 15). The pH and Ca^{++} also are quite different. After changes in alveolar concentrations of one or more of these ions after the start of air breathing might reflect the nature of the transport processes that drive the absorption of fetal lung fluid at birth. Therefore, we have applied the alveolar micropuncture method to newborn lambs, first to see if the alveolar subphase in 1- to 3-day-old lambs is similar to that in mature rabbits, and second to measure the rate of change of the composition of the alveolar subphase after the onset of air breathing.

MATERIALS AND METHODS

Time-dated, pregnant ewes of Western mixed breed were anesthetized at 129 to 144 days gestation with ketamine (30 mg/kg) given intramuscularly, followed by additional doses intravenously to maintain anesthesia. After a midline incision and hysterotomy, the uterus was externalized and covered with blankets and a heating pad to minimize heat losses. The head and neck of the fetus were delivered and a tight fitting, latex glove was placed over the fetal head to prevent spontaneous respiration. After local anesthesia (1% lidocaine subcutaneously) and a midline incision of the neck, the trachea was located and clamped. A sample of fetal lung fluid was removed by inserting a 16-gauge needle between two of the tracheal rings and then passing a catheter through the needle and into the airway. Five to 6 ml of fetal lung fluid were removed and set aside for analysis. Partial pressure of gasses and pH were measured (Radiometer, ABL-1) within 10 min on a portion of the tracheal fluid sample collected anaerobically. A snugly fitting catheter was placed and tied securely in the tracheal lumen and, then this tracheal catheter was clamped so that no lung fluid escaped. Catheters were placed in the right carotid artery and jugular vein. Arterial pressures and heart rate were measured continuously, and intermittent blood samples from the catheter provided information about the steady state gas exchange of the lamb. The arterial catheter was infused at 6 ml/h with lactated Ringer's solution with 1 U heparin/ml added. The venous catheter was infused intermittently with less than 3 ml/h of lactated Ringer's solution with 10 U/ml of heparin added. While still connected to the placenta, the lamb was moved to a warming table inside of a Faraday cage. It was dried and wrapped in blankets to minimize evaporative heat losses. A rectal temperature probe was inserted to continuously monitor body temperature. After injecting 1% lidocaine, an incision was made at the level of the third intercostal space and the right upper lobe exposed. D-Tubocurarine (1 mg) was injected intravenously to prevent spontaneous respiratory efforts. At this point mechanical ventilation of the lung started (Harvard,

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model 681, tidal volume = 10 ml/kg) and simultaneously the umbilical cord was clamped. Supplemental oxygen was added to the inspired gas in an effort to maintain arterial partial pressure of oxygen above 100 torr, and the ventilator rate was adjusted to achieve a $PaCO_2$ of 35 to 40 torr. Additional anesthesia was given to the lamb every 60 min (ketamine 10 mg/kg intramuscularly).

Alveolar micropuncture began as soon as possible after the onset of ventilation of the lung. A small plastic restrainer, described previously (14), was attached to the lung by suction and an adhesive (Super Glue, Duro). An electrolyte solution warmed to 39 ± 1° C bathed the lung surface. This solution contained 136 mEq/liter Na^+, 4.0 mEq/liter K^+, 3.0 mEq/liter Ca^{++}, 110 mEq/liter Cl^-, and 25 mEq/liter lactate with a pH = 7.0. A cotton wick in the restrainer that was saturated with the electrolyte solution made electrical contact with the visceral pleural surface. A calomel cell touching the wetted cotton wick served as the reference side of the measuring circuit. A chloride-selective microelectrode or a 3 M KCl electrode was attached to a micromanipulator and connected to an electrometer (World Precision Instruments, model FD 223).

