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Pulmonary Surface Activity in Induced Pulmonary Edema *

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Induction of acute pulmonary edema in anesthetized dogs causes a large fall in compliance, out of proportion to lung volume (1), and a sharp increase in venous admixture that can be reversed by forcible inflation of the lungs (2). This pattern of abnormal function suggested alveolar closure (1, 2).

Since alveolar stability depends in large measure on the presence of normal pulmonary surface properties (3), altered surface forces were considered as an underlying mechanism. Cook and co-workers (1) reasoned that the decrease in alveolar diameter, resulting from the accumulation of intra-alveolar fluid, would account for an increase in total surface forces. We examined the possibility that pulmonary edema alters alveolar surface tension properties, in this way contributing to alveolar instability and to the failure of respiratory function. It can be shown that for alveoli, as for spherical surfaces, total surface forces equal twice the surface tension divided by the radius of curvature (4).

We induced pulmonary edema in anesthetized dogs by rapid intravenous infusion of dextran. Surface activity of lung extracts was measured and correlated with morphologic changes. There was a regional loss or impairment of surface activity in the edematous lung, associated with areas of atelectasis and hemorrhage. Pressure-volume relations, determined in some excised lobes, confirmed premature alveolar closure. The explanation for the reduced alveolar surface activity in pulmonary edema was not fully established. Foaming contributed significantly, but was not required, and the presence of surfactant inhibitors seemed likely.

Methods

Procedure. The experiments were conducted on 24 mongrel dogs weighing 7 to 33 kg. The dogs were tracheostomized and anesthetized with 30 mg per kg pentobarbital intravenously. They were supine and breathed spontaneously or with the assistance of a Starling respirator, set to deliver ventilation in the normal tidal range. To duplicate the conditions of an earlier study of gas exchange in pulmonary edema (2), we gave the animals 100% oxygen to breathe before the induction of pulmonary edema; the total period of oxygen breathing was generally 1 to 2 hours and did not exceed 5 hours. Pulmonary edema was induced by intravenous infusion of 6% dextran solution in saline, at the rate of approximately 4 ml per kg per minute. The infusion was maintained until foam came out of the trachea. The animals were then killed with magnesium sulfate.

Post-mortem, the lungs were weighed separately and their gross morphologic features noted. In every case, one sample was taken from a dark, depressed part of the lung and another from a pink, relatively unaltered part. The samples were examined for surface activity, as detailed below. In seven instances, portions of these samples were also fixed in Bouin's solution and stained with hematoxylin and eosin, toluidine blue, and with periodic acid Schiff reagent for microscopic examination.

In ten experiments, a thoracotomy was performed, and the airway to one or two lobes was clamped after the animal had breathed 100% oxygen sufficiently long to wash out alveolar nitrogen. When absorption atelectasis was complete, the dextran infusion was begun. The occluded lobes thus became edematous, but remained nonventilated and hence did not foam. Comparison of surface tension of samples from these lobes and from edematous, unoccluded lobes permitted an estimation of the importance of foaming, as such, in altering alveolar surface tension. The possible influence of atelectasis alone was assessed by comparing pulmonary surface ten-

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sion characteristics in other lobes from the same lung before and after degassing.

Measurement of surface activity of pulmonary extracts. Lung extracts were prepared by mincing at least 0.5 g of lung tissue in 30 ml of 0.9% saline solution. (As small a sample as 0.4 g of pink edematous lung was capable of giving normal surface tension.) Although the weight of samples extracted varied in different experiments, approximately equal portions were compared in each experiment, or a larger sample was taken from the dark portions to make up for the possible effect of reduction in expanded lung tissue (Table I). The extracts were filtered through four layers of gauze onto the trough (12.8×5 cm) of a modified Wilhelmy balance. The balance measured tension in the surface film of extracts by the downward pull on a platinum strip, partially submerged in the fluid. This pull was recorded continuously as a function of surface area of the extract, which was varied automatically by a movable barrier (5). The balance was calibrated by known weights, a vertical excursion of 8 mm corresponding to a tension of 75 mg (or 10 dynes) per cm. Freshly poured extracts were allowed to age in the trough for 20 minutes and were then cyclically compressed to a minimal area of 8.5 cm^2, and re-expanded to a maximal area of 55 cm^2. Each cycle was completed in 10 minutes, and the procedure was continued for at least 2 hours, or until a reproducible tension-area relationship was obtained.

