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DEVELOPMENTAL CHANGES IN LUNG EPITHELIAL ION TRANSPORT AND LIQUID MOVEMENT

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INTRODUCTION

During fetal development, mammalian lungs are secretory organs that make breathing-like movements but have no role in respiratory gas exchange, which before birth is a function of the placenta. In utero the lungs are filled with liquid and receive less than 10% of the combined ventricular output of blood from the heart (102). In fetal sheep, however, this relatively meager blood supply is sufficient to deliver the substrate needed by the pulmonary epithelium to make surfactant and secrete into the lung lumen as much as 500 ml of liquid daily during the last third of gestation (4, 73). A recent report indicates that liquid production by the bronchopulmonary epithelium of human fetuses may occur as early as the sixth week of gestation, with resultant expansion of the lung lumen (70). The presence of an appropriate volume of this liquid within the fetal respiratory tract is essential for normal lung growth and development before birth (8, 75).

Rapid removal of liquid from potential airspaces during and soon after birth is a critical event in establishing a timely switch from placental to pulmonary gas exchange. For many years it was thought that mechanical compression of the chest during birth was responsible for squeezing out most of the lung

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liquid through the mouth, thereby facilitating inflation with air. In the past decade, however, several groups of investigators have presented evidence that the normal transition from liquid to air inflation is considerably more complex than the characteristic oral gush at delivery might suggest. The purpose of this review is to describe recent progress in our understanding of how lung liquid is formed during fetal life and how it is removed around the time of birth, with particular emphasis on developmental changes in ion transport and associated liquid movement across the respiratory epithelium.

FETAL LUNG EPITHELIAL ION AND LIQUID TRANSPORT

In Vivo Studies

Scientists have known for more than a century that fetal lungs are filled with liquid (95), but the source of that liquid was unclear until 1948, when Jost & Policard (55) discovered that ligating the trachea of fetal rabbits caused their lungs to become distended with liquid, which implied that the liquid came from within the lungs and was not aspirated from the amniotic sac, as others had suggested (6). Adams et al (2) observed that the composition of liquid drained from the trachea of fetal lambs differed considerably from that of plasma and of amniotic liquid sampled from the same animals. These and other investigators found that lung luminal liquid from fetal sheep contains almost no protein, a bicarbonate concentration of < 3 mEq/L, and a Cl^- concentration that is about 50% greater than the Cl^- concentration of fetal plasma (2, 3, 73). The K^+ concentration of luminal liquid exceeds that of plasma by about 1 to 2 mEq/L and increases further toward the end of gestation, when the lung epithelium releases surfactant into potential airspaces (73). Cotton et al (37) found that the composition of lung liquid in fetal dogs is similar to that of fetal lambs, except that the bicarbonate concentration of canine luminal liquid is not significantly different from that of plasma.

Strang and associates (22, 54, 80, 81) did extensive studies of water and solute movement in the developing lungs of fetal sheep. By sampling liquid from the trachea, lung lymphatics, and bloodstream of chronically-instrumented fetuses, they traced the movement of radiolabeled solutes between potential airspaces, interstitium, and pulmonary circulation. These and later studies showed that the fetal lung epithelium forms a tight barrier to macromolecules (80, 81, 87, 106), whereas the vascular endothelium has wider openings that allow passage of even large proteins (22, 80). Thus, the protein concentration of interstitial liquid, collected as lung lymph, is about 100 times greater than the protein concentration of liquid obtained from the fetal trachea. Olver & Strang (88) showed that despite this large transepithelial protein difference, active transport of Cl^- across the fetal lung epithelium

generates an electrical potential difference that averages ~ -5 mV, luminal side negative. The osmotic force generated by this process causes liquid to flow from the pulmonary microcirculation through the interstitium into potential airspaces.

As early as mid-gestation in fetal sheep, the pulmonary epithelium actively transports Cl^- in the direction of the lung lumen (87, 106). This secretory process can be inhibited by diuretics that block Na^+-K^+-$2Cl^-$ cotransport (26, 28, 111). This observation supports the concept that the driving force for transepithelial movement of Cl^- in the fetal lung is similar to the mechanism described for Cl^- transport across other epithelia (49, 120). According to this view, Cl^- enters the epithelial cell across its basal membrane linked to Na^+ and to K^+ (Na^+-K^+-$2Cl^-$). Na^+ enters the cell down its electrochemical gradient and is subsequently extruded in exchange for K^+ (3 Na^+ ions exchanged for 2 K^+ ions) by the action of Na^+-K^+-ATPase located on the basolateral surface of the cell. This energy-dependent process increases the concentration of Cl^- within the cell so that it exceeds its electrochemical equilibrium. As a result, Cl^- passively exits the epithelial cell through specific channels that are located in the apical membrane. While there is strong evidence supporting this mechanism for Cl^- secretion across airway epithelium (120), additional studies are needed to establish whether or not this sequence of events is responsible for Cl^- secretion across the distal epithelium of the fetal lung. Moreover, the specific properties of the apical Cl^- channels, their distribution on the various cells that make up the fetal respiratory tract epithelium, and the signals that regulate traffic through them remain unclear.

