Lymphology 12 (1979) 133–139

Pulmonary Oxygen Toxicity: Increased Microvascular Permeability to Protein in Unanesthetized Lambs

M.A. Bressack, M.D., D.D. McMillan, M.D., R.D. Bland, M.D.

Cardiovascular Research Institute and the Department of Pediatrics,
University of California San Francisco, San Francisco, California 94143

Summary

To study transvascular filtration of fluid and microvascular permeability to protein in the lung during prolonged hyperoxia, we measured lung lymph flow, protein transport, and simultaneous pulmonary vascular pressures of six lambs breathing 100 percent O_2 for five days. Lymph flow doubled, protein flow increased by 131 percent, and radioactive tracer studies demonstrated a clearcut increase in pulmonary microvascular permeability to protein after five days of continuous O_2 breathing.

Introduction

Breathing pure oxygen (O_2) for several days causes pulmonary edema (1). Many investigators have attributed this condition to "leaky" capillaries in the lung, a conclusion derived mainly from observations of endothelial cell damage seen with the electron microscope (2, 3). To assess more directly pulmonary microvascular permeability to protein in prolonged hyperoxia, we measured pulmonary lymph flow, transvascular movement of protein in the lung, and simultaneous pulmonary vascular pressures of six unanesthetized 2–4 week old lambs breathing 100 percent O_2 at one atmosphere continuously for five days. With no appreciable change in lung vascular pressures, pulmonary lymph flow increased progressively after 72 hours in O_2, and transport of protein from plasma into lymph more than doubled in five days, demonstrating a substantial increase in the permeability of the pulmonary microvascular membrane to substances of large molecular weight.

Methods

Preparation of Animals for Experiments. Using methods previously described (4, 5), we surgically prepared eight newborn lambs to collect lung lymph and measure average pulmonary arterial ($\bar{P}pa$), left atrial ($\bar{P}la$), and aortic ($\bar{P}ao$) pressures. Each lamb had two thoracotomies, the first at one to two days of age, and the second three to seven days later.

During the first operation we placed polyvinyl catheters (Tygon Tubing, Akron, Ohio, USA) in the thoracic aorta, pulmonary artery and both atria. In the second operation we resected the systemic contributions to the caudal mediastinal lymph node and inserted a heparin-impregnated (TDMAC processing, Polyscience Inc, Warrington, PA, USA) polyvinyl catheter (internal diameter .015″) into the efferent duct of that node. The lambs had at least three days to recover from surgery, and experiments did not begin until the lymph was free of visible blood and flowing at a steady rate.

Six lambs remained in 100 percent O_2 for five days after a 4-hour baseline period in air. Two control lambs had identical management except that they breathed air instead of pure O_2 for five days.

Experimental Protocol. The six lambs treated with O_2 weighed 3.6 ± .2 kg at birth and 8.1 ± 1.0 kg when they began breathing 100 percent O_2 (19 ± 2 days of age). Until the start of the experiments, the lambs were with their ewes for feeding and warmth. When studies began, the lambs remained in a sealed wood and lucite chamber, into which humidified O_2 flowed at 6–9 L/min, sufficient to keep the inspired O_2 concentration greater than 90 percent for five days. Ice within the box kept the temperature between 24 and

0024–7766/79 1500–0133 $ 02.00

26°C, and soda lime maintained the concentration of CO_2 below 4 torr. The lambs were out of O_2 for no more than five minutes each day, long enough to be weighed and transported from the box to a supporting canvas sling in which measurements were made. During the five days of O_2 exposure we fed the lambs commercial infant formula* providing 120–140 ml/kg/day and 80–90 cal/kg/day.

Measurements. At the beginning of experiments and at 24 hour intervals thereafter, we measured $\bar{P}pa$, $\bar{P}la$, and $\bar{P}ao$ continuously and collected lymph specimens every 30 minutes during a steady-state period of 3–4 hours. Pressure measurements were made with calibrated pressure transducers (Statham P23 Dc, Hato Rey, Puerto Rico) and a 6-channel amplifier-recorder (Grass Model 7B polygraph, 7DA E amplifiers, and 7P1B preamplifiers, Grass Instrument Co., Quincy, Mass., USA). Zero reference level for pressure recordings was a line drawn on the lamb's skin at the left atrium immediately after surgery. We measured the volume of lymph to the nearest .01 ml and obtained blood samples at the mid-point of each 30-minute lymph collection. We also recorded respiratory frequency, heart rate, arterial blood gas tensions and pH, and cardiac output by the indicator dilution method (6) using Cardio-Green dye (Hynson Wescott and Dunning, Inc, Baltimore, Maryland, USA). We calculated pulmonary vascular resistance as $\frac{\bar{P}pa - \bar{P}la}{\text{Cardiac Output}}$.

