Lung Water and Proton Magnetic Resonance Relaxation in Preterm and Term Rabbit Pups: Their Relation to Tissue Hyaluronan

GUNNAR SEDIN, PÉTER BOGNER, ERVIN BERÉNYI, IMRE REPA, ZOLTÁN NYÚL, AND ENDRE SULYOK

Department of Women's and Children's Health, Uppsala University, SE-75185 Uppsala, Sweden [G.S.];
Diagnostic Centre, Pannon University of Agriculture, H-7400 Kaposvár, Hungary [P.B., E.B., I.R.]; and
County Children's Hospital, H-7624 Pécs, Hungary [Z.N., E.S.]

ABSTRACT

The present study was performed to investigate simultaneously total lung water, T_1 and T_2 relaxation times, and hyaluronan (HA) in preterm and term rabbits. Attempts were also made to establish the relationship of HA to total lung water and to T_2-derived motionally distinct water fractions. Experiments were performed in fetal Pannon white rabbit pups at gestational ages of 25, 27, 29, and 31 d and at a postnatal age of 4 d. Lung tissue water content (desiccation method), T_1 and T_2 relaxation times (H^1-NMR method), and HA concentration (radioassay) were measured, and free and bound water fractions were calculated by using multicomponent fits of the T_2 relaxation curves. Lung water content and T_1 and T_2 relaxation times were highest at a gestational age of 27 d and then declined steadily during the whole study period. Similar trends and time courses were seen for the fast and slow components of the T_2 relaxation curve. The T_2-derived free water fraction remained unchanged at a gestational age of 25–29 d (~67%), but increased progressively to a value of 78.5 ± 7.9% at 31 d ($p < 0.001$) and to 83.4 ± 9.4% at the postnatal age of 4 d ($p < 0.01$). Opposite changes occurred in the bound water fraction. Lung HA concentration decreased with advancing gestation from 870.8 ± 205.2 μg/g dry weight at 25 d to 162.6 ± 32.4 μg/g dry weight at 31 d ($p < 0.001$), but it was increased 2-fold postnatally. HA correlated positively with total lung water ($r = 0.39$; $p < 0.001$) but not with the bound water fraction. It is suggested that the physiologic lung dehydration is associated with macromolecule-related reorganization of lung water and that the role of HA in this process needs to be further investigated. (*Pediatr Res* **48:** 554–559, 2000)

Abbreviations
H^1-NMR, proton nuclear magnetic resonance
MRS, magnetic resonance spectroscopy
HA, hyaluronan
AQP, aquaporin

During fetal life the potential air space of the lung is filled with fluid, which is generated by continuous epithelial secretion driven by active chloride transport. As parturition approaches, the lung fluid secretion decreases, and sodium transport–dependent reabsorption begins. This switch in transepithelial water movement culminates at birth and results in rapid clearance of lung fluid, which is critical for successful transition to air breathing (1–4).

The osmotic water permeability of the alveolar epithelium is developmentally regulated by and related to the expression of water-transporting proteins, called AQPs. Three AQPs have been identified in the lung: AQP1 in the capillary endothelium, AQP4 in the basolateral membrane of the airway epithelium, and AQP5 in the apical membrane of alveolar epithelial type I cells (5–8).

The specific localization of AQPs to endothelial and epithelial cells suggests water movement between the air space and the interstitial and capillary compartments. It takes place through individual water channels. The mRNA and protein expression of AQPs appears late in gestation, increases markedly at birth, and remains elevated during early postnatal life (9, 10). This developmental pattern of AQP expression parallels perinatal changes in the osmotic water permeability of the lung and the rate of lung water removal that occurs around birth (11).

In addition to active ion transport–driven AQP-mediated water transport, the physical state of tissue water is also an important determinant of perinatal lung water clearance. Namely, a fraction of tissue water is motionally constrained, *i.e.* bound to macromolecules and thus made unavailable for immediate transport across alveolar, microvascular, and airway barriers (12).

H^1-NMR relaxation measurement is a unique method of investigating the physical state of tissue water compartments of the fetal lung even without changes in the total water content of the lung. To our knowledge, the contribution of motionally distinct water fractions to the maturity-related alterations in the fetal and neonatal lung water metabolism has not yet been characterized. By applying the H^1-NMR technique, the behavior of water molecules, *i.e.* the water–macromolecular interaction, can be assessed quantitatively, and information can be obtained about the dynamics of the reduction in fetal lung water (13).