In this report, surface characteristics of lung extracts are described in these terms: *1*) minimal surface tension (γ min), in dynes per cm: tension on maximal compression of film; *2*) maximal surface tension (γ max), in dynes per cm: tension at full expansion of the film; and *3*) extract stability index ($\bar{S}$): change in tension/average tension $= 2\ (\gamma \text{ max} - \gamma \text{ min})/(\gamma \text{ max} + \gamma \text{ min})$.

The meaning of these measurements was discussed by Clements, Hustead, Johnson, and Gribetz (6) and will be referred to later in this paper. Since there is no numerical value that expresses the degree of tension-area hysteresis, i.e., the area within the loop described by the surface balance, tracings from representative experiments are here reproduced.

Pressure-volume characteristics of lungs: "expansion index." Pressure-volume relations were determined from the degassed state in ten edematous lobes excised post-mortem. After removing the lungs carefully from the chest, lobes that appeared grossly boggy and hemorrhagic were cannulated via the airway and then degassed in a vacuum chamber (6). The cannula in the airway was then connected by means of a T-tube, either to a water manometer or to an air syringe. Each lobe was inflated by adding small volumes of air. After each increment, 2 minutes was allowed for equilibration of air throughout the lobe before the airway (transpulmonary) pressure was recorded. This procedure was repeated until further attempts to introduce air resulted in little or no increase in volume. If an air leak was detected at any time, the study was discarded. The deflation curve was determined similarly by withdrawing air in small volumes and recording pressure at each step. Pressure-volume relations were also examined in ten normal lobes serving as controls, and the expansion index for each lobe was calculated as $(V_5 - V_D)/(V_{max} - V_D)$, where V_5 is the total volume of air in the lobe at a deflation pressure of 5 cm H_2O, V_{max} is the maximal volume of the lobe, and V_D, the dead space of the lobe, assumed to be 10% of its V_{max} (6).

Clements and associates (6) have found this ratio to be a useful measure of alveolar stability. It correlates inversely with minimal surface tension and directly with the stability index of lung extracts. At a transpulmonary deflation pressure of 5 cm H_2O, which corresponds to resting end-expiratory pressure, an unstable lung has a relatively greater number of alveoli that have already closed, and hence, a smaller alveolar volume relative to maximal alveolar volume, i.e., a smaller expansion index. Gruenwald (7), and Johnson, Permutt, Sipple, and Salem (8) have suggested the use of somewhat different numerical ratios that also measure alveolar instability from changes in the deflation curve. The latter authors emphasized the importance of pressure-volume determina-

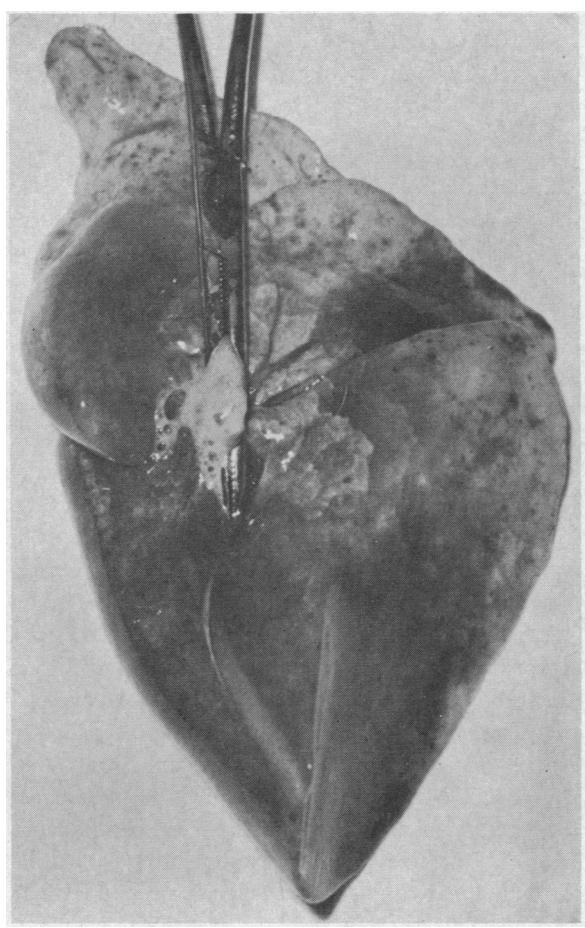

FIG. 1. PHOTOGRAPH OF EDEMATOUS LUNG SHOWING DARK, HEMORRHAGIC AREAS AND RELATIVELY UNALTERED, LIGHT AREAS. Dorsal and lower portions of lung are predominantly dark. Bronchus contains foam.