In five lambs we injected ^{125}I-human albumin (Mallinkrodt, San Francisco, CA, USA) intravenously and measured the time for the lymph specific activity to reach half that of plasma, both before they breathed O_2 and again after five days in O_2. We obtained two baseline samples of blood and lymph, then injected ^{125}I-albumin, 3–5 ml/kg, into the right atrium, and collected lymph samples every 15 minutes for three hours, and blood samples at the mid-point of each collection period.

* Similac, Ross Laboratories, Columbus, Ohio, USA: SMA, Wyeth Laboratories, Philadelphia, PA, USA.

Analytic Methods. We centrifuged lymph and blood samples and measured the concentration of protein in the supernatant fluids by the Biuret method (7) and of albumin by the Bromcresol green method (8). We measured PaO_2, $PaCO_2$, pH and inspired gas concentrations with a Radiometer acid-base analyzer (Copenhagen, Denmark).

For the radioactive tracer studies, we transferred 0.1 ml of each sample of lymph and plasma into test tubes, measuring the radioactivity within each sample for one minute in a Searle 512 multichannel pulse-height analyzer (Searle Analytic, Inc, Des Plaines, Ill., USA). To obtain specific activity for each sample we expressed the number of counts per minute as a function of the concentration of albumin in the sample. We constructed a regression line by plotting lymph:plasma specific activity as a function of time after injection, yielding the time at which lymph specific activity reached half that of plasma (defined as half-equilibration).

Postmortem Studies. At the end of experiments, we gave the lambs sodium pentobarbital (Diamond Laboratories, Des Moines, Iowa, USA), 20 mg/kg intravenously, resected the lungs at an inflation pressure of 25 cm H_2O, and immediately froze them in liquid N_2. We examined sections of lung under a microscope, made photomicrographs, and homogenized the remaining lung to measure extravascular water content (9). In each case we calculated dry lung weight, exclusive of blood, so that we could express pulmonary lymph flow relative to lung tissue weight. Protein flow – protein concentration in lymph x lymph flow rate – is expressed relative to both dry lung tissue and plasma protein concentration.

Statistical Analysis. We used the paired *t*-test (10) to compare measurements made during the baseline period and the first four hours in O_2; we also compared results of the first four hours to those obtained on the fifth day of O_2 breathing, with $p < .05$ indicative of a significant difference. All data are given in the text as the mean ± one standard error from the mean.

Results

General. The weights of the lambs did not change significantly throughout the experiments. Five lambs survived the duration of the study; one died between the fourth and fifth day in O_2. After 4–5 days in O_2, all the lambs became lethargic, weak, and had retractions of the chest wall and blood-tinged lymph. We observed no change in vascular pressures, lymph flow, protein concentrations or the clinical appearance of the two control lambs during their five days in the chamber.

Acute Effects of Oxygen Breathing. As shown in the upper portion of Tables 1 and 2, PaO_2 and $PaCO_2$ increased and $\bar{P}pa$, pulmonary vascular resistance and heart rate decreased during the initial four hours in O_2. There were no significant acute changes in respiratory frequency, $\bar{P}ao$, $\bar{P}la$, cardiac output, lymph flow, or protein concentrations.

Chronic Effects of Oxygen Breathing. Alveolar-arterial O_2 gradient and respiratory frequency did not change significantly during five days of O_2 breathing. As Table 1 shows, heart rate and $PaCO_2$ increased, while PaO_2 and pH decreased in all lambs by the fifth day. Cardiac output did not change significantly, but pulmonary vascular resistance increased after five days in O_2.

Figure 1 shows the results of a typical experiment in which a lamb breathed O_2 for five days. Following the initial decrease of $\bar{P}pa$, there was no appreciable change in vascular pressures, but lymph flow increased beginning on the fourth day, and the concentration of protein in lymph increased progressively from the third to the fifth day.