The measurements of ionic concentration and alveolopleural PD proceeded as before (14, 15) (Fig. 1) with microelectrodes selective for Cl^-, and with a nonselective KCl electrode. There were some measurements made with K^+- and H^+-sensitive electrodes. Before each alveolar measurement, a baseline potential was recorded by immersing the microelectrode tip in the calibrating fluid on the lung surface. Then respiratory movements were stopped at end-inspiration. With continuous observation through a stereomicroscope (M5Apo, Wild), the microelectrode tip penetrated the pleura and entered the alveolar space. After recording a stable potential for 20 s, the electrode was withdrawn, ventilation of the lung resumed, and the baseline potential remeasured. Electrode position was controlled with a micromanipulator (Leitz). No alveolus was punctured more than once.

Serum electrolytes were measured on samples drawn from the arterial catheter at the onset of ventilation and at the end of each experiment. Samples of serum were analyzed by atomic absorption spectrophotometry.

At the end of each experiment the lambs were killed by anesthetic overdose followed by a lethal injection of KCl solution. The ewes were killed similarly immediately after cord clamping.

Eight lambs were studied 24 to 72 h after spontaneous birth. They were anesthetized with intramuscular ketamine (30 mg/kg), supplemented at 60-min intervals, and alveolar micropuncture performed as outlined above. For the pH measurements the solution bathing the lung surface was bubbled with 6.5% CO_2 in air and the pH adjusted to 7.0 with HCO_3^-. This was necessary because of the dependence of alveolar pH on $PaCO_2$ (14). Alveolar $[Cl^-]$, $[K^+]$, and pH were measured with the corresponding ion-selective microelectrodes, and the alveolopleural PD was measured with a KCl microelectrode.

The criteria for an acceptable alveolar measurement were as before (14, 15): the electrode potential must arrive at and maintain a steady state, there must be no bleeding, and the baseline electrode potential must not change more than 1 mV during the measurement. Acceptable measurements produced tracings similar to Figure 1. Approximately one-third of micropuncture attempts did not meet these criteria.

The microelectrodes were constructed as previously described (15) with resins selective for Cl^-, K^+, and H^+. Each electrode was calibrated at 39° C with electrolyte solutions of the appropriate ion at four concentrations with a range encompassing that expected for the alveolar measurements. A linear regression calculation yielded a slope, intercept, and regression coefficient from a semilog plot of the calibration data from each electrode. The slope was always more than 50 mV/log 10 difference in concentration. The intercept was close to zero, and the regression coefficients were always more than 0.999. This calibration line was used to convert data from the alveolar measurements (Fig. 1) into concentrations. The PD between the pleural surface and the alveolar lining was measured with a KCl electrode (1 to 2 × 10^{-6} m beveled tip). This PD had to be taken into account in the calculation of alveolar ion concentrations. The reference electrode for all measurements was a calomel cell in contact with the visceral pleura through a small cotton wick saturated with Ringer's solution. The electrical circuit was the same as in our previous report (14). The electrometer (model FD-223, W-P Instruments, Inc.) had an input impedance of 10^{15} ohm. All experiments took place in a Faraday cage that was carefully grounded to minimize electromagnetic noise. This cage was 4′ × 6′ × 4′ and consisted of conductive screens on four sides and the top and a ⅛″ aluminum sheet on the bottom.

RESULTS

Alveolar chloride decreased very rapidly after the onset of air breathing (Figs. 2–4). Inasmuch as it was impossible to obtain a true time zero measurement of alveolar chloride, initial chloride concentration was taken as that in the lung fluid removed from the airway before onset of ventilation. There was a tendency for Cl^- to be higher in the tracheal fluid from the term lambs (Fig. 4), but no difference was discernible after that. The decrease in

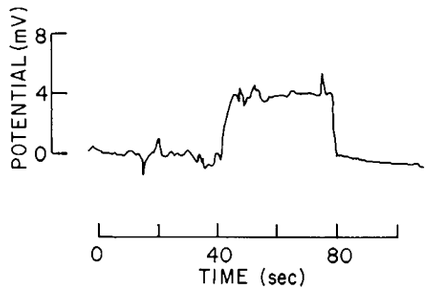

Fig. 1. A typical tracing with a chloride-sensitive microelectrode during alveolar micropuncture. The baseline potential, taken as zero, was generated with the microelectrode in the electrolyte solution on the lung surface. At about 40 s an alveolus was punctured and a new steady state potential achieved within 10 s. The microelectrode was withdrawn from the alveolus at about 80 s, returning to the surface solution and the baseline potential.

