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Effect of Mechanical Ventilation and Barotrauma on Pulmonary Clearance of ^{99m}Technetium Diethylenetriamine Pentaacetate in Lambs

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ABSTRACT. We studied the effect of positive pressure mechanical ventilation on the pulmonary clearance (k) of ^{99m}technetium diethylenetriamine pentaacetate (^{99m}Tc-DTPA) in lambs. Twelve lambs were anesthetized and ventilated with air at 25 breaths/min and with end expiratory airway pressure at 2 cm H_2O for a minimum period of 8 h. Four lambs received normal pressure ventilation with peak airway pressure at 17 ± 3 cm H_2O and eight received high pressure ventilation with peak airway pressure at 41 ± 2 cm H_2O. Three ^{99m}Tc-DTPA clearance studies were done in each lamb; at the start and after 4 and 8 h of mechanical ventilation. All clearance measurements were made at normal peak airway pressure 16 ± 3 cm H_2O. Baseline clearance rate, k, (n = 12) was 1.2 ± 0.5% (SD). During normal pressure ventilation, k increased from 1.0 ± 0.5 to 2.2 ± 0.8% after 4 h and to 2.7 ± 0.8% after 8 h. During high pressure ventilation, k increased from 1.4 ± 0.4 to 3.6 ± 1.3% after 4 h and to 4.8 ± 1.2% after 8 h. To see if 8 h of mechanical ventilation was associated with other evidence of lung epithelial injury, we determined the movement of I^{125} labeled albumin from the interstitium into the airspace, lung water content, lung histology, and lung lavage constituents in the lambs at the end of the study. All variables were similar between ventilated and four unventilated lambs, except the percentage of alveolar macrophages recovered by lavage, which was more in the ventilated lambs. We conclude that positive pressure mechanical ventilation results in a continuous increase in the pulmonary clearance of ^{99m}Tc-DTPA and the duration and magnitude of positive pressure applied tended to increase the value of k. (*Pediatr Res* 27: 70–74, 1990)

Abbreviations

^{99m}Tc DTPA, ^{99m}Technetium diethylenetriamine pentaacetate
PAW, peak airway pressure
NPV, normal pressure ventilation
HPV, high pressure ventilation
PEEP, positive end expiratory pressure

Since the description of bronchopulmonary dysplasia in premature infants with hyaline membrane disease after mechanical ventilation (1), it is apparent that ventilator-induced barotrauma can also occur in the absence of acute lung injury (2, 3). Hyperventilation with assisted mechanical ventilation is frequently used as a therapeutic maneuver to reduce pulmonary vascular resistance in newborn infants with pulmonary hypertension (4) and to reduce cerebral edema in newborn and pediatric patients in the intensive care unit. In these cases where hyperventilation is used to produce hypocarbia and alkalosis, the lung parenchyma may be structually normal to begin with, but subsequently become injured from prolonged barotrauma (5). High peak airway pressures have been shown in animals to injure both the airway (6) and alveolar epithelium (7, 8), as well as the capillary endothelium (9).

The measurement of the pulmonary clearance of aerosolized solutes such as ^{99m}Tc-DTPA has been used in the assessment of the integrity of the pulmonary epithelium. In a variety of lung diseases such as adult respiratory distress syndrome (10), interstitial lung disease (11), and *Pneumocystis carinii* infection (12) where damage to the pulmonary epithelium is present, the detection of an accelerated clearance rate of ^{99m}Tc-DTPA has been attributed to an increase in pulmonary epithelial permeability. However, other maneuvers such as an increase in lung volume (13) and acute exposure to cigarette smoke (14) have also resulted in an increase in clearance of ^{99m}Tc-DTPA, in the absence of any substantial evidence of lung epithelial injury. In our study, we determined the effect of positive pressure mechanical ventilation on the rate of clearance of ^{99m}Tc-DTPA from the lungs of healthy neonatal lambs. We found that positive pressure mechanical ventilation resulted in an increase in the rate of clearance of ^{99m}Tc-DTPA; the higher the peak airway pressure and the longer the duration of ventilation, the greater the increase in the clearance rate of ^{99m}Tc-DTPA. This alteration in lung epithelial permeability to ^{99m}Tc-DTPA was not associated with an histologic change in the epithelial membrane, nor with an increased permeability of the epithelium to protein.