HA, the major macromolecule that limits water movements, has a great number of hydrophilic residues that bind water and form viscous hydrated gels, and it also has the ability to immobilize water molecules by osmotic forces (14–17). As a large amount of HA has been detected in fetal and neonatal tissues, it was considered of importance to investigate the role of HA in the control of lung tissue water during the perinatal period.

The present study, using *in vitro* H^1-NMR relaxometry, was therefore performed to determine simultaneously total lung water, the motionally distinct water fractions, and HA concentration in lungs of preterm and term rabbit pups. Attempts were also made to establish the relationship of HA to total lung water and also to the motionally constrained bound water fraction.

METHODS

Experiments were performed in fetal Pannon white rabbit pups at gestational ages of 25 ($n = 17$), 27 ($n = 14$), 29 ($n = 17$), and 31 d ($n = 12$). An additional group of 18 full-term (31 d) newborn rabbits was studied at a postnatal age of 4 d. These animals were nursed by their mothers and suckled *ad libitum*. The rabbit pups were born of does with known conception times by elective cesarean section under epidural anesthesia with 1.5–2.0 mL of 0.125% bupivacaine after pretreatment with 15–20 mg/kg ketamine.

Experimental procedures. Immediately after delivery, the animals were killed by an overdose of s.c. pentobarbital, and the lungs were removed as quickly as possible. Lung samples were separated by lobes and used to determine the tissue water content, NMR relaxation times, and HA concentration. To prevent influence of postmortem changes in water compart-

ments, NMR measurements were made promptly after killing the animals, and samples for HA determinations were kept frozen at $-70°$ until analyzed.

HA determination. The lung tissue was weighed and frozen and then freeze-dried for 74 h. The dried lung was reweighed, and the water content was calculated as a wet to dry weight ratio. The dried lung tissue was then digested with pronase (protease p-5147, *Streptomyces griseus*, Sigma Chemical Co., St. Louis, MO, U.S.A.) in a 2.5 U/mL buffer solution [0.05 M Tris (hydroxymethyl) aminomethane, 0.01 M $CaCl_2$, pH 7.2] at 55°C overnight (>16 h). One unit of enzyme was added for each 10 mg of dry lung material. The digestion was terminated by heating to 100°C for 10 min in a water bath. The HA content was determined with a radiometric assay kit [HA 50, Pharmacia, Uppsala, Sweden; (18)].

Magnetic resonance spectroscopy. Tissue samples of approximately 200 mg were placed in 5-mm-diameter NMR glass tubes and incubated at 40°C for 5 min to reach thermal equilibrium with the magnet temperature. MRS was performed on a Bruker Minispec PC 140 portable MR spectroscope (Bruker Co., Karlsruhe, Germany) operating at 0.96 T (40 MHz). A 386 AT personal computer was used as a storage scope to adjust the 90° and 180° pulses. T_1 relaxation time was measured by an inversion recovery method with eight different time intervals between the 180° and 90° pulses. Repetition time was $5 \times T_1$. T_2 relaxation time was obtained by using the Carr-Purcell-Meiboom-Gill sequence; 1000 echoes with an echo time of 1 ms was applied. Each point was the average of five measurements. The data were transferred to the PC for storage and analysis.

Mathematical analysis. To approximate tissue water fractions quantitatively according to their mobility, multicomponent analysis of the T_2 relaxation decay curves was applied by a nonlinear least-squares method (19). The free induction decay of the proton relaxation process follows an exponential function. This function can be described by a multiexponential equation provided that in the tissue studied there are water compartments with different rates of relaxation and that these compartments are not interdependent at the time of the measurements. The multiexponential nature of the T_2 decay curve indicates that protons of water molecules do not undergo rapid exchange between the compartments. In contrast, when water proton relaxation follows a pattern of monoexponential decay,

Table 1. *Lung water and HA content, T_1 and T_2 relaxation times, and the fast (T_{21}) and slow (T_{22}) components of T_2 relaxation time in fetal and neonatal rabbit pups (mean ± SD)*

