Experimental Lung Research

ISSN: 0190-2148 (Print) 1521-0499 (Online) Journal homepage: https://www.tandfonline.com/loi/ielu20

Hyperoxia-Induced Alterations of Rat Alveolar Lavage Composition and Properties

Marvin R. Balaan, Linda Bowman, Harakh V. Dedhia & Philip R. Miles

To cite this article: Marvin R. Balaan, Linda Bowman, Harakh V. Dedhia & Philip R. Miles (1995) Hyperoxia-Induced Alterations of Rat Alveolar Lavage Composition and Properties, Experimental Lung Research, 21:1, 141-156, DOI: 10.3109/01902149509031750

To link to this article: https://doi.org/10.3109/01902149509031750

HYPEROXIA-INDUCED ALTERATIONS OF RAT ALVEOLAR LAVAGE COMPOSITION AND PROPERTIES

Marvin R. Balaan □ Section of Pulmonary and Critical Care Medicine, West Virginia University, Morgantown, West Virginia, USA

Linda Bowman □ Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Harakh V. Dedhia □ Section of Pulmonary and Critical Care Medicine, West Virginia University, Morgantown, West Virginia, USA

Philip R. Miles □ Department of Physiology, West Virginia University, and Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

□ *Although lethal exposures of most animal species to oxygen result in a reduced amount of surfactant phospholipids (PL), hyperoxia in rats leads to elevated levels of PL on the alveolar surface. Because of this different response, a study was made of the amount, composition, surface properties, and subfraction distribution (obtained by differential centrifugation) of alveolar lavage materials from rats exposed to >95% oxygen for 64 h. The exposures lead to severe lung damage, which includes the appearance of pleural effusion, pulmonary edema, and increased protein levels on the alveolar surface. However, the PL levels of lavage fluid are increased two- to threefold, and the PL composition is altered. In O_2-exposed rats, only 39(±1)% of the phospholipid is disaturated phosphatidylcholine (DSPC), the major surface active component of surfactant, as compared to 46(±1)% DSPC in lavage from control animals. The distribution of PL and DSPC in subfractions of lavage materials obtained by differential centrifugation is approximately reversed following hyperoxia. In lavage from control animals, 36% of the PL is in the heavier, more dense subfractions and 64% is in the lighter, less dense subfractions, while 72% is heavier and 28% lighter in lavage from O_2-exposed animals. Measurements of surface properties with the Wilhelmy balance indicate that the ability of the lavage materials to reduce surface tension is impaired following hyperoxia. Thus, lethal exposures of rats to oxygen lead to increased amounts of surfactant on the alveolar surface, but the surface properties of the surfactant are impaired, probably due to reduced levels of DSPC, increased amounts of protein, and alterations in its physical form.*

Keywords *lung surfactant, surface properties, surfactant subfractions*

Received 28 November 1993; accepted 1 April 1994.

This work was supported in part by NIH Biomedical Research Grant No. 2S07RR05433 and the West Virginia University Medical Corporation.

Address correspondence to Marvin R. Balaan, MD, Section of Pulmonary and Critical Care Medicine, West Virginia University School of Medicine, Morgantown, WV 26506-9166, USA.

0190-2148/95 $10.00 + .00

It is well known that exposure of mammals to 100% oxygen under normobaric conditions eventually leads to death and that the lungs are the primary site of damage [1]. Morphological evidence from earlier studies suggests that the primary site of lung injury is the pulmonary microvasculature [2]. However, more recent studies have shown that there are other pathophysiological changes in the lungs. Matalon and coworkers [3–5] have demonstrated that exposure to 100% oxygen results in a gradual increase in the permeability of the blood–air barrier to lipid insoluble molecules. Thus, there may also be damage to the alveolar epithelium and pulmonary interstitial space. Prolonged exposure to toxic levels of oxygen has been shown to result in a lung injury that is similar to that seen in the adult respiratory distress syndrome (ARDS). Some of the pathophysiologic features of ARDS appear to be related to alterations of pulmonary surfactant, a mixture of lipids and proteins that lines the alveolar surface and prevents their collapse [6]. Therefore, there has been considerable interest in the effects of oxygen exposure on the surfactant system.

The exposure of most animal species to 100% oxygen seems to result in a decrease in the amount of pulmonary surfactant on the alveolar surface. For example, Gross and Smith [7] have shown that exposure of mice for 72 h leads to a reduction in the level of surfactant phospholipids, and the authors suggest that this is due to a decrease in surfactant synthesis. Holm et al. [8] demonstrated that exposure of rabbits for 64 h results in a lowering of total surfactant phospholipids, no change in the profile of individual phospholipids, and an impaired ability of surfactant to reduce surface tension. Other studies with rabbits have demonstrated that the hyperoxia-induced changes in surfactant phospholipid levels may be due to alterations in surfactant biosynthesis by type II cells [9]. Engstrom et al. [10] showed that surfactant replacement attenuates the effects of exposure to oxygen on alveolar permeability in rabbits, another indication that hyperoxic injury results in a surfactant-deficient state. Finally, when baboons are exposed to 100% oxygen for 6 days, there is a decrease in surfactant disaturated phosphatidylcholines (DSPC), the major surface active component of lung surfactant. Furthermore, there is a decrease in DSPC as a percentage of phospholipids and in phosphatidylglycerol, and the ability of the alveolar lavage materials to reduce surface tension is impaired [11]. Thus, all of these studies suggest that hyperoxic lung injury is accompanied by a reduction in the amount of surfactant on the alveolar surface, and some suggest that surfactant function is impaired.

