Depletion of Alveolar Surface Active Material by Transbronchial Plasma Irrigation of the Lung

SIDNEY LEVITSKY,* M.D., CHARLES A. ANNABLE, MAJ., USA,
BENJAMIN S. PARK, CAPT., USAR,** ALLEN L. DAVIS, LTC., USAR,
PAUL A. THOMAS, COL., USA

From the Thoracic Surgery Service, Valley Forge General Hospital, Phoenixville, Pennsylvania 19460

THE descriptive but ill defined terms "shock lung" and "wet lung" have been used to characterize a clinical picture of deteriorating lung compliance and inadequate blood oxygenation associated with pink frothy tracheobronchial fluid in patients subjected to severe trauma with shock. This problem has been emphasized by experiences in the management of severely wounded patients in Vietnam.[6, 13, 18] The pathologic and hemodynamic evolution of the entity have been simulated and studied in experimental animals.[5, 16] Henry and co-workers[9] concluded that a disturbance in the lung surfactant system may contribute to the progressive pulmonary difficulty in these patients.

Five lung specimens secured at postmortem from casualties who died of wounds in Vietnam were received and studied in this laboratory. The fresh specimens were immediately frozen and transported within 29 days of the patients' demise. These tissues were examined to correlate histologic features, water content and surface activity of minced extracts with the clinical course between wounding and death (Table 1). Hyaline membranes appeared in several edematous lungs secured from patients with clinical manifestations of "shock lung" during life (Fig. 1). Demonstration of abnormal surface activity in extracts prepared from these specimens gave direction to the experimental effort.

The normal rate of production and consumption of pulmonary surfactant has not been established. Henry and others[9] suggested that the anoxic injury to surfactant producing alveolar lining cells results in a state of surfactant depletion. This exhaustion of alveolar surface activity may be hastened by events subsequent to the initial pulmonary insult. Simmons and coauthors[12] found pulmonary edema in a large number of casualties dying after resuscitation in Vietnam. It was postulated that leakage of protein rich fluid from the vascular space into the alveoli in the "shock lung" occurs frequently. Said and coworkers[15] observed that pulmonary surfactant activity was adversely affected by pulmonary edema. The therapeutic requirement to assist ventilation and provide an oxygen enriched atmosphere to sustain life confuses this problem. These apparently beneficial measures may singly or in concert contribute to further depletion, deterioration or destruction of pulmonary surfac-

Submitted for publication February 24, 1970.

* Present address: Dept. of Surgery, Abraham Lincoln School of Medicine, University of Illinois College of Medicine, P. O. Box 6998, Chicago, Ill. 60680.

** Deceased March 15, 1969, Republic of Korea.

Supported by Department of Army Medical Research Grant Number 62110A 3A062110A826 00 106.

The principles of laboratory and animal care as established by the National Society for Medical Research were adhered to in these experiments.

TABLE 1. *Pathology, Water Content and Surface Activity of Pulmonary Tissues Collected from Five Patients Dying of Wounds in Vietnam*

Case Number	Histopathology	% Lung Water	Surface Tension		$\bar{S}$
			Maximal	Minimal	
1	None, normal lung*	80	29	5	1.42
2	Pulmonary embolism without infarction	84	24	7	1.10
3	Pulmonary edema	81	25	14	0.57
4	Pulmonary edema, hyaline membranes	83	32	19	0.51
5	Pulmonary edema, hyaline membranes	85	43	24	0.54

* The patient died immediately after wounding.
Normal lung values for stability Index ($\bar{S}$) in this laboratory are 1.33 ± 0.16.

tant.[7,11,12] Therefore these experiments attempted to accumulate data to provide a clearer understanding of early surfactant changes in "shock lung."

Experimental Design

This investigation assumed that the initial pulmonary insult resulted in transudation of edema fluid through the cell membrane barriers of the alveolus and pulmonary surfactant was floated away from the alveolar surface. Furthermore mechanical agitation and administration of oxygen provoked removal, deactivation or destruction of surfactant. Therefore the experiments were conducted in the following sequence. First, the surface activity of tracheobronchial fluids and lung extracts were measured after creating acute pulmonary edema in experimental animals. Second, surface tension measurements were made of washings and lung extracts after *in vitro* transbronchial irrigation with 0.9% sodium chloride solution, canine plasma and 4% human albumin solution. Third, similar measurements were made of lung extracts 24 hours after *in vivo* transbronchial irrigations with 0.9% sodium chloride solution or canine plasma. Fourth, and finally, surface tension measurements were made of lung extracts following *in vivo* transbronchial irrigations in animals maintained on positive pressure ventilation with or without oxygen enrichment for 12 hours.

