# Surfactant Deficiency in Rats without a Decreased Amount of Extracellular Surfactant

DONALD MASSARO, LINDA CLERCH, DONALD TEMPLE, and HORST BAIER, *Veterans Administration Hospital, Miami, Florida 33125; Pulmonary Division, Oak Asthma Research and Treatment Facility, and Division of General Medicine, University of Miami School of Medicine, Miami, Florida 33136*

ABSTRACT Low volume ventilation without periodic large inflations leads to diminished alveolar stability and to the accumulation of increased amounts of airway disaturated phosphatidylcholine (DSPC) in large aggregates that sediment at 1,000 *g;* surfactant in this form lowers surface tension less rapidly than surfactant present in the 1,000-*g* supernatant fraction. These observations led to the present work in which we tested the notion that alveolar instability may develop in the presence of an undiminished quantity of total airway surfactant, if the amount of surfactant found in the 1,000-*g* supernatant fraction is diminished.

Pulmonary compliance fell and the alveolar-arterial $O_2$ gradient widened in normothermic rats during constant ventilation in the resting tidal volume range, and, in hyperthermic rats (~39°C) similarly ventilated but with the addition of periodic sighs. The total amount of airway DSPC was undiminished in each group, but in each less DSPC was present in the 1,000-*g* supernatant fraction compared with controls. Alveolar instability and hypoxemia also developed in hyperthermic rats during low volume ventilation without periodic sighs. Although the total amount of airway DSPC was decreased in these rats, enough remained to theoretically form a continuous monomolecular film over the entire alveolar surface at functional residual capacity; however, there was insufficient surfactant in the 1,000-*g* supernatant fraction to form such a continuous film.

These findings demonstrate that the mode of ventilation, and moderate hyperthermia, may lead to decreased alveolar stability despite the presence of normal amounts of airway surfactant, and, by inference, indicate the extracellular form or state of surfactant has an important effect on alveolar stability.

## INTRODUCTION

Ventilation in the resting tidal volume range without occasional deep breaths, leads to a fall in pulmonary compliance, to the development of alveolar atelectasis, and to hypoxemia (1–3). The available evidence indicates the atelectasis and decreased compliance result from increased surface tension in the alveolar lining film (4), but, the reason the surface tension increases is unknown. Constant low volume ventilation also results in the accumulation of increased proportions of airway disaturated phosphatidylcholine (DSPC)[1] as large aggregates that sediment when lung lavage returns are centrifuged at 1,000 *g* (5, 6). Surfactant in these large aggregates lowers surface tension less rapidly than surfactant not sedimented at 1,000 *g* (6). In the present work we wished to determine whether alveolar instability develops in the presence of an undiminished amount of total DSPC in lung lavage returns (airway DSPC) if a decreased proportion of the DSPC is found in the 1,000-*g* supernatant fraction.

## METHODS

*Animals.* Male Long-Evans hooded rats (Charles River Breeding Laboratories, Wilmington, MA) were allowed food

This work was presented in part at the Annual Meeting of the Association of American Physicians, Wash., DC, May 1982, and appeared in abstract form in 1982. *Clin. Res.* 30: 570*a*. (Abstr.)

Dr. D. Massaro was a Medical Investigator of the Veterans Administration at the time of this study. Address all correspondence to Dr. D. Massaro at the Pulmonary Division R120, University of Miami School of Medicine, Miami, FL 33101.

*Received for publication 15 June 1982 and in revised form 31 January 1983.*

[1] *Abbreviations used in this paper:* A-a $O_2$ gradient, alveolar-arterial $O_2$ gradient; $C_0$ min, compliance at time zero; $C_{60}$ min, compliance after 60 min ventilation; CTV, constant tidal volume; CTV + S, constant tidal volume plus sighs; DSPC, disaturated phosphatidylcholine.

(rodent laboratory chow, Ralston-Purina Co., St. Louis, MO) and water ad lib. They were anesthetized with pentobarbital sodium (50 mg/kg$^{-1}$ body wt, i.p.), and at the end of the experiment killed by exsanguination.

*Ventilation.* Anesthetized rats were given 15 mg of tubocurarine chloride in 0.5 ml of 0.15 M NaCl i.p. The trachea was quickly incised, a plastic endotracheal tube inserted and tied into place, and ventilation begun with humidified air, delivered by a Harvard small animal ventilator (Harvard Apparatus Co., Inc., S. Natick, MA), at either room temperature (~22°C) or ~39°C. The nomogram of Kleinman and Radford (7) was used to determine the tidal volume appropriate for the animal's body weight and a respiratory rate of 40 breaths · min$^{-1}$. Sighed rats also received a three times tidal volume inflation once each minute. Rectal temperature was measured.