Other ion transport mechanisms also may contribute to fetal lung liquid formation. The low bicarbonate concentration of this liquid led Olver & Strang (88) to suggest that the pulmonary epithelium of fetal sheep might actively transport bicarbonate out of the lung lumen, perhaps in exchange for Cl^-. The fact that acetazolamide, a carbonic anhydrase inhibitor, slows secretion of lung liquid in fetal sheep supports this notion (5). In view of the low pH of this liquid (6.27 ± 0.01), it is also possible that the fetal pulmonary epithelium actively secretes H^+. Lubman et al (64) presented evidence that an active H^+-ATPase may exist on the plasma membrane of cultured alveolar epithelial cells from adult rats, and Shaw and co-workers (107) recently reported the presence of a Na^+-H^+ exchanger on the apical membrane of lung epithelial cells from fetal sheep. Nord et al (78) identified the presence of a Na^+-H^+ exchange pathway in the plasma membrane of type II alveolar epithelial cells obtained from adult rats, and these investigators postulated that this cation exchange mechanism might be important in maintaining intracellular pH and in shifting Na^+ out of the lung lumen. Nord et al (79) also described a Cl^--HCO_3^- exchange mechanism capable of modulating in-

tracellular pH in adult rat type II cells. It is possible that this anion exchange mechanism might influence the Cl^- concentration of liquid that bathes the surface of mature lung epithelial cells. The possible role that these counter-transport systems might have in acidifying and regulating the rate of formation of fetal lung liquid needs to be defined.

There is also no clearcut explanation for the increase in K^+ concentration that occurs in luminal liquid of fetal lambs late in gestation, which Mescher and associates (73) suggested might be linked to surfactant release just before birth. Recent studies have shown that K^+ channels are present on the basolateral membrane of respiratory epithelial cells and that these may play a role in Cl^- secretion (61). The existence of such channels on the basal aspect of the epithelium, however, would not explain the high concentration of K^+ measured in liquid that is secreted across the apical membrane of the fetal respiratory epithelium. The prenatal change in the concentration of K^+ in luminal liquid more likely reflects increased Na^+-K^+-ATPase activity of lung epithelial cells near birth (17).

The concentration of Na^+ in fetal lung liquid is almost identical to that of plasma (2, 3, 37, 73). In studies performed with chronically-catheterized fetal sheep, intraluminal delivery of amiloride (10^{-4}M), a Na^+ transport inhibitor, had little or no effect on the rate of production of lung liquid (26, 85). This finding indicates that Na^+ absorption by the respiratory tract epithelium is not an important modulator of liquid production in the fetal lung, at least not until very late in gestation. Consistent with this view, Butcher et al (25) showed that Na^+ uptake by apical plasma membrane vesicles isolated from lung epithelial cells of preterm fetal lambs was relatively insensitive to amiloride.

In Vitro Studies

In vivo studies, such as those described above, cannot distinguish between the bioelectric and ion transport properties of distal lung epithelium vs airway epithelium. Several groups of investigators have done in vitro experiments to help clarify this issue. In studies using excised trachea from fetal and adult sheep, Cotton et al (39) showed that the electrical potential difference across the adult airway epithelium was greater (lumen more negative) than it was across fetal epithelium under both open-circuit and short-circuit conditions. Their results indicated that the fetal trachea secretes Cl^-, whereas the adult trachea absorbs Na^+. In studies from another laboratory, however, Na^+ absorption exceeded Cl^- secretion in tracheas from both fetal and adult sheep (86). It is unclear why these two laboratories obtained different results using similar techniques and tissues from the same species. Both groups found that isoproterenol stimulated Cl^- secretion in fetal as well as adult airways. In subsequent studies, Cotton et al (37, 38) observed Cl^- secretion under both open-circuit and short-circuit conditions in tracheal and bronchial segments obtained from fetal dogs. Zeitlin et al (122) measured the bioelectric proper-

ties of cultured monolayers of tracheal epithelial cells obtained from fetal and adult rabbits. Their results were consistent with the concept that the fetal airway epithelium secretes Cl^-.

In studies designed to compare ion transport properties of proximal and distal portions of the fetal respiratory tract epithelium, Krochmal and co-workers (60) demonstrated that cultured cysts of alveolar and tracheal tissue from fetal rats produced luminal liquid with a higher Cl^- concentration than that of the external bathing solutions. In the same studies, bumetanide, which inhibits Na^+-K^+-$2Cl^-$ cotransport, decreased the transepithelial potential difference in cultured explants of lung obtained from immature fetal rats (14–16 day gestation). McCray & Welsh (71) found that cultured epithelial cells from 18 day fetal rat lung formed cystic, alveolar-like structures with a transepithelial electrical potential difference that averaged -2 to -3 mV, luminal side negative. Exposure of these cultured cell aggregrates to bumetanide decreased the size and number of cysts, thus implying that cation-dependent Cl^- transport contributed to liquid secretion within these fluid-filled cysts. In related studies, bumetanide inhibited the transepithelial potential difference of these cultured cell cysts (69).