MATERIALS AND METHODS

We studied 20 lambs, aged 7 ± 3 d and weighing 3 to 11 kg. Twelve lambs were used for ventilation and postmortem studies and eight lambs for postmortem studies alone.

Animal preparation. After medication with ketamine hydrochloride (25 mg/kg intramuscularly) and 2% lidocaine subcutaneously, catheters were placed in the aorta and inferior vena cava. In eight of the lambs a 5F thermodilution balloon catheter (Gould Statham thermodilution catheter ND SP 5003 for use with cardiac output computer ND SP 1425, Gould Instruments, Oxnard CA) was placed in the pulmonary artery. Animals were allowed to recover for at least 1 d before the ventilation study. On the day of the study, the lambs appeared healthy and well

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hydrated with normal heart rate, systemic arterial blood pressure, and arterial blood gas tensions. All lambs received antibiotics (penicillin 200 000 IU/mL and streptomycin 250 mg/mL; Pfizer, NY, 1 mL intramuscularly) at the time of catheter placement.

Experimental protocol for ventilation study. Lambs were sedated with ketamine hydrochloride (25 mg/kg intramuscularly), intubated with a cuffed endotracheal tube and placed upright in a canvas sling under a radiant warmer. A piston type mechanical ventilator (Harvard) was used for ventilation. Rectal temperature was kept constant at 38.5 ± 1°C. Lambs were ventilated with air at a rate of 25 breaths/min and with 2 cm H_2O PEEP. The tidal volume was adjusted to maintain arterial pH between 7.35–7.45 and arterial P_{CO_2} between 35–45 torr. Once adjusted, tidal volume was kept constant in each lamb during baseline and subsequent serial ^{99m}Tc-DTPA clearance studies.

Heart rate and vascular pressures were recorded continuously using pressure transducers (Gould Statham P23) connected to a recorder (Beckman Polygraph) and airway pressure was monitored continuously at the endotracheal tube, using a Pneumogard (Nova-metrics Medical System, Inc). Arterial pH and gas tensions were measured every 15–30 min using standard electrode techniques (Radiometer BMS 3MIC2, Copenhagen, Denmark). Blood hematocrit, total solids (Refractometer), and glucose concentration (Dextrostix) were monitored every 15–30 min. We measured cardiac output in triplicate by thermal indicator dilution at hourly intervals. The lambs received pentobarbital sodium, 5–10 mg/kg body wt intravenously, to maintain anesthesia and pancuronium bromide 0.05 mg/kg intravenously to maintain paralysis as necessary. Adequacy of anaesthesia was judged by the presence of a stable heart rate and blood pressure with no fluctuations during handling of the animal. Each lamb received a continuous infusion of 10 mL/kg $^{-1}\cdot h^{-1}$ of normal saline or Ringer's lactate solution during the study period.

Normal pressure mechanical ventilation studies (n = 4). After a baseline clearance study, we continued mechanical ventilation for a total of 11 h. Repeat ^{99m}Tc-DTPA clearance studies were done after 4 h and again after 8 h of normal pressure ventilation.

High pressure mechanical ventilation studies (n = 8). After determining the baseline ^{99m}Tc-DTPA clearance rate, tidal volume was increased to yield a peak airway pressure between 40–45 cm H_2O. The respiratory rate (25 breaths/min) and PEEP (2 cm H_2O) were kept constant in all the lambs. During HPV, to maintain P_{aCO_2} between 35–45 torr, carbon dioxide was bled into the inflow tube of the ventilator. After 4 h of HPV, tidal volume was reduced to baseline, and after 20–30 min of stabilization, a second ^{99m}Tc-DTPA clearance study was performed. At the completion of this study, tidal volume was increased again and HPV continued for another 4 h, after which tidal volume and airway pressures were once again returned to baseline and a third radioaerosol clearance study performed. All experimental protocols are reviewed and approved by our institutional review board for animal care.

