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Properties of lavage material from excised lungs ventilated at different temperatures

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Abstract

We studied the phospholipid (PL) and protein contents, the PL composition, and some of the surface properties of lavage materials obtained from freshly excised rat lungs and excised lungs which had been ventilated at different temperatures (22, 37, and 42° C). Ventilation (60 breaths/min) was carried out at constant tidal volume with periodic sighs for one hour. Although there is slightly more lavageable PL and protein in lungs ventilated at 22° C than in freshly excised lungs, there is no difference in the PL composition or surface properties of lavage materials from these lungs. However, as the temperature at which lungs are ventilated is increased to 37° and 42° C, there is(are): 1) a reduction in lavage fluid PL, 2) a reduction in the relative amounts of total phosphatidylcholines (PC) and disaturated PC (DSPC), the major surface active component of pulmonary surfactant, 3) an increase in unsaturated PC, and 4) increases in total protein and nonsedimentable protein (100,000 *g*; 2 hr) in the lavage materials. There are also differences in the surface properties of the lavage materials from lungs ventilated at higher temperatures when compared with freshly excised lungs or lungs ventilated at 22° C, probably as a result of the changes in composition. Maximal surface tension is greater for lavage materials from lungs ventilated at 37° C. For lungs ventilated at 42° C, maximal and minimal surface tension values are increased. These results demonstrate that there are differences in the composition and surface properties of alveolar lavage materials from excised lungs ventilated at different temperatures.

Keywords: Mammals; (rat); Surfactant; (pulmonary; composition); Temperature; (pulmonary surfactant)

1. Introduction

Several different investigators have reported that the mechanical properties of excised lungs are dependent upon the temperature at which the studies are performed. For example, pressure-volume (P-V) curves are influenced by temperature. Clements and Trahan (1963) reported that the hysteresis of P-V loops in excised rat lungs is reduced with increasing temperature (up to 49° C). In contrast, Lempert and Macklem (1971) demonstrated increased hysteresis of P-V loops with increasing temperature (between 21° and 37° C) in excised rabbit lungs. Nagao et al. (1977) reported similar results with rabbit lungs; i.e., with decreasing temperature (between 21° and 4° C) there was a reduction in total lung capacity and an increase in recoil pressure at 80% of total lung capacity during lung inflation, leading to a decrease in hysteresis. It has also been reported by Frazer et al. (1982) that temperature affects gas trapping in

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excised rat lungs. In addition, other investigators have reported minor mechanical changes in response to temperature alterations (Gruenwald, 1964; Horie et al., 1974). Although these effects seem to be quite variable, the evidence does indicate that the mechanical properties of excised lungs are affected by temperature.

One very important determinant of the mechanical properties of lungs is pulmonary surfactant, a mixture of lipid, protein, and carbohydrate components which lines the alveoli and prevents their collapse by lowering surface tension forces. Recently, while working with excised rat lungs ventilated at different temperatures, we were surprised to find some alterations in the amount and composition of alveolar lavage materials. In our opinion, it may be important to describe these changes in surfactant for two reasons. First, these data may help in explaining why the mechanical properties of excised lungs are altered with changes in temperature. Second, there are occasions when investigators may want to assess the mechanical properties of excised lungs following treatment or exposure of the animals to various substances. It is possible that the temperature at which these measurements are made may influence the mechanical properties and/or the properties of pulmonary surfactant beyond those changes caused by treatment of the animals.

The objective of our experiments was to study the composition and some of the surface properties of alveolar lavage materials obtained from freshly excised rat lungs and from excised lungs which were ventilated for one hour at different temperatures (22°, 37°, and 42° C). The lungs were ventilated at constant tidal volume with periodic sighs. The specific measurements made include 1) the total amounts of lavage phospholipid and protein, 2) the distribution of some individual lavage phospholipids, and 3) some of the in vitro surface properties of the lavage materials. A preliminary report of these results has appeared previously (Miles et al., 1990).