Age (d)	Wet to dry weight ratio	HA (µg/g dry wt)	T_1 (ms)	T_2 (ms)	T_{21} (ms)	T_{21} (%)	T (ms)	T_{22} %
Fetal								
25	8.9 ± 0.5	870.8 ± 205.2	1300 ± 100	191.4 ± 25.8	97.7 ± 11.7	32.7 ± 7.3	246.6 ± 39.4	67.3 ± 7.3
27	10.1 ± 0.5†	573.2 ± 219.2†	1430 ± 80*	248.5 ± 19.2†	113.6 ± 9.7*	34.0 ± 5.5	325.3 ± 41.2†	66.0 ± 5.5
29	8.8 ± 0.4	274.9 ± 56.2†	1400 ± 50*	215.0 ± 16.8*	102.6 ± 7.9	31.8 ± 6.1	271.7 ± 27.1	68.2 ± 6.1
31	7.7 ± 0.7†	162.6 ± 32.4†	1290 ± 130	165.2 ± 15.4*	73.5 ± 13.3†	21.6 ± 8.0*	190.3 ± 24.0*	78.5 ± 7.9*
Postnatal								
4	5.9 ± 0.3†	347.3 ± 70.8†	1030 ± 80†	109.3 ± 12.2†	50 ± 13.4†	16.6 ± 9.4†	121.2 ± 20.6†	83.4 ± 9.4†

* $p < 0.01$.
† $p < 0.001$.

there is a rapid exchange of protons between tissue water and macromolecules, proving that the water compartments are interdependent (20, 21).

We applied biexponential fitting for estimation of the bound and free water compartments. Two components of the T_2 relaxation curve were derived from the following expression:

$$F = k_1 \times e^{-t/T_{21}} + k_2 \times e^{-t/T_{22}}$$

where F is the function of T_2 relaxation, k_1 and k_2 represent the relative contributions of the two sets of protons, and T_{21} and T_{22} are the relaxation times of the different components.

Statistical analysis. Data are expressed as mean $\pm$ SD of the mean. For statistical analysis, the Mann-Whitney test and least-squares linear regression analysis were used. The study was approved by the Institutional Ethics Committee on Animal Experiments.

RESULTS

Lung water expressed as wet-to-dry weight ratio did not change with advancing gestation from 25 d (8.9 $\pm$ 0.5) to 29 d (8.8 $\pm$ 0.4). It then started to decline, reaching 7.7 $\pm$ 0.7 ($p < 0.001$) at 31 d, and this was followed by a marked fall to 5.9 $\pm$ 0.3 ($p < 0.001$) at 4 d postnatally. The trends and time courses of T_1 and T_2 relaxation times proved to be quite similar; there was a transient rise from their initial values of 1300 $\pm$ 100 ms and 191.5 $\pm$ 25.8 ms (25 d) to 1430 $\pm$ 80 ms ($p < 0.01$) and 248.5 $\pm$ 19.2 ms ($p < 0.001$), respectively, at 27 d of gestation. Later on, a steady decline occurred in both values, but the extent and rate of the decline appeared to be more pronounced for T_2 than for T_1 relaxation time (10% *versus* 33% at a gestational age of 31 d and 28% *versus* 56% 4 d after birth at term).

Using biexponential analysis of the T_2 relaxation curves, distinction could be made between their fast (T_{21}) and slow (T_{22}) components that corresponded to the bound and free water fractions, respectively. Both T_{21} and T_{22} increased initially up to the 27th day of gestation and then decreased progressively during the rest of the age period studied. As a result, the relative contribution of the bound water fraction represented by T_{21} amounted to 31% to 34% of the total lung water in the period between 25 and 29 d of gestation, decreased significantly before delivery (21.6 $\pm$ 8.0% at 31 d; $p < 0.01$), and decreased even further (16.6 $\pm$ 9.4%) until a postnatal age of 4 d after birth at term ($p < 0.001$). The maturity-related changes in the HA concentration in the immature lung are also shown in Table 1. The highest HA concentrations were observed in the most immature animals, and with advancing gestational age, there was a progressive reduction from 870.8 $\pm$ 205.2 μg/g dry weight at 25 d to 162.6 $\pm$ 32.4 μg/g dry weight at 31 d of gestation ($p < 0.001$). It is of interest that the HA concentration increased 2-fold to 347.3 $\pm$ 708 μg/g dry weight postnatally ($p < 0.001$) in spite of the concomitant abrupt decrease in lung tissue water, especially in the bound fraction. (Table 1).