There is one animal species in which the response to hyperoxia is different from the others. Exposure of rats to the sublethal dose of 85%

oxygen for 3 to 7 days results in a five- to tenfold increase in surfactant DSPC and surfactant protein A [12–14]. Young et al. [12] have associated the increases in surfactant DSPC with type II cell hyperplasia. The effects of exposing rats to a lethal dose, i.e., greater than 95% oxygen, seem to be a little different. For example, there appears to be no type II hyperplasia [15], but there is a 50% increase in alveolar lavage phospholipids [16]. Since the response of the rat to hyperoxia is unique among the animal species tested, in that there seems to be an increase in lung surfactant, we decided to do some additional studies. Our results also indicate that exposures of rats to lethal doses, i.e., greater than 95% oxygen for 64 h, results in an increase in alveolar lavage phospholipids. Therefore, the objective of this investigation was to study the composition, surface properties, and subfraction distribution (obtained by differential centrifugation) of alveolar lavage materials obtained from the lungs of rats exposed to greater than 95% oxygen for 64 h.

MATERIALS AND METHODS

Exposure of Animals and Isolation of Alveolar Lavage Materials

Male Sprague-Dawley rats (300–400 g) obtained from Hilltop Labs (Scottsdale, PA, USA) were used in all experiments. The animals were placed in plexiglass chambers constructed with partitions so that individual housing for four rats was provided. Free access to food and water was available. One group of animals was exposed to oxygen at atmospheric pressure, and the other group (control) was exposed to air. The flow rate in the chambers was 12–15 L per minute, which provided for 7–10 complete gas changes per hour. Thus, the level of CO_2 in the chambers was always less than 0.15%. The oxygen concentration in the appropriate chambers was monitored with an oxygen analyzer (Model 04066; Instrumentation Laboratories; Lexington, MA, USA), and the level never fell below 97%. Animals were exposed continuously for 64 h and then sacrificed for study of alveolar lavage materials.

Following the exposures, animals from both groups were anesthetized with sodium pentobarbital (200 mg/kg body weight) and exsanguinated by cutting the abdominal aorta, and the heart and lungs were removed. After the heart was carefully dissected away, the lungs and trachea were blotted and weighed. Alveolar lavage materials were obtained by tracheal lavage (5 mL per gram lung; 3 lavages) with Ca^{2+}-free phosphate-buffered medium (145 mM NaCl, 5 mM KCl, 9.35 mM Na_2HPO_4, 1.90 mM NaH_2PO_4, pH 7.4). There was no significant difference between control and oxygen-exposed rats in the lavage returns, which were greater than 80%. Alveolar macrophages were removed from

the lavage fluid by centrifugation at 300*g* for 5 min. Because a significant amount of surfactant can be trapped in the alveolar macrophage pellet [17], these cells were washed three times with phosphate-buffered medium by alternate resuspension and centrifugation at 300*g* for 5 min. All supernatants from the washes were combined and spun at 12,000*g* for 10 min. This pellet was then resuspended in the lavage fluid so that it was included in the alveolar lavage materials. These materials were then stored at 4°C until used for measurements described below.

Measurement of Phospholipid and Protein Contents

The total amount of phospholipid (PL), amounts of some individual phospholipids, and the protein content of alveolar lavage materials from control and oxygen-exposed animals were measured. Protein measurements were also made on samples of supernatants and pellets obtained from 100,000*g* (1 h) centrifugations of alveolar lavage materials. The total amount of phospholipid present in the lavage was measured as the phosphorus present in lipid extracts (chloroform/methanol; 2:1; v/v) of the material [18]. PL content was obtained by multiplying the lipid phosphorus values by 25 [19]. Disaturated phosphatidylcholines (DSPC) were isolated from some samples according to the method of Mason et al. [20]. Some individual phospholipids were identified by using two-dimensional thin-layer chromatography [21]. Total PL was expressed as milligrams per gram lung and the individual phospholipids were expressed as a percentage of the total. The total protein contents of samples of whole lavage materials, lavage pellets, and lavage supernatants were determined by the method of Lowry et al. [22] with 1% sodium dodecyl sulfate added to reduce interference by lipids [23]. These results were expressed as milligrams protein per gram lung.