2. Methods

2.1. Treatment of excised lungs

Male Sprague-Dawley rats (250–400 grams; Hilltop Laboratories, Scottdale, PA) were anesthetized with sodium pentobarbital (150 mg/kg body weight) and the heart and lungs were rapidly removed. The trachea and lungs were trimmed free of the heart and connective tissue and weighed. The lungs were divided into two groups, freshly excised and ventilated lungs. Alveolar lavage materials were obtained immediately, as described below, from the freshly excised lungs. For ventilated lungs the trachea was cannulated with polyethylene tubing (outer diameter = 2.42 mm, inner diameter = 1.67 mm), the tubing was connected to a 15-gauge needle which had been inserted into a rubber stopper, and the lungs were suspended in a glass plethysmograph which served as the incubation chamber. The bottom was covered with a layer of phosphate-buffered medium (145 mM NaCl, 5 mM KCl, 9.35 mM Na_2HPO_4, and 1.9 mM NaH_2PO_4; pH 7.4) to maintain the humidity. The lungs were ventilated for one hour during which time the glass plethysmographs were immersed in water baths maintained at 22°, 37°, or 42° C. The temperature of the air surrounding the lungs was monitored with a thermometer located very close to the lung surface and did not vary by more than ± 1° C of the desired temperature.

The lungs were mechanically ventilated (Harvard Apparatus Rodent Respirator; Model 681; South Natick, MA) at a rate of 60 breaths per min, with humidified air and with an end-expiratory pressure of +4 cm H_2O. The humidified air traveled through a coil immersed in the same water bath which housed the plethysmograph before entering the lungs, so that the air entering the lungs was approximately the same temperature as the lungs. Tracheal pressure was measured with a pressure transducer (Model 239E; Setra Systems, Inc., Natick, MA) and monitored with an oscilloscope. The tidal volume used for each experiment was determined from the nomogram published by Kleinman and Radford (1969). Tidal volume was measured by attaching the barrel of a syringe (10 cm^3), which had a liquid film centered between the ends, to the plethysmograph. Since there was no detectable curvature of the film surface, its displacement during an inflation-deflation cycle was equal to the volume of gas displaced from the plethysmograph, or tidal volume. The tidal volume for each experiment was set at the beginning of the experiment by adjusting the stroke volume of the ventilator while measuring the volume of gas dis-

placed from the plethysmograph. At 15 min intervals, the exhaust line of the ventilator was clamped until tracheal pressure reached 25 cm H_2O and then it was released. This maneuver was used to simulate a periodic sigh because it has been shown that ventilation without occasional deep breaths results in alteration of the alveolar lining layer (Thet et al., 1979).

2.2. Isolation of alveolar lavage materials

Alveolar lavage materials were obtained from the freshly excised lungs and from ventilated lungs following incubation at the appropriate temperature. Quantitative tracheal lavage was performed by a technique which we have used previously (Miles et al., 1986), i.e., three lavages (each with 5 ml per gram of lung weight) with an ice-cold HEPES-buffered solution containing 145 mM NaCl and 10 mM N-2-hydroxyethyl-piperazine-N′-2-ethane-sulfonic acid (HEPES), pH 7.4. (The lung weights do not change by more than 0.1 gram during incubation.) Alveolar macrophages were separated from the lavage materials by centrifugation at 300 *g* for five min. The cells were then washed three times and all supernatants were saved. The supernatants from the cell washes were spun at 12,000 *g* for 10 min and this pellet was resuspended in the lavage materials. This was done because we have found that 5–10% of the total amount of lavage phospholipids can be obtained by washing alveolar macrophages (Miles et al., 1986). The lavage materials were then saved (at 4° C) for analysis of composition and surface balance studies.

2.3. Measurements of phospholipid and protein composition

The total amount of phospholipid (PL), amounts of some individual phospholipids, and the protein content of alveolar lavage materials from freshly excised and from ventilated lungs maintained at different temperatures were measured. Some measurements were also made on samples of supernatants and pellets obtained from 100,000 *g* (two hours) centrifugations of alveolar lavage materials. The total amount of phospholipid was measured as the phosphorus present in lipid extracts (chloroform/methanol; 2:1, v/v) of the materials (Bartlett, 1959). Disaturated phosphatidylcholines (DSPC) were isolated from some samples according to the method of Mason et al. (1976) and measured as lipid phosphorus. Some individual phospholipids were identified by using two-dimensional thin-layer chromatography (Poorthuis et al., 1976). In all cases, the phospholipid content was obtained by multiplying lipid phosphorus values by 25 (Oyarzun and Clements, 1978). The protein contents of samples of whole lavage materials, lavage pellets, and lavage supernatants were determined by the method of Lowry et al. (1951) with 1% sodium dodecyl sulfate added to reduce interference by lipids (Lees and Paxman, 1972). The results were expressed as mg PL or protein per gram of lung.