TABLE I

*Surface characteristics of lung extracts in acute pulmonary edema**

Dog	Survival time	Lung wt	Predominantly airless parts: Sample wt	γ max	γ min	$\bar{S}$	Relatively unaltered parts: Sample wt	γ max	γ min	$\bar{S}$
	min	*% of control*	*g*	*dynes/cm*				*dynes/cm*		
1	42	407	1.5	40	17	0.80	1.5	40	6	1.47
2	26	273	0.8	48	23	0.70	1.2	48	19	0.88
3	87	326	1.5	50	20	0.85	1.5	50	20	0.85
4	19	494	1.5	50	23	0.73	1.1	48	10	1.31
5	63	361	3.0	55	35	0.44	2.0	50	8	1.62
6	31	336	1.0	53	19	0.94	0.5	50	7	1.47
7	58	329	3.0	52	28	0.60	0.8	47	3	1.76
8	39	658	2.8	50	30	0.50	0.5	43	4	1.70
9	141	281	1.0	50	30	0.50	1.2	52	20	0.89
10	46	249	1.4	52	25	0.70	1.3	40	7	1.40
11	30	402	2.0	48	25	0.63	1.9	35	10	1.11
12	14	432	2.7	45	22	0.69	2.0	48	22	0.75
13	26	171	1.5	48	20	0.82	0.5	40	11	1.14
14	149	280	4.3	48	22	0.74	2.4	36	8	1.27
15	86	255	2.2	45	12	1.16	2.0	30	8	1.16
16	38	240	3.0	38	7	1.38	1.7	30	5	1.43
17	115	182	2.4	46	7	1.47	0.9	40	5	1.56
18	35	378	1.0	55	25	0.75	1.1	45	7	1.46
19	28		1.5	50	25	0.67	2.5	40	6	1.48
20	60	214	0.7	50	30	0.50	0.9	50	8	1.45
21	17	170	1.5	55	30	0.59	1.7	45	8	1.40
22	49	152	2.2	35	15	0.80	1.0	40	8	1.33
23	31	159	2.8	40	7	1.40	2.0	35	5	1.50
24	42	240	2.3	48	30	0.46	1.5	45	8	1.40
Mean	53	300		48†	22†	0.78†		43	9	1.32
SD	±37	±121		±5.3	±7.9	±0.30		±6.9	±5.4	±0.27

* γ max and γ min are, respectively, maximal and minimal surface tensions; $\bar{S}$ is extract stability index.
† Significantly different from corresponding values in the following column ($p < 0.001$).

tions in evaluating the magnitude and extent of surface tension alterations.

Statistical treatment. In calculating the *t* test for statistical significance (p), results on samples taken from the same lung or from the same animal were paired, but those on samples from different animals were not.

Results

Gross and microscopic appearance of lungs (Figure 1). The trachea and major airways contained thin, bloody fluid and foam. The lungs weighed from 152 to 658% (mean 300 ± 121%) of normal weight, predicted on the basis of 8.3 g per kg. They showed dark, somewhat depressed areas, as well as pink, relatively unaltered areas. The dark parts were liver-like, and when sectioned yielded blood but no foam; the pink parts were lighter, and foam could be expressed from their cut surface. The lower and dorsal portions of both lungs showed the greatest preponderance of dark, hemorrhagic areas. Lung lobes that had been rendered gas-free and maintained without ventilation were uniformly dark, hemorrhagic, and foamless.

Microscopically, most alveoli in the pink portions of the lungs appeared normal in size and shape. In contrast, alveolar spaces in the dark portions were smaller and filled with exudate and red cells or were completely obliterated.