Aerosol generation and administration. ^{99m}Tc pertechnetate was eluted from a molybdenum generator and ^{99m}Tc bound to DTPA. ^{99m}Tc binding to DTPA was checked on random urine samples after administration of aerosol and was always found to be >95% bound by thin-layer chromatography. ^{99m}Tc-DTPA is freshly prepared in our institution for each study, *i.e.* ^{99m}Tc is eluted from a molybdenum generator at our institution and bound to DTPA by our radiopharmacist just 20 min before each aerosol clearance study. ^{99m}Tc-DTPA was diluted in 2 mL of 0.9% saline solution that was then aerosolized with 10 L/min of compressed air for 3 min through an OEM acorn nebulizer into a 30-L balloon. The droplets from this system have been previously sized and were found to have an aerodynamic mass median diameter of 1.8 microns and a geometric standard deviation of 1.65 (15). Radioactivity in the lung was detected using a 2 × 2″ collimated sodium iodide probe positioned next to the right lateral chest. The probe was interfaced to a preamplifier, amplifier and single channel analyzer (Canberra, Meriden, CT). Counts were displayed on a timer scale. To protect against regional variation in aerosol clearance rates (15), the probe was always positioned adjacent to the right lung by aligning the probe with position markers on the restraining sling. The intake from the ventilator circuit was opened to the balloon and the radioaerosol administered for 10 min or up to 20 000 counts/100 s, whichever came first. After inhalation, radioactivity was monitored at 100-s intervals for 30 min. Background radioactivity was subtracted from the data obtained and the recorded counts were corrected for radioactive decay. The logarithm of the counts for each interval was taken and the slope of the best fit line was determined from the peak of activity to 10 min after the peak by the least squares method. After 10 min, due to appearance of the isotope in blood, there is an apparent slowing of clearance; hence, the first 10 min of data were analyzed. The slope of this line is expressed in units of percent decline per min (k).

POSTMORTEM STUDIES

To see if our experimental protocols of mechanical ventilation had resulted in any other evidence of lung injury, we determined lung epithelial permeability to protein, lung water content, lung histology, and the composition of lung alveolar subphase constituents in four lambs after 8 h of NPV and in all eight lambs after 8 h of HPV. Similar measurements were made in eight other lambs; four that received no mechanical ventilation and four that received only 30 min of mechanical ventilation.

Bronchoalveolar lavage. In all 20 lambs, we injected ^{125}I-labeled human albumin 3–5 μCi/kg body wt intravenously at least 16 h before the time of death. This was done to allow sufficient time for ^{125}I-albumin to attain nearly complete equilibrium between the vascular and interstitial compartments, but little in the airspaces (16).

Just before death with overdose of intravenous pentobarbital, 10 mL of heparinized venous blood was collected for measurement of radioactive ^{125}I-albumin content in the plasma. The lungs were removed intact and the left lung excised with its bronchus. After obtaining the wet lung wt, we cannulated the left lung and lavaged it using 60–100 mL of normal saline at room temperature. Full distension of left lung, by visual inspection, occurred at infusion pressures of <25 cm H_2O. Each time, the saline was reinfused three times and withdrawn. Two subsequent washes were performed in a similar manner and the lavage fluid collected. Recovery of the instilled fluid was between 85 to 95%. Lavage fluid was stored at −20°C for later analysis. After the lavage, the left lung was dried in an oven at 70–80°C for 72 h to obtain the dry lung wt. Lung wet wt to dry weight ratio was calculated. The sample of venous blood collected terminally was centrifuged at 1000 × *g* for 10 min, the plasma collected and radioactivity of ^{125}I-albumin in the plasma sample determined.