Measurement of Surface Tension in Vitro

The relationship between surface tension and surface area was determined for samples of alveolar lavage materials obtained from control animals and from rats that had been exposed to oxygen. Measurements were made with a Kimray Greenfield surfactometer (Kimray Medical Associates, Oklahoma City, OK, USA) according to the method described by King and Clements [24]. The balance had a Teflon trough (length 11.5 cm, width 5 cm) with a tight-fitting Teflon barrier that allowed variation of the surface area. The actual measurements were made at a temperature of 22°C. The most reproducible results were obtained by preparing the balance in the following manner. The trough was filled

with 20 mL of Hepes-buffered medium (140 mM NaCl, 10 mM Na Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate), pH 7.4) which also contained 2.5 mM $CaCl_2$ and 0.5 mM EDTA. This medium was used for all experiments with the surface balance. Dipalmitoyl phosphatidylcholine (DPPC) was dissolved in chloroform-methanol (20:1, v/v) so that the final concentration was 1 mg/mL, and 10 μL of this solution was gently placed on the surface of the subphase. After 10 min, we compressed and spread the DPPC monolayer three or four times. The material was then aspirated from the balance and the trough was rinsed 2–3 times with distilled water. The balance was primed in this manner before each measurement was made. Although treatment of the balance walls with lanthanum, dipalmitoyl PC, and/or distearoyl PC has been used to prevent leakage of surface films in some instances [25, 26], we found no differences in our measurements when these treatments were used.

Lavage materials were prepared for study in the surface balance by vortexing samples in Hepes-buffered medium containing Ca^{2+} and EDTA. The final concentration of phospholipids in the balance was 80 μg/mL, i.e., approximately the same amount as in undiluted lavage fluid from control animals. Twenty milliliters of the vortexed sample was placed in the trough of the surface balance. In all samples the final concentrations of Ca^{2+} and EDTA were 2.5 and 0.5 mM, respectively. All samples were allowed to equilibrate for 10 min. The relationship between surface tension and surface area was determined by compressing and expanding the surface between areas of 52.5 cm^2 (100%) and 7.9 cm^2 (15%). The time required for this cycle was 170 s. After recording the relationship between surface tension and surface area, we determined the values for maximum surface tension and minimum surface tension.

Phospholipid and DSPC Levels in Subfractions of Alveolar Lavage Fluids

Subfractions of alveolar lavage materials were first prepared by Magoon et al. [27]. These investigators obtained the subfractions from rabbit alveolar lavage by differential centrifugation and suggested that the smaller, less dense subfractions represent older surfactant materials, while larger, more dense subfractions are newer surfactant materials. We obtained subfractions of alveolar lavage materials from control and oxygen-exposed rats immediately following their recovery according to methods we reported previously [17]. Briefly, alveolar macrophages were removed from the lavage fluid by centrifugation at 300*g* for 5 min. The

cells were washed three times with phosphate-buffered medium by alternate resuspension and centrifugation at 300*g* for 5 min. A pellet, designated as P_1, was obtained by centrifuging the supernatants from the cell washes at 12,000*g* for 10 min. The cell-free lavage fluid was then spun at 1000*g* for 20 min in order to obtain a second pellet, P_2. This supernatant was then centrifuged at 60,000*g* for 60 min to obtain a third pellet, P_3, and a supernatant, S_3. The total phospholipid and DSPC contents of each of these four subfractions were measured as described above.

Statistical Analyses

All comparisons of statistical significance were made by using the unpaired Student *t* test. $p < .05$ was taken as the limit to indicate significance.

RESULTS

Body Weights, Lung Weights, and Alveolar Lavage Proteins

The effects of exposing rats to almost 100% oxygen for 64 h on body weights, lung weights, and alveolar lavage protein levels are shown in Table 1. Although O_2-exposed animals appear to lose weight during the

Table 1 Body weights, lung weights, and alveolar lavage proteins in control and oxygen-exposed rats

Measurement	Control animals	O_2-exposed animals
Body weight (g)		
Prior to exposure	366 (±15)	384 (±20)
After exposure	369 (±14)	362 (±20)
Lung wet weight (g)	1.82 (±0.07)	2.80 (±0.21)*
Lung dry weight (g)	0.38 (±0.02)	0.40 (±0.02)
Lung wet weight/dry weight	4.75 (±0.08)	7.01 (±0.07)*
Alveolar lavage proteins		
mg/g lung wet weight	2.92 (±0.23)	14.81 (±2.50)*
mg/g lung dry weight	13.80 (±1.00)	104.40 (±18.50)*
Distribution of lavage proteins following 100,000*g* (1 h) centrifugation (mg/g lung dry weight)		
Pellet	3.30 (±0.47)	14.08 (±2.79)*
Supernatant	10.55 (±0.93)	90.31 (±19.31)*

Note. The numbers shown are means (±SEM) of values obtained from five to seven different animals in each treatment group. *A significant difference ($p < .05$) from the corresponding value for control animals.

exposure period, there are no significant differences in the weights of unexposed and exposed animals, probably due to large variabilities within the groups. There are some differences in lung weights. The wet weights of lungs from O_2-exposed animals are approximately 50% greater than those from control rats. Lung dry weights do not differ between the two treatment groups. Thus, it follows that lung wet weight to dry weight ratios are approximately 50% greater in the O_2-exposed group. The increases in lung wet weights and in the wet to dry weight ratios suggest the accumulation of alveolar material and/or edema fluid caused by damage to the pulmonary air–blood barrier. The amount of protein in alveolar lavage materials is increased by five- to eightfold in O_2-exposed animals, depending on whether the levels are normalized to lung wet or dry weights. Proteins were divided into sedimentable and nonsedimentable fractions by centrifugation at 100,000*g* for 1 h. There is a greater than eightfold increase in nonsedimentable protein, which may be derived mainly from leakage across the barrier, and a more than fourfold increase in sedimentable protein.