In order to estimate protein contamination with serum proteins, we measured albumin in the 100,000 *g* (2 hr) pellets and supernatants of the alveolar lavage materials from freshly excised lungs and lungs ventilated at different temperatures. These measurements were made according to a method reported by Doumas et al. (1971). Briefly, the sample (0.1 ml) was added to 2 ml of 0.3 mM bromcresol green (BCG; pH 4.20; Sigma Chemical Co., St. Louis, MO), and the tube was gently inverted to mix the contents. The absorbance at 628 nm was then read with a spectrophotometer. Values were reported as mg of albumin by using different amounts of bovine serum albumin as standards.

2.4. Measurement of surface tension in vitro

The relationship between surface tension and surface area was determined for samples of alveolar lavage materials obtained from freshly excised lungs and from ventilated lungs maintained at different temperatures. Measurements were made with a Kimray Greenfield surfactometer (Kimray Medical Associates, Oklahoma City, OK) according to the method described by King and Clements (1972). The balance had a Teflon trough (length 11.5 cm, width 5 cm) with a tight-fitting Teflon barrier which allowed variation of the surface area. The actual measurements were made at a temperature of 22° C. In order to maintain a constant temperature, the balance was housed in a plexiglass chamber along with a radiator through which water of the appropriate temperature

was pumped. The chamber also contained brass wool to maintain temperature stability. Temperature was monitored with a thermistor probe located approximately two cm above the surface of the trough. In all cases, temperature of the air and subphase did not vary by more than 1° C in either direction of the desired level.

The most reproducible results were obtained by preparing the balance in the following manner. The trough was filled with 20 ml of HEPES-buffered medium which also contained 2.5 mM $CaCl_2$ and 0.5 mM EDTA. This medium was used for all experiments with the surface balance. Dipalmitoyl phosphatidylcholine (DPPC) was dissolved in chloroform-methanol (20:1, v/v) so that the final concentration was one mg/ml, and 10 μl of this solution was gently placed on the surface of the subphase. After 10 min, we compressed and spread the DPPC monolayer three or four times. The material was then aspirated from the balance and the trough was rinsed 2–3 times with distilled water. The balance was primed in this manner before each measurement was made. Although treatment of the balance walls with lanthanum, dipalmitoyl PC, and/or distearoyl PC has been used to prevent leakage of surface films in some instances (Hildebran et al., 1979; Goerke and Gonzales, 1981), we found no differences in our measurements when these treatments were used.

Lavage materials were prepared for study in the surface balance by vortexing samples in HEPES-buffered medium containing Ca^{2+} and EDTA. The final concentration of phospholipids in the balance was approximately 100 μg per ml for lavage materials, i.e., the same amount as in undiluted lavage fluid. Twenty ml of the vortexed sample was placed in the trough of the surface balance. In all samples the final concentrations of Ca^{2+} and EDTA were 2.5 mM and 0.5 mM, respectively. All samples were allowed to equilibrate for 10 min. The relationships between surface tension and surface area were determined by compressing and expanding the surface three times between areas of 52.5 cm^2 (100%) and 7.9 cm^2 (15%). The time required for each cycle was 170 sec. After recording curves of the relationship between surface tension and surface area, we determined maximum surface tension, minimum surface tension, and hysteresis area was measured with a planimeter.

2.5. Data analysis

An unpaired Student's t test was used to test differences between group values. The significance level was set at $P < 0.05$.

