pH-Dependent Changes in Surface Activity of Lung Extracts[1] (34507)

ALTON I. SUTNICK, MALCOLM M. CRONLUND,[2] AND LOUIS A. SOLOFF
(Introduced by Irving Millman)

The Institute for Cancer Research, Fox Chase, and Temple University Medical Center, Philadelphia, Pennsylvania

The low surface tension of the alveolar lining material on compression of surface area has been adequately and repeatedly demonstrated (1, 2). This property of pulmonary surfactant produces a stabilizing effect on the alveoli, preventing their total collapse on expiration (3, 4). Any interference with this surface activity would be expected to result in atelectasis.

The finding that surface activity is often decreased in specimens of lung tissue contiguous to areas involved with pneumonia, as well as in infected specimens themselves (5), led us to speculate that some surfactant antagonist is produced in the infectious process. This might be a product of the infecting organism, a result of cell breakdown, or a response of the host to the infection. Potassium hydroxide is a volume-active substance, known to increase the surface tension of distilled water (6, 7). This study was initiated to test the hypothesis that local potassium release might be responsible for altering the surface tension-lowering effect of pulmonary surfactant in pneumonia. It was clear that a systematic investigation of the influence of pH changes on surface activity was also needed in order to evaluate the hydroxyl ion as well. The results of these experiments are reported here.

Materials and Methods. Surface tension was measured of a series of aqueous solutions of potassium hydroxide, potassium chloride, and sodium hydroxide in concentrations varying from saturated solutions to solutions of very low density. All surface tension measurements were performed on a modified Langmuir-Wilhelmy surface-film balance (8). This method depends upon the exertion of the force of surface tension of a liquid upon a frosted platinum rider lowered into its surface. The liquid is placed in a polytetrafluoroethylene (Teflon[3]) trough, and a movable barrier compresses the surface to 20% of the original area at a controlled rate of speed. The surfaces of the solutions were carefully cleaned by aspiration, and compressed by the barrier in 1-min cycles. The surface tension was continuously monitored, and a constant value was obtained when the surface was kept clean. The pH of all preparations was measured with a Beckman pH meter.

The effect of abnormalities in ionic environment of normal lung tissue extracts was determined by the use of lungs obtained from normal dogs and rabbits. Three-gram specimens were first minced and extracted in 0.9% sodium chloride solution. They were filtered through two thicknesses of gauze into the Teflon trough of the surface-film balance. The surface tension was then measured during cyclic compression in 15-min cycles to 20% of the original area with subsequent reexpansion. The minimal surface tension was used to compare differences in surface activity.

Additional specimens of these same normal dog lungs were extracted in various concentrations of potassium hydroxide, potassium

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[2] Present address: Department of Biochemistry, College of Medicine, University of Iowa, Iowa City, Iowa 52240.

[3] DuPont, Wilmington, Delaware.

TABLE I. Surface Tensions of Aqueous Potassium Hydroxide, Potassium Chloride, and Sodium Hydroxide Solutions.

Concentration (g/100 ml)	Surface tension (dynes/cm)
KOH	
1.0	71.4
6.5	75.0
10.0	76.0
25.0	86.2
28.0	81.6
35.0	93.1
41.2	93.0
50.0	95.2
104.8	104.3
135.2	108.8
KCl	
0.0375	72.5
0.100	72.5
1.0	74.3
5.0	74.0
10.0	74.0
25.0	80.5
Saturated solution	85.6
NaOH	
1.0	70.0
5.0	74.0
10.0	80.0
25.0	87.0
50.0	96.0
Saturated solution	98.0

chloride, and sodium hydroxide solutions. Surface tension was similarly measured during surface compression and expansion. Thus, each lung served as its own control. In some cases, different specimens of lung tissue from the same animal were used for several different extractions.

The pH of saline extracts of seven normal dog lungs was altered by the addition of 10% sodium hydroxide, and surface activity was again measured. These extracts were subsequently neutralized with 0.1 *N* hydrochloric acid in an attempt to reverse the effect of a rise in pH. The opposite of this was also done, with an initial fall in the pH produced by 0.1 *N* hydrochloric acid, followed by neutralization with 10% sodium hydroxide. These lungs were also extracted in potassium carbonate and sodium bicarbonate solutions in an effort to evaluate the effects of other alkalinizing agents. Hydrochloric acid was used in the latter cases to reverse any abnormalities produced. Rabbit lungs were extracted in 1/15 *M* Sørensen phosphate buffer solution with pH ranging from 4.5 to 9.1.