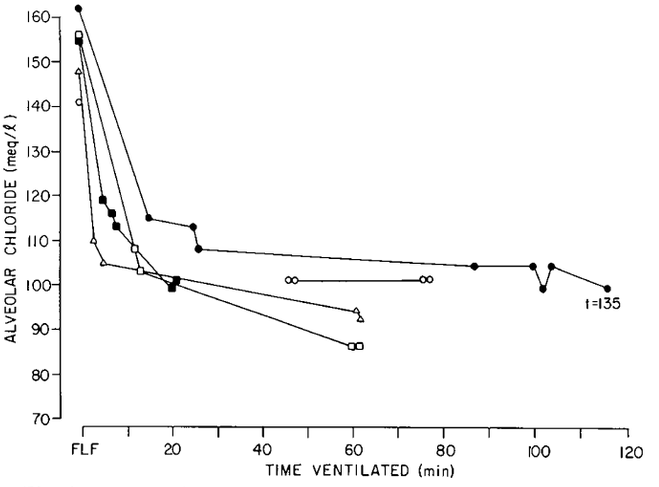

Fig. 2. Decrease in alveolar chloride in five term lambs during mechanical ventilation. The time zero value is the concentration of chloride in the fetal lung fluid sample taken from each of these lambs before starting ventilation. The remaining *points* were obtained by alveolar micropuncture with chloride-sensitive microelectrodes after starting mechanical ventilation of the lungs.

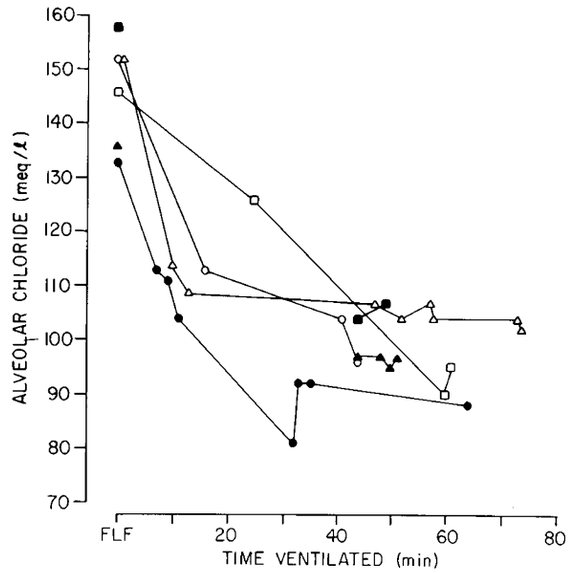

Fig. 3. Decrease in alveolar chloride in six preterm lambs (129 to 131 days). *Points* were obtained as in Figure 2.

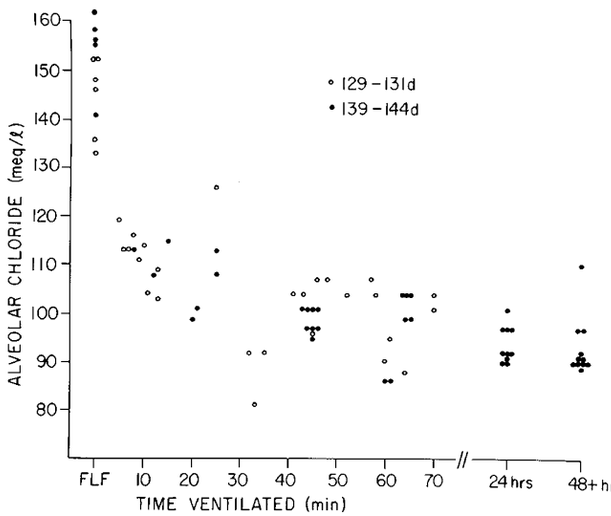

Fig. 4. Cumulative data are shown. Again, the time zero time *points* are the concentrations of chloride in fetal lung fluid removed before mechanical ventilation started. There was no difference in the rate of decrease between 139- to 144-day (●) and 129- to 131-day (○) lambs. The *points* at 24 and 48+ h were obtained in seven spontaneously delivered lambs.