Measurement of albumin leak from interstitium into airspace. ^{125}I-albumin radioactivity in the lavage fluid was counted in a γ-counter and the total radioactivity in the lavage fluid was divided by the radioactivity in 1 mL of plasma. The albumin leak from the interstitium into the airspace was then expressed as plasma equivalents, *i.e.* mL of plasma per g dry lung (17).

Bronchoalveolar lavage fluid analysis. Total lipids were extracted from an aliquot of the lavage fluid, using a mixture of chloroform and methanol (18) and expressed as mg/g dry lung. Total proteins were measured in another aliquot of lavage fluid (Lowry's method), and expressed as mg/g dry lung. A third aliquot of the lavage fluid was centrifuged at 300 × *g* for 20 min. The cell pellet was resuspended in PBS solution and total cell count determined by standard hemacytometer counting techniques. Cell smears were air-dried and stained with modified Wright's stain and differential cell counts were done. Cells were classified as alveolar macrophages, mononuclear cells, or neutrophils. Cells that had the characteristic morphology with vacuoles were counted as alveolar macrophages. Erythrocyte contamination was minimal.

Lung histology. The right lung was rapidly frozen in liquid nitrogen at an airway pressure of 25 cm H_2O. A 1-cm block of tissue was fixed by freeze substitution (19), the tissue embedded in paraffin, and 4-μm thick sections were cut and stained with hematoxylin and eosin for light microscopy.

DATA ANALYSIS

Results are expressed as mean ± 1 SD. Data from the ventilation studies were analyzed with an analysis of variance for repeated measurements and a significant difference was determined using the Student Newman-Keuls test. For the postmortem studies, a significant difference among groups was determined by an analysis of variance and the Student Newman-Keuls test for multiple comparisons. We accepted a $p < 0.05$ as significant.

RESULTS

^{99m}Tc-DTPA Clearance Studies. Normal pressure ventilation. Baseline ^{99m}Tc-DTPA clearance rate in the four lambs was 1.01 ± 0.53%. After normal pressure ventilation for 4 and 8 h, k increased to 2.19 ± 0.85 and 2.74 ± 0.83%, respectively (Fig. 1). The clearance rate increased 170% after 8 h of ventilation.

High pressure ventilation. Baseline ^{99m}Tc-DTPA clearance rate in the eight lambs was 1.39 ± 0.46%. After high pressure ventilation with normocarbia for 4 and 8 h, k increased to 3.65 ± 1.36 and 4.8 ± 1.26%, respectively (Fig. 2). The clearance rate increased by 245% after 8 h of ventilation. The absolute values of k after 4 and 8 h of NPV are different from that after 4 and 8 h of HPV. But when we express the data as a percent increase in clearance rate from baseline, the difference in the percent increase in clearance rate from baseline in the two groups after 8 h of ventilation does not reach statistical significance.

Cardiorespiratory variables in the two groups of lambs during all ^{99m}Tc-DTPA clearance studies and during the period of mechanical ventilation are shown in Tables 1 and 2, respectively. During the radioaerosol clearance studies, all variables measured were similar between the two groups of lambs (Table 1). Pulmonary artery pressure and arterial P_{O_2} was higher during the high pressure ventilation than during normal pressure ventilation. Heart rate, mean aortic pressure, and cardiac output were similar between the groups.

Postmortem Studies. Lung epithelial permeability to albumin. The albumin leak from the interstitium into the airspaces, which we expressed as plasma equivalents, *i.e.* mL of plasma per g dry lung, was similar in the unventilated lambs and lambs that were ventilated for 30 min or for 8 h with normal or high pressure (Table 3).

Bronchoalveolar lavage constituents. Total lipid and total protein content in the lavage fluid was similar between the unventilated and ventilated lambs. The total cell count was also similar.