To analyze the influence of total lung water on the relaxation times and the components of T_2 relaxation, the lung water content was studied as a function of T_1 and T_2 relaxation times and also of the fast and slow components of T_2 relaxation. Lung water was found to correlate positively with T_1 ($r = 0.87$, $p < 0.001$) and T_2 relaxation times ($r = 0.93$, $p < 0.001$) and also with the T_2-derived bound water fraction ($r = 0.67$, $p < 0.001$; Fig 1). As shown in Figure 2, the HA concentration correlated with the lung water content ($r = 0.39$, $p < 0.001$) but not with the T_1 ($r = 0.15$, $p > 0.05$) or T_2 ($r = 0.22$, $p > 0.05$) relaxation times. The fast component of T_2, representing the bound water fraction, also proved to be independent of lung HA ($r = 0.24$, $p > 0.05$).

When the association of tissue HA with lung water characteristics determined by H^1-NMR relaxometry was analyzed separately at different study ages, again no significant relationship was found.
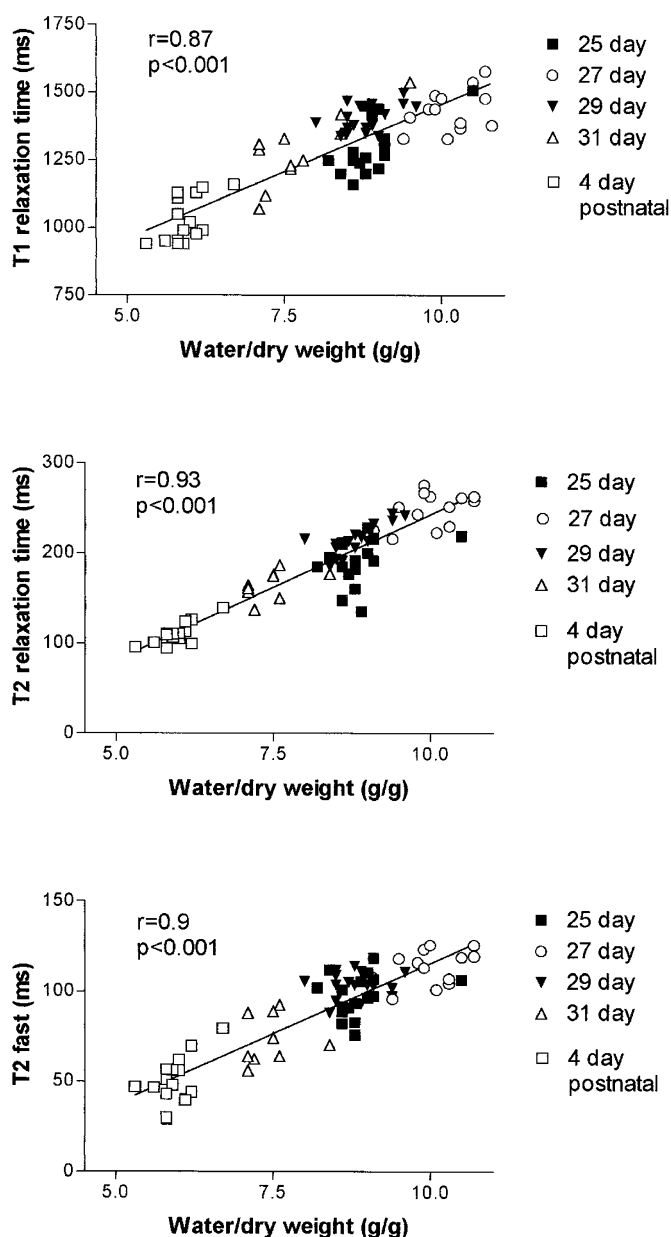

Figure 1. Relationship of lung water content to T_1 relaxation time, T_2 relaxation time, and the fast component of T_2 relaxation time in preterm and term rabbit pups studied at birth and in term rabbit pups studied at 4 d postnatally.