Surface properties of edematous lungs (Table I). The predominantly airless parts showed a significant increase in γ min ($p < 0.001$) and in γ max ($p < 0.001$) and a significant decrease in $\bar{S}$ ($p < 0.001$) with respect to the remainder of the lung. In addition to these differences, surface tension of extracts from dark, airless portions exhibited less hysteresis with change in surface area (Figure 2).

Pulmonary edema induced in atelectatic, nonventilated lobes: effect of foaming (Table II). In lobes that had been degassed *in vivo* and kept nonventilated during the induction of edema, there was a significant increase in γ max ($p < 0.05$) and in γ min ($p < 0.05$), and a significant decrease in $\bar{S}$ ($p < 0.05$), relative to control. These changes in surface properties, however, were less

marked than in the lobes that were permitted to foam ($p < 0.05$).

Effect of atelectasis without pulmonary edema. The possible effect of degassing alone was studied in seven lobes, made atelectatic for periods of 5 minutes to 3 hours 19 minutes. γ min and $\bar{S}$ were not significantly different from control values ($p > 0.4$) although γ max was higher ($p < 0.01$).

Changes in "expansion index" (Table III). Mean "expansion index" in nine edematous lobes (mean lung weight, 258 ± 74% of control) was 0.35 ± 0.08, as compared to 0.63 ± 0.06 in eight normal control lobes ($p < 0.001$).

Discussion

Critique of the methods. There is now considerable theoretical and experimental evidence that alveoli of adult mammalian lungs are endowed with a surface-active material (surfactant) that ensures their stability. In this study, as in most other studies thus far, evaluation of surfactant function was based on certain quantitative differences in surface tension properties of lung extracts. Thus the ability of a given extract to reach a low surface tension (lower than 20, and usually below 10 dynes per cm) on compression of its surface area, a large degree of tension-area hysteresis (fat loop), and a high $\bar{S}$ (> 0.85) indicate the presence of a "sufficient" concentration of surfactant in the surface film of extract. This, in turn, is taken to mean that normal surface tension properties existed during life, i.e., that the

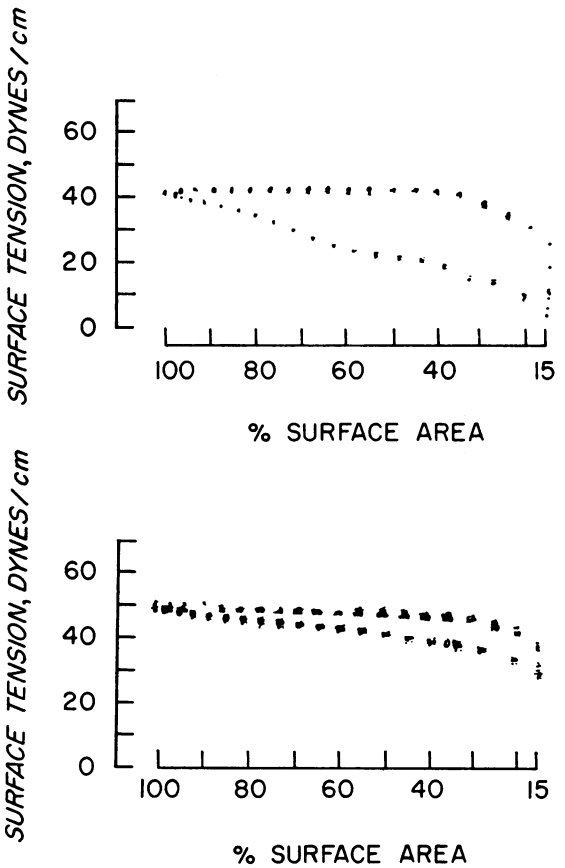

FIG. 2. SURFACE TENSION-SURFACE AREA DIAGRAMS ON EXTRACTS OF PINK, RELATIVELY UNALTERED SAMPLE (UPPER PLATE) AND DARK, PREDOMINANTLY AIRLESS SAMPLE (LOWER PLATE) OF EDEMATOUS LUNG. Tracing in the lower plate shows higher maximal and minimal surface tensions and less hysteresis. Only final reproducible tracing is shown in each figure; other cycles were deleted for clarity.