The pH of saline extracts of 26 additional rabbit lungs was measured to compare with their respective minimal surface tensions. Lung tissue pH of 10 of these specimens was determined by direct measurement using IL electrode No. 14133 (Instrumentation Laboratory, Inc., Watertown, Massachusetts).

Results. Increasing concentrations of aqueous potassium hydroxide solutions produced gradual increases in the surface tension of distilled water (Table I). Very little change was produced by potassium chloride solutions even in high concentrations (Table I). That of sodium hydroxide was much more marked, approaching that of potassium hydroxide (Table I).

Extracts of normal dog and rabbit lung specimens demonstrated normal surface activity in accordance with previously determined standards by this method, with minimal surface tension below 10 dynes/cm (Table II). These specimens all demonstrated a wide hysteresis loop and a steep slope of the curve, indicating significant surface activity (Fig. Ia).

There was no significant alteration in sur-

TABLE II. Minimal Surface Tension of Extracts of 30 Normal Dog Lungs and 13 Normal Rabbit Lungs in 0.9% Sodium Chloride (dynes/cm).

Dog lungs		Rabbit lungs
3.4	4.6	0
3.4	5.7	9.6
9.1	6.8	5.9
5.7	3.4	5.9
3.4	4.6	5.5
9.1	5.7	1.0
8.0	5.7	3.6
5.7	9.1	5.8
9.1	5.7	1.9
9.1	2.3	6.8
3.4	6.9	6.7
8.0	6.9	6.7
3.4	5.7	6.6
9.1	4.6	
2.3	6.0	

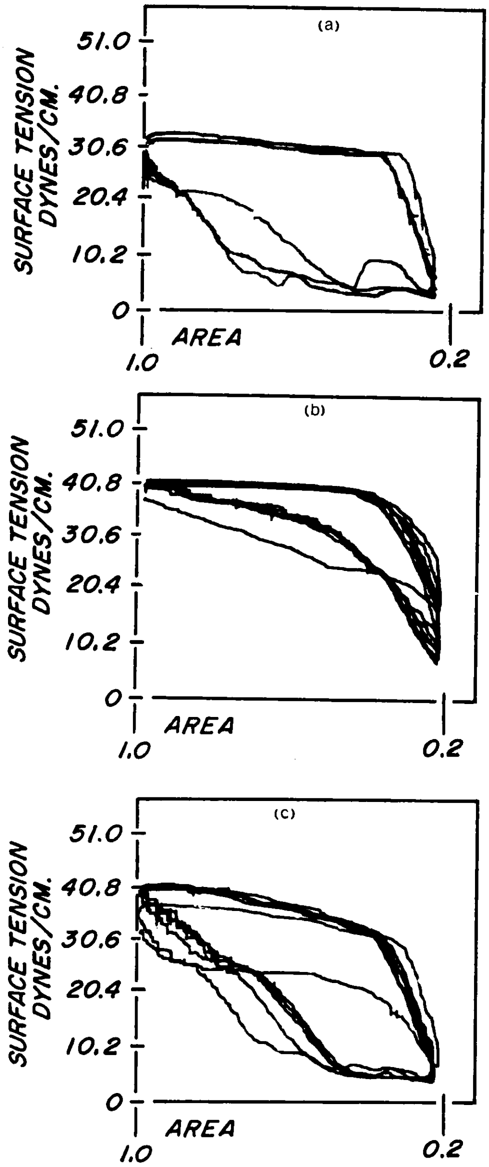

FIG. 1. a. Surface tension-area plot of normal lung extracted in 0.9% sodium chloride solution, with characteristic low minimal surface tension, wide hysteresis loop, and steep slope of curve. b. Change of surface tension-area plot to more gradual slope and narrower hysteresis loop with increase in pH. c. Return to normal slope of one specimen upon neutralization.

TABLE III. Minimal Surface Tension of Extracts of Normal Dog Lungs.