Figure 2. Relationship of lung HA concentration to brain water content, T_1 and T_2 relaxation times, and the fast component of T_2 relaxation time in preterm and term rabbit pups studied at birth and in term rabbit pups studied at 4 d postnatally.
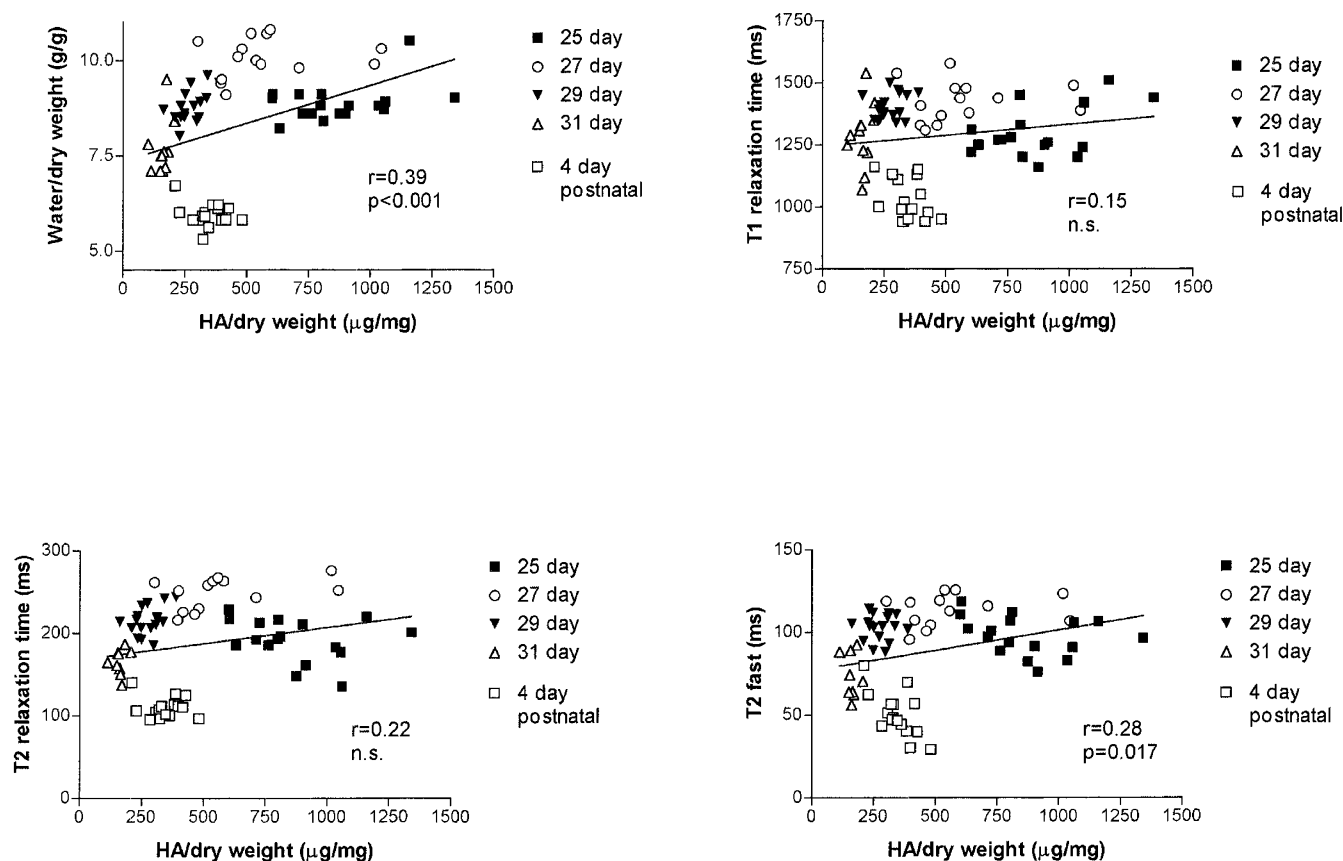
DISCUSSION

The present study confirms previous observations that the removal of fluids from the fetal lung begins before birth to permit effective pulmonary gas exchange in postnatal life. Furthermore, our results provide new insight into the changes in physical properties of lung water by demonstrating quantitatively a perinatal reduction in the bound fractions of lung tissue water and a simultaneous increase in the free fractions. Additionally, this study has shown a significant positive correlation between lung water content and tissue HA concentration, but has failed to demonstrate an association of HA with the motionally constrained bound water fraction. These data suggest a macromolecule-related reorganization of lung water in which process HA is not the only factor that may play a role.

H^1-NMR relaxation measurements have already been applied to analyze the physical state of tissue water in experimental lung injury induced by oxygen, endotoxin, irradiation, and norepinephrine administration. Lung edema formation has been found to be associated with prolongation of T_1 and T_2 relaxation times (22–25).