Recently, McCray and co-workers (70) used electrophysiological and morphometric techniques to assess ion transport properties and liquid formation in cultured explants of first trimester human fetal lung. Microelectrode studies performed after 3 to 8 days of tissue culture demonstrated an electrical potential difference that averaged -4 mV (lumen negative) across the bronchopulmonary epithelium of these explants. Both bumetanide (5×10^{-4}M) and ouabain (10^{-3}M)) caused a reduction in transepithelial potential difference, consistent with the aforementioned model of cation-dependent Cl^- transport driven by Na^+-K^+-ATPase. Morphometric studies showed a progressive increase in luminal diameter of acinar tubules within these explants of primitive lung tissue during time in culture. Exposure of the explants to either isoproterenol or an analogue of adenosine 3'5'-cyclic monophosphate (cAMP) caused significant increases in both luminal size and transepithelial potential difference; the latter was inhibited by bumetanide.

Taken together, these studies indicate that both proximal and distal portions of the fetal respiratory tract epithelium secrete Cl^-, which drives liquid toward the lung lumen. It is likely that most of this liquid forms in the distal lung, where the total surface area is many times greater than it is in the conducting airways.

LUMINAL LIQUID FORMATION AND FETAL LUNG GROWTH

Formation and retention of liquid within the lumen of the fetal lung has an important effect on lung growth and development before birth. As liquid is

secreted across the pulmonary epithelium, it flows from terminal respiratory units through conductive airways into the oropharynx, from which it is either swallowed or discharged into the amniotic sac. The upper airway functions as a one-way valve, inhibiting entry of amniotic liquid, but allowing outward flow of lung liquid (1, 23). In fetal lambs, prolonged obstruction of tracheal outflow expands the lungs, increases their DNA content, and leads to a decrease in the number of surfactant-producing (type II) cells, whereas continuous and unimpeded removal of luminal liquid decreases lung size and DNA content, increases apparent tissue density and capillary volume, and stimulates proliferation of type II cells (8, 75). Conditions that interfere with normal production of lung liquid, such as pulmonary artery occlusion (115), herniation of abdominal contents into the thoracic cavity (96), and uterine compression of the fetal chest from chronic leakage of amniotic liquid (43), also inhibit lung growth. These observations underscore the importance of liquid expansion of potential airspaces in the development of normal lung structure before birth, which in turn may influence lung function after birth.

The volume of liquid in the potential airspaces of fetal lambs increases from 4 to 6 ml/kg body weight at mid-gestation to more than 20 ml/kg near term (54, 80, 81, 87). The hourly flow rate of tracheal liquid increases from ~2 ml/kg body weight at mid-gestation to ~5 ml/kg at term (59, 73, 87). This increased production of luminal liquid during development probably reflects an increase in pulmonary microvascular and epithelial surface area that is associated with proliferation and growth of lung capillaries and respiratory units (104). The observation that pulmonary artery occlusion reduces tracheal liquid production in fetal lambs by at least 50% indicates that the pulmonary circulation, rather than the bronchial circulation, is the major source of this liquid (108, 113). Intravenous infusion of saline solution at a rate sufficient to increase lung microvascular pressure and to double lung lymph flow in fetal lambs had no effect on the flow of liquid across the pulmonary epithelium (27, 88). Thus, transepithelial Cl^- secretion appears to be the major driving force responsible for the production of luminal liquid in the fetal lung.

DECREASE IN LUNG LIQUID BEFORE BIRTH

Lung water content remains fairly constant at 90–95% of total lung weight during the last one-third of gestation in fetal sheep (89). Likewise, the rate of production of fetal lung liquid, expressed per kg body weight, does not change significantly from 120 days gestation until near term (73). Kitterman et al (59) discovered that secretion of liquid into the trachea of fetal lambs begins to decrease 2 to 3 days before spontaneous birth. Dickson and coworkers (44) confirmed this finding and showed that the volume of liquid in the lung lumen diminishes before the onset of labor in lambs. Brown et al (23)

reported that luminal liquid in fetal sheep may be absorbed rapidly late in labor and immediately after delivery.

Studies done with fetal rabbits showed that lung water content was ~25% greater after preterm delivery (28 days gestation) than it was at term (31 days gestation) (15). In related studies, rabbits that were born at term after the onset of labor had less water in their lungs than did rabbit pups that were delivered operatively without prior labor (15, 18, 21). There was no significant difference in the amount of water contained in the lungs of pups that were born vaginally vs those that were delivered by cesarean section after labor began.

The effect of labor on lung water content was even more striking in fetal sheep than it was in rabbits (20). Extravascular lung water was 45% less in lambs during labor than it was in lambs that had no labor, and there was a further 38% reduction in extravascular lung water measured in lambs that were studied 6 hr after birth. Morphometric analysis of sections of frozen lung obtained from fetal lambs showed that the decrease in lung water that precedes birth is the result of a decrease in the volume of liquid in potential airspaces relative to the volume in the interstitium.

Perks et al (93) recently reported a late-gestation decrease in the rate of liquid secretion in excised lungs of fetal guinea pigs. It is unknown what causes this reduction in fetal lung liquid secretion before birth, but several studies indicate that hormonal changes, which occur in the fetus just before and during labor, might signal changes in lung epithelial ion transport, which in turn might reverse the direction of liquid flow out of the lumen into the circulation.