Alveolar Lavage Phospholipids

Exposure of rats to oxygen leads to some changes in the alveolar lavage phospholipid (PL) level and composition, and the results are shown in Table 2. Total PL content of lavage from lungs of unexposed rats is 1.59 (±0.18) mg per gram of wet lung weight, a value similar to one we published previously [28]. The amount of total PL, expressed in

Table 2 Alveolar lavage phospholipids (PL) from control and oxygen-exposed animals

Measurement	Control animals	O_2-exposed animals
Total PL		
mg/g wet lung weight	1.59 (±0.18)	2.71 (±0.08)*
mg/g dry lung weight	7.31 (±0.59)	19.02 (±0.41)*
Distribution of individual PL (% of total)		
Disaturated phosphatidylcholines (DSPC)	46 (±1)	39 (±1)*
Total phosphatidylcholines (PC)	76 (±1)	72 (±3)
Phosphatidylglycerols (PG)	11 (±1)	14 (±2)
Phosphatidylethanolamines (PE)	4 (±1)	5 (±1)
Sphingomyelins (SM)	3 (±1)	3 (±1)
Phosphatidylinositols (PI)	2 (±1)	2 (±1)
Phosphatidylserines (PS)	2 (±1)	2 (±1)
Others	2 (±1)	2 (±1)

Note. The numbers shown are means (±SEM) of values obtained from five to seven different animals in each treatment group. *A significant difference ($p < .05$) from the corresponding value for control animals.

this manner, is increased by about 70% in lungs from O_2-exposed animals. However, because lung weights are probably increased due to edema formation, we also expressed the results as total PL per gram of dry lung weight. In this case, there is a 2.6-fold increase in lungs from exposed rats. There are also some major differences in the phospholipid composition of the lavage materials following exposure of the animals to oxygen. Disaturated phosphatidylcholines (DSPC), the major surface active component of lung surfactant, are affected by the exposures. When expressed as a percentage of total PL, DSPC is reduced from 46 to 39% following O_2 exposures. There are no significant differences in other individual PL, although there may be some trends. For example, the mean value for phosphatidylcholines appears to be decreased while that for phosphatidylglycerols seems to be increased by the exposures. These results show that exposure of rats to oxygen leads to changes in the amount and composition of surfactant PL. There is an increase in total surfactant PL, but the DSPC, expressed as a percentage of the total PL, is reduced.

Surface Properties of Alveolar Lavage Materials

There are some differences in the amounts of DSPC and protein in alveolar lavage materials from control and oxygen-exposed animals. Because these substances are known to influence surface properties, we studied the surface activity of lavage materials from lungs of these two groups of rats. The results are summarized in Table 3. In these experiments, comparisons were made by using equal amounts of lavage fluid phospholipids; i.e., final concentrations in the balance were 80 μg PL/mL. The maximal value for surface tension (γ max) is approximately 45% greater for alveolar lavage materials from oxygen-exposed rats than those from control animals. When the surfaces are compressed to 15% of the original surface area, the values for the minimal surface tensions (γ min) attained are 19 and 4 dynes/cm for lavage from O_2-exposed and

Table 3 Surface properties of alveolar lavage materials from control and oxygen-exposed rats

Measurement	Control animals	O_2-exposed animals
Minimum surface tension (dynes/cm)	4 (±1)	19 (±3)*
Maximum surface tension (dynes/cm)	38 (±2)	55 (±2)*
Stability Index	1.70 (±0.11)	1.00 (±0.11)*

Note. The numbers shown are means (±SEM) of values obtained from six different animals in each treatment group. *A significant difference ($p < .05$) from the corresponding value for control animals.

control rats, respectively. We also calculated a stability index (γ max $-$ γ min/½ (γ max + γ min), which was first proposed by Clements et al. [29] and which may provide a measure of the surface tension lowering ability of the lavage material samples. The stability index is reduced by 40% for alveolar lavage fluid from rats exposed to oxygen. These results suggest that the ability of alveolar lavage materials to reduce surface tension may be impaired following exposure of animals to oxygen.