T_1 relaxation time is assumed to be related to the tissue water content, whereas T_2 relaxation time is mainly influenced by water–macromolecular interaction (26). Changes in T_2 relaxation therefore suggest an alteration in the free and bound fractions of lung tissue water.

In a very recent study using time domain reflectometry, Miura *et al.* (27) investigated the dynamic structure of tissue water in normal lungs and in pulmonary edema induced by oleic acid in rats. They found that free water accounted for 59% of the total tissue water in the normal lung and that there was an increase in free water to 76% in pulmonary edema. Interestingly, free water in the lung tissue had the same physical properties as pure water when assessed by relaxation time measurements.

In our H^1-NMR study, decomposition of T_2 relaxation curve into components with fast and slow relaxing rates yielded a reliable estimate of the free water fraction. The relative contribution of free water to the total lung water was smaller than that to the brain water (28), but considerably larger than the relative contributions to the water contents of the skin, skeletal muscle, and liver in newborn rabbits (12).

An important finding in our experiment is that the maturity-related reduction in the lung water is accompanied by a paradoxical increase in the free water fraction from 67% at 25–29 d of gestation to 78% at 31 d and 83% at a postnatal age of 4 d after birth at term. The increased free water in the face of the physiologic dehydration of the lung tissue can be interpreted as indicating reorganization of lung water, possibly as a result of quantitative or qualitative changes in tissue macromolecules.

Our experimental model does not allow anatomic localization of tissue water fractions and does not reveal the underlying mechanism of fetal lung water elimination. H^1-NMR investigation of oleic acid–induced pulmonary edema with intraalveolar exudates, however, has shown significant prolongation

of both the fast and slow components of the T_2 decay curves, which has been claimed to be accounted for by cellular, interstitial, and alveolar fluid accumulation (29). Fetal lung dehydration, on the other hand, is associated with shortening of both components of T_2 relaxation, with a more pronounced reduction in the fast component. This observation can be interpreted as indicating that water molecules are first liberated from the tissue store and set free for subsequent transport across the pulmonary membranes irrespective of their tissue localization. Furthermore, the marked postnatal rise in the free water fraction is accompanied by a simultaneous increase in pulmonary blood flow, and intravascular water has been shown to contribute to the augmentation of the slow component of T_2 in the lung (29).

HA has been claimed to be the major macromolecular compound controlling water mobility and water balance in the lung. Hällgren *et al.* (30) observed accumulation of HA in the small airways in patients with adult respiratory distress syndrome and proposed that it contributed to water immobilization and generation of interstitial and alveolar edema. In agreement with this contention, a strong positive correlation has been reported between tissue HA and the water content of the lung (31, 32).

During fetal and neonatal life, higher HA concentrations have been found in the lung tissue of preterm rabbits (33) and preterm infants (34) than in rabbits and infants born at term, and the role of HA as a determinant of the tissue water content during pulmonary adaptation has been established. In this context, it is worthy to note that an increased lung HA concentration and water content have been demonstrated in neonatal respiratory distress syndrome (35, 36) and in preterm and term rabbit pups during exposure to oxygen breathing (37).

The developmental changes in lung HA observed in this study are almost identical to those described by Allen *et al.* (33) and seem to support their conclusion that, during gestation, the major function of HA is to facilitate morphogenesis, whereas in early postnatal life it is involved in the regulation of lung fluid balance. However, the apparent dissociation of tissue HA and lung water, in particular the bound fraction of tissue water, argues against this conclusion. To reconcile these seemingly conflicting contentions, one may assume that during the perinatal period HA may undergo still only partly defined molecular alterations related to its water-binding capacity. These may include maturity-related changes in HA molecular size (38), changes in its electrical charge, variations in its conformation with subsequent (un)covering of active, polar sites of the molecular surface (39), and also hormonally regulated enzymatic degradation (40, 41). It is possible, furthermore, that the relative importance of other macromolecules in reorganizing lung water becomes more significant. In this context, it is of interest that the intermittent mechanical strain that may be associated with fetal breathing movements differentially regulates gene and protein expression of extracellular matrix molecules (laminin, procollagen I, collagen type I and IV, biglycan, metalloproteinases 1, 2, and 3, and tissue inhibitor of metalloproteinase) in fetal lung cells (42).