alveolar chloride was seen in all experiments, but only in six of 11 lambs studied after cesarean section were we able to obtain enough points (4 or more) to calculate a rate constant for a single experiment (Table 1).

Assuming simple first order kinetics, the change in alveolar chloride can be expressed by the equation $-d(C-C_f)/dt = k(C-C_f)$, where C is the alveolar chloride concentration at time t, C_f is the steady state concentration of alveolar chloride, and k is a constant. Integrating this expression between t = 0 and t yields the expression $\ln[(C-C_f)/(C_o-C_f)] = -kt$, where C_o is the initial chloride concentration in the alveolus (assumed equal to the tracheal fluid concentration). Linear regression analysis performed on a semilogarithmic plot of the data yielded k and a regression coefficient. There were six experiments in which we obtained four or more chloride measurements by alveolar puncture. Assuming that time zero corresponded to the onset of ventilation resulted in a good fit for first order kinetics (Table 1).

Table 1. *Parameters based on simple kinetic model of decrease in alveolar chloride with air breathing*

Gestation (day)	k (min^{-1})	$t_{½}$ (min)	*r*	*n*
142	0.062	11.1	0.955	9
131	0.061	11.4	0.908	4
131	0.115	6.0	0.992	8
129	0.053	13.1	0.911	9
143	0.11	6.3	0.981	6
142	0.047	14.7	0.928	4
Mean	0.075	10.4	0.945	

Rate constants, $t_{½}$ and regression coefficients were similar in all six, despite the differences in gestational age (Table 1). Recalculating the kinetic parameters for these experiments using the alternative assumption that time zero corresponded to the time of delivery of the lamb's body resulted in somewhat different parameters. The time of delivery was 48.8 ± 16.4 min (mean ± SD, *n* = 6) before the onset of ventilation. Using the delivery time for each experiment as time zero, the correlation coefficients decrease from 0.945 ± 0.036 to 0.889 ± 0.070 (mean ± SD, *n* = 6). Under this assumption, k = 0.0292 ± 0.010 min^{-1} (mean ± SD) and $t_{½}$ = 24.7 ± 10.3 min.

The alveolopleural PD in the first few hours after birth was significantly different (unpaired, two-tailed *t* test) from that measured in the 1- to 3-day-old lambs. The alveolopleural PD in the lambs delivered by cesarean section was −1.8 ± 0.5 mV (mean ± SD, *n* = 41 in 11 lambs) in the first minutes and hours after delivery. The alveolopleural PD in the 1- to 3-day-old lambs was −2.9 ± 0.5 mV (*n* = 32 in eight lambs). The PD did not change measurably during any experiment as long as vital signs were stable. As the preterm lambs developed unstable vital signs, the measurements of PD became erratic. Those data are not reported and the experiments were terminated when this occurred.

There was no difference in the alveolopleural PD by gestational age. The PD in the 129- to 131-day-old lambs was −1.7 ± 0.5 mV (mean ± SD, *n* = 19 in six lambs). In the 139- to 144-day-old lambs it was −2.0 ± 0.4 mV (*n* = 22 in five lambs).

The serum concentration of Cl^- in the cesarean section lambs was 104 ± 4 mEq/liter (mean ± SD) at the start and 108 ± 3 mEq/liter at the end of 10 experiments. The serum sodium concentration was 143 ± 3 mEq/liter at the start and 145 ± 4 mEq/liter at the end of the same 10 experiments.