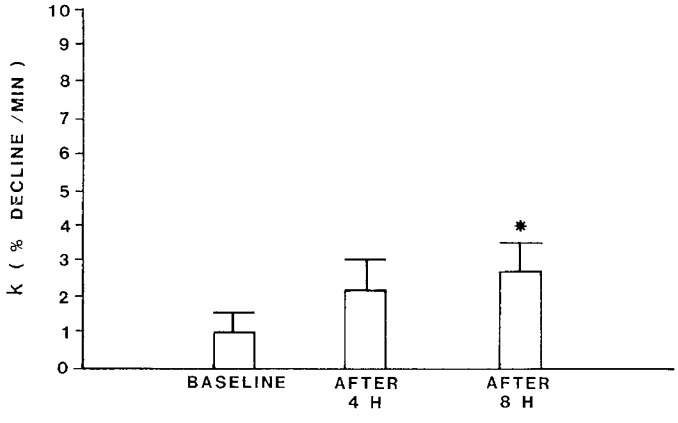

Fig. 1. Clearance rates of ^{99m}Tc-DTPA in lambs during baseline, and after 4 and 8 h of normal pressure ventilation. Values are mean ± SD, $*p < 0.05$, compared with baseline and 4-h values.

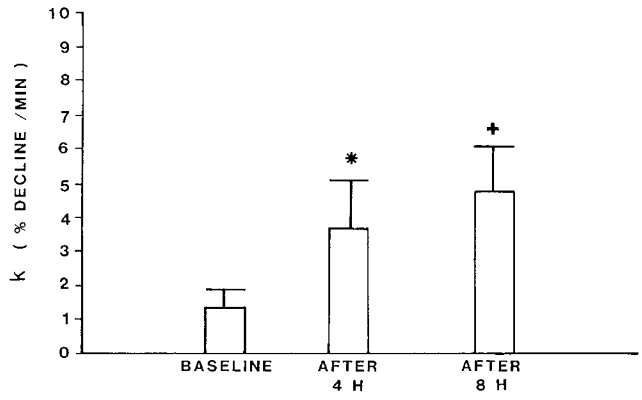

Fig. 2. ^{99m}Tc-DTPA clearance rates in eight lambs during baseline and after 4 and 8 h of high pressure ventilation. Values are mean ± SD. $*p < 0.05$, compared with baseline, $+p < 0.05$, compared with baseline and 4 h values.

Table 1. *Cardiorespiratory variables in two groups of lambs during ^{99m}Tc-DTPA clearance studies (mean ± SD)*

Group	Normal pressure ventilation	High pressure ventilation
pHa	7.40 ± 0.07	7.38 ± 0.03
$Paco_2$ (Torr)	41 ± 3	42 ± 3
Pao_2 (Torr)	79 ± 13	84 ± 5
Heart rate (bpm)	220 ± 17	218 ± 29
Mean aortic pressure (cm H_2O)	91 ± 15	88 ± 6
Cardiac output (mL/kg/min)	360 ± 116	396 ± 70
Pulmonary artery pressure (cm H_2O)	21 ± 3	21 ± 4
Tidal vol (mL)	130 ± 25	150 ± 15
Peak airway pressure (cm H_2O)	15 ± 3	16 ± 2

Table 2. *Cardiorespiratory variables in two groups of lambs during period of mechanical ventilation (mean ± SD)*

Group	Normal pressure ventilation	High pressure ventilation
pHa	7.37 ± 0.03	7.39 ± 0.03
$Paco_2$ (Torr)	39 ± 3	35 ± 2
Pao_2 (Torr)	81 ± 12	112 ± 10*
Heart rate (bpm)	223 ± 26	224 ± 23
Mean aortic pressure (cm H_2O)	89 ± 13	91 ± 8
Cardiac output (mL/kg/min)	352 ± 114	371 ± 95
Pulmonary artery pressure (cm H_2O)	20 ± 2	25 ± 4*
Peak airway pressure (cm H_2O)	17 ± 3	41 ± 2*

* Different from normal pressure ventilation.