Table 2 is a summary of data related to microvascular fluid and protein transport for the six experimental lambs. Soon after O_2 breathing began, $\bar{P}pa$ decreased and then remained constant during the succeeding days in O_2. In all cases, the concentration of protein in lymph increased and the plasma protein concentration decreased over five days, producing an increase in the protein concentration of lymph relative to that in plasma. Lymph flow increased by an average of 100 percent and lymph protein flow by 131 percent by

Tab. 1 Respiratory and circulatory measurements in 6 lambs breathing air, then 100% O_2 for five days

	Respiratory Frequency	pH	$PaCO_2$	PaO_2	Aortic Pressure	Heart Rate	Cardiac Output	Pulmonary Vascular Resistance
	breaths/min		torr		torr	breaths/min	L/min/kg	torr/L/min/kg
Baseline	45 ± 3	7.42 ± .01	38 ± 1	85 ± 3	83 ± 2	183 ± 4	.33 ± .03	50.6 ± 2.7
Hours in O_2								
0– 4	43 ± 5	7.42 ± .01	42 ± 1$^+$	402 ± 12$^+$	84 ± 4	159 ± 5$^+$	.31 ± .03	46.9 ± 3.0$^+$
24– 28	40 ± 4	7.41 ± .01	42 ± 3	368 ± 23	81 ± 3	161 ± 8		
48– 52	37 ± 3	7.43 ± .01	42 ± 4	382 ± 27	82 ± 3	168 ± 10		
72– 76	40 ± 3	7.42 ± .02	45 ± 4	388 ± 27	83 ± 3	177 ± 9	.26 ± .02	47.4 ± 4.0
96–100	41 ± 3	7.32 ± .03	69 ± 8	353 ± 26	81 ± 3	193 ± 10	.26 ± .01	57.6 ± 6.2
120–124	33 ± 3	7.23 ± .03*	99 ± 7*	301 ± 34*	80 ± 3	194 ± 10*	.26 ± .02	61.7 ± 4.4*

Mean ± SE of the mean

$^+$ Significant difference between measurements obtained during the baseline period and those obtained during the first four hours in O_2, $p < .05$

* Significant difference between measurements obtained during the first four hours in O_2 and those obtained between 120–124 hours in O_2, $p < .05$

Tab. 2 Vascular pressures, protein concentrations and lung lymph flow in six lambs breathing air, then 100% O_2 for five days

	$\bar{P}pa$	$\bar{P}la$	Protein Concentrations		Lymph Flow	Protein Flow	Protein Ratios		
			Lymph	Plasma			Albumin/Globulin Lymph	Plasma	Lymph/Plasma
	torr	torr	g/dl		ml/h[a]	mg/h[b]			
Baseline	20 ± 1	2 ± 1	3.26 ± .27	5.29 ± .18	.09 ± .02	.52 ± .07	1.00 ± .05	.56 ± .03	.62 ± .04
Hours in O_2									
0– 4	17 ± 1[+]	3 ± 1	3.17 ± .26	5.24 ± .19	.09 ± .02	.54 ± .07	.98 ± .04	.56 ± .04	.60 ± .02
24– 28	16 ± 1	2 ± 1	3.17 ± .26	5.18 ± .13	.09 ± .02	.52 ± .06	1.00 ± .06	.59 ± .03	.60 ± .03
48– 52	15 ± 1	2 ± 1	3.31 ± .23	5.18 ± .19	.07 ± .01	.46 ± .03	.96 ± .05	.60 ± .04	.63 ± .02
72– 76	16 ± 1	2 ± 1	3.46 ± .26	5.14 ± .13	.12 ± .02	.81 ± .06	.87 ± .07	.60 ± .05	.67 ± .03
96–100	17 ± 1	1 ± 1	3.48 ± .28	4.92 ± .20	.16 ± .01	1.14 ± .06	.73 ± .07	.55 ± .04	.70 ± .02
120–124	18 ± 1	1 ± 1	3.54 ± .27*	4.85 ± .21*	.18 ± .02*	1.25 ± .09*	.65 ± .06*	.55 ± .04	.72 ± .02*

Mean ± SE of the mean

a Per g of dry lung tissue

b Per g of dry lung tissue, per g of plasma proteins · dl^{-1}

+ Significant difference between measurements obtained during the baseline period and those obtained during the first four hours in O_2, $p < .05$

* Significant difference between measurements obtained during the first four hours in O_2 and those obtained between 120–124 hours in O_2, $p < .05$

