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Albumin fetus
fetal pulmonary fluid neonate

Lamb Fetal Pulmonary Fluid. I. Validation and Significance of Method for Determination of Volume and Volume Change

EMILE M. SCARPELLI,[40] SALVATORE CONDORELLI, AND ERMELANDO V. COSMI

WITH THE TECHNICAL ASSISTANCE OF FRANKLIN A. TAYLOR, JR.

Pulmonary Division, Department of Pediatrics, and Department of Anesthesiology, Albert Einstein College of Medicine, Bronx, New York, USA, and the University of Rome, Rome, Italy

Extract

An indicator-dilution method is described for measuring fetal pulmonary fluid, FPF, volume (Ve), and volume change with time ($\dot{V}s$) in the lamb fetus *in utero*. The indicator, albumin, which is the predominant protein of normal FPF, was administered directly to FPF in the form of radio-iodinated human serum albumin (RISA). We have shown that (*1*) RISA is disbributed homogeneously throughout the FPF compartment, (*2*) it is not altered within FPF, (*3*) it does not alter the functional characteristics of FPF, and (*4*) it remains within the FPF compartment throughout the period of measurement. Ve varies directly with the weight of the fetus so that Ve per kilogram was 31.6–35.6 ml/kg in four of five fetuses. The Ve per kilogram was lower in one fetus at the start of the experiment but increased rapidly to 29.0 ml/kg within 45 min. Thus the relaxation volume of the fetus *in utero* is in the range of functional residual capacity (FRC) and thoracic gas volume of the air-breathing neonate, which indicates that the pulmonary transformation at birth is, in essence, an isovolumic change in state wherein the fetal liquid-lung becomes the neonatal air-lung. The rate at which FPF is formed ($\dot{V}s$) is about 1.5 ml/hr·kg; however, the rate may change in so far as both rapid increases and rapid decreases of FPF volume were observed. When breathing movements were induced in the fetus by stimulation of the sciatic nerve, FPF volume decreased rapidly ($\dot{V}s$ = –3.0 ml/hr·kg), which indicates that negative intrathoracic pressures promote its resorption. When FPF volume fell transiently or was low initially, there was subsequent rapid restoration of volume to the range of anticipated FRC.

Speculation

Validation of a reliable method for measuring Ve and $\dot{V}s$ of FPF *in utero* provides a basis for study of molecular transformations within this compartment during gestation, *e.g.*, our investigation of the fate of FPF phosphatidylcholine in the accompanying report. Since FPF is the analog of the alveolar lining layer of the air-breathing animal, studies of the former (FPF) may give insight into the fluid and molecular dynamics of the latter which, to date, have been quite elusive because the lining layer cannot be sampled directly. Our observation that negative intrathoracic pressure alone seems to promote resorption of FPF under the conditions of our experiments suggests that this may be an important mechanism for the formation of the air-lung at birth. Since relaxation volume of the fetus is practically the same as that of the neonate, we have proposed that alveolar surface tension of the neonate is close to 0.

The liquid that fills the lungs of mammalian fetuses (7, 17, 20, 28), fetal pulmonary fluid (FPF), is produced locally (2). Its chemical profile is qualitatively and/or quantitatively different from that of other liquid compartments of the fetus (2, 3, 11), although certain reported similarities to amniotic fluid suggested (21, 22) the possibility of "amniotic fluid tests of lung maturation" (8, 13). Fetal pulmonary fluid contains components of the pulmonary surfactant system that are products of pulmonary synthesis and secretion (21). The fate of these components has not been documented, whereas it has been suggested (22) that they may take part in the formation of the alveolar lining layer at birth. Fetal pulmonary fluid is resorbed at birth (4, 6, 16), as the liquid-lung of the fetus becomes the air-lung of the neonate.

The present report describes a method for measuring FPF volume and rate of volume change *in situ* by means of a radio-iodinated serum albumin ([^{131}I]RISA) dilution technique in which the fetus is not removed from his natural environment. Others have measured FPF volume in the exteriorized fetus using an inulin dilution technique (19). We have chosen albumin because it is a normal constituent of FPF (21). In the present paper the [^{131}I]RISA dilution method is validated, FPF volume determinations are presented, and their significance is discussed. The fate of FPF phosphatidylcholine, determined on the basis of FPF dilution, is described in the accompanying report (26).