Distribution of Phospholipids in Subfractions of Alveolar Lavage Materials

It is known that alveolar lavage materials can be separated into various subfractions by differential centrifugation and that the age of the materials may be related to the density of the subfractions. Smaller, less dense subfractions are thought to represent older surfactant, while larger, more dense subfractions are newer surfactant materials. Therefore, we obtained four subfractions from the alveolar lavage fluids of both control and O_2-exposed animals. These subfractions consisted of three pellets, P_1 (300*g*), P_2 (1000*g*), and P_3 (60,000*g*), and a supernatant remaining after the centrifugation at 60,000*g* (S_3). The order of the materials from most to least sedimentable is $P_1 > P_2 > P_3 > S_3$. The distribution of phospholipids (PL) in each of the subfractions, expressed as a percent of total lavage PL, was measured and the results are shown in Figure 1. In lavage materials from control animals, 40% of the PL is in subfraction P_3, 20–25% is in each of subfractions P_2 and S_3, and only 14% is in subfraction P_1. This distribution is similar to that which we reported previously for alveolar lavage from untreated rats [17]. However, the phospholipid distribution for alveolar lavage from animals exposed to oxygen is much different in that most is found in the heavier, more dense subfractions. More than 70% of the PL is found in the heavier subfractions (P_1 and P_2) and less than 30% in the lighter subfractions (P_3 and S_3) following exposure to oxygen. This result is the reverse of that obtained in control animals, i.e.; approximately 35% of the PL is in the heavier subfractions and 65% is in the lighter subfractions. These results indicate that the physical form of the phospholipids in lavage materials from lungs of animals exposed to oxygen is much different from that in lungs of control rats.

Since disaturated phosphatidylcholines (DSPC) represent the major surface active component of surfactant, we also measured its distribution in each of the lavage subfractions. These results are summarized in Table 4. The distribution of DSPC in the subfractions is similar to the distribution of total phospholipids; i.e., most of the DSPC is in the heavier

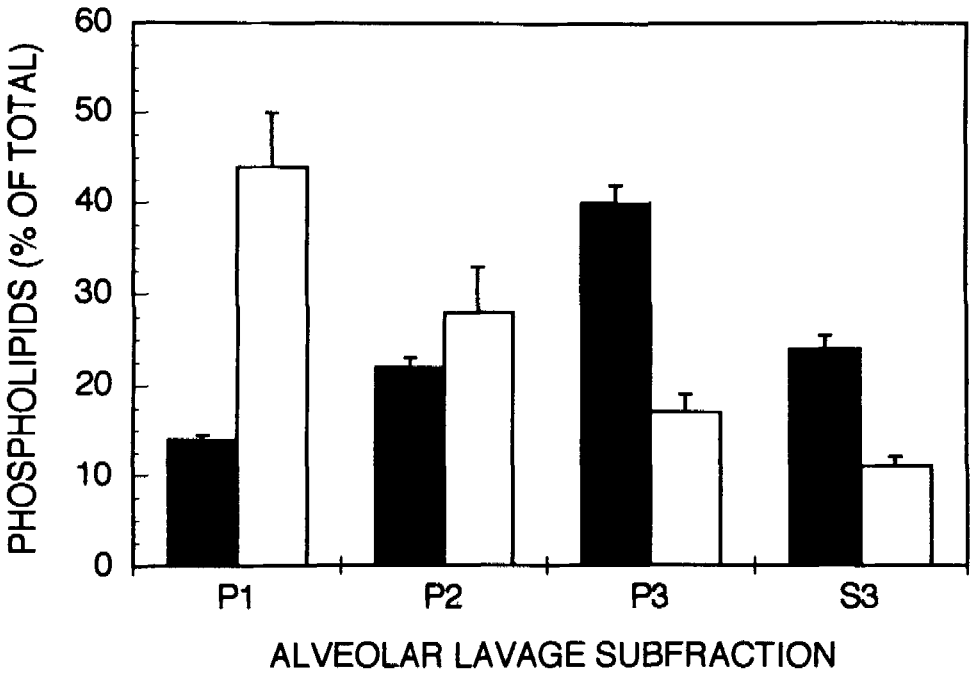

Figure 1. The distribution of phospholipids in subfractions of alveolar lavage materials from control (solid bars) and oxygen-exposed (open bars) animals. Subfractions of the lavage materials were obtained by differential centrifugation as described under Materials and Methods. The order from most to least sedimentable is P_1 (300*g* pellet) > P_2 (1000*g* pellet) > P_3 (60,000*g* pellet) > S_3 (60,000*g* supernatant). The phospholipid content of each subfraction was measured, and the results are expressed as a percentage of the total phospholipids in the lavage. The values shown are means ± SEM for six different animals in each treatment group.

subfractions obtained from animals exposed to oxygen, while most DSPC is in the lighter subfractions from control animals. In fact, the ratios of DSPC in lavage from animals exposed to oxygen to the DSPC in lavage from control animals is similar in all subfractions to these ratios for total phospholipids. Thus, exposure of rats to oxygen also leads to alterations in the physical form of DSPC in alveolar lavage materials.