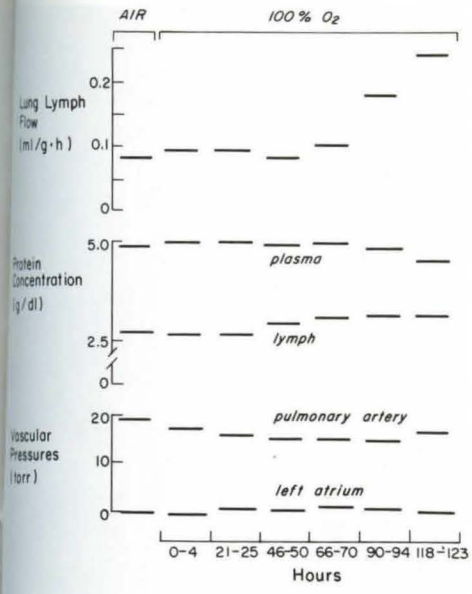

Fig. 1 Pulmonary lymph flow, protein concentrations, and average vascular pressures of a lamb breathing air, then 100 percent O_2 for 5 days. Lymph flow, measured per gram of dry lung tissue, exclusive of blood, was greater than the baseline flow rate by the fourth day in O_2, and the concentration of protein in lymph increased progressively beginning on the third day in O_2

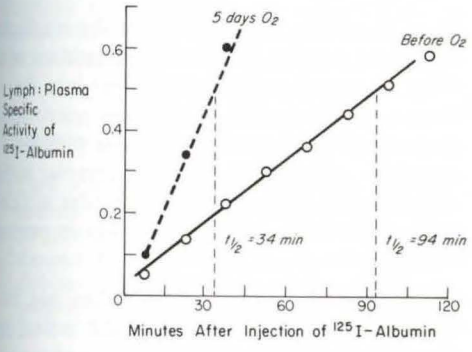

Fig. 2 Lymph:plasma specific activity of ^{125}I-albumin as a function of time after intravascular injection of the radioactive protein tracer in one lamb. Before O_2 exposure, the specific activity in lymph was one-half that in plasma 94 minutes after injection; after five days in O_2, half-equilibration was 34 minutes

the fifth day in O_2 – these were consistent findings. [Albumin] : [globulin] in plasma did not change during the course of experiments, but this ratio decreased significantly in lymph, by an average of 35 percent.

Radioactive Tracer Studies. Figure 2 shows a typical experiment in which we injected ^{125}I-albumin into the right atrium of a lamb and sampled lymph and blood at 15 minute intervals for two hours. There was a linear rise of lymph:plasma specific activity after injection. Before O_2 breathing, half-equilibration occurred 94 minutes after injection. Following five days in O_2, half-equilibration was 34 minutes. For the five lambs studied in O_2 with radioactive tracer injections, half-equilibration averaged 101 ± 4 min before O_2 and 44 ± 4 min after five days in O_2. This significant difference was the result of a more rapid increase of specific activity in lymph; O_2 produced no change in the rate of decrease of specific activity in plasma.

Postmortem Studies. Extravascular lung water content per g of dry lung tissue, exclusive of blood, averaged 5.78 ± .28 for the six lambs, significantly greater than the 4.82 ± .11 g of water/g dry lung tissue of control animals breathing air (5).

Five of six lambs given O_2 had extensive cuffs of fluid surrounding blood vessels of the lung, confirming the presence of pulmonary edema. Figure 3 illustrates a photomicrograph of a section of such a lung, adjacent to that of a normal lamb.

Discussion

DeLemos et al. (11) showed that newborn lambs breathing pure O_2 acquire pulmonary edema within three to four days. Our results confirm this observation. Pulmonary edema developed in all experimental lambs within five days of O_2 exposure. Extravascular lung water content was 20 percent above normal, there were large cuffs of fluid surrounding pulmonary blood vessels, and lung lymph flow doubled.

Several reports have suggested that O_2 causes pulmonary edema by changing permeability