METHODS

Anesthesia was induced in three pregnant ewes at or near term (>135 days gestation) with Na pentobarbital, 15 mg/kg. Anesthesia was induced in a fourth ewe (>135 days gestation) with Na Pentothal, 15 mg/kg. The ewes were tracheostomized, placed on a volume-controlled ventilator (30), and maintained with N_2O:O_2 75:25 throughout the experiments. Arterial blood was maintained at a P_{O_2} of 85–100 mm Hg, P_{CO_2} of 35–45 mm Hg, and pH of 7.38–7.42.

PREPARATION OF FETUS

The uterus was exposed transabdominally and the neck of the fetus was marsupialized to the uterine wall by methods reported previously (10). The fetal trachea was exposed through a longitudinal neck incision and a glass cannula was inserted at the level of the second or third cartilagenous ring. FPF spontaneously filled the dead space of the cannula which was then closed with a three-way stopcock. Catheters were advanced to the ascending aorta (AAo) from the left carotid artery and to the right atrium (RA) from the jugular vein. (Catheter position was verified at postmortem examination.)

The procedure took less that 15 min for each fetus. Five fetuses were prepared in this manner including twins, *fetuses 1* and *2*, and two singletons, *fetuses 3* and *4*, of pentobarbital-induced ewes, and one of twins, *fetus 5*, of the Pentothal-induced ewe. The left lower leg of *fetus 5* was delivered through the uterine wall as preparation of the trachea was being attended by another operator. The sciatic nerve was exposed, severed, and covered with paraffinated gauze.

[^{131}I]RISA DILUTION

Human [^{131}I]RISA (hereafter written RISA) (31), 1–2 μCi, in 1.0 ml 0.15 M NaCl, was injected into the tracheal cannula (32). The RISA was mixed with FPF by moving 20 ml FPF back and forth between syringe and lung at a rate of 20 cycles/min. Thus, except for the 1.0 ml of RISA in saline, FPF volume was not altered by the mixing procedure itself. Adequate clearance of anatomical dead space was insured since 20 ml approximated the anticipated tidal volume. Intrapulmonary pressure fluctuations were less than 5 cm H_2O during mixing. Transfer of FPF between lung and syringe was done continuously in *fetuses 1* and *2*, for 10 min out of every 15 min in *fetus 3*, and for 5 min out of every 15 min in *fetuses 4* and *5*. Samples of FPF, 1.0–3.5 ml, and of RA and AAo blood, 1.0 ml each, were obtained at 3, 15, 30, and 45 min after administration of RISA. Additional samples were taken at 60 and 75 min (*fetus 4*), at 60, 75, and 90 min (*fetus 5*), and at 60 and 90 min (*fetuses 1* and *2*).

STABILITY OF RISA

In order to verify molecular integrity and stability of RISA in FPF during the course of the experiment, aliquots of RISA from the original injectate and aliquots of the 90-min FPF samples were separated by polyacrylamide disc electrophoresis, 7.0% gel, by methods described previously (25). The FPF gels revealed several protein bands (Fig. 1) as anticipated from previous studies in our laboratory (21), with albumin the largest band. The albumin band, the proteins of the separation gel (excluding albumin), and the spacer gel were isolated and prepared for radioisotope survey.

DISTRIBUTION OF RISA

Fetus 5 was killed *in utero* with an overdose of Na pentobarbital after the 90-min FPF sample was taken and the main bronchus to each lobe was tied. Each lobe was then severed with FPF intact and weighed. Several pieces from each lobe were cut directly into vials and weighed in the vials before radioisotope survey.