POSSIBLE ROLE OF HORMONES IN LUNG LIQUID CLEARANCE AT BIRTH

Several investigators have studied the influence of catecholamines on fetal lung liquid production. Enhorning et al (47) found that injection of a β-adrenergic agonist into pregnant rabbits reduced the amount of water in the lungs of their pups. In studies performed with fetal sheep late in gestation, Walters & Olver (116) discovered that intravenous infusion of epinephrine or isoproterenol, but not norepinephrine, caused either a decrease in secretion or reabsorption of liquid from the lung lumen, an effect that β-adrenergic blockade with propranolol prevented. Lawson et al (62) confirmed the inhibitory effect of epinephrine on secretion of fetal lung liquid and also found that intravenous epinephrine led to an increase in the concentration of surfactant in lung liquid. Brown et al (23) reported a positive correlation between plasma concentrations of epinephrine and absorption of lung liquid during labor in fetal lambs. Olver et al (85) showed that intraluminal administration of amiloride, a diuretic that inhibits epithelial Na^+ absorption, blocked the

effect of epinephrine on lung liquid absorption in fetal sheep. This finding suggests that β-adrenergic agonists stimulate Na^+ uptake by the respiratory tract epithelium, which in turn drives liquid from the lung lumen into the interstitium where it can be absorbed into the bloodstream or removed through lymphatics (20).

Tracheal instillation of dibutyryl adenosine 3'5'-cyclic monophosphate (DBcAMP) also causes absorption of lung liquid in fetal sheep late in gestation (10, 117). Recent studies showed a synergistic effect of terbutaline, a β-adrenergic agonist, and aminophylline, a phosphodiesterase inhibitor, in switching lung liquid secretion to absorption in fetal lambs (31). In these studies, addition of amiloride to the lung liquid prevented its absorption. These findings support the view that as birth approaches conditions that stimulate release of cAMP in the lung may trigger absorption of luminal liquid through a Na^+-dependent process.

The effects of both intrapulmonary DBcAMP and intravenous epinephrine on net production of lung luminal liquid increase with advancing gestational age, and both responses are attenuated by prior removal of the thyroid gland (10). Barker et al (12) demonstrated that replacement therapy with triiodothyronine after thyroidectomy restored the inhibitory effect of epinephrine on lung liquid formation in fetal sheep. Recently the same investigators presented evidence that treatment of preterm fetal sheep with triiodothyronine and hydrocortisone, given together, may stimulate early maturation of epinephrine-induced absorption of lung liquid (11).

The reduction in fetal lung liquid secretion that occurs in lambs before birth might be related in some cases to a rise in plasma epinephrine concentration late in labor (23). However, lung water content in fetal sheep often decreases before there is any detectable release of catecholamines (20, 44, 59). Several investigators have reported that the concentration of β-adrenergic receptors in pulmonary tissue increases late in gestation (35, 118, 121), which may make the lungs more responsive to the effects of epinephrine during labor (23).

At least two reports have indicated that lung liquid absorption near birth does not depend on epinephrine. McDonald et al (72) showed that irreversible blockade of β-adrenergic receptors in fetal rabbits did not prevent the normal reduction in lung water that occurs during parturition, and Chapman et al (30) showed that inhibition of β-adrenergic activity with propranolol did not prevent absorption of lung liquid in fetal lambs late in labor.

A number of other hormones have been shown to inhibit production of fetal lung liquid. Perks & Cassin (90, 92) reported that intravenous infusion of arginine vasopressin reduced lung liquid secretion and sometimes caused absorption of liquid from the lung lumen of fetal goats. The reported effect was dose-related and increased with advancing gestational age. Other studies have yielded similar results in fetal sheep close to term (91, 101, 112). It

should be noted, however, that the dose of vasopressin needed to cause lung liquid absorption in these studies produced plasma concentrations of the hormone that exceeded the concentrations usually found during labor (63, 109, 112). Nevertheless, there is some evidence that release of both epinephrine and vasopressin at birth may be additive in stimulating lung liquid absorption (92).

Intravenous infusions of epidermal growth factor (57), atrial natriuretic factor (29), and prostaglandin E_2 (58) also reduce tracheal liquid production in fetal lambs. It is noteworthy, however, that inhibition of prostaglandin synthesis with meclofenamate did not block the decrease in lung liquid secretion observed in sheep during the 2 to 3 days before birth (114). Thus, the importance of epinephrine, prostaglandins and other hormones in regulating the formation and removal of lung liquid before, during and after birth remains uncertain.

Recent work by Zeitlin et al (123) indicates that changes in progesterone and estrogen late in gestation may have an important effect on ion transport in cultured airway epithelial cells obtained from rabbits. These studies provide evidence that steroid hormones of pregnancy stimulate Cl^- secretion by tracheal epithelium; in the absence of these hormones, Na^+ absorption is the predominant ion transport process of airway epithelial cells.