However, the percentage of alveolar macrophages was significantly higher in the lambs that were mechanically ventilated for at least 8 h, with a proportionate decrease in the percentage of monocytes. The percentage of neutrophils was not different.

Lung water content. The lung wet to dry wt ratio was similar in all the lambs. The mean wet to dry ratio was 5.0 ± 0.1.

Lung histology. Figure 3 shows representative photomicrographs of sections of lung obtained from an unventilated lamb (*A*) and from a lamb after 11 h of high pressure mechanical ventilation (*B*). We could detect no gross abnormality in the lungs obtained from lambs after 11 h of normal or high pressure mechanical ventilation. The lungs were well inflated, the alveolar septal walls were not thickened and there was no evidence of interstitial or airspace edema.

DISCUSSION

The rate of clearance of inhaled radioaerosol particles, such as ^{99m}Tc-DTPA, from the airspaces of the lungs has been used by

Table 3. *Summary data for postmortem studies in unventilated and ventilated lambs (mean ± SD)*

Group	Unventilated ($n = 4$)	30 min of NPV ($n = 4$)	8 h of NPV ($n = 4$)	8 h of HPV ($n = 8$)
Albumin leak from interstitium to airspace (Plasma equivalents; mL/g dry lung)	0.05 ± 0.05	0.16 ± 0.08	0.10 ± 0.05	0.12 ± 0.07
Lung lavage constituents				
Total lipids (mg/g dry lung)	29.7 ± 9.1	24.4 ± 2.1	17.4 ± 9.8	21.1 ± 13.0
Total proteins (mg/g dry lung)	17.6 ± 9.3	14.0 ± 8.3	15.0 ± 6.2	12.8 ± 5.7
Total cell count ($\times 10^6$ cells/g dry lung)	8.2 ± 2.0	11.9 ± 1.4	11.7 ± 5.7	9.6 ± 6.1
Alveolar macrophages (%)	45.1 ± 5.5	37.4 ± 8.7	66.7 ± 3.5*	71.2 ± 6.0*
Monocytes (%)	50.7 ± 6.1	60.2 ± 11.5	29.8 ± 6.5*	25.7 ± 6.7*
Neutrophils (%)	3.9 ± 0.9	4.7 ± 3.2	2.7 ± 1.7	3.5 ± 2.3

* Different from unventilated and 30 min ventilated lambs, $p < 0.05$. NPV, normal pressure ventilation; HPV, high pressure ventilation.

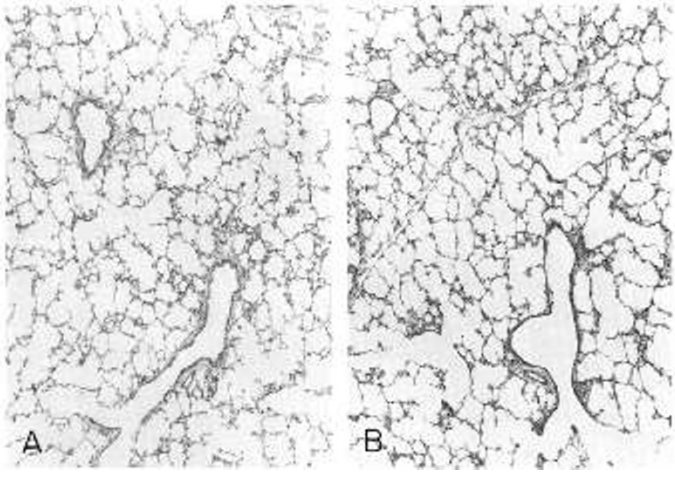

Fig. 3. Sections of lung obtained from an unventilated lamb (*A*) and from a lamb after 11 h of high pressure mechanical ventilation (*B*). There is no evidence of alveolar atelectasis, septal wall thickening and interstitial or alveolar edema in either lung.