*Lung lavage, measurement of phospholipids, and isotopic studies.* Lungs were lavaged five times through the trachea; for each lavage we used 5 ml of ice-cold 0.15 M NaCl · g$^{-1}$ of lung weight. In some experiments the lavage returns were centrifuged at 1,000 *g* for 20 min and the pelleted and supernatant material separately analyzed (6). Lipids were extracted from the lavage returns and from the remaining lung tissue by the method of Bligh and Dyer (8); they were dried under nitrogen and brought to a known volume with chloroform/methanol (2:1, vol/vol). DSPC was then isolated (9), and, when isotopes were used, the radioactivity in DSPC was measured in a liquid scintillation counter. We added [$^{14}$C]dipalmitoyl lecithin to unlabeled lungs before extraction to estimate and correct for losses during the isolation of DSPC.

To test the effect of hyperthermia on the release of DSPC into the airways rats were given 100 $\mu$Ci of D-[$^{14}$C(U)]glucose (sp act 2.2 $\mu$Ci · $\mu$M$^{-1}$) subcutaneously in 0.5 ml of 0.15 M NaCl 45 min before the induction of anesthesia. The percentage of the total [$^{14}$C]DSPC (lung tissue + lavage returns) in the airway of rats killed 45 min after the injection of [$^{14}$C]glucose was considered the time-zero value. This figure was subtracted from the percentage of total [$^{14}$C]DSPC present in lavage returns from rats killed after an additional hour.

*Lung mechanics.* To assess lung recoil during ventilation, rats were given a three times tidal volume inflation at the start of each mode and condition of mechanical ventilation. We then measured airway pressure over 5 s while the lung was inflated with air to the volume to be used as tidal volume; the measurements, but not the large inflation, were repeated after 60 min. We also determined the effect of different modes and conditions of in vivo ventilation on the descending pressure-volume relations of saline-filled excised lungs, using the technique described by Frank et al. (10). Briefly, lungs were degassed by ventilation for 10 min with 100% $O_2$ and then clamping the trachea while the heart continued to beat. The lungs were excised and inflated to a transpulmonary pressure of 10 cm $H_2O$ with 0.15 M NaCl at 37° or at 39°C. Saline was removed to lower the pressure by 1.0- or 0.5-cm decrements measured with a water manometer.

*Blood gases and pH.* Blood was drawn from the abdominal aorta after 60 min of ventilation. The $PO_2$, $PCO_2$, and pH of these samples were measured in a Radiometer BMS$_3$-MK$_2$ analyzer (Radiometer America Inc., Westlake, OH) at 37°C; the results from hyperthermic rats were converted to values appropriate for 39°C (11). Alveolar-arterial (A-a) $O_2$ gradients were calculated assuming a respiratory quotient of 0.8 for normothermic, and 1.0 for hyperthermic rats (12).

*Lung weight and lavage proteins and cells.* The dry weight of the lung was determined by heating lungs at 60°C until two weights, 24 h apart, were the same. Cells in lavage returns were counted using a hemocytometer. The protein content of lavage returns was measured by the method of Bradford (13) using bovine serum albumin as standard.

*Statistical analysis.* For each parameter measured or calculated from measurements, the values for individual rats were averaged per experimental group and the standard error of the group mean calculated. An analysis of variance was used to test for significant differences between more than two group means (14).

## RESULTS

*Development of hyperthermia.* Rats placed in a chamber where the temperature was ~39°C experienced a rise in temperature within 30 min to 39.2±0.3°C ($n$ = 21) for sighed animals and to 39.2±0.2°C ($n$ = 10) for unsighed rats. The temperatures were unchanged after an additional 30 min. Rats exposed to room temperature had a rectal temperature of 36.9±0.06°C at the beginning of the ventilatory period and 36.9±0.03°C at the end of the ventilatory period; the sighed ($n$ = 10) and unsighed ($n$ = 21) group of rats had the same temperature (36.9°C).