Materials

Unselected heart worm free mongrel dogs of both sexes weighing from 10 to 20 Kg. were used. Pentobarbital, 30 mg. per Kg., was administered intravenously to each animal before beginning an experiment. Ventilatory support was varied with each experiment and will be described in the appropriate section. *In vivo* isolation of the right and left lungs for irrigations and independent ventilation was accomplished with either a transoral double lumen metal tracheal divider[24] or a transtracheal double lumen silastic tracheal divider.*

Surface tension measurements were made with a Wilson self-cycling, direct writing surface tension balance. The method of preparing minced lung extracts and measuring surface tension was previously reported from this laboratory.[21,22] Results are expressed as maximal surface tension, minimal surface tension (dynes per cm.) and stability index ($\bar{S}$) calculated as 2 (maximal surface tension − minimal surface tension)/(maximal surface tension + minimal surface tension).

Statistical analysis of the data accumu-

* Supplied by U. S. Catheter & Instrument Corp., Glens Falls, New York.

lated during experiments 2 and 3 was performed on a CEIR Basic computer programmed to compare two groups of paired data on an equal variance model. Analysis of the data accumulated for experiment 4 was made by the signed-ranks analysis of matched pairs differences.

Methods and Results

Experiment 1. Pulmonary edema was acutely induced in six animals by intravenous infusion of 6% low molecular weight dextran (average molecular weight 40,000) in sodium chloride given at a rate of 4 ml./Kg./min.[15] Before starting the infusion in a peripherally cannulated vein a catheter was placed in the superior vena cava through the jugular vein for central venous pressure measurement. A cuffed endotracheal tube was placed and ventilation was controlled with a piston respirator. The rapid intravenous infusion was continued until frothy pink fluid appeared in the endotracheal tubing which occurred when the central venous pressure exceeded 39 cm. of water. A collection of tracheal fluid was made by gravity drainage. The animal was sacrificed, the chest was opened and representative lung specimens were secured for extract preparation.

The results of this experiment are tabulated (Table 2). A 50 ml. aliquot of gravity collected pulmonary edema fluid was placed directly in the measuring trough of the surface tension balance and lung specimens were extracted for surfactant as described. The measured surface tensions and calculated stability indices of the edema fluid and lung extracts were not significantly different. Surfactant activity was evident in both specimens. Similar examination of the dextran solution used to precipitate pulmonary edema did not have surface activity comparable to that of either the edema fluid or lung extracts.

Experiment 2. *In vitro* transbronchial irrigations were made with collapsed canine lung lobes removed from healthy animals. Paired lobes from the same lung were

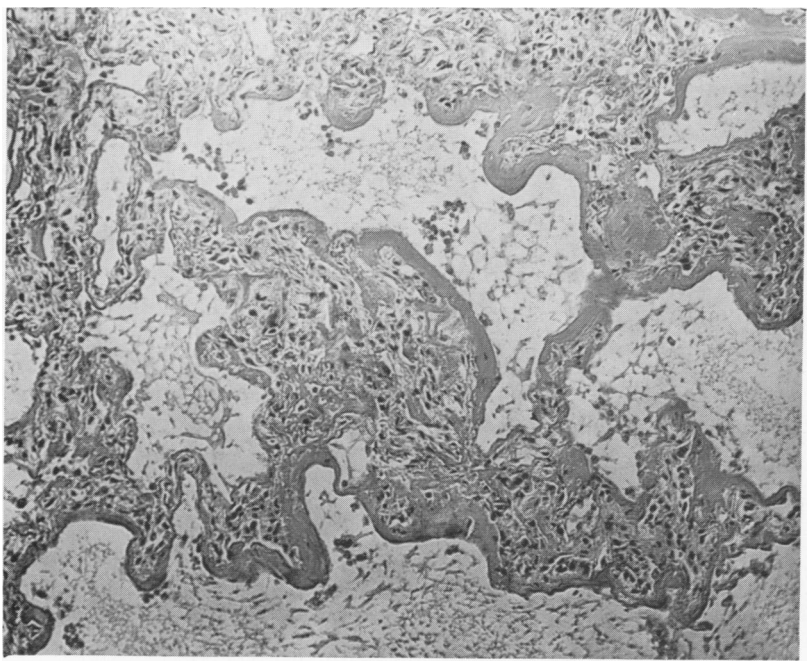

FIG. 1. (Photomicrograph, mag. ×40.) This section was prepared from pulmonary tissues of patient 5 (Table 1). Note the remarkable hyaline membranes formed over the alveolar surfaces. These changes have been observed frequently to lesser degrees in patients with similar clinical pictures.