SURVEY FOR RADIOACTIVITY

Duplicate aliquots of FPF, plasma, and RISA injectate were pipetted directly into a 5-ml tube to which 2 ml 0.15 M NaCl were added. The tubes were placed in plastic vials. The pulmonary tissue from each lobe of *fetus 5* was counted directly. The γ activity (33) was expressed either as counts per minute per milliliter (FPF and plasma) or counts per minute per gram of pulmonary tissue. Bands from polyacrylamide gels were homogenized, placed in plastic vials, and counted.

EFFECT OF BREATHING *IN UTERO*

After 45 min of RISA dilution, the central end of the sciatic nerve of *fetus 5* was stimulated repetitively for 3 min (1 impulse/sec, 0.6 msec, 6 V). These stimuli initiated regular respiratory movements and for the last minute of stimulation fetal breathing was synchronous with the sciatic stimuli, *i.e.*, 1 breath/sec. Thereafter the spontaneous respiratory movements of the fetus, which were intermittent, continued until the end of the RISA dilution experiment at 90 min. All fetal respirations were made against a closed trachea, with the tracheal cannula connected to a transducer. Tracheal pressure and sciatic stimuli were recorded on a six-channel polygraph (34).

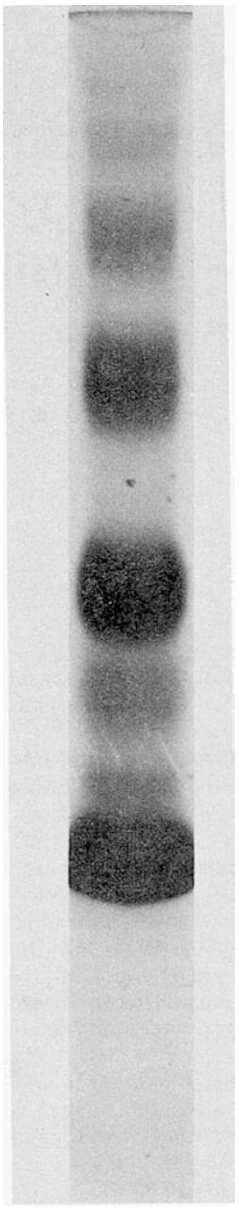

Fig. 1. Polyacrylamide gel electrophoresis of fetal pulmonary fluid into which radio-iodinated human serum albumin had been injected. The large band at the front (bottom of gel) is albumin; the other, slower moving large dense band migrates like transferrin.

ASSESSMENT OF RISA TRANSFER AFTER BIRTH

Fetuses 1, 2, and *3* were delivered upon completion of the RISA dilution studies and blood samples were taken 30 and 60 min after the onset of breathing. Because of the pentobarbital anesthesia, controlled ventilation was required for *fetus 1* using a Bird Mark VII ventilator (frequency = 22/min; end-inspiratory pressure = 25 cm H_2O). *Fetuses 3* and *4* breathed spontaneously at 70–75 breaths/min.

RESULTS

A general description of the fetuses and a summary of our findings are given in Table 1.

Table 1[1]

Fetus	Body wt, kg	Ve, ml	Ve/kg	V̇s, ml/hr	V̇s/kg	Condition
1	3.2	112.0	35.0	4.0	1.25	Quiet
2	3.2	114.0	35.6	5.8	1.81	Quiet
3	3.0	74.0	24.7	16.5	5.50	Quiet
4	2.8	96.0	34.3	3.5	1.25	Quiet
5	4.0	126.5	31.6	6.0	1.50	Quiet
2	3.2	118.0 → 111.0	36.9 → 34.7	−28.0	−8.75	? Cause (45 → 60 min)
2	3.2	111.0 → 118.0	34.7 → 36.9	14.0	4.38	"Recovery" (60 → 90 min)
3	3.0	74.0 → 87.0	24.7 → 29.0	16.5	5.5	"Recovery" (0 → 45 min)
5	4.0	126.5 → 121.8	31.6 → 30.5	−12.0	−3.0	Breathing (45 → 90 min)

[1] Ve: fetal pulmonary fluid (FPF) volume by extrapolation of Vs-time plot to zero time; V̇s: rate of change of FPF volume; arrows denote periods during which Vs increased or decreased (negative sign) most rapidly.