Surface tension (dynes/cm)	Concentration (g/100 ml)	Surface tension (dynes/cm)
In 0.9% sodium chloride	In aqueous potassium chloride solution	
9.1	0.0375	6.8
8.0	0.0375	8.0
4.6	0.100	8.0
4.6	1.0	6.8
4.0	10.0	17.0
4.0	25.0	17.0
3.4	Saturated solution	5.7
5.7	Saturated solution	4.6
5.7	Saturated solution	14.8
6.0	Saturated solution	15.0
In 0.9% sodium chloride solution	In aqueous potassium hydroxide solution	
9.1	0.028	25.0
8.0	0.028	26.1
5.7	0.028	13.6
9.1	0.028	17.0
9.1	0.028	34.0
3.4	0.028	31.7
8.0	0.028	13.6
9.1	1.048	14.8
3.4	1.352	18.2
4.0	5.0	16.0
5.0	10.0	35.0
5.7	28.0	25.0
3.4	28.0	26.1
4.0	50.0	18.0
5.0	50.0	14.0
5.0	Saturated solution	15.0
In 0.9% sodium chloride solution	In aqueous sodium hydroxide solution	
3.4	0.020	31.7
5.7	0.020	14.8
3.4	0.020	13.6
6.8	0.020	27.2
4.0	1.0	15.0
5.0	5.0	14.0
6.0	10.0	35.0
5.0	10.0	14.0
5.0	25.0	14.0
5.0	50.0	18.0
5.0	50.0	14.0
5.0	Saturated solution	15.0

face forces of the extracts in potassium chloride solutions of low concentrations, and even high concentrations had only a moderate effect (Table III). Lung tissue extracted in various concentrations of potassium hydroxide, however, demonstrated quite a marked alteration in surface forces (Table III). Even in very low concentrations, there was an increase in minimal surface tension. An at-

TABLE IV. Minimal Surface Tension (dynes/cm) of Normal Dog Lung Tissue.

Extracted in:		
0.9% Sodium chloride	Potassium carbonate (69 mg/100 ml)	Sodium bicarbonate (42 mg/100 ml)
5.7	13.6	—
5.7	—	28.4
4.6	27.2	—
5.7	18.2	15.9
3.4	29.5	26.1
9.1	12.5	20.4

tempt was made to reverse this change in surface forces by neutralizing the extract with 0.1 *N* hydrochloric acid. Despite an effective change in pH from a mean of 8.5 to 3.3, there was no significant change in surface activity. A similar response was noted when the lungs were extracted in sodium hydroxide solution (Table III). There was a rise in minimal surface tensions of all specimens.

Other alkalizing ions had a similar effect upon the surface activity of the film. Potassium carbonate and sodium bicarbonate were both effective in raising minimal surface tension (Table IV). In one of these, complete reversal to normal surface activity was obtained on acidification.

Of the seven normal saline extracts to which 10% sodium hydroxide was added (Table V), four demonstrated a marked increase in minimal surface tension (A, B, C, D). In two of these, we could demonstrate a progressive change as pH was increased. In the three additional specimens (E, F, G), the slope of the curve became more gradual as the pH increased, but there was no change in the minimal surface tension (Fig. 1b). Neutralization of one of these extracts (E) resulted in a return to a normal-appearing curve (Fig. 1c). This reversibility occurred only in this and one other specimen.

Acidification was also shown to alter the surface activity of lung extracts, by virtue of similar changes in the tension-area diagram of eight specimens with the addition of 0.1 *N* hydrochloric acid (Table VI).

The pH of our normal saline extracts was distinctly below that of blood, with a range of 6.1-7.2, and a mean of 6.7. The mean pH change necessary to alter surface activity was +1.0 or —1.5 pH units.

The mean pH of normal lung tissue specimens was 7.0, with a range of 6.7-7.3 (Table VII). There was generally no marked change in surface activity with phosphate buffer solutions over a range of —2.1 pH units to +1.2 pH units from that of the saline extracts (Table VIII). The buffering capacity of lung appears to be such that this range becomes —1.3 - +1.1 pH units when the tissue is extracted with the various buffers. In one phosphate buffer lung extract a pH change from 6.6 to 7.6 resulted in an increase of minimal surface tension from 6.5 to 18.1 dynes/cm.

There was a slight positive correlation between the pH and the minimal surface tension in the 26 lungs studied with buffer solution ($r = +0.2918$, Table IX); this was not quite statistically significant for this size sample. Those extracts with pH between 5.3

TABLE V. Alteration of Surface Forces by Addition of 10% Sodium Hydroxide.