TABLE 2. *Surface Activity of Edema Fluid and Lung Extracts*

Exp. Number	Edema Fluid Surface Tension			Lung Extract Surface Tension		
	Maximal	Minimal	$\bar{S}$	Maximal	Minimal	$\bar{S}$
101	22	2	1.67	31	6	1.35
102	19	1	1.80	38	5	1.54
103	24	3	1.56	36	8	1.45
104	23	2	1.68	35	8	1.26
105	31	7	1.27	38	10	1.17
106	29	12	0.83	29	9	1.05
		Mean	1.47			1.30
		Std. Dev.	±0.36			±0.18

used for nine comparative determinations after irrigation with either 0.9% sodium chloride solution or pooled canine plasma. An additional five canine lung lobes were irrigated *in vitro* with 4% human albumin solution. The lobes were prepared by placing a glass cannula in the lobe bronchus. The irrigating solution was introduced by gravity flow into the lobe until an end pressure of 15 to 20 cm. of water was achieved. The irrigating solution was poured out of the lobe and collected for surface tension measurement. Approximately two thirds of the volume introduced was recovered and a 50 ml. aliquot was placed in the trough of the balance. The lung lobes were then inflated with air and a portion excised for preparation of an extract.

The $\bar{S}$ decreased to 0.86 ± 0.23 in the plasma irrigated lung lobes while the saline filled lobes has a $\bar{S}$ of 1.26 ± 0.38 ($p < 0.01$) (Table 3). There was no significant difference between the $\bar{S}$ of the bronchial returns and the lung extracts of the saline irrigated lobes which were 1.35 ± 0.30 and 1.26 ± 0.38, respectively. In plasma irrigated lung lobes, however, the bronchial returns had a $\bar{S}$ of 1.70 ± 0.16 and the lung extract a $\bar{S}$ of 0.86 ± 0.23 ($p < 0.01$). In addition normal surfactant activity was demonstrable in the irrigation returns after instillation of 4% human albumin solution *in vitro* (Table 4). Examination of the 0.9% sodium chloride solution, pooled canine plasma and 4% human albumin solu-

TABLE 3. *Surface Activity of Returns and Extracts After* in vitro *Transbronchial Saline and Plasma Irrigations*

Exp. Number	Saline Irrigation						Plasma Irrigation					
	Bronchial Return			Lung Extract			Bronchial Return			Lung Extract		
	Max.	Min.	$\bar{S}$	Max.	Min.	$\bar{S}$	Max.	Min.	$\bar{S}$	Max.	Min.	$\bar{S}$
107	29	10	0.97	35	15	0.80	22	1	1.83	35	16	0.75
108	23	2	1.68	34	2	1.78	23	1	1.83	39	11	1.12
109	23	3	1.54	36	6	1.43	28	5	1.39	40	20	0.67
110	33	4	1.57	40	9	1.27	20	2	1.64	37	17	0.74
111	23	3	1.54	35	8	1.26	23	2	1.68	36	12	1.00
112	25	7	1.13	46	7	1.47	22	2	1.67	38	13	0.90
113	41	4	1.64	37	3	1.70	23	3	1.54	38	8	1.30
114	40	15	0.91	27	12	0.77	25	1	1.85	36	18	0.67
115	30	8	1.16	29	12	0.83	24	1	1.84	34	18	0.62
	Mean		1.35			1.26			1.70			0.86
	Std. Dev.		±0.30			±0.38			±0.16			±0.23

tion used in these irrigations did not disclose surface activity comparable to that of either the bronchial returns or lung extracts.