3. Results

3.1. Composition of alveolar lavage materials

The total phospholipid (PL) and protein contents of lavage materials from freshly excised lungs and from excised lungs ventilated for one hour at different temperatures were determined. The results are shown in Table 1. There is approximately 30% more total PL in lavage materials from lungs ventilated at 22° C than from freshly excised lungs. When lungs

Table 1
Total phospholipid (PL) and protein contents of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures

	Freshly excised lungs	Ventilation temperature		
		22° C	37° C	42° C
Total PL (mg/gm Lung)	1.28(±0.09)	1.67(±0.06) [a]	1.40(±0.13) [b]	1.09(±0.07) [b,c]
Total Protein (mg/gm Lung)	2.31(±0.34)	4.68(±0.58) [a]	7.04(±1.16) [a,b]	8.39(±0.97) [a,b]
Lavage Returns (%)	83(±1)	85(±1)	84(±1)	82(±2)

Lavage returns were calculated by dividing the total volume of lavage fluid obtained from the lungs by the original volume put into the lungs. The numbers shown are mean values (±SEM) for five experiments. [a] Significantly different from freshly excised lungs. [b] Significantly different from lungs ventilated at 22° C. [c] Significantly different from lungs ventilated at 37° C.

are ventilated at different temperatures, there is a progressive reduction in the phospholipid content with increasing temperature; i.e., there is 35% less lavageable PL at 42° than at 22° C. The total protein content is also altered. There is more lavage protein in lungs ventilated at 22° C than in freshly excised lungs, and the protein is increased when lungs are ventilated at higher temperatures. The results do not appear to be influenced by differences in lavage returns. These results demonstrate that there are differences in the total phospholipid and protein contents of lavage materials from freshly excised lungs and from lungs ventilated at different temperatures.

Since there are changes in the total phospholipid content, we measured the distribution of some individual PL in the lavage materials. The results are shown in Table 2. The phospholipid distribution in lavage fluid from lungs ventilated at 22° C is the same as that from freshly excised lungs. However, increases in the temperature of ventilated lungs result in alterations of the PL profile. The most dramatic effect is on the distribution of disaturated phosphatidylcholines (DSPC), the major surface active component of pulmonary surfactant. In materials from freshly excised lungs or from lungs ventilated at 22° C, DSPC accounts for 46–47% of the total PL. When excised lungs are ventilated at 37° and 42° C, the DSPC is reduced to 38% and 34%, respectively. At the same time, the unsaturated phosphatidylcholines are increased by approximately 5–6%. Therefore, the overall effect of increasing temperature to 37° C or above is a 5–7% reduction of the total PC. The fact that there is a reduction in the percentage of total PC suggests that there must be an increase in one or more other PL components. When lungs are ventilated at 37° and 42° C, there appear to be slight increases in the relative lavage fluid content of phosphatidylethanolamines (PE) and sphingomyelins (SM) over that from freshly excised lungs or from lungs ventilated at 22° C. There also appears to be an increase in phospholipids which were not identified (others) with increasing temperature. Although the relative increases in PE, SM, and other PL are not statistically significant, the magnitude of the overall increase is similar to the decrease in total PC. The results of these experiments indicate that the composition of PL is similar in lavage from freshly excised lungs and from lungs ventilated at 22° C. However, the PL composition of lavage from lungs ventilated at 37° C or 42° C is different. There is a decrease in total PL, a decrease in total PC, a dramatic reduction in the relative lavage content of DSPC, the major surface active component of surfactant, and an increase in the relative lavage content of unsaturated PC at higher temperatures.

The total protein content of lavage materials is

Table 2
Phospholipid (PL) composition (%) of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures

	Freshly excised lungs	Ventilation temperature		
		22° C	37° C	42° C
Total PC	74(±2)	74(±2)	69(±2)	67(±2) [a,b]
DSPC	46(±3)	47(±1)	38(±1) [a,b]	34(±1) [a,b]
Unsat. PC	28(±4)	27±3)	31(±3)	33(±1) [a]
PG	12(±1)	11(±1)	12(±1)	13(±2)
PE	4(±1)	4(±1)	6(±1)	7(±1)
SM	3(±1)	3(±1)	5(±1)	4(±1)
PI	3(±1)	3(±0)	3(±1)	3(±1)
LPC	2(±1)	2(±1)	2(±1)	2(±1)
PS	0.7(±.3)	0.6(±.5)	1.6(±.8)	1(±1)
Others	0.9(±.6)	0.8(±.6)	1.8(±.9)	2.8(±.8)

Phospholipids measured include phosphatidylcholines (PC), disaturated phosphatidylcholines (DSPC), unsaturated (unsat.) PC, phosphatidylglycerols (PG), phosphatidylethanolamines (PE), sphingomyelins (SM), phosphatidylinositols (PI), lysophosphatidylcholines (LPC), phosphatidylserines (PS), and others (not identified). The results for each are expressed as a percent of the total phospholipid pool. The numbers shown are mean values (±SEM) for five experiments. [a] Significantly different from freshly excised lungs. [b] Significantly different from lungs ventilated at 22° C.