Dog lung extract	pH	Minimal surface tension (dynes/cm)
A	6.1	3.4
	6.8	13.6
	7.6	21.5
B	6.7	9.1
	7.0	18.2
C	6.6	5.7
	7.0	10.2
	7.5	17.0
	8.0	20.4
D	7.2	3.4
	7.9	15.9
E	7.1	2.3
	9.2	8.0
	6.6	4.6
F	6.8	4.6
	7.1	3.4
	7.5	4.6
	8.5	5.7
G	6.7	6.8
	7.6	5.7

TABLE VI. Alteration of Surface Forces by Addition of 0.1 N Hydrochloric Acid.

Dog lung extract	pH	Minimal surface tension (dynes/cm)
A	6.9	4.6
	5.1	18.2
B	6.8	5.7
	6.2	5.7
	5.9	5.7
	5.2	14.8
C	6.7	5.7
	4.8	17.0
D	6.6	9.1
	4.9	20.4
E	6.9	5.7
	5.2	12.5
F	6.9	3.4
	6.0	3.4
G	6.9	5.7
	6.3	12.5
H	6.9	5.7
	6.0	12.5

and 6.8 had a mean minimal surface tension of 6.7 dynes/cm, while those from pH 6.9 to 8.0 were 12.6 dynes/cm. This difference was nearly significant ($p = 0.065$).

Discussion. This study has shown that there is an influence of pH changes on the surface activity of extracts of normal mammalian lung tissue. The effect of potassium ion, if any, is very small.

Although the effects of inorganic solutes on the surface tension of pure water have been known for a long time (6), it was the studies of the surface tension of potassium hydroxide solutions by Dunlap and Faris (7) which suggested an investigation of the possible effect of potassium hydroxide on pulmonary surfactant. Since they used the sessile drop method for determining surface tension (9), we first repeated some of their observations with the modified Langmuir-Wilhelmy surface-film balance. Surface tension was measured on a series of aqueous potassium hydroxide solutions, including the range covered in the previously mentioned study. Our results were quite similar to theirs, showing increasing concentrations of potassium hydroxide to produce gradual increases in surface tension over that of distilled water.

TABLE VII. pH Measurements of Lung Tissue of 10 Normal Rabbits.

6.7	7.0
6.9	7.1
6.9	7.1
7.0	7.2
7.0	7.3

TABLE VIII. The Effect of pH Changes with Buffer Extraction on Minimal Surface Tension (γ_{min}) of Lung Extracts.

Rabbit lung	Extracted in:[a]	Extract pH	γ_{min} (dynes /cm)
A	Saline	6.8	0.9
	7.0	6.6	10.4
	8.0	7.9	1.4
B	Saline	6.7	0.4
	6.6	6.65	0.7
	6.6	6.6	1.6
C	Saline	6.7	0
	6.0	6.05	0
	6.5	6.0	1.4
D	Saline	6.6	6.5
	4.5	5.3	4.2
	9.1	7.6	18.1
	9.1	7.7	18.1

[a] "Saline" indicates extraction in unbuffered saline; numbers indicate pH of 1/15 M Sørensen phosphate buffer solution used to make extract.

The question of whether this phenomenon is a function of potassium or hydroxyl ion concentration was approached by studying the effect of various concentrations of potassium chloride and sodium hydroxide. The effect of potassium chloride solution was a modest one, even in high concentrations. That of sodium hydroxide was much more marked, similar to that of potassium hydroxide. This implied that, while there may be some influence of potassium ion on the surface tension, the major effect of potassium hydroxide solutions was that of the hydroxyl ion.

This did not eliminate the possibility that potassium might exert a greater effect on lung tissue, and the effect of similar alterations in ionic environment of normal lung tissue extracts remained to be determined. Speci-

TABLE IX. Relationship of pH Changes to γ_{min} of Saline and Buffer Lung Extracts.

Rabbit lung extracts in order of pH

pH	Minimal Surface Tension γ_{min} (dynes/cm)		
5.3	4.2		
6.0	1.4		
6.05	0		
6.3	20.9		
6.6	1.6		
6.6	4.6		
6.6	6.5		
6.6	15.3	Mean = 6.7	
6.65	0.7	Median = 4.6	
6.65	13.4		
6.7	13.6		
6.8	0		
6.8	0.9		
6.8	4.5		
6.8	13.6		$p = 0.065$
6.9	0.9		
6.9	19.1		
6.9	28.2		
7.0	6.4		
7.0	10.4		
7.0	11.8	Mean = 12.6	
7.1	11.6	Median = 11.8	
7.5	12.8		
7.7	18.1		
7.9	1.4		
8.0	18.1		

Correlation coefficient (r) = +0.2918

mens of normal lungs were extracted in potassium chloride solutions of various concentrations, ranging from 5 meq of potassium per liter to saturated solutions. There was no significant alteration in surface forces of the extracts in potassium chloride solutions of low concentrations, and even high concentrations had only a moderate effect. Two extracts in saturated solution exhibited normal surface activity. This suggested that the potassium ion has only a moderate effect upon pulmonary surfactant, and provided evidence against the release of this ion producing the abnormalities found in pneumonia.