DISCUSSION

In the experiments reported in this paper, rats were given a lethal exposure to oxygen. We find an increase in the total amount of alveolar lavage phospholipids in O_2-exposed animals, confirming some earlier studies [16, 30]. In addition, we report some new findings, including changes in phospholipid composition, surface properties, and physical properties of alveolar lavage materials from rats exposed to oxygen. Dur-

Table 4 Distribution of phospholipids (PL) and disaturated phosphatidylcholines (DSPC) in subfractions of alveolar lavage materials

Subfraction	Measurement		
	Control animals	O_2-exposed animals	O_2-exposed/control
	Total PL (%)		
P_1	14 (±1)	44 (±6)*	3.14
P_2	22 (±1)	28 (±5)	1.27
P_3	40 (±2)	17 (±2)*	0.42
S_3	24 (±2)	11 (±1)*	0.46
	DSPC (% Total PL)		
P_1	6 (±1)	18 (±3)*	3.00
P_2	10 (±1)	10 (±2)	1.00
P_3	19 (±1)	6 (±1)*	0.32
S_3	11 (±1)	5 (±1)*	0.46

Note. The numbers shown are means (±SEM) of values obtained from five different animals in each treatment group. *A significant difference ($p < .05$) from the corresponding value for control animals.

ing the latter stages of the exposure period, the O_2-exposed animals exhibit a decrease in motor activity and in feeding and drinking. These rats also have retraction of the chest musculature and cyanosis of the muzzle and paws. There is also evidence to suggest severe lung damage, including the appearance of pleural effusion. In the oxygen-exposed rats, the lungs are boggy and the ratio of lung wet weight to dry weight is increased (Table 1). There is, however, no difference in the lung dry weights of control and oxygen-exposed rats. These results suggest that the increase in lung wet weight is due mostly to accumulation of interstitial and/or alveolar fluid, i.e., pulmonary edema. The marked increase in alveolar lavage proteins, especially nonsedimentable proteins, suggests a loss in the integrity of the pulmonary air–blood barrier. Other investigators have demonstrated such an increase in the permeability of this barrier following exposure of rabbits [3–5] and rats [31] to 100% oxygen. Thus, these results demonstrate that our exposure conditions produce very severe lung injury.

Although the O_2-exposed animals appear to have lethal lung injuries, there seems to be an increase in the amount of surfactant phospholipids on the alveolar surface (Table 2). Other investigators have also reported increases in lavage phospholipids [16, 30] following exposures of rats to lethal levels of oxygen. This is a unique response among different animal species, and one that is somewhat surprising. One might expect a reduction in pulmonary surfactant levels given the lethal lung injury which occurs. Indeed, exposure of other animals, such as rabbits [8], mice

[7], and baboons [11], to lethal doses of oxygen does result in a decreased level of surfactant phospholipids on the alveolar surface.

One area of concern in our experiments is the technique used for quantitative lavage of the lungs. The volume of fluid used for lavage was dependent on lung weight and was greater for O_2-exposed animals due to the increased lung weight caused by pulmonary edema. Thus, elevated phospholipid levels may have been due to the larger volume of fluid. However, we measured the concentrations of phospholipids and found them to be greater in alveolar lavage fluid from O_2-exposed animals than from controls. In addition, we corrected for differences in lung size by expressing the results as milligrams PL per gram of lung. Another concern is that the larger volume of fluid may have caused surfactant release due to greater expansion of lungs in oxygen-exposed rats. In this regard, we used equal volumes of fluid (10 mL; three lavages) to lavage lungs from a smaller group of control and exposed animals and also found an increase in alveolar PL in O_2-exposed animals (data not shown). It should be pointed out that investigators who have reported decreases in surfactant PL levels in other animals following exposure to oxygen [7, 8, 11] used equal volumes of fluid to lavage lungs. Therefore, our results show that, no matter which way we did the experiments, rats exposed to lethal levels of oxygen have elevated levels of surfactant phospholipids on the alveolar surface.

The composition of alveolar lavage materials is altered in two major ways following exposure of rats to oxygen. First, disaturated phosphatidylcholines (DSPC), the major surface tension lowering component of lung surfactant, are reduced when expressed as a percentage of the total phospholipids (Table 2). Effects of exposing rats to oxygen on the DSPC levels expressed in this manner have not been previously reported. The second major alteration is a greatly increased protein level in lavage from oxygen-exposed rats. Undoubtedly, the majority of the proteins, especially those in the nonsedimentable fraction, are derived from blood and appear on the alveolar surface as a result of lung injury. Some of these, however, may be surfactant proteins. In this regard, it has been shown that exposure of rats to 85% oxygen for up to 7 days leads to an increase in surfactant protein A levels in lung lavage [14].

These alterations in the composition of lung lavage materials from O_2-exposed rats probably account, at least in part, for the impairment in its surface activity. The results obtained from surface balance studies show that lavage materials from O_2-exposed rats exhibit higher maximal surface tensions than do materials from control animals, and the minimal surface tension values achieved during compression of the surface are greater than those from control animals (Table 3). As a result, the sta-

bility indices for lung lavage from O_2-exposed animals are reduced. One major reason for the reduced surface activity is probably the decrease in the proportion of DSPC, the major surface active component of lung surfactant. In addition, it has been known for some time that protein contamination has adverse effects on the ability of lung surfactant to reduce surface tension [32, 33]. Thus, although there is more surfactant phospholipid in lungs from O_2-exposed rats, its surface activity is impaired, probably due, at least in part, to alterations in the DSPC and protein levels.