Experiment 3. Five animals were prepared for *in vivo* left lung irrigations. Ventilation was assisted with a cuffed endotracheal tube using 100% oxygen for 15 minutes. The endotracheal tube was removed and the metal, double lumen tracheal divider was inserted to the carina to isolate the right and left lungs. The occluding balloons were inflated and selective ventilation of the right lung with oxygen was initiated. The left lung was allowed to become atelectatic over the next 15 minutes as the residual alveolar oxygen was absorbed. The animal was positioned with the left lung dependent and 150 ml. of pooled canine plasma was introduced by gravity flow. A 15-minute period of contact was followed by gravity drainage of the plasma from the lung by repositioning the animal; approximately two thirds of the volume instilled was recovered. The tracheal divider was removed and replaced by a cuffed endotracheal tube. Mechanical ventilation with room air was continued until the animal recovered from anesthesia. Twenty-four hours later the animals were sacrificed and specimens of both right and left lungs were secured for extraction and measurement of surface activity. In addition histologic sections were prepared and reviewed.

TABLE 4. *Surface Activity of Returns after* in vitro *Transbronchial 4 Per Cent Human Albumin Irrigations*

Exp. Number	Surface Tension (dynes/cm.) Maximal	Minimal	$\bar{S}$
116	24	2	1.70
117	21	8	0.90
118	25	8	1.03
119	23	7	1.06
120	30	6	1.34
		Mean	1.21
		Std. Dev.	±0.32

The surface activity of the non-irrigated (control) right lung extracts was normal whereas the activity of the left lung extracts was abnormal (<0.001 (Table 5). On gross inspection the irrigated left lungs had patchy areas of atelectasis confirmed by histologic examination.

Experiment 4. Sixteen dogs were prepared for this experiment. Pentobarbital was administered intravenously and an endotracheal tube was placed as before for ventilation with 100% oxygen. During this 15-minute period of oxygenation a femoral artery and vein were cannulated to monitor arterial pressure and provide access for

TABLE 5. *Surface Activity of Extracts after* in vivo *Transbronchial Plasma Irrigation of the Left Lung*

Exp. Number	Surface Tension (dynes/cm.) Right Lung maximal	minimal	$\bar{S}$	Left Lung maxinal	minimal	$\bar{S}$
121	28	8	1.12	33	15	0.75
122	30	3	1.64	33	10	1.07
123	29	12	0.83	32	18	0.56
124	30	6	1.33	28	10	0.95
125	25	3	1.57	36	17	0.73
		Mean	1.30			0.81
		Std. Dev.	±0.33			±0.20
			$p < 0.001$			

intravenous drug and fluid administration. The endotracheal tube was removed and a tracheal divider placed to isolate the right and left lungs. Metal dividers passed orally were used in nine dogs and silastic dividers inserted through a tracheostomy were used in seven. Again the right lung was ventilated with oxygen while the left lung was permitted to become atelectatic by absorbing the residual oxygen over a 15-minute period. These 16 animals were divided randomly into groups of four to compare plasma vs. no plasma irrigation and oxygen vs. room air ventilation. Subsequent to the programmed treatment of the isolated left lung the animals were lightly anesthetized with supplemental intravenous administrations of pentobarbital, curare and flaxidil for 12 hours. A single irrigation of the isolated left lung with 150 ml. of either 0.9% sodium chloride solution or canine plasma was performed as in the previous experiment. After 15 minutes, the solution was recovered by gravity drainage. In this experiment the tracheal divider was not removed but the isolated lungs were independently ventilated for the post-irrigation period of 12 hours to the time of sacrifice. Separate respirators were used to ventilate the right lung at a rate of 14 to 16 per minute and the left lung at an independent rate of 6 to 8 per minute. Half of the animals were ventilated with room air and half with 100% oxygen for this 12-hour period. The animals were then sacrificed and specimens of each lung were secured for study.

In a control group of eight animals plasma irrigation of the left lung was not performed. Treatment was as follows: in four animals, no irrigation, in two the left lungs remained unventilated throughout the 12-hour interval, and in two the lungs were ventilated independently. In the other four animals left lungs were irrigated with 0.9% sodium chloride solution. Comparison groups for evaluating room air and oxygen ventilation were established by equal numbers of animals as irrigation controls and plasma treated subjects. During this experiment it was observed that a remarkable outpouring of pink watery fluid from the plasma irrigated lungs persisted for several hours. The total volume recovered frequently exceeded the 150 ml. instilled.

At the time of sacrifice there were severe changes in the plasma irrigated lungs, particularly marked in the left lower lobes which received the greater proportion of irrigating solution. The lungs were airless, discolored and heavy with fluid. In contrast after saline irrigation patchy changes were sometimes apparent but involvement of even the most dependent aspect of lower lobes was minimal. Specimens taken from both lungs included portions of the left lower lobes. Surface tension measurements were made and the water content of each specimen was determined by obtaining wet and dry weight.