Lung tissue extracted in various concentrations of potassium hydroxide demonstrated a marked alteration in surface forces. Even in very low concentrations, there was an increase in minimal surface tension. Attempts to reverse these abnormalities by neutralization were unsuccessful.

If this phenomenon were due to hydroxyl ion or the pH, there should be a similar response to sodium hydroxide and other alkali. A similar group of studies with lungs extracted in sodium hydroxide solutions produced results quite similar to those with potassium hydroxide. There was a marked rise in minimal surface tension of all specimens. The addition of 10% sodium hydroxide to normal saline lung extracts produced similar changes. In the three specimens not demonstrating a significant rise in minimal surface tension, the change to a much more gradual slope of the curve might be considered evidence of a decrease in surface activity. Although one specimen demonstrated a return to a steeper slope upon neutralization with 0.1 N hydrochloric acid, the other two did not.

Current evidence indicates that surfactant is dipalmitoyl lecithin (10–13). It is not unreasonable to assume that a rather complex organic molecule may be sensitive to pH changes. Scarpelli, Gabbary, and Kochen (14) have indicated the necessity of maintaining a minimum ionic concentration in the subphase to preserve surface activity. They also pointed out that there is no significant difference between the effect of sodium chloride and potassium chloride solutions at low concentrations, which we had previously noted as well (15). Their studies did not include more concentrated solutions, nor did they attempt to alter pH.

Our studies with lung extracts demonstrate that alteration of the pH of the subphase may result in abnormalities of surface activity. This is more likely to occur with the addition of strong acids and bases. When pH was altered with buffer solutions instead of acids and bases, the range was narrower, and the effect upon surface tension was not so marked. Perhaps some buffering effect accounts for the reported lack of influence of pH changes on surfactant in other studies (16). It has also been said that an acid pH promotes normal surface activity (17). Our studies likewise indicate some correlation between pH of lung extracts in saline and minimal surface tension on compression. The saline extracts of lung tissue had pH ranges below those seen normally in blood; this was also true of direct pH measurements of intact lung. Evidence has been presented that the normal pH of extravascular lung tissue is slightly acid. Effros and Chinard (18), using indicator techniques, have reported a pH of 6.71 $\pm$ 0.09; Hyde *et al.* (19), using a method of dissociation of weak acids and bases (20), found pH= 6.92 $\pm$ 0.09 at pCO_2 40 mm Hg. The implication of these observations is of a functional role of an acid pH in the lung. This may be true for other tissues as well, since lung pH values were similar to those reported for intracellular muscle tissue (21).

The present study emphasizes the importance of pH changes in altering surface activity as measured by the surface film balance. The pH of saline extracts of normal lung and the lung tissue itself is lower than that of blood. A slightly acid pH has some correlation with lower minimal surface tension. The significance of these findings for the intact animal or in clinical medicine is uncertain; it is clear that pH affects surface activity and is important in defining experimental conditions. Investigation is warranted of the possibility of a functional role of pH in the lung.

Summary. We have studied the effect of various KOH concentrations and pH alterations on dog and rabbit lung tissue extracts. Extracts in KOH had a higher minimal surface tension than saline extracts. Most KCl extracts were normal. Twelve lungs were extracted with NaOH, and an additional seven saline extracts were alkalinized with 10% NaOH solution. Sixteen of these showed a marked rise in minimal surface tension. In the three that did not, the contour of the tension-area curve changed markedly, with more gradual slope and narrower hysteresis loop. Minimal surface tension was consistently raised with addition of K_2CO_3 and $NaHCO_3$ as well. Similar abnormalities were produced by lowering pH with 0.1 *N* HCl.

The pH of 26 lung extracts was also altered with phosphate buffer solution. Minimal surface tension changes were not so extreme, but there was a slight positive correlation of pH with minimal surface tension. The pH of saline extract of normal lung tissues and of the lung itself was slightly lower than normal blood pH.

This study demonstrates an influence of pH changes on surface activity of normal lung extract. The possibility of a functional role of pH in the lung must be considered.

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