One interesting finding in our study is the effect of lethal exposures to oxygen on the distribution of phospholipids (PL) and DSPC in subfractions of rat alveolar lavage materials. In lavage from control animals, 36% of the PL is in the heavier subfractions (P_1 and P_2), while 64% is in the lighter subfractions (P_3 and S_3). The distribution is reversed in lavage from O_2-exposed rats; i.e., 72% of the PL is in heavier and 28% is in lighter subfractions (Figure 1). It has been proposed by Magoon et al. [27] that heavier subfractions represent newer surfactant materials, and lighter subfractions represent older surfactant, and that the former are metabolic precursors for the latter. Since exposures of rats to high levels of oxygen lead to increased amounts of lavage PL, the newly synthesized materials may accumulate in the heavier subfractions (P_1 and P_2). In this regard, the absolute amounts of PL in the lighter subfractions (P_3 and S_3) appear to be similar in lavage from control and exposed animals. The altered physical form of lavage materials from O_2-exposed rats may also lead to impairment of surface activity. Although we did not measure surface activity of the subfractions, Magoon et al. [27] did make such measurements on rabbit lavage subfractions isolated with centrifugation speeds similar to those used in this study. They found that lavage materials which sediment at $\leq 500g$ are the least surface active, although their subfraction did contain some cells. In our experiments, exposure of rats to oxygen leads to a shift in the distribution of PL and DSPC so that there is a dramatic increase in these substances (i.e., a threefold increase in each, expressed as a percentage of the total) in the 300*g* pellet. Thus, these alterations in physical properties may contribute to the impairment in surface activity of the lavage materials.

To the best of our knowledge, the effects of exposing rats or any other animal species to oxygen on the physical forms of alveolar lavage phospholipids have not been determined. However, such measurements have been made for some other types of lung injury. These reports include *N*-nitroso-*N*-methylurethane (NNNMU)-induced lung injury in dogs [34] and rabbits [35] and adult respiratory distress syndrome in humans [36]. In each of these types of lung injury, there is an increase in

the lighter, less dense subfractions of the alveolar lavage materials and a reduction in the lavage phospholipid content. These results are the reverse of what we find in O_2-exposed rats, i.e., an increase in heavier subtypes and PL levels. There is one other report of increases in heavier surfactant subtypes following lung injury. That result was obtained in mice after radiation-induced lung injury [37]. Gross [38] suggested that the heavier subfractions accumulate in the lavage from radiation-injured mice because the enzyme responsible for conversion of the heavy subtype to the lighter subtypes is inhibited. Such inhibition by something in the alveolar lavage may also be responsible for the excessive amount of the heavy subtypes in our experiments.

In summary, exposure of rats to lethal levels of oxygen results in severe lung damage, including the appearance of pleural effusion, pulmonary edema, and a dramatic increase in proteins on the alveolar surface. Yet, in spite of this lung damage, the amount of phospholipids on the alveolar surface is actually increased by two- to threefold. This response is unique among all other animal species that have been tested for oxygen-induced lung injury. Although there may be more surfactant on the alveolar surface of O_2-exposed rats, there is evidence to suggest that its quality and surface activity are adversely affected. The composition is altered so that there is less DSPC (expressed as a percentage of total PL), the major surface active component of surfactant, and a greatly increased amount of proteins, most of which are probably derived from plasma and crossed the air–blood barrier following injury. In addition, the physical forms of the surfactant PL have been altered so that the heavier subtypes are more abundant. Both the change in composition and the alterations in the physical form of surfactant probably contribute to its impaired ability to reduce surface tension.

REFERENCES

1. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: a review. Pharmacol Rev. 1971;23:37–133.
2. Kistler GS, Caldwell PRB, Weibel ER. Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lung. J Cell Biol. 1967;32:605–628.
3. Matalon S, Egan E. Effects of 100% O_2 breathing on permeability of alveolar epithelium to solute. J Appl Physiol. 1981;50:859–863.
4. Matalon S, Egan E. Interstitial fluid volumes and albumin spaces in pulmolnary oxygen toxicity. J Appl Physiol. 1984;57:1767–1772.
5. Matalon S, Cesar M. Effects of 100% oxygen breathing on the capillary filtration coefficient in rabbit lungs. Microvasc Res. 1985;29:7–80.
6. Hopewell PC, Murray J. The adult respiratory distress syndrome. In: Schiebel EM, Moser KM, eds. Respiratory Emergencies. St. Louis: Mosby; 1977:101–128.
7. Gross NJ, Smith DM. Impaired surfactant phospholipid metabolism in hyperoxic mouse lungs. J Appl Physiol. 1981;51:1198–1203.