RISA DILUTION AND FPF VOLUME

FPF volume was calculated for each FPF sample by the formula: $Vs = \frac{I_A - S_A}{C_A}$ = milliliters, where Vs = calculated FPF volume at time sample was obtained; I_A = amount (counts per minute) of RISA injected initially; S_A = amount (counts per minute) of RISA removed in previous sample(s), if any; C_A = concentration of RISA (counts per minute per milliliter) in sample.

Equilibration of RISA distribution in FPF occurred 15–30 min after its initial administration into the lung. The rate of cycling or rinsing did not appear to affect this time. Equilibration was determined as the time at which the rate of change of RISA concentration or, as we shall express it here, the rate of change of calculated FPF volume (V̇s) became constant, *i.e.*, fit a straight line plot (Fig. 2). For *fetuses 2, 3,* and *5*, V̇s before 15 min fell below the linear plot and the same was true for V̇s of *fetuses 1* and *4* before 30 min. The linear plot was extrapolated to time zero (- - - in Fig. 2), and the point at which this intercepted the volume axis was read as the volume in milliliters of FPF (Ve) at the time of the initial injection of RISA. The slope, dV/dt or V̇s, provided an index of the rate of change of volume; a positive slope indicating net increase in volume and a negative slope indicating net decrease in volume (Figs. 2 and 3).

Ve ranged from 74.0 to 126.5 ml. When related to body weight, Ve ranged from 24.7 to 35.6 ml/kg (Table 1). Four of the fetuses (*fetuses 1, 2, 4,* and *5*) had Ve per kilogram of 31.6–35.6; *fetus 3* had the lowest Ve per kilogram, 24.7, which, however, increased rapidly to 29.0 in 45 min.

The initial V̇s of *fetuses 1, 2, 4,* and *5* ranged from 3.5 to 6.0 ml/hr. When related to body weight, V̇s per kilogram ranged from 1.25 to 1.81 ml/hr·kg in these fetuses (Table 1). *Fetus 3,* which had the highest Vs per kilogram (5.5 ml/hr·kg), was the fetus with the lowest initial Ve per kilogram (24.7). In *fetus 5,* V̇s per kilogram became negative (−3.0 ml/hr·kg) after 45 min, indicating loss of FPF from the lung. This coincided with the onset of regular breathing movements against the closed trachea. The V̇s per kilogram also became negative (−8.75 ml/hr·kg) in *fetus 2* after 45 min; however, it resumed a positive but much higher slope (4.38 ml/hr·kg) from 60 to 90 min.

CORRELATIVE STUDIES

Radioactivity of the FPF-filled lobes of *fetus 5,* surveyed at the end of the experiment, revealed that of the total radioactivity in the lung, expressed as counts per minute per gram, 19.3% was in the right upper lobe, 20.4% in the right middle lobe, 20.4% in the right lower lobe, 19.7% in the left upper lobe, and 20.2% in the left lower lobe.

None of the blood samples, including those from RA and AAo, contained γ activity. Even blood samples obtained up to 60 min after delivery contained no detectable RISA activity.

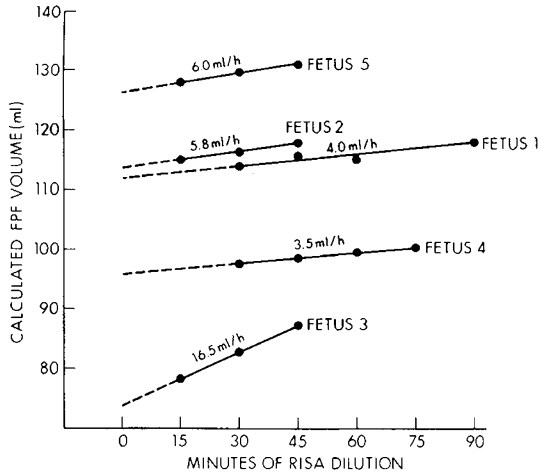

Fig. 2. Broken lines (- - -) are extrapolations to zero time; the fetal pulmonary fluid volume that was actually calculated during these periods fell below the broken lines (see text). *RISA*: radio-iodinated human serum albumin.