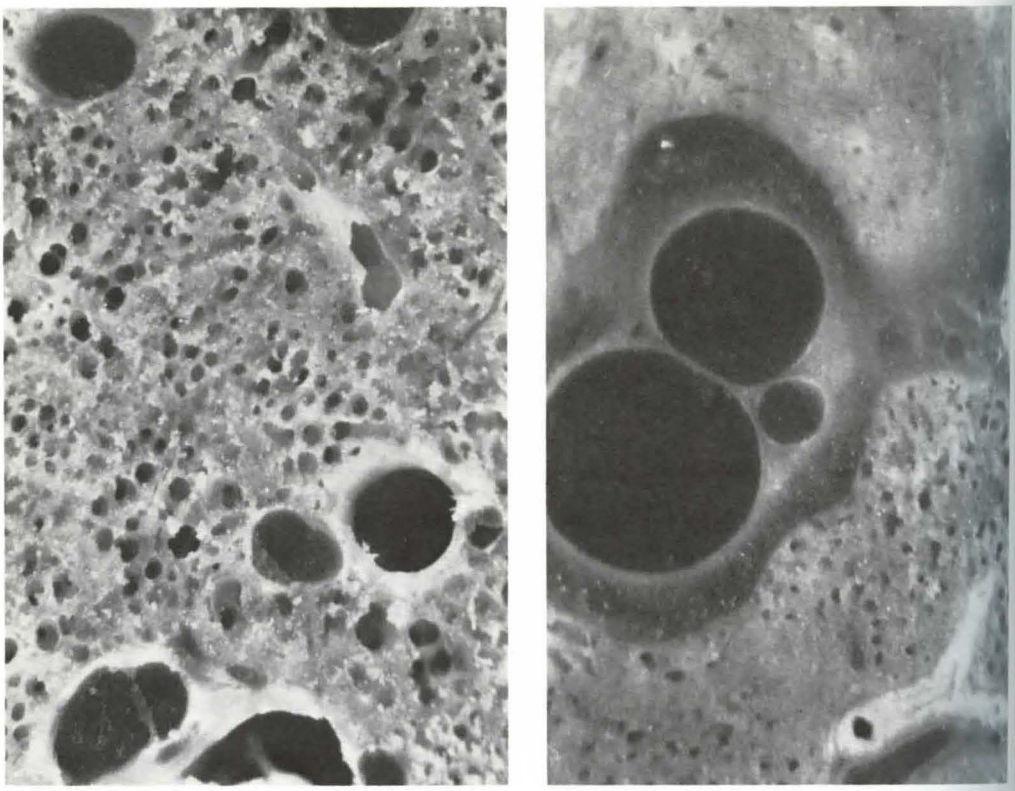

Fig. 3 Sections of lung, frozen in liquid nitrogen during inflation with gas at 25 cm H_2O pressure. The one on the left is from a normal lamb and the one on the right from a lamb killed after five days in 100 percent O_2. Note the large cuffs of fluid surrounding pulmonary blood vessels and the reduced aeration in the lung damaged by O_2. Magnification 5X

to protein in the microciculation of the lung. Anatomic studies by *Kistler,* using rats (3), and by *Kapanci,* using monkeys (2), showed early endothelial cell injury and interstitial edema. *Valimaki* et al. (12) injected polyvinylpyrrolidone (PVP: molecular weight 30,000–40,000) intravascularly into rats breathing 90–95 percent O_2 for up to 60 hours and showed that PVP accumulated in the interstitium of the lung and could be harvested in endobronchial extracts. They found that the yield in lung washings correlated directly with duration of O_2 breathing. Likewise, *Niinikoski* et al. (13) found increased protein in endobronchial saline extracts of rat lungs after O_2 exposure. *Jamieson* et al. (14), using Evans blue dye as an indicator, demonstrated leakage of plasma into the lungs of rats exposed to hyperbaric O_2. These observations, however, do not indicate the mechanism by which protein enters the interstitium of the lung; increased protein movement into the lung could be caused by elevated microvascular pressure (15), reduced lung tissue pressure (possibly induced by increased alveolar surface tension) (16, 17), or greater vascular surface area in the lung, without a change in permeability.

Steady-state pulmonary lymph flow and the concentration of protein in lymph reflect net transvascular movement of water and protein in the lung (18), assuming that the concentration of protein in lymph approximates that in the pulmonary interstitium (19, 20). The method described by *Staub* et al. (4) therefore provides an *in vivo* assessment of lung fluid dynamics and pulmonary microvascular permeability to solutes of large molecular

weight; it permits recognition of changes in ung fluid filtration caused by increased micro- ascular pressure vs. changes caused by altered icrovascular permeability. With this tech- ique we demonstrated a permeability change nd documented its time of onset in six lambs reathing 100 percent O_2 for five days.

Lymph protein flow increased by an average f 131 percent above baseline, and there was significant reduction in the half-equilibration f ^{125}I-albumin, without a change in lung ascular pressures. These findings clearly re- lect an O_2-induced change in pulmonary icrovascular permeability to protein. The 5 percent decrease of [albumin] : [globulin] n lymph and the progressive 20 percent in- rease in the concentration of protein in ymph relative to that in plasma are further vidence that O_2 altered the normal sieving roperties of the pulmonary microvascular embrane, causing pulmonary edema despite normal intravascular filtration pressure.