CHANGES IN LUNG EPITHELIAL ION TRANSPORT AT BIRTH

While the stimulus for lung liquid absorption near birth remains unclear, studies performed with fetal and adult sheep (32, 67, 68, 85) and with isolated, perfused rat lungs (13, 14, 40, 53) indicate that active Na^+ transport across the mature pulmonary epithelium drives liquid from the lung lumen into the interstitium, with subsequent absorption into the vasculature. Perks & Cassin (91) found that liquid expansion of the lungs of fetal goats caused a decrease in secretion or induced absorption of luminal liquid, with associated flux of Na^+ and Cl^- in the direction of the interstitium and K^+ toward the lung lumen. In studies done with fetal sheep before and during labor, Chapman et al (30) found that Cl^- concentration often decreases in fetal lung liquid while K^+ concentration increases as liquid is absorbed from the lungs of lambs during labor. Using a direct micropuncture technique to measure alveolar liquid Cl^- concentration in lambs before and after birth, Nielson (76) found that the large Cl^- gradient between lung luminal liquid and plasma decreased rapidly with the onset of air breathing. This change was associated with a threefold increase in the Ca^{2+} concentration of alveolar liquid (77).

Chapman et al (32) recently did studies with chronically-catheterized fetal sheep that showed a consistent reduction in lung liquid formation associated

with the onset of labor, leading to liquid absorption in about one half of the studies. There was also a consistent 5 mV increase (lumen more negative) in transpulmonary electrical potential difference during labor. Whereas β-adrenergic blockade with propranolol did not inhibit lung liquid absorption during labor in these studies (30), intrapulmonary administration of amiloride during labor reversed lung liquid absorption and caused a decrease (lumen less negative) in transpulmonary electrical potential difference (32).

Cotton et al (37, 38) studied transepithelial ion fluxes and the bioelectric properties of excised airways obtained from fetal and newborn dogs. There was consistent Cl^- secretion in fetal tissues, whereas lobar bronchi from 3- to 6-week old pups exhibited net Na^+ absorption. Luminal application of amiloride reduced short-circuit current of neonatal bronchi by almost 50%, but had no effect of fetal bronchi.

Collectively, these studies provide firm evidence that the distal respiratory tract epithelium switches from a predominantly Cl^--secreting membrane before birth to a predominantly Na^+-absorbing membrane after birth. Consistent with this notion, O'Brodovich et al (83) reported that tracheal instillation of amiloride at birth caused a delay in lung liquid clearance in newborn guinea pigs.

Transport of Na^+ from the luminal side of the respiratory tract epithelium to the abluminal side may occur by several mechanisms, including diffusion through apical membrane channels (66, 82), Na^+-H^+ exchange (78, 107), and substrate-anion cotransport, whereby transcellular movement of Na^+ is coupled to specific substrates, such as D-glucose (9, 13) or amino acids (24). The importance of these pathways across the pulmonary epithelium during fetal life is unknown, but recent studies indicate that the lung epithelium of fetal sheep can take up Na^+ coupled to D-glucose, a process that phlorizin inhibits (9). The physiological significance of this observation is unclear, however, as liquid in the lungs of fetal sheep normally contains almost no glucose (9).

STUDIES OF CULTURED RAT ALVEOLAR TYPE II CELLS

Adult Studies

In vitro studies of ion transport and the bioelectric properties of cultured alveolar type II cells harvested from lungs of adult rats have shown that the same cells that secrete surfactant into the airspaces near birth also may pump Na^+ in the opposite direction, thereby generating the driving force for absorption of liquid from the lung lumen (34, 36, 51, 52, 65, 110). Mason et al (65) discovered that monolayers of cultured rat type II cells, when mounted in an Ussing-type chamber, maintained a transepithelial electrical potential differ-

ence, luminal side negative, that increased in response to β-adrenergic stimulation with terbutaline and decreased in response to luminal amiloride or abluminal ouabain. Although type II cells occupy only a small portion of the surface area of terminal airspaces in the mature lung, the presence of numerous microvilli on the luminal aspect of these cells increases their absorptive surface area (119). In addition, recent morphometric studies indicate that there are almost three times as many type II cells per unit tissue mass lining the interior of the newborn lung as there are lining the interior of the adult lung (99). Thus it is reasonable to postulate that Na^+ transport by these cells might be important in liquid clearance during and after birth.

It is also possible that Na^+/pump activity on type I lung epithelial cells might have a role in liquid removal from the airspaces, but studies by Schneeberger & McCarthy (105) showed little or no Na^+-K^+-ATPase on type I cells of adult rat lungs, although it was present on the basolateral surface of type II cells. More definitive assessment of the possible contribution of type I cells to the formation and clearance of fetal lung liquid awaits improved methods to isolate these cells in sufficient purity and number to permit in vitro study of their ion transport properties.

Fetal Studies

Two groups of investigators have examined the bioelectric properties of monolayers of cultured lung epithelial cells obtained from near-term fetal rats (18–21 days gestation, term = 22 days) (84, 100). In both studies the cells were grown for 2 to 6 days on either collagen-coated nitrocellulose or polycarbonate filters, which then were mounted in Ussing chambers that contained buffered salt solution exposed to 95% air and 5% CO_2 at 37°C. The transepithelial potential difference (PD) across these epithelial cell monolayers averaged −2 to −4 mV, luminal surface negative; PD was more negative with monolayers of cells obtained from fetuses at 21 days gestation than it was for monolayers of cells obtained from fetuses at 18 days gestation (100). Short-circuit current (I_{sc}) averaged 5 to 8 $\mu Å/cm^2$ and I_{sc} increased significantly as gestation increased from day 18 to day 21. Resistance of these cell monolayers averaged 240 to 500 ohms/cm^2. Under basal conditions, inhibitors of Cl^- transport had no significant effect on the bioelectric properties of these sheets of cells, whereas Na^+ transport inhibitors (ouabain, amiloride, and benzamil) caused significant reductions in both PD and I_{sc} without affecting resistance. Terbutaline increased the PD and I_{sc} across these monolayers, and these effects were reversed by subsequent exposure to amiloride. In the studies performed by Rao & Cott (100), amiloride had a greater effect in reducing I_{sc} in monolayers of cells obtained from fetal rats at 21 days gestation than it had on monolayers of cells taken from fetuses at 18 days gestation. Thus under the aforementioned experimental conditions, con-