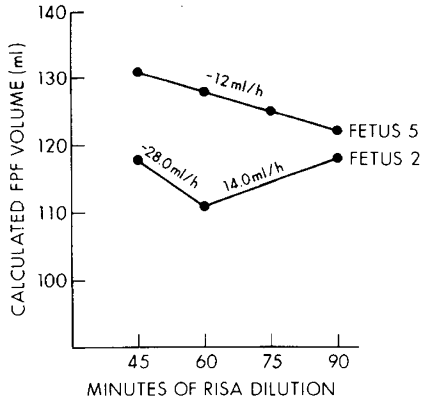

Fig. 3. Periods during which V̇s was greatest. *FPF*: fetal pulmonary fluid; *RISA*: radio-iodinated human serum albumin.

Survey for radioactivity in the albumin band, the proteins of the separation gel (which included other proteins of FPF; see Fig. 1), and the spacer gel from disc electrophoresis of FPF containing RISA revealed over 90% of the activity in the albumin band, less than 9% primarily in the transferrin-like protein of the separation gel, and a trace in the spacer gel. Over 95% of the total activity was contained within the albumin band of the RISA injectate. RISA contained a second

protein, a faint band with the electrophoretic mobility of the transferrin-like protein of FPF (Fig. 1). Less than 4% of the γ activity was in this band.

DISCUSSION

To our knowledge this is the first report of the measurement of both FPF volume and rate of change of volume in the fetal lamb *in utero*. Our fetuses were at or near term, with trachea cannulated and closed to the exterior. The indicator used, albumin, is normally the major protein of FPF (21).

COMMENT ON METHOD

For an indicator-dilution technique to be reliable, certain criteria must be met.

1. The indicator must be distributed evenly throughout the liquid compartment. Our finding that each lobe of the liquid-filled lung contained virtually the same amount of RISA activity per unit weight (*fetus 5*) demonstrates that the distribution of RISA was indeed homogeneous. Thus we may assume that all liquid-filled segments of the lung, which were in communication with the trachea, received a properly proportioned share of the indicator. The apparent time required to achieve optimal distribution varied from 15 to 30 min among the fetuses, whereas the rate at which syringe-mixing was performed, *i.e.*, continuously or 5–10 min out of every 15 min, did not seem to be important.

2. The indicator must not be altered, metabolically or otherwise, within the liquid compartment. The distribution of radioactivity, as determined by surveying the proteins separated by disc gel electrophoresis, was the same in both the RISA injectate and in the proteins of FPF after 90 min, *i.e.*, over 90% was in the protein with the electrophoretic mobility of albumin and less than 9% was in the transferrinlike protein. RISA distribution was the same in those experiments in which protein-phosphatidylcholine was also given (32). According to these studies, therefore, both ^{131}I binding and the proteins had not been altered after RISA was dispersed in FPF.

3. The indicator must not alter the functional characteristics of the liquid compartment. The amount of protein added to FPF as RISA, *i.e.*, 20–40 μg (31), was minimal with respect to the overall protein content of FPF. Normally, FPF protein concentration is greater than 200 μg/ml in the term fetus (personal observations and Reference 3), so that our subjects' FPF protein content was greater than 14.8–25.3 mg to begin with. In the studies in which protein-complexed phosphatidylcholine (32) was administered simultaneously with RISA, the additional 50 mg protein should not have influenced osmolality or osmotic pressure to any significant degree. Thus, it may be calculated that FPF osmolality was increased by about 0.01 mOsmol/liter, giving an increase of less than 0.2 mm Hg osmotic pressure. The effect of these changes on liquid transfer during the 90 min of observation would be negligible.