*Body and lung weights.* Since the amount of airway or tissue DSPC is usually given relative to lung weight, undetected weight increases due to edema could be misleading. We therefore chose rats of approximately equal size and measured the effect on lung weight of the ventilatory and thermal conditions to be used in our studies on DSPC. The body weight (mean±SD) of rats used in these experiments were: "fresh"[2] group = 227±13 ($n$ = 20); constant tidal volume (CTV), 37°C = 233±14 ($n$ = 10); CTV, 39°C = 228±8 ($n$ = 14); constant tidal volume plus sighs (CTV + S), 37°C = 225±24 ($n$ = 8); CTV + S, 39°C = 213±32 ($n$ = 10). Although rats of the last group are somewhat lighter than rats comprising the other four groups there were no statistically significant ($P > 0.05$) differences in mean weight between any group. However, lungs of CTV-ventilated normothermic rats, and lungs of rats of both sighed groups, were heavier than lungs from fresh rats; the differences between fresh lungs and each sighed group were statistically significant (Fig. 1). The wet weight of lungs from hyperthermic CTV-ventilated rats was the lowest of any group, and the differences were statistically significant compared with each group except fresh lungs. The wet-to-dry weight ratio of lungs from CTV-ventilated normothermic, and of lungs from each sighed group, were significantly higher than that of fresh lungs (Fig. 1 B). In contrast, the ratio for lungs from hyperthermic

[2] "Fresh" denotes lungs removed from rats immediately after the onset of surgical anesthesia without a period of mechanical ventilation.

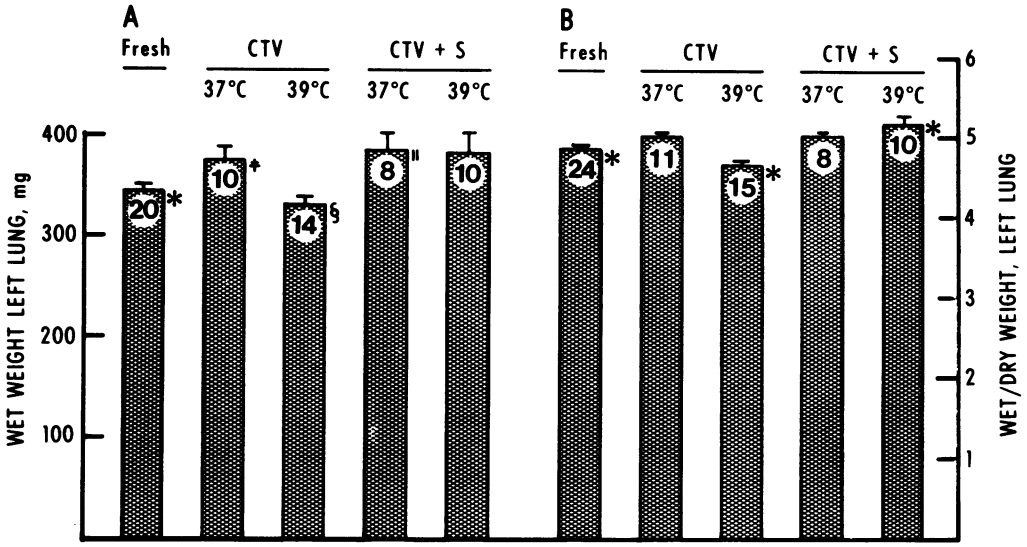


FIGURE 1 Lung weights. Fresh refers to lungs removed immediately after spontaneously breathing rats were killed. CTV denotes lungs from anesthetized rats that had been ventilated at constant tidal volume at 40 breaths · $min^{-1}$ for 60 min; CTV + S refers to similarly ventilated rats that were given a three times tidal volume sigh once each min. Mean±SE are given. (A) *$P > 0.05$ vs. CTV, 39°C and <0.05 vs. CTV, 37°C and vs. each sighed group; ‡$P < 0.05$ vs. CTV, 39°C and >0.05 vs. each sighed group; §$P < 0.05$ vs. each sighed group; ‖$P > 0.05$ vs. CTV + S, 39°C. (B) *$P < 0.05$ vs. each other group.

CTV-ventilated rats was significantly lower than that of each other group.

*Phospholipid studies.* The intergroup differences in lung weight just described were noted among rats of very similar size. These differences must therefore be ascribed to the effect of experimental conditions on lung weight rather than to intergroup variation in lung size or alveolar surface area at the start of the experiment. Because of these findings we selected rats of almost identical size for use in our studies on DSPC. The body weight of rats used to determine the effect of ventilatory mode and temperature of DSPC were "fresh" = 230±22 (mean±SD, $n$ = 22); CTV, 37°C = 230±10 ($n$ = 24); CTV, 39°C = 230±15 ($n$ = 24); CTV + S, 37°C = 236±14 ($n$ = 16); CTV + S, 39°C = 231±19 ($n$ = 17) ($P > 0.05$ between groups). In view of the similar body weight of rats in the different groups we have expressed the measured amount of DSPC in lavage returns and in lung tissue, as micrograms per lung. We have designated the DSPC in the lavage returns as airway rather than alveolar DSPC since the returns include material from alveoli and conducting airways.