altered following ventilation of excised lungs at different temperatures (Table 1). In order to determine if this protein is sedimentable or nonsedimentable, we measured the protein in the pellet and in the supernatant following a 100,000 *g* (two hours) centrifugation of lavage materials. The results are shown in Table 3. There is more protein in the lavage pellet from ventilated than from freshly excised lungs. However, the protein content of the lavage pellet from ventilated lungs is reduced with increasing temperature of incubation between 22° and 42° C. There is also more protein in the lavage supernatant from ventilated than from freshly excised lungs, and this protein level is increased with increasing temperature of ventilation. Some, but not all, of this protein is albumin, which may have leaked into the alveolar spaces from the capillaries. The amount of albumin in the lavage supernatants increases with increasing temperature of ventilation. There is no albumin in any of the lavage pellets. In an attempt to determine if cell damage and/or contamination with whole blood contributes to the increase in nonsedimentable protein, we measured lactate dehydrogenase and hemoglobin levels in the lavages and found no differences in any of the samples (data not shown). The results of these experiments demonstrate that the protein content of lavage materials is altered when the temperature at which excised lungs are ventilated is increased. In general, there is an increase in total lavage protein, a reduction in sedimentable lavage protein, and an increase in nonsedimentable lavage protein, which contains some albumin, with increasing temperature of ventilation. There are no differences in the relative distribution of sedimentable (approximately 85% of the total PL) and nonsedimentable (approximately 15% of the total PL) phospholipids in any of the samples.

3.2. Surface balance studies with alveolar lavage materials

There are some differences in the amounts and in the compositions of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures. Therefore, we decided to study the surface activity of lavage materials obtained from these lungs. These experiments were done by placing approximately equal amounts of lavage phospholipid (final concentration of approximately 100 μg PL per ml) in the Wilhelmy surface balance. The studies were carried out with the balance temperature maintained at 22° C. The relationship between surface tension and surface area was measured by compressing and expanding the surface three times after the materials had been in the balance for ten minutes. We measured maximal and minimal surface tensions and hysteresis area for the third cycle.

Typical surface tension vs. area relationships for lavage materials from lungs ventilated at 22° C or at 42° C are shown in Fig. 1. The differences in the tracings illustrate that the surface properties of lavage materials from lungs ventilated at different temperatures are not the same. The results of all these experiments are shown in Table 4. The surface properties of lavage materials from freshly excised lungs and from lungs ventilated at 22° C, i.e., materials with identical phospholipid compositions, are not

Table 3
Total protein and albumin contents of pellets and supernatants from 100,000 *g* (2 hr) centrifugations of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures

Total protein or albumin (mg/gm lung)	Freshly excised lungs	Ventilation temperature		
		22° C	37° C	42° C
Pellet:				
Total Protein	0.67(±0.16)	1.29(±0.08) [a]	0.97(±0.23)	0.90(±0.06) [b]
Supernatant:				
Total Protein	1.67(±0.27)	3.57(±0.44) [a]	6.07(±1.01) [a,b]	7.49(±0.30) [a,b]
Albumin	0.04(±0.01)	0.52(±0.07) [a]	0.66(±0.10)[a]	2.85(±0.34) [a,b,c]

The numbers shown are mean values (±SEM) for five experiments. [a] Significantly different from freshly excised lungs. [b] Significantly different from lungs ventilated at 22° C. [c] Significantly different from lungs ventilated at 37° C.