fluent monolayers of lung epithelial cells harvested from late-gestation fetal rats appear to absorb Na^+ rather than secrete Cl^-. These results are similar to those obtained with cultured type II cells derived from lungs of adult rats (65).

In their studies of the bioelectric properties of fetal rat lung epithelial cells at different stages of development, Rao & Cott (100) showed no significant effect of bumetanide on I_{sc} across monolayers of cells from day 21 (mature) fetuses, whereas bumetanide partially inhibited I_{sc} of cell monolayers from day 18 (immature) fetuses. These observations, which confirm the presence of Cl^- transport in preterm lung epithelial cells, should pave the way for further studies to determine the specific ion transport properties of the various fetal lung epithelial cells and to define the conditions that may convert at least some of these cells from Cl^--secretors to Na^+-absorbers.

DEVELOPMENTAL CHANGES IN LUNG EPITHELIAL CELL Na^+-K^+-ATPase

To study the possible relationship between epithelial cell ion transport and the change in lung liquid volume that occurs during and after birth, Bland & Boyd (17) measured total and ouabain-sensitive rubidium ($^{86}Rb^+$) uptake as an index of Na^+-K^+ exchange and Na^+-K^+-ATPase activity in freshly isolated lung epithelial cells obtained from fetal, newborn, and adult rabbits. $^{86}Rb^+$, which mimics transmembrane movement of K^+, entered fetal cells at ~5% of the rate measured for adult cells. Ouabain, a cardiac glycoside that inhibits Na^+-K^+-ATPase, blocked >80% of the $^{86}Rb^+$ uptake by fetal cells and ~50% of the uptake by adult cells. The rate of $^{86}Rb^+$ entry into cells obtained from newborn pups was three to four times greater than it was in fetal cells; ouabain blocked ~ two-thirds of the $^{86}Rb^+$ uptake in newborn cells. Amiloride also inhibited $^{86}Rb^+$ uptake in both adult and newborn cells. These observations support the concept that changes in epithelial cell cation flux may contribute to the shift of lung luminal liquid that occurs around the time of birth.

In related studies, ouabain-sensitive $^{86}Rb^+$ uptake, an index of Na^+-pump activity, was similar in cells harvested from fetal rabbits and from newborn pups that had respiratory distress after premature birth (17). In contrast, there was a threefold or greater increase in ouabain-sensitive $^{86}Rb^+$ uptake in cells derived from term fetuses that experienced labor. These findings suggest that events associated with labor may stimulate Na^+-K^+-ATPase activity in lung epithelial cells, possibly contributing to the decrease in lung water that occurs in fetal rabbits during labor. The stress of premature birth and subsequent respiratory failure, however, does not increase lung epithelial cell cation flux, an observation that may help to explain the lung liquid retention often associated with premature birth (16, 45).

To see if these developmental changes in epithelial cell cation flux were the result of an increase in the number of Na^+ pumps or in the activity of these pumps, Chapman et al (33) measured the number of ouabain-binding sites (Na^+ pumps) and ouabain-sensitive $^{86}Rb^+$ uptake (to derive a turnover number) of type II lung epithelial cells obtained from fetal, newborn, and adult rabbits. They measured cell binding of radiolabeled ouabain and cell uptake of $^{86}Rb^+$ in the presence of 10^{-4}M ouabain, and by Scatchard analysis of $^3H^+$-ouabain binding, they determined a dissociation constant (K_d) and a maximal number of binding sites/cell (U_{max}). Na^+-K^+-ATPase turnover number was calculated from measurement of ouabain-sensitive $^{86}Rb^+$ influx and U_{max}. Na^+ pump number was the same in fetal and newborn cells, but turnover number was four times greater in newborn cells than it was in fetal cells, indicative of increased pump activity after birth. Adult cells had almost three times the number of Na^+ pumps as did fetal and newborn cells. This difference was sufficient to account for the postnatal tripling of ouabain-sensitive $^{86}Rb^+$ uptake. Turnover number was not significantly different in newborn and adult cells. These findings indicate that Na^+ pump activity increases at birth, whereas the number of Na^+ pumps increases after birth.