Hyperthermic rats ventilated at CTV had significantly less airway DSPC than each other group; there were no differences of airway DSPC among the other groups (Fig. 2 A). There were no intergroup differences in the amount of tissue DSPC (Fig. 2 B). These results were not altered if expressed per gram of body weight (data not shown). The quantity of airway DSPC relative to total DSPC (airway + tissue) was significantly lower in hyperthermic CTV-ventilated rats than in each other group (Fig. 2 C).

The CTV mode of ventilation markedly increased the percentage of airway DSPC that pelleted at 1,000 $g$ compared with the fresh group. The temperature differences between the CTV groups did not lead to a statistically significant difference in the proportion of DSPC pelleted (Fig. 3). However, sighs during ventilation diminished the percentage of DSPC pelleted, and did so to a greater extent in normo- than in hyperthermic rats.

The amount of [$^{14}$C]DSPC present in lavage returns, and in lung tissue, was similar in the CTV groups, as was the percentage of total [$^{14}$C]DSPC (lavage + tissue) present in lavage returns: CTV-ventilated normothermic rats ($n$ = 14) had 27,135±1,327 and 1,060±113 dpm of [$^{14}$C]DSPC in lung tissue and lavage returns respectively, the percentage in the returns was 3.2±0.2% of the total; CTV-ventilated hyperthermic rats ($n$ = 14) had 29,975±1,043 and 1,153±83 dpm of [$^{14}$C]DSPC in lung tissue and lavage returns respectively, 3.7±0.2% of the total was in the lavage returns; $P > 0.05$ between groups for all values.

*Compliance measurements.* Except for sighed normothermic rats, compliance fell in all groups during

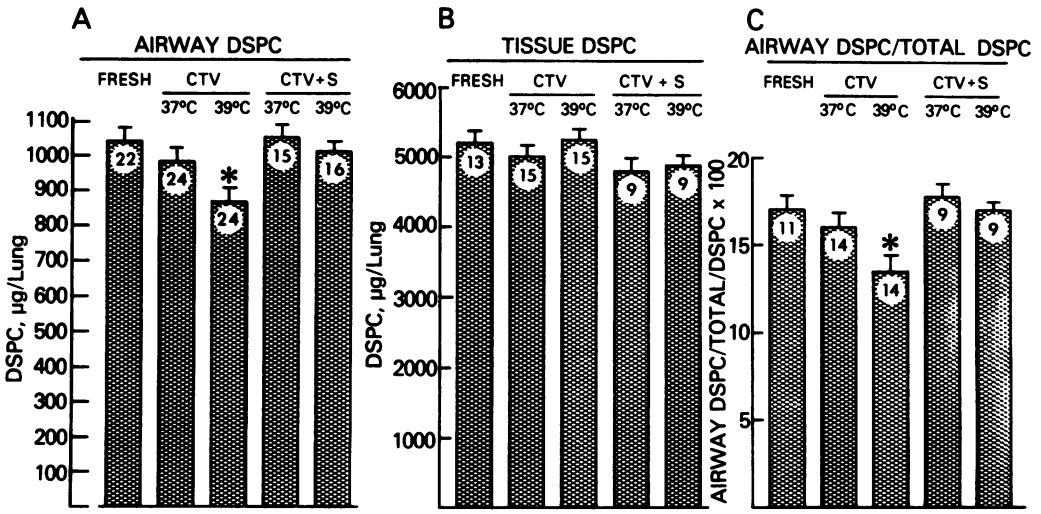


FIGURE 2 DSPC in airways and tissue. Designations are defined in the legend to Fig. 1. (A) $*P < 0.05$ compared with each other group. (B) $P > 0.05$ for each mean; (C) $*P < 0.05$ compared with each other group.

mechanical ventilation (Table I). Furthermore, combining the data in Figs. 2, 3, and Table I shows that a substantial decrease in compliance developed in the CTV + S, 39°C group and the CTV, 37°C group in the absence of a statistically significant fall in total airway DSPC (Fig. 4).

The decreases in compliance, which developed during mechanical ventilation, were not associated with differences in the recoil of excised saline-distended lungs compared to the recoil of lungs from normothermic rats ventilated at CTV with sighs (Table II). In addition, there were no intergroup differences in the absolute volume of saline-distended lungs at any transpulmonary pressure (data not shown).