TABLE II

*Separate effects of foaming and of atelectasis on pulmonary surface properties**

	Normal control			Bronchial occlusion				Bronchial occlusion and edema			Edema without bronchial occlusion (predominantly airless parts)		
Dog	γ max	γ min	$\bar{S}$	Time	γ max	γ min	$\bar{S}$	γ max	γ min	$\bar{S}$	γ max	γ min	$\bar{S}$
				min									
1								48	11	1.26	52	25	0.70
2				14	43	20	0.73	45	22	0.69			
3	33	6	1.39	20	45	16	0.95	45	25	0.57			
4	32	7	1.28	30	40	12	1.08	50	30	0.50	48	25	0.63
5								50	26	0.63			
6	42	12	1.11	12	45	10	1.28	48	14	1.11	48	20	0.82
7	35	7	1.29	13	40	7	1.40	45	10	1.27	50	25	0.75
8	45	8	1.40	5	45	16	0.95	40	10	1.20	50	30	0.50
9	40	6	1.48	15	45	7	1.46				55	30	0.59
				79	45	7	1.46						
10	40	15	0.91	139	40	5	1.56						
				199	45	6	1.53						
Mean	38	9	1.27		43	11	1.24	47	18	0.90	51	26	0.67
SD	±4.9	±3.4	±0.2		±2.4	±5.2	±0.32	±3.2	±7.7	±0.31	±2.7	±3.7	±0.11
p					<0.01	NS	NS	<0.05	<0.05	<0.05	<0.01	<0.0005	<0.005

* Symbols as in Table I.

TABLE III

Mean and standard deviation of expansion index in eight normal and nine edematous lobes

Normal lobes	Edematous lobes
0.63 ± 0.06	0.35 ± 0.08 $p < 0.001$

lung was capable of lowering alveolar surface tension and of varying it over a wide range during the respiratory cycle. Conversely, a high γ min (> 20 dynes per cm), a low $\bar{S}$ (< 0.85), and a small degree of tension-area hysteresis (thin loop) characterize an inactive extract and usually imply a lack of surfactant in the lung during life.

This means of determining whether lung tissue has normal or abnormal surface tension properties is indirect and does not depend on a quantitative estimate of the specific surface-active lipoprotein. Further, an inactive extract is not always due to deficient surfactant but could result from the presence of inhibitors in the extract (9), from improper extraction (10), or from the use of inadequate sample. Despite these limitations, the methods have proved valuable and have confirmed theoretical predictions of the influence of pulmonary surface forces on pulmonary structure and function (11).

Possible mechanisms of impaired pulmonary surface activity in pulmonary edema. The finding of decreased pulmonary surface activity may be due to one or more of these mechanisms affecting surfactant metabolism: *1*) inadequate synthesis, *2*) inactivation, and *3*) excessive depletion.

Pulmonary edema could conceivably interfere with surfactant function on all three counts. By impairing the integrity and nutrition of alveolar cells, it could interfere with their ability to synthesize surfactant. Impaired synthesis, however, was probably of little importance in the acute preparation examined here, since at least 15 hours was required for surface activity of lung extract to become distinctly abnormal after occlusion of the pulmonary artery (12).

A second mechanism by which pulmonary edema could alter surface tension properties is suggested by the observation, reported by Tierney, that rinsing an excised rat lung with dilute serum altered pressure-volume relations in a manner consistent with altered surface properties (13). In addition, Abrams and Taylor have recently demonstrated that fibrinogen can inactivate the surface-active lipoprotein of the lung (14). Leakage of serum and plasma into the alveolar spaces, could, therefore, alter pulmonary surface tension properties by inhibiting the activity of surfactant.

Finally, to the extent that pulmonary edema foam is rich in surface-active material (15), which appears from studies with labeled fatty acids to be derived from pulmonary surfactant (16), foaming leads to loss of normal alveolar surfactant. It could also enhance the mechanical damage to the alveolar cells and the surface-active lining layer and contribute to atelectasis by obstructing the airways. That foaming contributed to the reduction in surface activity was demonstrated by the findings that in eight lobes in which foaming was prevented, surface properties were within the normal range in four, and were, on the average, less marked than in the lobes that were left unoccluded ($p < 0.05$).