Data accumulated from these determinations have been tabulated (Table 6). In each paired determination the water content and minimal surface tension of the right lung represented the control for that experimental animal. Plasma irrigated lungs contained more water and minimal surface tension of extracts was not as low as that of right lung specimens, whereas there was no difference for non-plasma treated lungs. There was no significant difference between lungs ventilated with 100% oxygen and those ventilated with room air for 12 hours. Histologic examination of the lungs confirmed the gross appearance. All right lung specimens were normal except for rarely encountered alveoli containing fluid. The left lungs from non-plasma irrigated specimens were normal including those of the saline irrigation groups. In plasma irrigated lungs intra-alveolar fluid accumulated in the specimens after room air ventilation. In lung specimens examined after plasma irrigation and oxygen ventilation atelectasis and some inflammatory cell infiltration with fluid appeared.

TABLE 6. *Water Content and Extract Surface Activity after* in vivo *Lung Irrigations and 12 Hours Forced Ventilation with Room Air or Oxygen*

Exp. No.	Lung Treatment		% Lung Water		Surface Tension Minimal	
	Irrigant (Left Lung)	Ventilation	Right	Left	Right	Left
126	None	Room air	81	82	11	11
127	None	Oxygen	89	84	4	2
128	None	Room air*	71	76	5	8
129	None	Oxygen*	75	81	7	5
130	Saline	Room air	81	87	7	17
131	Saline	Room air	75	72	12	1
132	Saline	Oxygen	74	79	10	12
133	Saline	Oxygen	79	76	4	7
134	Plasma	Room air	85	90	6	18
135	Plasma	Room air	80	88	6	18
136	Plasma	Room air	76	83	11	19
137	Plasma	Room air	80	79	3	2
138	Plasma	Oxygen	78	93	2	18
139	Plasma	Oxygen	81	89	4	15
140	Plasma	Oxygen	75	91	8	18
141	Plasma	Oxygen	81	84	1	17

* Left Lung not ventilated.
$p < 0.05$ for increased water content and decreased surface activity in plasma irrigated lungs.

Discussion

Pulmonary mechanics and alveolar surface activity has been previously studied in patients and in experimental animals with pulmonary edema.[3, 17] Said and co-workers [15] found a decrease in surface active material in predominantly airless portions of the lung lobe following induced pulmonary edema. Surface active material in the bloody foam was not studied or compared to average lung samples. Other workers obtained surface active material from the tracheal effluent of excised rat lungs that were perfused with saline.[2] These reports agree with our findings in acutely induced pulmonary edema where an equivalent amount of surface active material was found in the lung extracts and tracheal fluid.

Other investigators have extracted surfactant from the lung by bronchial irrigation.[4, 25] There has been no previous attempt, however, to quantitate effects of various irrigating fluids on surface activity. Although both saline and plasma irrigations extract surfactant from the lung, plasma appears to be more efficient (Table 3). In addition, plasma bronchial irrigations both *in vitro* and *in vivo* markedly deplete the lung lobe of surface active material (Table 4).

The relatively large quantities of surfactant in bronchial returns after induced pulmonary edema and saline or plasma lavage suggest that surface active material is physically removed rather than inactivated. Johnson and others [10] found decreases in surface active material in lobes filled with saline and amniotic fluid and concluded that intra-alveolar fluid inactivates or displaces surface active material from the alveolar lining membrane. Using intravenous injections of labeled palmitate Harlan and co-workers [8] showed that transudation of plasma into alveoli with the onset of foaming in pulmonary edema inhibits surfactant production. Other authors postulate inactivation of surfactant by plasma during pulmonary edema, respiratory distress syndrome of newborn babies and oxygen poisoning.[14] Fibrinogen, oleic acid, and cholesterol have been shown to inactivate sur-

factant.[20, 23] Their presence in plasma may assist in decreasing surfactant levels in the lung during pulmonary edema but does not account for elevated levels in the bronchial returns.

The third experiment demonstrated a lingering effect of plasma irrigation on pulmonary surfactant extending to 24 hours. The duration of this effect is disturbing if, in addition, the metabolic productivity of the alveolar lining cell has been disturbed by anoxic injury.[9] A mechanical washout of pulmonary surfactant from the alveolar surface would be expected to result in temporary alveolar instability lasting only until resurfacing could be affected. However, the disturbance of forces acting at the air liquid interface with surfactant depletion would lead to continued fluid accumulation setting up a vicious cycle. Continued appearance of larger volumes of tracheobronchial fluid after plasma irrigation in the fourth experiment suggests such a mechanism to a limited extent.