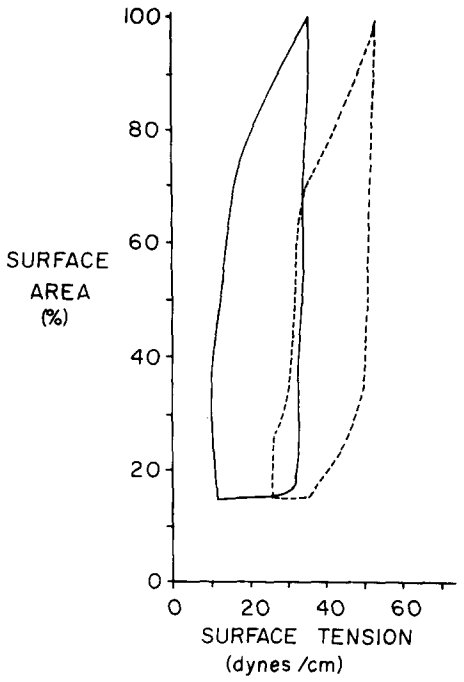

Fig. 1. Relationships between surface tension and surface area for lavage materials from lungs ventilated for one hour at 22° C (—) and at 42° C (---). Alveolar lavage materials were suspended in HEPES-buffered medium containing 2.5 mM $CaCl_2$ and 0.5 mM EDTA so that the final phospholipid concentrations in the trough were 102 and 99 mg/ml for materials from lungs ventilated at 22° C and 42° C, respectively. The samples were vortexed and placed in the surface balance which was maintained at 22° C. After the materials had been in the balance for ten minutes, the surface was compressed and expanded three times. These tracings are from the third cycle.

different. These values for maximal and minimal surface tension are similar to those for rabbit lung lavage reported by Lempert and Maklem (1971). However, when the measurements are made for materials from lungs ventilated at 37° C, minimal surface tension remains unchanged but maximal surface tension is increased. When the measurements are made for materials from lungs ventilated at 42° C, both maximal and minimal surface tensions are increased. The increase in the value for minimal surface tension is most striking. In general, hysteresis areas are similar for all samples of lavage materials. We also calculated a stability index, which was first proposed by Clements et al. (1961) and which may provide a measure of the surface-tension lowering ability of the lavage material samples (Table 4). The only significant difference is the lower stability index for materials from lungs ventilated at 42° C. The results of all these experiments demonstrate that the surface properties of lavage materials obtained from lungs ventilated at 37° and 42° C differ from the surface properties of lavage materials from freshly excised lungs or from lungs ventilated at 22° C.

4. Discussion

The results of these experiments show that there are differences in the quantity and composition of alveolar lavage phospholipids (PL) obtained from

Table 4
Surface properties of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures

	Freshly excised lungs	Ventilation temperature		
		22° C	37° C	42° C
Maximal surface tension (dynes/cm)	35(±1)	37(±2)	46(±1) [a,b]	46(±3) [a,b]
Minimal surface tension (dynes/cm)	8(±2)	8(±2)	9(±1)	19(±3) [a,b,c]
Hysteresis area (cm^2)	11.6(±0.9)	11.1(±0.4)	12.9(±0.4)	10.0(±0.4) [c]
Stability index	1.24(±0.09)	1.26(±0.12)	1.33(±0.06)	0.84(±0.10) [a,b,c]

The amount of lavage materials present were 109(±3), 104(±4), 102(±5), and 99(±6) μg phospholipid per ml for materials from freshly excised lungs, lungs ventilated at 22° C, lungs ventilated at 37° C, and lungs ventilated at 42° C, respectively. The numbers shown are mean values (±SEM) for five experiments. [a] Significantly different from freshly excised lungs. [b] Significantly different from lungs ventilated at 22° C. [c] Significantly different from lungs ventilated at 37°C.

freshly excised lungs and excised lungs ventilated at different temperatures. Although there is more lavageable PL obtained from lungs ventilated at 22° C than from freshly excised lungs, the PL compositions of lavage materials from these lungs are the same. As the temperature at which lungs are ventilated is increased to 37° C and 42° C, there is a reduction in the quantity of lavageable PL and alterations in the PL composition. In general, there is a reduction in the relative amounts of disaturated phosphatidylcholines (DSPC) and total phosphatidylcholines (PC) and an increase in the relative amount of unsaturated PC.

For the most part, the reasons for the differences in quantity and composition of the lavage materials remain unknown. It is known that increased ventilation of lungs enhances the release of pulmonary surfactant materials into the alveolar space (Oyarzun and Clements, 1977, 1978). That may be the reason that there is more lavageable PL in lungs ventilated at 22° C than in freshly excised lungs. However, why is the PL level reduced with increasing temperature of the ventilated lungs? There are several possibilities, although the mechanism is not known. These include 1) a reduction in surfactant release, 2) an increase in the degradation or clearance of surfactant, or 3) both of the above. Also, why is the PL composition altered with increasing temperature? Although it is not certain, there seem to be at least three possibilities: 1) alterations in synthesis of individual PL, 2) differential rates of degradation and/or clearance for various PL, or 3) both of the above. More studies are required to sort through these possibilities.