In conclusion, the physiologic lung dehydration that occurs during late fetal and early postnatal life is a complex process, one which involves water movements that take place through specific epithelial and endothelial channels and are driven by active ion transport and generation of free water. Our H^1-NMR relaxation study has demonstrated that elimination of lung fluid is associated with an increase in free water at the expense of the bound water fraction. The underlying mechanisms of the release of water molecules from macromolecular bindings remain to be established. HA does not appear to be directly involved in this process.

REFERENCES

1. Bland RD, Nielson DW 1992 Developmental changes in lung epithelial ion transport and liquid movement. Annu Rev Physiol 54:373–394
2. Bland RD 1990 Lung epithelial ion transport and fluid movement during the perinatal period. Am J Physiol 259:L3–L37
3. Pitkänen OM, O'Brodovich HM 1998 Significance of ion transport during lung development and in respiratory disease of the newborn. Ann Med 30:134–142
4. Folkesson HG, Norlin A, Baines DL 1998 Salt and water transport across the alveolar epithelium in the developing lung: correlations between function and recent molecular biology advances. [review] Int J Mol Med 2:515–531
5. Nielsen S, Smith BL, Christensen EI, Agre P 1993 Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 90:7275–7279
6. Folkesson HG, Matthay MA, Hasegawa H, Kheradmand F, Verkman AS 1994 Transcellular water transport in lung alveolar epithelium through mercury-sensitive water channels. Proc Natl Acad Sci USA 91:4970–4974
7. Folkesson HG, Matthay MA, Frigeri A, Verkman AS 1996 High transcellular water permeability in microperfused distal airways: evidence for channel-mediated water transport. J Clin Invest 97:664–671
8. King LS, Nielsen S, Agre P 1997 Aquaporins in complex tissues: I. Developmental patterns in respiratory and glandular tissues of rat Am J Physiol 273:C1541–C1548
9. King LS, Nielsen S, Agre P 1996 Aquaporin-1 water channel protein in lung: ontogeny, steroid-induced expression, and distribution in rat. J Clin Invest 97:2183–2191
10. Umenishi F, Carter EP, Yang B, Oliver B, Matthay MA, Verkman AS 1996 Sharp increase in rat lung water channel expression in the perinatal period. Am J Respir Cell Mol Biol 15:673–679
11. Carter EP, Umenishi F, Matthay MA, Verkman AS 1997 Developmental changes in water permeability across the alveolar barrier in perinatal rabbit lung. J Clin Invest 100:1071–1078
12. Berényi E, Szendrõ Zs, Rózsahegyi P, Bogner P, Sulyok E 1996 Postnatal changes in water content and proton magnetic resonance relaxation times in newborn rabbit tissues. Pediatr Res 39:1091–1098
13. Fullerton GD 1992 Physiologic basis of magnetic relaxation. In: Stark DD, Bradley WG (eds) Magnetic Resonance Imaging. Mosby Year Books, St. Louis, pp 88–108
14. Comper WD, Laurent TC 1978 Physiological function of connective tissue polysaccharides. Physiol Rev 58:269–281
15. Granger HJ 1981 Physiological properties of the extracellular matrix. In: Hargens AR (ed) Tissue Fluid Pressure and Composition. Williams and Wilkins, Baltimore, pp 43–61
16. Lamberg SI, Stoolmiller AC 1974 Glycosaminoglycans: a biochemical and clinical review. J Invest Dermatol 63:433–449
17. Wiederhielm CA, Fox JR, Lee DR 1976 Ground substance mucopolysaccharides and plasma proteins: their role in capillary water balance. Am J Physiol 230:1121–1125
18. Laurent UBG, Tengblad A 1980 Determination of hyaluronate in biological samples by a specific radioassay technique. Anal Biochem 109:386–394
19. Mulkern RV, Bleier AR, Adzamil IK, Spencer RGS, Sándor T, Jolesz FA 1989 Two-site exchange revisited: a new method for extracting exchange parameters in biological systems. Biophys J 55:221–232
20. Hazlewood CF, Chang DC, Nichols BL, Woessner DE 1974 Nuclear magnetic resonance transverse relaxation times of water protons in skeletal muscle. Biophys J 14:583–606
21. Cole WC, LeBlanc DA, Jhingran SG 1993 The origin of bioexponential T_2 relaxation in muscle water. Magn Reson Med 29:19–24
22. Shioya S, Haida M, Fukuzati M, Ono Y, Tsuda M, Ohta Y, Yamabayashi H 1990 A 1-year time course study of the relaxation times and histology for irradiated rat lungs. Magn Reson Med 14:358–368
23. Shioya S, Haida M Tsuji C, Ohta Y, Yamabayashi H, Fukuzaki M, Kimula Y 1988 Acute and repair stage characteristics of magnetic resonance relaxation times in oxygen-induced pulmonary edema. Magn Reson Med 8:450–459
24. Shioya S, Haida M, Tsuji C, Ono Y, Miyairi A, Fukuzaki M, Ohta Y, Yamabayashi H 1990 T_2 of endotoxin lung injury with and without methylprednisolone treatment. Magn Reson Med 15:201–210
25. Shioya S, Tsuji C, Fukuzaki M, Haida M, Tanigaki T, Kurita D, Ohta Y, Yamabayashi H 1996 Magnetic resonance relaxation times in acute hydrostatic pulmonary edema induced by noradrenaline in rats. Lung 174:235–241