In the final experiment an attempt was made to isolate a specific oxygen related effect on pulmonary surfactant. Over a 12-hour period, ventilation with 100% oxygen did not appear particularly harmful. When combined with plasma lobar instillation, however, there was a definite increase in water content and a decrease in surface activity ($p < 0.05$).

Present methods for measuring surfactant in the lung are indirect and depend on changes in the surface area of a film balance.[1] Steim and co-workers[19] isolated a mixture of lipids that appears to satisfy the physical properties expected of lung surfactant and is a step towards direct measurement of surfactant.[19] All measurements performed in these experiments were indirect and thus it is difficult to compare results in the normal range. Nevertheless, statistically, patients diagnosed as having the "shock lung syndrome" and lung lobes irrigated with plasma have decreased alveolar surface activity.

The pulmonary insult which initiates the events terminating in pulmonary insufficiency, "shock lung" or "wet lung," is illusive and may be related to hypoperfusion, hypoxia, embolization, overzealous resuscitation, retained secretions, release of vasoactive substances or humoral agents in any relationship. The common response of pulmonary tissues, however, is leakage of fluid from the intravascular space to the alveolar interstitium and finally into the alveolus to obliterate the air liquid interface. Pulmonary surfactant is depleted by mechanical removal probably aided by an affinity for protein carried in the plasma. Mechanical agitation of the now free surfactant bearing fluid by assisted ventilation further depletes the surface active substance. Resurfacing of the alveolus and establishment of the air liquid interface is prohibited by metabolic depression of the alveolar cell which produces surfactant and may result in an irreversible terminal state.

Summary

Pulmonary complications associated with periods of inadequate tissue perfusion has been identified as the "shock lung" syndrome. In its most severe form there is evidence of hypoxemia secondary to arteriovenous shunting and accumulation of serosanguineous intrabronchial fluid. In three severely wounded Vietnam battle casualties with evidence of this syndrome in whom there was histological confirmation of edema and hyaline membrane formation, the Surface Tension Stability Index (STSI) was decreased to 0.52 ± 0.02 (Normal $= 1.33 \pm 0.16$). This suggested that the intra-alveolar plasma containing fluid found in this syndrome could be a factor depleting surface active material.

Experimental production of pulmonary edema by dextran infusion in six dogs produced a STSI of 1.40 ± 0.36 in the fluid and 1.30 ± 0.18 in the lung extracts. Thus while acute pulmonary edema fluid contained surfactant, there was no evidence of alveolar depletion. In nine experiments, ad-

jacent ipsilateral lung lobes were irrigated *in vitro* with either saline or pooled canine plasma. The STSI decreased to 0.86 ± 0.23 in plasma irrigated lung lobes while saline filled lobes had a STSI of 1.26 ± 0.38 ($p < 0.01$). In five animals the right and left lungs were isolated from each other with double lumen tracheal dividers and left lungs were irrigated *in vivo* with plasma. Twenty-four hours later the animals were sacrificed and right and left lungs showed STSI of 1.30 ± 0.33 and 0.81 ± 0.20 ($p < 0.001$), respectively. Addition of a highly concentrated oxygen atmosphere for a 12-hour period of mechanically assisted ventilation did not produce discernable surfactant abnormalities in addition to those observed after plasma irrigation.

These human and animal studies support the hypothesis that a plasma factor, when introduced into the alveoli contributes to the removal of surface active material and may explain the apparent irreversibility of the "shock lung" syndrome.

Acknowledgment

The authors acknowledge the technical assistance provided by the personnel of the Surgical Research Laboratory of the Temple University School of Medicine where these experiments were conducted.