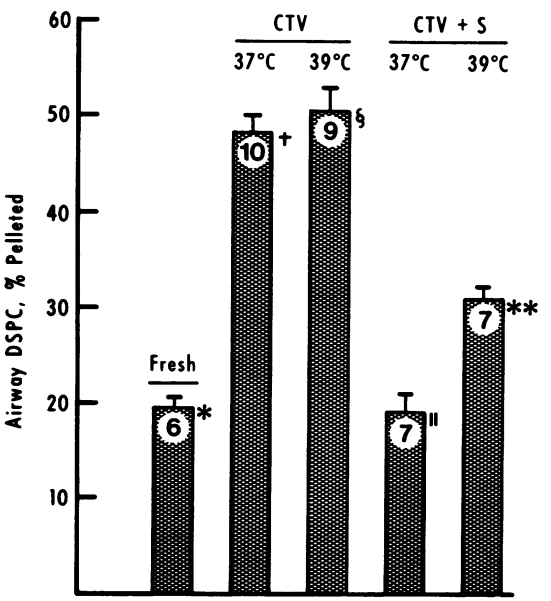


FIGURE 3 Pelleting of airway DSPC. Designations are defined in the legend to Fig. 1. $*P < 0.05$ compared with each group except normothermic sighed rats; $\ddagger P > 0.05$ vs. CTV, 39°C rats and <0.05 vs. each other group; $\S P > 0.05$ vs. CTV, 37°C rats and <0.05 vs. each other group; $\|P > 0.05$ vs. fresh group and <0.01 vs. each other group; $**P < 0.05$ vs. each other group.

TABLE I
*Compliance Measurements*

| Condition | Time-zero compliance | $\frac{C_{60}\ min}{C_0\ min}$ |
|---|---|---|
| | *ml/cm $H_2O$/g body wt* × $10^{-3}$ | % |
| CTV | | |
| 37°C (9) | 1.8±0.1* | 59.5±1.0‡ |
| 39°C (10) | 1.9±0.1 | 52.2±2.0§ |
| CTV + S | | |
| 37°C (11) | 1.7±0.0 | 100.4±2.6‖ |
| 39°C (10) | 1.8±0.1 | 81.2±2.7 |

Mean±SE are given; figures in parentheses indicate the number of rats.

* $P > 0.05$ between all values in this column.

‡ $P < 0.05$ vs. CTV, 39°C and <0.01 vs. each sighed group.

§ $P < 0.01$ vs. each sighed group.

‖ $P < 0.01$ vs. CTV + S, 39°C.

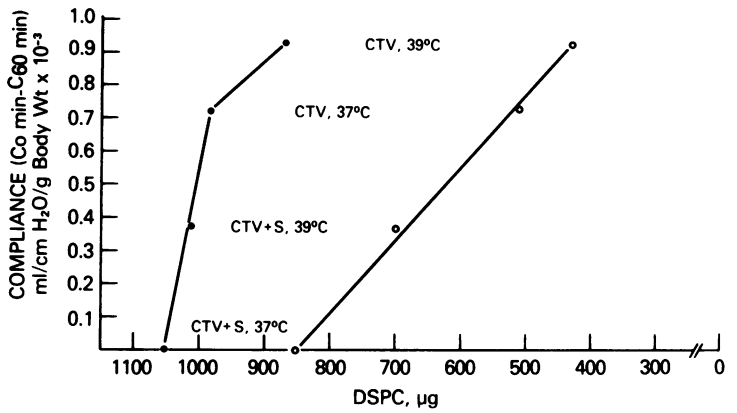


FIGURE 4 Relation between airway DSPC and change of compliance. This figure was drawn using the data in Figs. 2 and 3 and Table I. $C_0$ and $C_{60}$ refer to compliance (ml/cm $H_2O$/g body wt $\times 10^{-3}$) at the start ($C_0$) and after 60 min ($C_{60}$) of ventilation. DSPC, micrograms per lung (●); DSPC micrograms per lung in 1,000-*g* supernatant fraction (○).

*Blood gas tensions and pH and A-a $O_2$ gradient.* Hypercapnia was present in hyperthermic rats ventilated at CTV (Table III). Hypoxemia, a widened A-a $O_2$ gradient, and acidosis developed in all groups of rats except normothermic sighed rats.

*Protein and cells in lavage returns of mechanically ventilated rats.* The ventilatory or thermal conditions did not result in differences in the quantity of protein or cells in the lavage returns (Table IV).