Recently, Pinter et al (94) reported that Na^+-K^+-ATPase enzyme activity was decreased in late-gestation fetal rats that had been treated with streptozotocin to induce a diabetic-like state. In situ hybridization experiments showed that the specific signal for Na^+-pump $\alpha 1$ subunit message was strongest on columnar epithelial cells of air-conducting structures in both normal and streptozotocin-treated fetuses. There was also heavy labeling above cuboidal cells lining the developing alveoli. The investigators speculated that reduced amounts of pulmonary Na^+-K^+-ATPase $\alpha 1$ subunit mRNA and Na^+-K^+-ATPase activity in streptozotocin-induced diabetic rats might contribute to the increased risk of respiratory distress previously reported for infants of diabetic mothers.

CLEARANCE OF FETAL LUNG LIQUID AT BIRTH

There are two components of the process by which liquid in potential airspaces drains from the lungs during and after birth: transepithelial flow into the interstitium, followed by flow of liquid into the bloodstream, either directly into the pulmonary circulation or through lymphatics that empty into the systemic venous system. Development of effective respiratory gas exchange and lung volume soon after birth (74) makes it likely that the shift of liquid from airspaces into the interstitium occurs quickly, after which there is slower uptake into the pulmonary circulation or lung lymphatics (20, 98). In sheep, absorption of liquid from the lung lumen often begins during labor and accelerates immediately after birth (23, 32).

Several conditions may contribute to this liquid absorption, including a reduction in net Cl^- secretion and a corresponding increase in Na^+ uptake by the respiratory tract epithelium around the time of birth. The fact that the Na^+ transport inhibitor, amiloride, reverses lung liquid absorption in fetal sheep during labor (32) and slows the rate of lung liquid clearance in newborn guinea pigs (83) underscores the importance of this change in facilitating liquid removal from the lung lumen near birth. In studies performed with fetal lambs at the start of breathing, Egan et al (46) demonstrated a transient postnatal increase in hydraulic conductivity and small solute permeability of the pulmonary epithelium, which may contribute to an increase in bulk flow of liquid from potential airspaces into the intersitium. Air inflation also decreases hydrostatic pressure in the pulmonary interstitium, which may help to siphon liquid out of the lung lumen into the tissue (48, 97). Moreover, it is possible that biophysical changes that occur soon after birth, such as somatic nerve stimulation (103) and small decreases in body temperature (50), may hasten removal of liquid from the lung lumen. As plasma protein concentration increases during the few days before spontaneous birth (15, 20), the resultant increase in protein osmotic pressure of plasma may help to draw liquid into the pulmonary circulation.

Pattern and Time Course of Liquid Clearance

Removal of liquid from the lungs continues for several hours after birth. Studies done with fetal and newborn rabbits showed that pulmonary blood volume increases with the onset of breathing, whereas the postnatal decrease in extravascular lung water does not occur until 30 to 60 min after birth (21). When breathing begins, air inflation shifts residual liquid from potential airspaces into distensible perivascular spaces around large pulmonary blood vessels and bronchi. Accumulation of liquid in these connective tissue spaces, which are distant from sites of respiratory gas exchange, allows time for small blood vessels and lymphatics to remove the displaced liquid with little or no impairment of pulmonary function. In rabbits delivered at term gestation, perivascular cuffs of fluid are of maximal size 30 min after birth, at which time they may store up to 75% of the total volume of extravascular water in the lungs (21). These fluid cuffs normally disappear by 6 hr after birth.

The pattern of lung liquid clearance near birth is similar in lambs (20). As liquid secretion decreases before birth, luminal volume also decreases, with a corresponding reduction in the caliber of potential airspaces. After breathing starts, residual liquid flows into the interstitium and collects around large pulmonary blood vessels and airways. These perivascular cuffs progressively decrease in size as aeration of terminal respiratory units improves postnatally. Thus, clearance of fetal lung liquid in mature lambs is complete 6 hr after normal vaginal delivery. The process is slower in preterm lambs (19, 45), as it is in preterm rabbits (7, 15).

Pathways for Lung Liquid Removal

Potential routes for removal of pulmonary liquid at birth include lung lymphatics, the circulation, the pleural space, the mediastinum, and the upper airway. In studies designed to assess the role of lymphatics in removing fetal lung liquid at birth, Humphreys et al (54) measured pulmonary lymph flow for 1 hr before birth and for 2 hr after the start of mechanical ventilation of lambs delivered by cesarean section. There was a two- to threefold increase in lymph flow after breathing began, and the investigators concluded that pulmonary lymphatics drain ~40% of the liquid contained in potential airspaces of mature lambs before birth. The postnatal increase in lymph flow was less in premature lambs than it was in lambs delivered at term. These were acute studies performed on anesthetized fetuses immediately following extensive surgery, during which the trachea was occluded.

To reexamine the role of lymphatics in removing liquid from the lungs before and after birth, Bland et al (20) measured vascular pressures, pulmonary lymph flow, and concentrations of protein in lymph and plasma of five unanesthetized fetal lambs before, during, and after spontaneous vaginal delivery at an average gestational age of 139 days (term = 147 day). Lymph flow increased postnatally in all five lambs, but the increase was transient and small, returning to the prenatal level within 3 hr. The results of these studies suggested that the amount of excess liquid drained postnatally by pulmonary lymphatics accounted for ~11% of the residual liquid in the lungs at birth. The concentration of protein in lymph decreased with the onset of ventilation in all five lambs, which suggests that when breathing began, residual liquid in potential airspaces, which contains almost no protein, entered the interstitium and thereby reduced the protein concentration in lymph. With subsequent absorption of liquid into the bloodstream, the concentration of protein in lymph returned to its baseline level. Lymph protein clearance did not change significantly during the course of these studies; this is not surprising, since liquid from potential airspaces contains <0.3 mg protein/ml. These studies, coupled with parallel studies of lung liquid clearance conducted with older newborn lambs (20), indicate that lung lymphatics normally drain only a small fraction of liquid in potential airspaces.