4. The indicator must remain within the liquid compartment throughout the period of measurement. Our repeated surveys of fetal blood demonstrate that none of the RISA had entered the circulation. Thus, we may be reasonably sure that RISA was not transferred into the lymphatics or capillaries of the lung. Even after 60 min of air-breathing, *i.e.*, after birth, RISA could not be detected in the circulation. However, there is the possibility that RISA may have been taken up by alveolar epithelial cells or that it otherwise entered the pulmonary interstices. We did not look into this possibility directly, but reference to the experience of others indicates that during the 90-min period of observation this was not a complicating factor. Thus, it has been reported that uptake of intralumenal horseradish peroxidase (mol wt 40,000) by pulmonary epithelial cells of rabbits at the onset of breathing is "notoriously sluggish" (14). These same studies demonstrated that interstitial uptake of horseradish peroxidase could be detected by electron microscopy in only one of four neonates after 90 min and in none before that time. In addition, it was reported that horseradish peroxidase was only rarely incorporated into transport vacuoles of type I cells. Others have also reported that the lumenal surface of alveolar epithelium of neonatal mice is impermeable to horseradish peroxidase (27). Conversely, horseradish peroxidase administered into the circulation of neonatal mice passes through interendothelial clefts of the capillaries and diffuses into the interstitium (27). The impermeability of fetal alveolar epithelium to circulating albumin is suggested from experiments (2) in which RISA was injected into the blood of fetal lambs and was not recovered in FPF. Thus it appears that relative impermeability of the perinatal lung is established at the free or lumenal surface of the alveolar epithelium and that transfer of large molecules from this surface is a slow process that should not have affected the RISA-dilution experiments of the present study. The fact that we observed increasing as well as a decreasing RISA concentrations with time is in accord with this conclusion.

FPF VOLUME (Ve)

Using an inulin dilution method, Normand *et al.* (19) have reported that the mean FPF volume of the exteriorized fetal lamb is 30 ml/kg and that the mean rate of formation of FPF is about 2.2 ml/hr·kg. In a study of lamb fetuses *in situ* (5), in which either a tracheal cannula was exteriorized for collection of FPF or an artificial tracheal loop was formed from which flow was monitored with an electromagnetic flow probe, the mean flow of FPF was reported to be 9.4 ml/hr (exteriorized cannula) and 6.6 ml/hr (tracheal loop). By intermittent emptying of a rubber container attached to the fetal trachea *in situ*, Enhörning and Adams (12) reported FPF flow to range from 1.56 to 7.8 ml/hr·kg. The latter methods, however, did not permit quantification of FPF volume.

The Ve per kilogram as determined in the present experiments ranged from 31.6 to 35.6 ml/kg in four of the five fetuses. If comparisons may be made with the newborn infant whose average FRC is 30 ml/kg and thoracic gas volume is 36 ml/kg (18), we may say that the lung of the lamb fetus *in situ* contains a volume of liquid between these two. Thus, the idea that lung volume at FRC of the neonate is markedly greater than lung volume of the term fetus *in utero* must be reconsidered. Indeed, our studies indicate that the lung volumes are about the same and, therefore, that the pulmonary essence of birth is an isovolumic change in state, that is, from liquid-lung to air-lung (36). The one fetus whose Ve was somewhat below this range at 24.7 ml/kg (perhaps because of expulsion of FPF before tracheal cannulation), gained fluid most rapidly ($\dot{V}s$ = 16.5 ml/hr) and after 45 min had increased the volume of FPF to 29.0 ml/kg.

Our findings indicate that the relaxation volume of the lung of the fetus *in situ* is at or only slightly above the relaxation volume, or FRC, of the neonate. In addition, whereas intrapleural pressure at FRC in the fetus is virtually atmospheric, it is slightly below atmospheric pressure (about -1.5 to -2.0 cm H_2O) in the early neonate (7). Thus, at comparable lung volumes, the air-lung-throax system of the neonate produces a net retractive force 1.5–2.0 cm H_2O greater than that of the liquid-lung-thorax system of the fetus. Negative intrapleural pressure at relaxation volume at birth could be caused by decreased lung and/or thoracic cage compliance. It has been shown that compliance of the thorax tends to decrease with age after birth, whereas pulmonary compliance tends to increase, so that the principal reason for increasing subatmospheric intrapleural pressure is the change in chest wall compliance (7). However, another factor must be