8. Holm BA, Notter RH, Siegle J, Matalon S. Pulmonary physiological and surfactant changes during injury and recovery from hyperoxia. J Appl Physiol. 1985;59:1402–1409.
9. Holm BA, Matalon S, Finkelstein JN, Notter RH. Type II pneumocyte changes during hyperoxic lung injury and recovery. J Appl Physiol. 1988;65:2672–2678.
10. Engstrom PC, Holm BA, Matalon S. Surfactant replacement attenuates the increase in alveolar permeability in hyperoxia. J Appl Physiol. 1989;67:688–693.
11. King RJ, Coalson JJ, Seidenfeld JJ, Anzueto AR, Smith DB, Peters JI. O_2- and pneumonia-induced lung injury, II: properties of pulmonary surfactant. J Appl Physiol. 1989;67:357–365.
12. Young SL, Crapo JD, Kremers SA, Brumley GW. Pulmonary surfactant lipid production in oxygen-exposed rat lungs. Lab Invest. 1982;46:570–576.
13. Nogee LM, Wispe JR. Effects of pulmonary oxygen injury on airway content of surfactant-associated protein A. Pediat Res. 1988;24:568–573.
14. Nogee LM, Wispe JR, Clark JC, Whitsett JA. Increased synthesis and mRNA of surfactant protein A in oxygen-exposed rats. Am J Respir Cell Mol Biol. 1989;1:119–125.
15. Crapo JD, Barry BE, Foscue HA, Shelburne J. Structural and biochemical changes in rat lungs occurring during exposures to lethal and adaptive doses of oxygen. Am Rev Respir Dis. 1980; 122:123–143.
16. Välimäki M, Pelliniemi TT, Niinikoski J. Oxygen-induced changes in pulmonary phospholipids in the rat. J Appl Physiol. 1975;39:780–787.
17. Miles PR, Bowman L, Tucker J, Reasor MJ, Wright JR. Alterations in rat alveolar surfactant phospholipids and proteins induced by administration of chlorphentermine. Biochim Biophys Acta. 1986;877:167–178.
18. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem. 1959;234:466–468.
19. Oyarzun MJ, Clements JA. Control of lung surfactant by ventilation, adrenergic mediators, and prostaglandins in the rabbit. Am Rev Respir Dis. 1978;117:879–891.
20. Mason RJ, Nellenbogen J, Clements JA. Isolation of disaturated phosphatidylcholines with osmium tetroxide. J Lipid Res. 1976;17:281–284.
21. Poorthuis BJHM, Yazaki PJ, Hostetler KY. An improved two-dimensional thin-layer chromatography system for the separation of phosphatidylglycerol and its derivatives. J Lipid Res. 1976;17:433–437.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193:265–275.
23. Lees MB, Paxman S. Modification of the Lowry procedure for the analysis of proteolipid protein. Anal Biochem. 1972;47:184–192.
24. King RJ, Clements JA. Surface active materials from dog lung, II: composition and physiological correlations. Am J Physiol. 1972;223:715–726.
25. Hildebran JS, Goerke J, Clements JA. Pulmonary surface film stability and composition. J Appl Physiol: Respirat Environ Exercise Physiol. 1979;47:604–611.
26. Goerke J, Gonzales J. Temperature dependence of dipalmitoyl phosphatidylcholine monolayer stability. J Appl Physiol: Respirat Environ Exercise Physiol. 1981;51:1108–1114.
27. Magoon MW, Wright JR, Baritussio A, Williams MC, Goerke J, Benson BJ, Hamilton RL, Clements JA. Subfraction of lung surfactant: implications for metabolism and surface activity. Biochim Biophys Acta. 1983;750:18–31.
28. Miles PR, Bowman L, Miller MR. Alterations in the pulmonary microsomal cytochrome P-450 system after exposure of rats to silica. Am J Respir Cell Mol Biol. 1993;8:597–604.
29. Clements JA, Hustead RF, Johnson RP, Gribetz I. Pulmonary surface tension and alveolar stability. J Appl Physiol. 1961;16:444–450.
30. Niinikoski J, Nikkari T, Kulonen E. Pulmonary oxygen toxicity: composition of endobronchial saline extracts of rats during exposure to oxygen. Aerospace Med. 1971;42:525–528.
31. Royston B, Webster N, Nunn J. Time course of changes in lung permeability and edema in the rat exposed to 100% oxygen. J Appl Physiol. 1990;69:1532–1537.
32. Notter RH, Taubold R, Mavis RD. Hysteresis in saturated phospholipid films and its potential relevance for lung surfactant function in vivo. Exp Lung Res. 1982;3:109–127.

33. Seeger W, Stohr G, Wolf HRD, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol. 1985;58:326–338.
34. Liau DF, Barrett R, Bell ALL, Ryan SF. Functional abnormalities of lung surfactant in experimental acute alveolar injury in the dog. Am Rev Respir Dis. 1990;136:395–401.
35. Lewis JF, Ikegami M, Jobe AH. Altered surfactant function and metabolism in rabbits with acute lung injury. J Appl Physiol. 1990;69:2303–2310.
36. Petty TL, Reiss OK, Paul GW, Silvers GW, Elkins ND. Characteristics of pulmonary surfactant in adult respiratory distress syndrome associated with trauma and shock. Am Rev Respir Dis. 1977;115:531–536.
37. Gross NJ. Surfactant subtypes in experimental lung damage: radiation pneumonitis. Am J Physiol (Lung, Cell, Mol Physiol). 1991;260:L302–L310.
38. Gross NJ. Inhibition of surfactant subtype convertase in radiation model of adult respiratory distress syndrome. Am J Physiol (Lung, Cell, Mol Physiol). 1991;260:L311–L317.