There are also differences in the total amounts of lavage proteins and their distribution following centrifugation of the lavage materials from these lungs. Since the first step in purification of pulmonary surfactant is centrifugation, it is likely that the sedimentable proteins are surfactant proteins. In this regard, it is interesting to note that the relative changes in amounts of sedimentable protein obtained from the lavage materials from excised lungs follow patterns similar to changes in the levels of phospholipids. The source of all of the nonsedimentable proteins is unknown. It does not seem that they arise from cell damage or contamination with whole blood because LDH and hemoglobin levels are not elevated (data not shown). Some, but not all, of the nonsedimentable protein is albumin, which may have leaked into the alveolar spaces from capillaries (Table 3).

There are also differences in the surface properties of lavage materials from freshly excised lungs and excised lungs ventilated at different temperatures. These alterations in surface properties are undoubtedly related to the phospholipid and protein composition of the lavage materials. The surface properties of lavage materials from lungs ventilated at 22° C and from freshly excised lungs are identical, and the phospholipid compositions of these samples are similar. When lungs are ventilated at 37° C, the value for maximal surface tension of lavage fluid is elevated. Ventilation at 42° C produces increases in the values for maximum and minimum surface tension of lavage fluid. The lavage materials from excised lungs ventilated at higher temperatures (37° and 42° C) contain less DSPC, total PC, and sedimentable protein (perhaps surfactant protein) than materials from freshly excised lungs. Furthermore, the lavage contains more unsaturated PC and nonsedimentable proteins, some of which are probably serum proteins. It is well known that surface properties are affected by the PL composition, especially DSPC, and it has been shown that protein contamination affects surface properties (Notter et al., 1982; Seeger et al., 1985). In fact, Green et al. (1991) found that protein contamination of rat surfactant affects the surface properties following exposure of the animals to hydrogen sulfide.

There has been at least one other study in which alveolar surface tension has been measured following ventilation of excised lungs at different temperatures. Schürch et al. (1985) measured alveolar surface tension in individual alveoli over the entire pressure-volume loop at 22° C and 37° C in isolated, perfused rabbit lungs. They found that the maximum surface tension was not significantly different at the two temperatures. However, the calculated surface tension was consistently higher at 37° C than at 22° C between 40 and 70% of total lung capacity (TLC), and this difference was significant at 50% of TLC. Although the differences measured by Schürch et al. (1985) are smaller than those we measured, the changes are in the same direction; i.e., surface tension values are greater at higher temperatures.

One possible explanation of our results is that the

lung is slowly dying after being removed from the chest and this process is hastened at increased temperatures. The lungs are not maintained under physiological conditions. For example, there is no perfusion and no carbon dioxide in the alveolar air which could lead to pH changes. All of these factors could lead to cellular alterations and protein leakage across the alveolar barrier before there are changes in cell integrity and LDH levels. However, other investigators have used the same experimental approach to study lung mechanics and reported temperature-induced changes in mechanical properties. We have simply duplicated those conditions and reported changes in the composition and properties of alveolar lavage materials and suggest that these changes may be related to the alterations in lung mechanics.

In summary, there are differences in the quantity, composition, and surface properties of alveolar lavage materials from freshly excised lungs and from lungs ventilated at different temperatures. The data do show that lavage materials from freshly excised lungs and from lungs ventilated at 22° C are very similar. Although there is more phospholipid and protein in the lavage fluid from lungs ventilated at 22° C, the relative DSPC content, phospholipid composition, and surface properties are the same as those obtained with lavage materials from freshly excised lungs. Ventilation at higher temperatures (37° C and 42° C) results in alterations of lavage fluid content and surface properties. These changes may result in alterations of the mechanical properties of lungs ventilated at different temperatures. Furthermore, this suggests that in order to assess the mechanical properties of lungs following treatment or exposure of animals to some test substance, the measurements should be done at lower temperatures, e.g., 22° C. This would avoid changes in alveolar lavage material content and surface properties which may occur during the measurements of lung mechanics and not during treatment or exposure of the animals to the test substance.

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