The alveolar concentrations of Cl^-, K^+, and H^+ and the alveolopleural PD in the 1- to 3-day-old lambs were not different from those in mature rabbits (Table 2). Also, the composition of the tracheal fluid (Table 3) was similar to that reported elsewhere (5, 7).

A number of problems were encountered with the application of the alveolar micropuncture technique to newborn lambs. In preliminary experiments we found that with too frequent or prolonged measurements the lambs developed shock and died. Thus, the number and duration of measurements had to be limited and time for recovery allowed between measurements. Successful measurements were made from 5 to 135 min after the start of mechanical ventilation. Occasionally, two measurements were made in rapid succession, so that the lungs were not ventilated for a maximum of 30 s with each, but then 10 to 20 min were allowed for recovery.

The preterm lambs usually did not survive longer than 1 or 2 h because of progressive pulmonary insufficiency, often developing a pneumothorax or emphysema by the end of 1 h or more of ventilation. The initial ventilator peak pressure required to deliver the tidal volume of 10 ml/kg was higher ($p < 0.01$, unpaired, two-tailed *t* test) in the preterm (45 ± 9 cm H_2O) than in the term lambs (30 ± 9 cm H_2O). Over the course of the experiments the peak pressure for the term lambs decreased to about 20 cm H_2O. The peak inspiratory pressure in the preterm

Table 2. *Alveolar subphase composition in mature rabbits and 1- to 3-day-old lambs (mean ± SD)*

	K^+ (mEq/liter)	Cl^- (mEq/liter)	pH	PD (mV)
Lambs	6.2 ± 0.8	94 ± 3	6.99 ± 0.04	−2.9 ± 0.5
n	4	7	3	7
Rabbits*	7.3 ± 0.7	103 ± 5	6.94 ± 0.04	−3.5 ± 0.8
n	5	11	13	34

* Data from Refs. 13 and 14.

Table 3. *Composition of tracheal fluid in term and preterm lambs (mean ± SD)*

	129–131 day	139–144 day
Na^+ (mEq/liter)	151 ± 5	155 ± 2
K^+ (mEq/liter)	5.4 ± 0.9	7.4 ± 1.9
Ca^{++} (mEq/liter)	1.4 ± 0.3	1.2 ± 0.5
Cl^- (mEq/liter)	144 ± 7	153 ± 10
HCO^-_3 (mEq/liter)	2.5 ± 1.1	2.4 ± 1.0
pH	6.21 ± 0.15	6.41 ± 0.17
n	12	9

lambs decreased little or not at all, consistent with the expected course for hyaline membrane disease. In the first 30 min of the experiments the preterm lambs had a pulse of 135 ± 11 min^{-1} and an arterial pH = 7.31 ± 0.10, PaO_2 = 69 ± 25 torr, $PaCO_2$ = 36.6 ± 10 torr, systolic pressure = 67 ± 14 mm Hg, and diastolic pressure = 41 ± 12 mm Hg. The term lambs had a pulse = 139 ± 6 min^{-1} and an arterial pH = 7.42 ± 0.04, PaO_2 = 193 ± 50 torr, $PaCO_2$ = 39 ± 7 torr, systolic pressure = 89 ± 13 mm Hg, and diastolic pressure = 67 ± 15 mm Hg. Body temperature was 38 to 39° C when the lambs were placed on the warming table and maintained in that range throughout each experiment. The preterm lambs often deteriorated as lung damage progressed. The term lambs were healthier and could have been studied longer, but the measured concentrations quickly reached a steady state, so that longer studies were not done.

Alveoli could not be punctured until they could be seen clearly. They usually became visible about 5 min after starting mechanical ventilation. Fluid-filled alveoli were not visible. Attempts at blind puncture of these alveoli resulted in unstable potentials whose source was unknown, and therefore attempts to puncture alveoli before starting mechanical ventilation produced no usable data. Further, some alveoli might have continued to be fluid filled after the onset of ventilation and they would not have been seen. There was a clear impression that the size and number of alveoli visible through the pleura increased over the course of the experiment. No attempt was made to quantitate this apparent change. The static airway pressure during micropuncture was 15 to 30 cm H_2O in the preterm lambs and about 10 to 20 cm H_2O pressure in the term lambs.