References

1. Avery, M. E. and Mead, J.: Surface Properties in Relation to Atelectasis and Hyaline Membrane Disease. Amer. J. Dis. Child., **95**: 517, 1959.
2. Bondurant, S. and Miller, D. A.: A Method for Producing Surface Active Extracts of Mammalian Lungs. J. Appl. Physiol., **17**:167, 1962.
3. Cook, C. D., Mead, J., Schreiner, G. L., Frank, N. R. and Craig, J. M.: Pulmonary Mechanics during Induced Pulmonary Edema in Anesthetized Dogs. J. Appl. Physiol., **14**: 177, 1959.
4. Cook, W. A. and Webb, W. R.: Surfactant in Chronic Smokers. Ann. Thorac. Surg., **2**:327, 1966.
5. Cook, W. A.: Experimental Shock Lung Model. J. Trauma, **8**:793, 1968.
6. Gomez, A. C.: Pulmonary Insufficiency in Nonthoracic Trauma. J. Trauma, **8**:656, 1968.
7. Greenfield, L. J., Ebert, P. A. and Benson, D. W.: Effect of Positive Pressure Ventilation on Surface Tension Properties of Lung Extracts. Anesthesiology, **25**:312, 1964.
8. Harlan, W. R., Said, S. E. and Banejee, C. M.: Metabolism of Pulmonary Phospholipids in Normal Lung and during Acute Pulmonary Edema. Amer. Rev. Resp. Dis., **94**:938, 1966.
9. Henry, J. N., McArdle, A. H., Bounous, G., Hampson, L. G., Scott, H. J. and Gurd, F. N.: The Effect of Experimental Hemorrhage Shock on Pulmonary Alveolar Surfactant. J. Trauma, 7:691, 1967.
10. Johnson, J. W. C., Permutt, S., Sipple, J. H. and Salem, E. S.: Effect of Intra-alveolar Fluid on Pulmonary Surface Tension Properties. J. Appl. Physiol., **19**:769, 1964.
11. Lee, C. J., Lyons, J. H., Konisberg, S., Morgan, F. and Moore, F. D.: Effects of Spontaneous and Positive Pressure Breathing of Ambient Air and Pure Oxygen at One Atmosphere Pressure on Pulmonary Surface Characteristics. J. Thorac. Cardiovasc. Surg., **53**:759, 1967.
12. McClenahan, J. B. and Urthowski, A.: Effect of Ventilation on Surfactant, and Its Turnover Rate. J. Appl. Physiol., **23**:215, 1967.
13. Mills, M.: The Clinical Syndrome. J. Trauma, **8**:651, 1968.
14. Said, S. J.: The Lung as a Metabolic Organ. New Eng. J. Med., **279**:1330, 1968.
15. Said, S. I., Avery, M. E., Davis, R. K., Banerjee, C. M. and El-Gohary, M.: Pulmonary Surface Activity in Induced Pulmonary Edema. J. Clin. Invest., **44**:458, 1965.
16. Sealy, W. C.: Function and Structural Changes in Lung in Hemorrhagic Shock. Surg. Gynec. Obstet., **122**:754, 1966.
17. Sharp, J. T., Griffith, G. T., Bunnell, I. L. and Greene, D. G.: Ventilatory Mechanics in Pulmonary Edema in Man. J. Clin. Invest., **37**:111, 1958.
18. Simmons, R. L., Heisterkamp, C. A., Collins, J. A., Bredenburg, C. E. and Martin, A. M.: Acute Pulmonary Edema in Battle Casualties. J. Trauma, **9**:760, 1969.
19. Steim, J. M., Redding, R. A., Hauck, C. T. and, M.: Isolation and Characterization of Lung Surfactant. Biochem. & Biophys. Res. Commun., **34**:434, 1969.
20. Taylor, F. B., Jr. and Abrams, M. E.: Inhibition of Clot Lysis by a Surface Active Lipoprotein from Lung and Inhibition of Its Surface Activity by Fibrinogen. Physiologist, 7:269, 1964.
21. Thomas, P. A.: A Technique for Surface Tension Determination of Extracts from Lung Biopsy Specimens. J. Surg. Res., **6**:142, 1966.
22. Thomas, P. A. and Treasure, R. L.: Effect of N-acetyl-L-cysteine on Pulmonary Surface Activity. Amer. Rev. Resp. Dis., **94**:175, 1966.
23. Tierney, D. F. and Johnson, R. P.: Surface Tension of Lung Extracts. Fed. Proc., **22**: 339, 1963.
24. Trummer, M. J., Dooley, T. W. and Kist, J. D.: An Improved Tracheal Divider for Simultaneous Bronchospirometry in the Dog. Surgery, **56**:574, 1964.
25. Wetton, R., Valesquez, B. J. and DuBois, A. B.: Lung Washings in Living Dogs: Surface Activity in Sedimentary Fraction. J. Appl. Physiol., **22**:372, 1967.