Acknowledgements

We thank *L. Dong* and *R. Walker* for their technical assistance and *M. Biagini* for her secretarial help.

This work was supported by U.S. Public Health Service Pulmonary Specialized Center of Research grants HL 14201 and HL 19185. Dr. *Bressack* was a trainee supported by U.S. Public Health Service National Pulmonary Faculty Training Center grant HL 07159. Dr. *McMillan* received support from the Canadian Cystic Fibrosis Foundation, and currently is at the University of Calgary, Alberta, Canada.

References

1 *Clark, J.M., C.J. Lambertsen:* Pulmonary oxygen toxicity: a review. Pharmacol. Rev. 23 (1971) 37–133

2 *Kapanci, Y., E.R. Weibel, H.P. Kaplan, F.R. Robinson:* Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. Lab. Invest. 20 (1969) 101–110

3 *Kistler, G.S., P.R.B. Caldwell, E.R. Weibel:* Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lungs. J. Cell Biol. 32 (1967) 605–628

4 *Staub, N.C., R.D. Bland, K.L. Brigham, R.H. Demling, A.J. Erdmann, W.C. Woolverton:* Preparation of chronic lung lymph fistulas in sheep. J. Surg. Res. 19 (1975) 315–320

5 *Bland, R.D., D.D. McMillan:* Lung fluid dynamics in awake newborn lambs. J. Clin. Invest. 60 (1977) 1107–1115

6 *Rudolph, A.M.:* Congenital diseases of the heart, Year Book Medical Publishers, Inc, Chicago, Illinois (1974) 139–145

7 *Gornall, A.B., C.J. Bardawill, M.M. David:* Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177 (1949) 751–766

8 *Rodkey, F.L.:* Direct spectrophotometric determination of albumin in human serum. Clin. Chem. 11 (1965) 478–487

9 *Brigham, K.L., W.C. Woolverton, L.H. Blake, N.C. Staub:* Increased sheep lung vascular permeability caused by Pseudomonas bacteremia. J. Clin. Invest. 54 (1974) 792–804

10 *Snedecor, G., W. Cochran:* Statistical Methods, Iowa State University Press, Ames, Iowa (1972) 91–99

11 *DeLemos, R., J. Wolfsdorf, R. Nachman, A.J. Block, G. Leiby, H.A. Wilkinson, T. Allen, J.A. Haller, W. Morgan, M.E. Avery:* Lung injury from oxygen in lambs. Anesthesiology 30 (1969) 609–618

12 *Valimaki, M., J. Kivisaari, J. Niinikoski:* Permeability of alveolar-capillary membrane in oxygen poisoning. Aerospace Med. 45 (1974) 370–374

13 *Niinikoski, J., T. Nikkari, J.E. Kulonen:* Pulmonary oxygen toxicity: composition of endobronchial saline extracts of rats during exposure to oxygen. Aerospace Med. 42 (1971) 525–529

14 *Jamieson, D., H.A.S. van den Brenk:* Pulmonary damage due to high pressure oxygen breathing in rats. Austral. J. Exp. Biol. Med. Sci. 40 (1962) 309–314

15 *Erdmann, A.J., T.R. Vaughan, Jr., K.L. Brigham, W.C. Woolverton, N.C. Staub:* Effect of increased vascular pressure on lung fluid balance in unanesthetized sheep. Circ. Res. 37 (1975) 271–284

16 *Pattle, R.E.:* Properties, function, and origin of the alveolar lining layer. Proc. Roy. Soc. London 148 (1958) 217–240

17 *Morgan, T.E., T.N. Finley, G.L. Huber, H. Fialkow:* Alterations in pulmonary surface active lipids during exposure to increased oxygen tension. J. Clin. Invest. 44 (1965) 1737–1744

18 *Staub, N.C.:* Pulmonary edema. Physiol. Rev. 54 (1974) 687–811

19 *Nicolaysen, G.A., A. Nicolaysen, N.C. Staub:* A quantitative radioautographic comparison of albumin concentration in different sized lymph vessels in normal mouse lungs. Microvasc. Res. 10 (1975) 138–152

20 *Vreim, C.E., P.D. Snashall, R.H. Demling, N.C. Staub:* Lung lymph and free interstitial fluid protein composition in sheep with edema. Amer. J. Physiol. 230 (1976) 1650–1653

R.D.Bland, M.D., Cardiovascular Research Institute and the Department of Pediatrics Univ. of California, San Francisco, San Francisco, California 94143, USA