In subsequent related studies, Raj & Bland (98) found that left atrial pressure elevation delayed, but did not prevent, lung liquid clearance in lambs. Cummings et al (42) showed that reduction of plasma protein concentration in newborn lambs also slows the rate at which liquid from potential airspaces is removed from the lungs. These findings provide further evidence that the pulmonary microcirculation absorbs at least some, and perhaps most, of the residual liquid present in potential airspaces at birth. It is also possible that some liquid enters the bloodstream through the mediastinum and pleural space, though one study indicates that in normal lambs very little luminal liquid drains by way of the pleural space (41).

How important is the upper airway as a drainage route for lung liquid at birth? Karlberg et al (56) measured changes in thoracic pressure and volume in human infants during birth and concluded that chest compression associated with vaginal delivery drives liquid from the lungs into the oropharynx. Other studies, however, indicate that squeezing the thorax during spontaneous birth may not be a critical event in expelling fetal lung liquid. As noted above, animals in labor that are delivered by cesarean section after tracheal ligation have no more water in their lungs than do animals that are born vaginally (18, 20, 21). Moreover, studies of lung liquid dynamics in near-term fetal lambs have shown that late in labor, as luminal liquid is absorbed across the epithelium, the upper airway functions as a one-way valve, regulating outward flow of pulmonary fluid into the oropharynx and preventing entry of amniotic liquid into the lung lumen (23). Thus, while the conducting airways may serve as an escape route for lung liquid during delivery without prior labor, they probably play a minor role in liquid clearance during the normal birth process.

SUMMARY

During fetal life, the lungs are filled with liquid that flows from the pulmonary circulation across the epithelium in response to the osmotic force generated by Cl^- secretion of airway and distal lung epithelial cells. As birth approaches, net Cl^- secretion across the respiratory tract epithelium decreases, and this is associated with a reduction in the flow of liquid into the lung lumen. The cause for this change is unknown, but several recent studies indicate that it may be related to alterations in the hormonal milieu to which the lung epithelium is exposed late in gestation. The switch from placental to pulmonary gas exchange at birth requires rapid removal of liquid from the lung lumen. During labor and the immediate postnatal period, the pulmonary epithelium changes from a predominantly Cl^--secreting membrane to a predominantly Na^+-absorbing membrane, with resultant reversal of the direction of flow of lung liquid. There is considerable evidence that this change reflects an active metabolic process involving increased Na^+-K^+-ATPase activity in lung epithelial cells, which drives liquid from the lung lumen into the interstitium, with subsequent absorption into the pulmonary circulation. This Na^+-K^+-ATPase-dependent process persists in the bronchopulmonary epithelium of the mature lung and probably has an important role in clearance of alveolar edema associated with heart failure or lung injury.

LINGERING QUESTIONS

Despite the many documented advances in our understanding of the processes that regulate fluid balance in the developing lung, it is important to keep in

mind that our knowledge of these events is constantly changing. It is inevitable that ongoing research in this area will cause us to modify many of today's ideas tomorrow. A host of important questions remain unanswered.

What is the specific site and mechanism of Cl^- secretion in the fetal lung epithelium, and what regulates its activity? Is the Cl^- secretory mechanism defective in the developing lungs of fetuses afflicted with cystic fibrosis, and if so, why are the lungs often normal at birth? What is the relationship between surfactant flux and transepithelial ion movement in the fetal and newborn lung? What is the significance of the consistent acidity of fetal lung liquid? What are the cellular mechanisms that maintain the low pH of alveolar liquid before and after birth? What is responsible for the switch from a predominantly Cl^--secreting respiratory epithelium before birth to a predominantly Na^+-absorbing epithelium after birth? What controls the synthesis and activity of Cl^-, Na^+, and K^+ channels on the luminal surface of pulmonary epithelial cells before and after birth? How important are paracellular pathways in the transfer of ions and liquid across the respiratory tract epithelium before and after birth? What is the role of epithelial ion transport in keeping the air spaces relatively dry during air breathing? How do stressful conditions, such as hypoxia and shock, influence lung epithelial ion transport, either directly or indirectly, through their effects on hormone release or neuronal activation? How does pulmonary stretch or overexpansion affect transepithelial ion movement? What accounts for the apparent differences between mature airway epithelial cells and distal lung epithelial cells in their response to conditions that increase intracellular cAMP? What is the role of type I pulmonary epithelial cells in transporting Cl^- and liquid into the lung lumen before birth and in moving Na^+ and liquid in the opposite direction during labor and after birth? How do lung injury and repair influence epithelial ion transport?

These challenging questions are but a few of the important gaps in our knowledge that beg attention from inquisitive minds keen on discovering what, besides surfactant, eliminates the need for the placenta at birth.

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