considered, *i.e.,* surface tension: the liquid-lung of the fetus, because it has no air-liquid interface, has virtually 0 surface tension in the alveoli (29). With the establishment of an air-lung at birth, interfacial forces may become operative. If the assumption is made that the retractive force at relaxation volume in the neonate is caused by surface tension, one may estimate that surface tension is very low, *e.g.,* 3.75–5 dynes/cm (35). In fact, however, surface tension must be even lower, *i.e.,* to the extent to which thoracic cage retraction influences intrapleural pressure. These considerations support previous suggestions that surface tension at the air-hypophase boundary of the avleoli is near 0 (9, 23).

FPF VOLUME CHANGES ($\dot{V}s$)

Our studies indicate that FPF is formed normally at a rate of about 1.5 ml/hr·kg (range = 1.25–1.81 ml/hr·kg), which is close to the mean figure of 2.2 ml/hr·kg reported by Normand *et al.* (19) and to earlier estimates of mean $\dot{V}s$ (15, 21). A new finding here is that FPF volume may increase and also decrease at very rapid rates under certain circumstances. We did not attempt to determine the mechanisms responsible for these large positive and negative $\dot{V}s$, but certain observations may be significant.

1. The FPF volume decreased rapidly, −3.0 ml/hr·kg, when regular, spontaneous breathing movements against a closed tracheal outlet were induced in *fetus 5* by stimulation of the sciatic nerve (37). During inspiration, both intrapleural and intrapulmonary pressures were negative (up to −40 cm H_2O), so that venous flow to the heart and possibly also pulmonary lymph flow may have increased. It is of interest that, had the negative $\dot{V}s$ been sustained, all of the FPF would have been resorbed in about 10 hr. Thus, high negative intrathoracic pressure, as might be expected at birth, is associated with the removal of liquid from the lung even in the absence of air breathing, and therefore without the consequent changes in $Paco_2$ and Pao_2.

2. The FPF volume decreased in another fetus, *fetus 2,* over a 15-min period at a rate of −8.75 ml/hr·kg. The reason for this is not known (breathing was not recorded) but the observation reinforces the conclusion that FPF volume may fall transiently *in utero,* albeit there is a net increase with time. Similar periods of rapid fall may occur normally, for example when FPF is expelled periodically from the trachea into the posterior pharynx, as has been demonstrated in the intact lamb fetus *in situ* (1). As in *fetus 2,* this may be followed by a "recovery" period in which FPF volume increases at a rate that is very much higher than normal.

3. The FPF volume increased most rapidly, 5.5 ml/hr·kg, in *fetus 3,* whose Ve was the lowest to begin with (24.7 ml/kg). Possibly this was because of establishment of communication with new pulmonary segments with time, so that the measured increases in volume were only apparent, or perhaps this represented "recovery" from a previous episode in which FPF had been reduced. We have no answer at this time, but would point out that after 45 min, FPF volume was at the level of FRC (29.0 ml/kg).

SUMMARY

The RISA dilution method for the measurement of FPF volume and volume change with time has been validated in studies on the term lamb fetus *in utero.* We have shown that FPF volume varies directly with the weight of the fetus and that it ranged from 31.6–35.6 ml/kg in the five fetuses studied. This indicates that the relaxation volume of the liquid-filled lung of the fetus is of the same order as the functional residual capacity and thoracic gas volume of the air-lung of the neonate. The rate at which FPF is formed is about 1.5 ml/hr·kg; however, when the fetus breathed *in utero,* FPF volume decreased rapidly and after the fall increased at a more rapid rate than usual. The RISA dilution method may be used as the basis for study of molecular transformations within the FPF compartment as reported in the accompanying paper (26).