The ion-selective microelectrodes functioned well *in vivo* (Fig. 1). The time to reach steady state was short, the signal to noise ratio was excellent, and electrode drift was infrequent. The small baseline drift allowed, less than 1 mV, did not affect the difference between the baseline and intraalveolar potentials. The drift, when it occurred, was linear over the course of the measurements and was easily cancelled by drawing a straight line through the baseline and measuring the distance from that line to the steady state alveolar potential. The KCl electrode had a much lower impedance than the ion-selective microelectrodes, about 10^{-6} ohm compared to about 10^{-11} ohm. Consequently, the KCl electrode generated no measurable noise or drift.

DISCUSSION

Alveolar [Cl^-] decreased rapidly after the onset of air breathing in these lambs (Figs. 2–4; Table 1). The fact that the change took place in a matter of minutes, rather than hours or days, fits well with previous work (12, 13) and with the obvious need at birth to clear fluid rapidly from the airspaces. The fact that the decrease fit well with the assumption of a first order process reveals nothing about the relative importance of active or passive transport.

Although the simple kinetic analysis performed supports the assumption that the decrease followed first order kinetics and that time zero was nearer to the onset of ventilation than it was to delivery, it must be considered in light of its flaws. There was clustering of points and a wide gap between the assumed time zero values for alveolar Cl^- and the first alveolar measurements. The most critical points for performing good kinetic calculations are missing, those between "time zero" and the first successful alveolar measurements, when the apparent decrease in alveolar Cl^- usually was more than 50% complete. Again, the accuracy of the time zero points is questionable because there were no direct measurements of alveolar [Cl^-] in the fluid-filled lung before the onset of ventilation. Despite the lack of important early data points, it appears clear that the half-time for the change in alveolar chloride concentration was short, probably closer to 10 than it was to 25 min. In any case, the decrease in alveolar chloride after birth was very rapid, occurring in minutes rather than hours or days.

The choice of the fetal lung fluid concentration of chloride as the time zero value of alveolar chloride is reasonable but not assuredly correct. With our experimental approach we must assume the composition of tracheal fluid is similar to that in alveoli before the onset of air breathing. We were unable to obtain any direct measurements to validate this assumption because we could not puncture the fluid-filled alveoli. It is possible that the composition of the fluid secreted by the fetal alveoli is modified downstream by secretion or absorption across the airway epithelium (6, 16). In an attempt to address that issue, Adamson *et al.* (7) sequentially sampled fetal lung fluid in lambs and found no change in fluid composition. Of course, such sampling might not yield pure alveolar fluid, but if there were large differences in composition of airway and alveolar fluids, some change should have been found. Given this finding and the fact that the surface area for secretion is so much greater for alveoli than for airways, it probably is reasonable to assume that the tracheal fluid resembles the alveolar fluid in the fetal lamb.

However, even ignoring the tracheal fluid, there was a significant decrease in alveolar chloride concentration during the experiments. The concentration from 5 to 10 min after the onset of ventilation was 114 ± 3 mEq/liter, $n = 7$. From 40 to 70 min it was 99 ± 6 mEq/liter, $n = 28$. The difference was significant between these groups of points by a two-tailed, unpaired t test with $p < 0.02$. Further, the curve defined by the alveolar measurements (Figs. 2–4) describes a rapid process.