## DISCUSSION

*Alveolar instability.* The fall in compliance and the widened A-a $O_2$ gradient were taken as evidence of alveolar instability after we considered the possibility that these changes may have been due to intraalveolar edema, or to increased recoil due to tissue rather than surface forces. We rejected these alternative explanations because (*a*) the greatest fall in compliance, and

TABLE II
*Pressure-Volume Relations of Saline-filled Lungs*

| | Maximum lung volume, % | | | | | |
|---|---|---|---|---|---|---|
| | 37°C | | | 39°C | | |
| Ptp | Fr (6) | CTV (4) | CTV + S (3) | Fr (5) | CTV (5) | CTV + S (4) |
| *cm/$H_2O$* | | | | | | |
| 0 | 13.1±1.6 | 11.3±0.6 | 11.5±1.5 | 10.2±1.6 | 14.0±1.0 | 11.7±1.1 |
| 1 | 19.4±1.8 | 19.8±1.0 | 20.3±2.4 | 16.9±1.8 | 20.7±1.7 | 17.3±0.5 |
| 2 | 27.6±1.7 | 29.4±1.3 | 30.2±2.7 | 25.3±1.8 | 29.6±2.3 | 25.6±0.5 |
| 3 | 37.5±1.9 | 41.4±1.6 | 42.4±2.4 | 35.7±1.7 | 41.2±2.8 | 37.4±1.2 |
| 4 | 50.6±1.4 | 54.4±1.6 | 56.9±2.1 | 48.7±1.4 | 55.5±2.6 | 52.8±1.6 |
| 5 | 64.0±1.0 | 68.1±1.8 | 69.9±0.8 | 62.6±0.9 | 70.2±1.8 | 67.9±1.9 |
| 6 | 76.3±1.3 | 79.1±1.0 | 80.2±0.1 | 75.1±1.3 | 80.6±1.1 | 79.8±1.4 |
| 7 | 85.4±1.0 | 87.4±0.6 | 87.7±0.4 | 84.8±0.5 | 88.6±0.6 | 88.0±0.8 |
| 8 | 92.1±0.2 | 93.2±0.3 | 92.7±0.5 | 91.7±0.7 | 94.0±0.2 | 93.4±0.4 |
| 9 | 96.8±0.3 | 97.1±0.2 | 97.2±0.5 | 96.7±0.3 | 97.7±0.3 | 97.4±0.2 |
| 10 | 100 | 100 | 100 | 100 | | 100 |

Mean±SE are given; figures in parentheses indicate the number of rats. Fr denotes lungs, from rats killed without a 1-h period of mechanical ventilation, on which measurements were made at 37° or 39°C. Other rats were ventilated for 1 h in the mode and temperature indicated and the pressure-volume measurements then made at the same temperature. An analysis of variance did not reveal any intergroup differences at any transpulmonary pressure.

TABLE III
*Blood Gas Tensions and pH and A-a $O_2$ Gradient*

| Condition | $P_{CO_2}$ | $P_{O_2}$ | A-a $O_2$ | pH |
|---|---|---|---|---|
| | | *torr* | | |
| CTV | | | | |
| 37°C (14) | 42.1±2.0 | 74.7±2.6* | 25.8±2.1 | 7.33±0.02 |
| 39°C (14) | 48.9±2.1‡ | 56.8±3.1§ | 44.4±2.5‖ | 7.23±0.02¶ |
| CTV + S | | | | |
| 37°C (7) | 35.0±3.1 | 106.0±4.9§ | 2.5±1.6 | 7.38±0.07** |
| 39°C (10) | 42.0±2.4 | 70.9±3.9‡‡ | 37.1±3.0 | 7.31±0.02 |

Mean±SE are given; figures in parentheses indicate the number of rats.
* $P < 0.01$ vs. CTV, 39°C and vs. CTV + S, 37°C.
‡ $P < 0.05$ vs. each normothermic group and >0.05 vs. CTV + S, 39°C.
§ $P < 0.01$ vs. each other group.
‖ $P < 0.05$ vs. CTV + S, 39°C and <0.01 vs. each other group.
¶ $P < 0.01$ vs. each other group.
** $P < 0.05$ vs. each hyperthermic group.
‡‡ $P < 0.01$ vs. CTV, 39°C and CTV + S, 37°C.

the largest increase in A-a $O_2$ gradient, took place in rats (CTV, 39°C) whose lungs had the lowest wet-to-dry weight ratio; (*b*) there were substantial differences in compliance between groups (CTV, 37°C, CTV + S, 37°C, and CTC + S, 39°C) in the absence of differences in lung water as reflected by the ratio of lung wet-to-dry weight; (*c*) there was no evidence of increased alveolar-capillary permeability as indicated by the similar amounts of protein and cells in the lavage returns from the different groups; (*d*) fluid-filled alveoli, or alterations in tissue forces, would have led to changes in the relation between transpulmonary pressure and absolute volume of saline-distended lungs, and this was not found.