several investigators to provide some index of the permeability of lung epithelium. An acceleration in the clearance rate of ^{99m}Tc-DTPA has been detected in neonates and adults with conditions associated with lung epithelial injury, such as hyaline membrane disease (20), and adult respiratory distress syndrome (10). However, in these studies the subjects were receiving mechanical ventilation at the time of the aerosol clearance studies. Therefore the contribution of mechanical ventilation itself to the observed acceleration in ^{99m}Tc-DTPA clearance is unknown. Barotrauma from prolonged positive pressure mechanical ventilation is known to result in lung epithelial injury, especially in premature (1, 6) and mature neonates (5). The purpose of our study was to determine the effect of positive pressure mechanical ventilation on serial measurements of pulmonary clearance of ^{99m}Tc-DTPA in neonatal animals. In addition, we wished to determine whether the increase in clearance rate was associated with other evidence of lung epithelial injury. We found that continuous positive pressure ventilation resulted in a progressive acceleration in the clearance of ^{99m}Tc-DTPA from the lungs of neonatal lambs. The degree of acceleration in clearance rate was related both to the duration and magnitude of positive pressure applied.

In our experiments, after the initiation of mechanical ventilation and the completion of the baseline clearance study, we performed the next clearance study after an interval of 4 h. At this time, we found an increase in the clearance rate in all lambs. We do not know, however, at what point in time during this 4 h interval that the change in clearance rate occurred.

Several possibilities exist to explain the increased clearance of ^{99m}Tc-DTPA. The prolonged swings in airway pressure and lung volume produced by positive pressure mechanical ventilation may have perturbed the epithelium in some manner resulting in the increase in ^{99m}Tc-DTPA clearance rate. In sheep, marked swings in pleural pressure over a 30-min period did not result in acceleration of ^{99m}Tc-DTPA clearance (21). It is possible that such pleural pressure swings over longer periods of time in neonatal lambs may have resulted in changes in the rate of ^{99m}Tc-DTPA clearance.

Previous studies have investigated the effect of a rise in intrathoracic pressure on ^{99m}Tc-DTPA clearance from lungs of humans (13), sheep (21), and dogs (22). The data demonstrate that PEEP increases the rate of clearance of ^{99m}Tc-DTPA from lungs, particularly at high levels of PEEP, mainly by raising lung volume. However, this was not found to occur if the baseline clearance rate was already accelerated such as in human smokers (13). When lung volume is kept constant, increased intrathoracic pressure does not result in any significant change in ^{99m}Tc-DTPA clearance. It is possible that the application of as low as 2 cm H_2O PEEP to a relatively compliant neonatal lung may have resulted in increased lung volume, accounting for the relatively faster baseline k in our lambs as compared to that reported in adult sheep (21). However, what is important to note is that in the four lambs that received continuous normal pressure mechanical ventilation for more than 8 h, in which lung volume should have been fairly constant, ^{99m}Tc-DTPA clearance rate continued to increase. In our experiments we performed serial ^{99m}Tc-DTPA clearance studies in each lamb at the same airway pressure as the baseline study. Also, as lung parenchyma demonstrates hysteresis, sufficient time was allowed for equilibration after switching from high to normal peak airway pressure before delivery of the aerosol. Thus, lung volume was probably unchanged from the baseline in our studies.

The site of deposition of aerosol and size of the aerosol particles are of prime importance in interpreting the changes in ^{99m}Tc-DTPA clearance rates. Determining the exact site of deposition of radioaerosol droplets is not possible. Although large particles of more than 3 μm tend to settle in large airways, and particles less than 0.5 μm settle in airspaces or are exhaled, it is presumed that intermediate sized particles deposit in both compartments. Lourenco *et al.* (23) have demonstrated that 50% of 2-μm particles of labeled ferrous oxide deposit in nonciliated airways and in airspaces. Taplin and Chopra (24), using aerosol particles of 1–3 μm size, did not find any significant deposition in the large central airways using scintigraphic methods. In our study we generated an aerosol with an aerodynamic mass median diameter of 1.8 μm, and we believe that the aerosol particles were primarily deposited in small airways and alveoli. Oberdorster *et al.* (25) delivered large droplets of ^{99m}Tc-DTPA (4.1 μm), which presumably were deposited in large airways when dogs were ventilated with small tidal volumes (50 mL) and compared their clearance to small droplets (0.5 μm) delivered with normal tidal volumes (350 mL). The animals who received "dead-space" deposition of aerosol had a slower clearance rate than animals where droplets were probably in small airways and airspaces. Because we used normal tidal volumes that remained constant

during all ^{99m}Tc-DTPA clearance studies, it seems unlikely that the site of deposition changed during our studies.