In experimental preparations having certain features in common with the one we have described here, Pegg, Horner, and Wahrenbrock found altered surface properties in lungs of anesthetized, tracheostomized rats that had been submerged under fluid for several hours (17). And Johnson and associates reported abnormal surface tension and pressure-volume relations in dog lungs that had been ventilated *in vivo* while partially filled with saline or amniotic fluid (8). Similarly, Salisbury and co-workers showed that lavage of a lung lobe, i.e., its use as an artificial kidney, often resulted in edema, consolidation, and hemorrhage (18), although they did not measure surface tension. In all of these preparations, there was intra-alveolar fluid, the fluid was moving across the alveolar wall, or to and from the airways, and perfusion was preserved. This combination of factors suggested to us the possibility that surfactant, having been "dislodged" or "displaced" mechanically by the abnormal fluid movement, was then removed from the alveolar spaces, perhaps by lymph. For this reason, we examined the surface activity of lymph from the right lymph duct which, in the dog, contains most of the lymph flow from the lung (19). In four determinations, lymph surface activity was low (mean γ max 53 ± 4, mean γ min 35 ± 11 dynes

per cm) and did not increase with pulmonary edema. However, the possibility that inactivated surfactant was carried in lymph could not be excluded.

Interrelations between pulmonary edema, impaired surface activity, and alveolar closure. The demonstration that lungs with acutely induced pulmonary edema showed regional loss of surface activity and premature alveolar closure is best explained by postulating that pulmonary edema led to impaired surface activity, which in turn, led to alveolar closure. No other factors in the experimental conditions could explain the results. Breathing 100% oxygen has been reported to interfere with surfactant function in rabbits, but this was after a 3- to 4-day exposure (20).

The patchy "atelectasis" in edematous lungs was probably to a large extent a consequence of abnormal surface forces, since *a*) the induction of atelectasis alone did not significantly alter the surface activity of extracts, and *b*) a regional, rather than segmental or lobar, distribution of atelectasis is consistent with the pattern to be expected when it results from abnormal surface activity (13). The predominance of abnormal changes in dependent portions of the lung is attributable to an earlier and accelerated transudation of fluid due to greater hydrostatic forces.

Although loss of pulmonary surfactant could influence fluid balance in the pulmonary capillaries in favor of pulmonary edema and hemorrhage (21, 22), our results do not permit an evaluation of this factor. Intravenous infusion of dextran as given in this study causes a marked elevation in left atrial pressure (2) which, alone, could account for pulmonary edema. The possibility could not be ruled out, however, that the increase in alveolar surface forces due to pulmonary edema could have accentuated the rate of fluid transudation.

There is no reason to believe that the method we employed to induce pulmonary edema, i.e., intravenous infusion of dextran, was *per se* responsible for the changes in surface tension properties. We obtained similar results in several experiments in which pulmonary edema was induced by injection of alloxan, and failed to demonstrate *in vitro* any effect of dextran solution on normal lung extracts. If the mere presence of pulmonary edema, regardless of etiology, can alter alveolar surface properties, this could explain, at least in part, surface tension abnormalities that may be found in conditions complicated by pulmonary edema, e.g., vagotomy in guinea pigs (23) and oxygen poisoning.

Summary

1) The effect of pulmonary edema on surface activity of lung extracts was examined in 24 anesthetized dogs. Pulmonary edema was induced by intravenous infusion of dextran, and surface tension properties were measured on saline extracts of lungs. Pressure-volume relations were determined in excised lobes and compared with normal controls.

2) Dark, "atelectatic" portions of edematous lungs, scattered throughout, but most marked in dependent parts, showed significantly increased maximal and minimal surface tension ($p < 0.001$), and significantly decreased extract stability index ($p < 0.001$).

3) When edema was induced in degassed, nonventilated lung and no foaming occurred, surface properties were abnormal, but less so than in lung permitted to foam ($p < 0.05$).

4) Edematous lung lobes with considerable morphologic alteration showed a significantly reduced "expansion index" relative to normal lobes ($p < 0.001$).

5) We conclude that pulmonary edema leads to a regional impairment of pulmonary surface activity, associated with premature alveolar closure. The mechanism of altered surface activity was not explained fully; foaming was an important, but not essential, factor.

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