*Total amount and physical state of airway surfactant.* We studied four in vivo experimental conditions and failed to find a relation between changes in compliance and the total amount of airway DSPC. We did find a clear and direct relation between the extent of decrease of compliance and the diminished amount of airway DSPC present in the 1,000-*g* supernatant fraction (Fig. 4). Furthermore, although CTV-ventilated hyperthermic rats did develop alveolar instability in association with a diminished amount of airway surfactant, even in this circumstance, the total quantity of airway DSPC present is theoretically sufficient to form a monomolecular film over the entire alveolar surface at functional residual capacity (15); the amount of DSPC present in the 1,000-*g* supernatant fraction is not enough for this purpose.

TABLE IV
*Protein and Cells in Lavage Returns from Mechanically Ventilated Rats*

| Condition | Lavage protein | Lavage cells |
|---|---|---|
| | *μg/g body wt* | *cells/g body wt* × $10^{-4}$ |
| CTV | | |
| 37°C (4) | 10.1±2.0 | 1.6±0.3 |
| 39°C (4) | 8.8±1.0 | 1.5±0.6 |
| CTV + S | | |
| 37°C (5) | 11.1±1.0 | 1.6±0.1 |
| 39°C (5) | 10.2±0.9 | 1.5±0.6 |

Mean±SE are given. Figures in parentheses indicate the number of experiments. None of the intracolumn differences are statistically significant.

An inhibitor, or inactivator, of surfactant could decrease alveolar stability without diminishing the amount of airway surfactant and this could explain our results. However, we think the action of an inhibitor or inactivator is not a likely explanation for the alveolar instability because of the rapidity with which a large inflation reverses the increased aggregation (5, 6) and the decreased compliance (1, 2) that develop during low volume ventilation. We do not know if the altered surface properties (6) reflect the physical state or chemical composition of the material that pellets at low centrifugal forces (16).

A recent report (17) of the effect of pulmonary artery occlusion on the amount of airway surfactant has particular relevance to our work because it demonstrated the presence of increased amounts of airway surfactant shortly after pulmonary artery occlusion, at a time when alveolar instability is known to exist

(18). This paradox is not explained by the presence of inhibitors of surfactant (17). However, since pulmonary artery occlusion leads to low volume ventilation (16), the conditions are those that result in the accumulation of increased amounts of DSPC as large aggregates.

Our present studies, the prior work where we showed that sonication of pelleted material eliminated its sedimentability at 1,000 *g* and increased the rate at which it lowered surface tension (6), and the other studies cited (4, 17, 18), lead us to suggest that the accumulation of surfactant in the large aggregates decreases the available extracellular pool of rapidly adsorbing surfactant. This diminished pool results in incomplete replacement, during low or resting tidal volume breathing, of losses from the lining film engendered during prior expirations. A large inflation or sigh, deaggregates the surfactant that is sedimented at low centrifugal forces thereby increasing the pool of more rapidly adsorbing surfactant (5, 6), which in turn increases the rate at which losses from the surface film may be replaced by surfactant in the hypophase, thereby restoring alveolar stability. This explanation for the corrective action of a large breath does not preclude a contribution of surfactant, newly secreted in response to a large inflation (19–24), to the improved alveolar stability. However, the amount of surfactant secreted in response to a single large inflation (23) is substantially less than that generated by deaggregation of easily pelleted material (5, 6).

*Lung water.* Experimentally produced increases of alveolar surface tension have been shown to lead to pulmonary edema (25). We were therefore surprised to find less lung water in CTV-ventilated hyperthermic rats, the group that has the greatest loss of surfactant function, than in the other groups. However, Langmuir and Schaefer (26) have shown that when the surface pressure of a monomolecular film of fatty acids is raised to produce collapse of more than 1 or 2% of the surface film, the evaporation resistance of the film is greatly diminished, often to 10% of its value in the uncollapsed state. We have previously suggested and provided indirect evidence that the accumulation of increased amounts of DSPC in the form of large aggregates is due to the collapse of the alveolar surface film caused by the high surface pressure generated at low lung volumes (6). If the findings of Langmuir and Schaefer apply to the alveolar lining under CTV ventilation in hyperthermic animals, it indicates that surfactant deficiency, in addition to its well known effect on alveolar stability, may also result in extra water loss from the lung during hyperthermia. Thus, lung water loss and alveolar stability may both be importantly influenced by the extracellular state of surfactant.

## ACKNOWLEDGMENTS

We thank Drs. Lee Frank, Gary Sanford, and Philip Whitney for reviewing the manuscript.

This work was supported in part by Veterans Administration Research Funds and National Heart, Lung, and Blood Institute research grant HL20366.