The permeability of the lung epithelium to macromolecules, such as albumin, from the interstitium was not increased in the lambs after more than 8 h of mechanical ventilation. The lack of change in lung water content and lung histology in the ventilated lambs indicates that in these studies there was no gross disruption of the lung epithelium.

Inasmuch as the initial barrier to the movement of ^{99m}Tc-DTPA molecule is the alveolar liquid lining layer, it is possible that a thinning of this layer or a change in the composition of this layer resulted in the accelerated clearance of ^{99m}Tc-DTPA (26). The major constituents of the alveolar subphase are surfactant, a small quantity of plasma proteins and the alveolar cell population. We found no change in the total amount of lipid and protein recovered by alveolar wash, or in the total number of cells recovered. What we did find was an increase in the percentage of alveolar macrophages in the lambs that had 11 h of mechanical ventilation. Alveolar macrophages are capable of releasing oxygen radicals, that may be injurious to the lung epithelium. Alternatively, activated macrophages may release toxic oxygen radicals which may oxidize ^{99m}Tc DTPA into the smaller, more permeable molecule, ^{99m}Tc pertechnetate (27).

The baseline ^{99m}Tc-DTPA clearance rate (k) in neonatal lambs was 1.2 ± 0.5%, which is considerably faster than that reported in adult sheep (0.22 to 0.6%) (21, 28). O'Brodovich *et al.* (28) studied sheep during spontaneous breathing with 2 cm H_2O PEEP, whereas in our studies the lambs were mechanically ventilated with 2 cm H_2O PEEP. This degree of PEEP may bring about a greater volume change in a more compliant lung as in neonatal lambs as compared to adult sheep, accounting for the faster baseline clearance rate. Differences in ^{99m}Tc-DTPA clearance rates (k) have been reported among other species, k ranging from 1.0 to 3.2% in dogs (22, 29), 0.54 to 1.0% in rabbits (14, 30), and 0.25 to 1.8% in humans (13, 26). We calculated the clearance rates over the first 10 minutes after achieving peak counts. With time, an artifact due to accumulation of the indicator in tissues and blood may become prominent, resulting in apparent slowing of clearance. Some investigators correct for this accumulation of radioactivity in the pulmonary interstitium, blood, and chest wall (31). Controversy persists regarding the accuracy and need for such a correction (32, 33).

In summary, we found that positive pressure mechanical ventilation for as short a period as 4–8 h resulted in a continued increase in the pulmonary clearance of ^{99m}Tc-DTPA in neonatal lambs. We do not know the mechanisms by which positive pressure mechanical ventilation resulted in acceleration of ^{99m}Tc-DTPA clearance rate. Inasmuch as most of the resistance to diffusion of hydrophilic solutes is offered primarily by the epithelium, changes in the rate of clearance of the radioaerosol particles should predominantly reflect changes in epithelial permeability of the distal lung unit. Although there appears to be an increased permeability of the epithelium to small solute particles, we did not find an increase in the epithelial permeability to macromolecules such as albumin. It would seem that mild perturbations may result in an increased permeability of the lung epithelium to small solutes, with no change in epithelial permeability to large macromolecules. Finally, our findings emphasize the importance of careful interpretation of ^{99m}Tc-DTPA clearance rate, especially in patients receiving mechanical ventilation because mechanical ventilation per se is an important variable that affects lung clearance of ^{99m}Tc-DTPA.

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