26. Lorenzo AV, Jolesz FA, Wallman JK, Ruenzel PW 1989 Proton magnetic resonance studies of triethyltin-induced edema during perinatal brain development in rabbits. J Neurosurg 70:432–440

27. Miura N, Shioya S, Kurita D, Shigematsu T, Mashimo S 1999 Time domain reflectometry: measurement of free water in normal lung and pulmonary edema. Am J Physiol 276:L207–L212

28. Berényi E, Repa I, Bogner P, Dóczi T, Sulyok E 1998 Water content and proton magnetic resonance relaxation times of the brain in newborn rabbits. Pediatr Res 43:421–425

29. Shioya S, Haidi M, Fukuzaki M, Kurita D, Tsuji C, Ohta YM, Yamabayashi H 1997 Nuclear magnetic resonance study of lung water compartments in the rat. Am J Physiol 272:L772–L778

30. Hällgren R, Samuelsson T, Laurent TC, Modig J 1989 Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome. Am Rev Respir Dis 139:682–687

31. Bhattacharya J, Cruz T, Bhattacharya S, Bray BA 1989 Hyaluronan affects extravascular water in lungs of unanesthetized rabbits. J Appl Physiol 66:2595–2599

32. Nettelbladt O, Tengblad A, Hällgren R 1989 Lung accumulation of hyaluronan parallels pulmonary edema in experimental alveolitis. Am J Physiol 257:L379–L384

33. Allen SJ, Sedin EG, Jonzon A, Wells AF, Laurent TC 1991 Lung hyaluronan during development: a quantitative and morphological study. Am J Physiol 260:H1449–H1454

34. Sedin G, Gerdin B, Johnsson H, Jonzon A, Eriksson L, Hällgren R 1994 Hyaluronan and water content in the lung of preterm and term infants who died less than 24 hours after birth. Pediatr Res 36:37A(abstr)

35. Juul SE, Kinsella MG, Jakson JC, Truog WE, Standaert TA, Hodson WA 1994 Changes in hyaluronan deposition during early respiratory distress syndrome in premature monkeys. Pediatr Res 35:238–243

36. Juul SE, Kinsella MG, Wight TN, Hodson WA 1993 Alterations in non-human primate (*M. nemestrina*) lung proteoglycans during normal development and acute hyaline membrane disease. Am J Respir Cell Mol Biol 8:299–310

37. Johnsson H, Eriksson L, Jonzon A, Laurent TC, Sedin G 1998 Lung hyaluronan and water content in preterm and term rabbit pups exposed to oxygen or air. Pediatr Res 44:716–722

38. Dahl LB, Dahl IMS, Borresen A-L 1986 The molecular weight of sodium hyaluronate in amniotic fluid. Biochem Med Metab Biol 35:219–226

39. Ling GN 1992 A Revolution in the Physiology of the Living Cell. Krieger Publishing, Malabar, FL, pp 69–110

40. Ginetzinsky AG 1958 Role of hyaluronidase in the reabsorption of water in renal tubules: the mechanism of action of antidiuretic hormone. Nature 182:1218–1219

41. Göransson V, Odlind C, Hällgren R, Hansell P 1998 Physiological role of hyaluronan in renal papillary water handling? Eur J Physiol 435(Suppl):R213 (Abstract)

42. Xu J, Liu M, Post M 1999 Differential regulation of extracellular matrix molecules by mechanical strain of fetal lung cells. Am J Physiol 276:L728–L735