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30. Air Shields, Hatboro, Pa.
31. Albumotope (radio-iodinated (^{131}I)human serum albumin), 0.05 mCi/mg; Travelon Lab., Inc., Costa Mesa, Calif.
32. [*U*-^{14}C]Phosphatidylcholine complexed with 5% protein was injected simultaneously with RISA into FPF of *fetuses 1, 2,* and *3* (see Reference 25).
33. Automatic Gamma counter system, model 1085, Nuclear Chicago, Des Plaines, Ill.
34. Physiograph Six-B, Narco-Bio Systems, Houston, Tex.
35. $P = \frac{(2)(\gamma)}{(r)}$ (10), where γ = surface tension in dynes per centimeter; P = retractive pressure due to γ (assumed to be 1.5–2.0 cm H_2O); r = mean alveolar radius (assumed to be 50 μm). Substituting in equation gives γ = 3.75–5.0 dynes/cm.
36. We have also observed a transient state which begins at the onset of breathing and in which foam is present in the airways and presumably also in the periphery of the lung. Mixture of air with FPF would expectedly produce foam. Thus the transitional state between liquid-lung and air-lung is the stage of the "foam-lung."
37. We have been able to produce regular breathing movements in the fetus consistently by stimulation of the sciatic nerve. This is the topic of a separate study in our laboratory.
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40. Requests for reprints should be addressed to: E. M. Scarpelli, M.D., Department of Pediatrics, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, N.Y. 10461 (USA).
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Alveoli
fetal pulmonary fluid
fetus
lung
phosphatidylcholine clearance

Lamb Fetal Pulmonary Fluid. II. Fate of Phosphatidylcholine

EMILE M. SCARPELLI,[39] SALVATORE CONDORELLI, GIUSEPPE COLACICCO, AND ERMELANDO V. COSMI

WITH THE TECHNICAL ASSISTANCE OF FRANKLIN A. TAYLOR, JR., AND MARY LOUISE SKOVRON

Pulmonary Division, Department of Pediatrics, and Department of Anesthesiology, Albert Einstein College of Medicine, Bronx, New York, USA, and the University of Rome, Rome, Italy

Extract

Using the radio-iodinated human serum albumin ([^{131}I]-RISA) dilution method to measure lamb fetal pulmonary fluid (FPF) volume, we followed the disappearance of protein-complexed, ^{14}C-labeled phosphatidylcholine ([^{14}C]PC) during the first 90 min after its injection into FPF. The FPF samples were analyzed for total lipid ^{14}C activity and for distribution of ^{14}C in PC, other phospholipids (PL), fatty acids (FA), and neutral lipids (NL). For most sampling periods ascending aortic (AAo) and right atrial (RA) blood samples were obtained simultaneously with FPF and serum was analyzed for total lipid ^{14}C activity and for distribution of ^{14}C in total PL, FA, and NL. These studies indicate that (*1*) PC is cleared rapidly from FPF with an estimated half-time of 15–57 min; (*2*) FPF-PC may be metabolized to lyso-PC and FA within the fluid itself; and (*3*) FA derived from FPF-PC enter the pulmonary circulation, thus establishing a pulmonary arteriovenous FA gradient. The possible sites at which PC may be cleared from FPF are considered.

Speculation

The novel possibility is suggested that FPF contains appropriate enzymes (phospholipase(s)) for deacylation of PC and also that PC-degradative enzymes are active at the surface of the alveolar epithelial cells. By comparison with results of others regarding the half-life of PC in the air-lung, it appears that PC clearance outside the cell (*i.e.,* after secretion) occupies a relatively short period in the turnover of the molecule. Since the products of PC degradation appear in arterial blood as FA primarily, we may consider FPF as a possible source of serum FA.

Phospholipids, including surface-active phospholipids, are synthesized by the fetal lung and secreted into the FPF (19). With FPF they are expelled periodically from the lung of the lamb fetus and are swallowed (1). Whether or not they also enter the amniotic fluid compartment and add in a significant way to its phospholipid content is a moot question which, with regard to the lamb fetus, has received a negative answer (5). The metabolic fate of FPF phospholipids, of which phosphatidylcholine (PC) is the major component (2, 19), is not known. Whereas pulmonary PC of adult animals has been shown to have a half-life of about 12–14 hr (27, 28), the pathway(s) by which PC is degraded or removed from the alveoli have not been determined.