Was this rapid change due to active transport of ions across the alveolar epithelium? Previous studies have shown that alveolar chloride is not distributed passively in the air-filled lung (14, 15; Nielson DW, unpublished data) or in the fetal lamb lung (5). The alveolar chloride concentration at 40 to 70 min is not significantly different from that measured at 1 to 3 days after spontaneous birth. The alveolopleural PD in the ventilated lambs, −1.8 mV, could account for a difference in serum and alveolar chloride concentrations of about 7 mEq/liter with the alveolar concentration being greater than the interstitial concentration. According to the results herein, the relatively small gradient is in the opposite direction, consistent with some active process. Alternatively, some other anion could have diffused or been transported into the alveolar fluid, contributing to the decrease in alveolar [Cl^-]. However, it is unwise to conclude too much about the mechanisms of ion movement from this study.

Because we could only puncture those alveoli that were air filled we cannot be certain that all the alveoli filled with air. In fact, it appeared that the number of alveoli visible through the pleura increased with time. Thus, a significant volume of fluid might have remained in other alveoli and changes in concentra-

tion might have been much slower in these fluid-filled alveoli that we did not see and could not puncture. Because the volume of fluid in the air-filled alveoli was necessarily small, the epithelium in those alveoli might have been able to deal rapidly with that smaller challenge. However, if great discrepancies in alveolar concentrations existed as alveoli progressively filled with air, there should have been much more variability in the chloride concentration as alveoli were punctured randomly, rather than the smooth decrease observed (Figs. 2–4).

We cannot be certain that the stresses associated with delivery and preparation for micropuncture did not alter secretion at the alveolar level. A decrease in alveolar chloride secretion and concentration occurring before the start of mechanical ventilation would lead us to underestimate somewhat the length of time it takes for alveolar chloride to change from its fetal high. Epinephrine and other neurohumoral agents released by stress alter fetal lung fluid secretion in lambs (9–13). Efforts to minimize stress were successful in that body temperature was never more than 1° lower than the normal temperature for a newborn lamb, 39° C. This minimized cold stress, but other stresses might have affected alveolar secretion. However, others (5) found that the flow of fetal lung fluid after cesarean section was similar to that reported in studies of chronically catheterized fetal lambs (1, 11). This experience suggests that secretion by the alveolar epithelium is not greatly affected by the stress of a cesarean delivery.

It is possible but unlikely that the electrolyte solution on the lung surface significantly affected the alveolar Cl^- measurements. The concentration of Cl^- in that fluid was nearly equal to that in blood and, consequently, interstitium. Thus, there should have been no alteration of the electrochemical gradient driving chloride out of the alveolar space. Further, the concentration in the surface fluid was higher than the steady state concentration in the alveoli. In studies of mature rabbits, there was no apparent contamination of alveolar fluid by the surface buffer (14, 15).

Epithelial maturity certainly affects lung functions, such as in surfactant secretion, but no such effect was apparent herein. The higher ventilator pressures seen in the preterm lambs did lead to lung damage, manifest as air leaks near the end of those experiments, which might have changed epithelial permeability. However, because the greatest changes in alveolar chloride occurred in the first few minutes of all experiments, the preterm lungs had not yet sustained damage during the most critical time of comparison. Further, when interstitial air began to accumulate, it could be seen through the microscope, and alveoli in affected areas were not punctured.

The potential measured between the pleural surface and the alveolar interior in the first 135 min of air breathing, −1.8 mV, was less negative than the alveolopleural PD in the 1- to 3-day-old lambs, −2.9 mV, by an unpaired, two-tailed t test ($p < 0.01$). The less negative alveolopleural PD in the cesarean section lambs might be due to an increase in transepithelial conductance around the time of birth. Such a change is consistent with the transient increase in mean pore size with the onset of air breathing reported by Egan *et al.* (17).

Based on all of the above, we have learned that the alveolar subphase rapidly changes after the start of air breathing, achieving a mature composition within minutes. The composition of alveolar fluid measured with ion-selective microelectrodes is similar in both lambs and rabbits, suggesting that similar mechanisms of ion transport operate in or near the alveolar epithelium of both species. The mechanisms of this rapid change and bulk flow of fluid out of the air spaces are not defined by the results of this study. However, this information combined with that by other experimental approaches adds significantly to our understanding of the important changes that occur in lungs at birth.

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