## REFERENCES

1. Bernstein, L. 1957. The elastic pressure-volume curves of the lungs and thorax in the living rabbit. *J. Physiol (Lond.).* **138:** 473–486.
2. Mead, J., and C. Collier. 1959. Relation of volume history of lungs to respiratory mechanics in anesthetized dogs. *J. Appl. Physiol.* **14:** 669–678.
3. Hedley-Whyte, J., M. B. Laver, and H. H. Bendixen. 1964. Effect of changes in tidal ventilation on physiological shunting. *Am. J. Physiol.* **206:** 891–897.
4. Clements, J. A. 1967. The alveolar lining layer. *In* Ciba Foundation Symposium, Development of the Lung. A. V. S. de Reuck and R. Porter, editors. Little Brown and Company, Dubuque, IA. 202–237.
5. Thet, L. A., L. Clerch, G. D. Massaro, and D. Massaro. 1979. Changes in sedimentation of surfactant in ventilated excised rat lungs. Physical alterations in surfactant associated with the development and reversal of atelectasis. *J. Clin. Invest.* **64:** 600–608.
6. Massaro, D., L. Clerch, and G. D. Massaro. 1982. Surfactant aggregation in rat lungs: influence of temperature and ventilation. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **51:** 646–653.
7. Kleinman, L. I., and E. P. Radford, Jr. 1969. Ventilation standards for samll mammals. *J. Appl. Physiol.* **19:** 360–362.
8. Bligh, E. F., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* **37:** 911–917.
9. Mason, R. J., J. Nellenbogen, and J. A. Clements. 1976. Isolation of disaturated phosphatidylcholine with osmium tetraoxide. *J. Lipid Res.* **17:** 281–284.
10. Frank, N. R., E. P. Radford, Jr., and J. L. Whittenberger. 1959. Static volume-pressure interrelations of the lungs and pulmonary blood vessels in excised cats' lungs. *J. Appl. Physiol.* **14:** 167–173.
11. Severinghaus, J. W. 1971. Effect of temperature change on blood oxygen and carbon dioxide pressures: man and dog. *In* Respiration and Circulation. P. L. Altman and D. S. Dittmer, editors. Fed. Amer. Soc. Exp. Biol., Bethesda, MD. 219–221.
12. Shelley, W. B., and A. Hemingway. 1940. The effects of thermal polypnea on the energy metabolism, respiratory quotient, and water loss of dogs. *Am. J. Physiol.* **120:** 623–630.
13. Bradford, M. A. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–258.
14. Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics.* **11:** 1–42.
15. Brown, E. S., R. P. Johnson, and J. A. Clements. 1959. Pulmonary surface tension. *J. Appl. Physiol.* **14:** 717–720.
16. King, R. J., and M. C. MacBeth. 1981. Interaction of the lipid and protein components of pulmonary surfactant.

Role of phosphatidylglycerol and calcium. *Biochim. Biophys. Acta.* **647:** 159–168.
17. Baritussio, A., and J. A. Clements. 1981. Acute effects of pulmonary artery occlusion on the pool of alveolar surfactant. *Respir. Physiol.* **45:** 323–331.
18. Severinghaus, J. W., E. W. Swenson, T. N. Finley, M. T. Lategola, and J. Williams. 1961. Unilateral hypoventilation produced in dogs by occluding one pulmonary artery. *J. Appl. Physiol.* **16:** 53–60.
19. Oyarzún, M. J., J. A. Clements, and A. Baritussio. 1980. Ventilation enhances pulmonary alveolar clearance of radioactive dipalmitoyl phosphatidylcholine in liposomes. *Am. Rev. Respir. Dis.* **121:** 709–721.
20. Faridy, E. E. 1976. Effect of distention on release of surfactant in excised dogs' lungs. *Respir. Physiol.* **28:** 99–114.
21. Klass, D. 1979. Dibutyryl cGMP and hyperventilation promote rat lung phospholipid release. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **47:** 285–289.
22. Nicholas, T. E., and H. A. Barr. 1981. Control of release of surfactant phospholipids in the isolated perfused rat lung. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **51:** 90–98.
23. Hildebran, J. N., J. Goerke, and J. A. Clements. 1981. Surfactant release in excised rat lung is stimulated by air inflation. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **50:** 905–910.
24. Massaro, D., L. Clerch, and G. D. Massaro. 1982. Surfactant secretion: evidence that cholinergic stimulation of secretion is indirect. *Am. J. Physiol.* **243:** C39–C45.
25. Albert, R. K., S. Lakshinarayan, J. Hildebrant, W. Kirk, and J. Butler. 1979. Increased surface tension favors pulmonary edema formation in anesthetized dogs' lungs. *J. Clin. Invest.* **63:** 1015–1018.
26. Langmuir, I., and V. J. Schaefer. 1943. Rates of evaporation of water through compressed monolayers of water. *Franklin Institute Journal.* **235:** 119–162.