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Early Pulmonary Changes Associated with High-Frequency Jet Ventilation in Newborn Piglets

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ABSTRACT. To assess the short-term effects of high-frequency jet ventilation (HFJV) on the neonatal lung, 28 newborn piglets were studied. Nine piglets were unventilated except during brief pulmonary measurements, nine animals were conventionally ventilated (arterial CO_2 tension 35–45 torr, arterial O_2 tension 70–80 torr) for 4 h, and 10 piglets were ventilated with HFJV for the same period. Pulmonary function was analyzed using a computerized technique and tracheobronchial aspirates were examined for biochemical indicators of lung injury; after 4 h, bronchoalveolar lavage was obtained for surfactant composition and activity, and lung sections were examined by light and electron microscopy. Results showed that HFJV provided adequate ventilation at lower inspiratory pressure compared with conventional ventilation (8.6 ± 0.3 *versus* 13.8 ± 1.3 cm H_2O; $p < 0.01$), while pulmonary mechanics did not vary significantly among the three animal groups. Tracheobronchial aspirates from HFJV animals had higher elastase activity *versus* unventilated piglets (118.5 ± 14.1 *versus* 57.7 ± 8.4 μg/mL; $p < 0.01$), as well as higher albumin concentration *versus* unventilated animals (94.2 ± 18.7 *versus* 23.2 ± 6.5 μg/mL; $p < 0.01$). In addition, there were small but statistically significant differences between all three groups in the distribution of surfactant phospholipids in bronchoalveolar lavage, although biophysical activity was normal. Scanning electron microscopy revealed flattening of Clara cells in the terminal bronchioles of HFJV animals due to loss of glycogen and secretory granules. These data indicate that despite lower peak inspiratory pressures, HFJV can cause subtle biochemical changes in lungs. Further studies are indicated to determine if these changes precede significant lung injury. (*Pediatr Res* 27: 460–465, 1990)

Abbreviations

HFJV, high-frequency jet ventilation
CMV, conventional mechanical ventilation
EM, electron microscopy
PIP, peak inspiratory pressure
BAL, bronchoalveolar lavage
PMN, polymorphonuclear cell

Pulmonary barotrauma from conventional positive-pressure ventilation has been implicated in the pathogenesis of neonatal lung injury such as bronchopulmonary dysplasia (1, 2). This type of ventilation has the potential to damage cell membranes in the lung, causing release of inflammatory mediators that attract polymorphonuclear cells (3). These cells release proteolytic enzymes such as elastase, causing further lung injury with increased permeability to albumin and other small proteins. Merritt *et al.* (4) have demonstrated increased white blood cells, elastase, and proteins in tracheal aspirates of infants with respiratory distress syndrome who subsequently developed bronchopulmonary dysplasia. In animal models of acute lung injury, these biochemical changes can precede the occurance of significant morphologic and physiologic abnormalities (5, 6).

One possible way to decrease injury effects from mechanical ventilation is to adopt methodologies that permit adequate gas exchange with reduced peak airway pressure and presumably less barotrauma. Several recent studies have suggested that high-frequency ventilation produces more efficient gas exchange at lower airway pressures in infants with severe lung disease (7–11). Although it has been postulated that this type of ventilation may lead to less barotrauma and lung injury than CMV, this has not been well characterized in controlled studies. In our experiments, biochemical, biophysical, physiologic, and morphologic measurements are used to study pathophysiologic effects accompanying 4 h of CMV and HFJV in neonatal piglets. This timepoint is long enough to allow for inflammatory changes to be initiated and changes in surfactant to occur, but short enough to precede significant physiologic and morphologic abnormalities. These groups are compared with each other and with additional unventilated piglets to elucidate any associated lung injury changes and their relative magnitude.

MATERIALS AND METHODS

Physiologic studies. Twenty-eight term neonatal piglets were studied, with wt 1.3 ± 0.3 kg (mean ± SD) and ages of 1 to 4 d. Piglets were anesthetized with intraperitoneal pentobarbital (25 mg/kg), and umbilical or femoral arterial lines were placed. The piglets were then intubated with a 3.0- or 3.5-mm endotracheal tube (either conventional or hi-lo triple lumen jet tube), and were studied in one of three ventilation groups: *1*) nine animals that were unventilated except for a brief (<10 min) period of CMV while baseline biochemical and physiologic measurements were obtained; *2*) nine animals mechanically ventilated with room air on a Baby Bird ventilator (Bird Products Corp., Palm Springs, CA) (inspiratory time 0.5 s; flow rate 8 L/min; positive end expiratory pressure = 3–4 cm H_2O) for a 4 h study period (CMV group); PIP and frequency were varied to maintain arterial CO_2 tension 35–45 torr and arterial O_2 tension 65–75 torr; *3*) 10 animals ventilated with room air and the Life Pulse High-Frequency Jet Ventilator (Bunnell Inc., Salt Lake City, UT; positive end expiratory pressure 3–4 cm H_2O, inspiratory time 0.02 s; frequency 420 breaths/min) for 4 h. PIP was varied to

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Table 1. *Pulmonary mechanics in unventilated, conventionally ventilated, and high-frequency jet ventilated groups*

	PIP*	MAP	V_T	MV	C_{RS}	R_T	W_R
Unventilated	13.6 ± 0.9	3.9 ± 0.3	12.6 ± 1.9	416 ± 71	1.85 ± 0.23	53 ± 5	51 ± 13
CMV	13.6 ± 1.2	4.1 ± 0.3	13.7 ± 1.1	460 ± 73	1.79 ± 0.19	56 ± 11	47 ± 7
HFJV	8.6 ± 0.3†	4.0 ± 0.4	9.6 ± 0.9†	387 ± 35	1.96 ± 0.13	57 ± 11	39 ± 10

* Animal groups are unventilated, CMV, and HFJV. Variables are: PIP (cm H_2O); MAP, mean airway pressure (cm H_2O); V_T, tidal volume (mL/kg); MV, minute ventilation (mL/min/kg); C_{RS}, dynamic compliance, (mL/cm H_2O/kg); R_T, total resistance (cm H_2O/L/s); and W_R, resistive work (g·cm/kg). All values are given as mean ± SEM.

† Statistically significant, $p < 0.01$, compared to unventilated and CMV groups.

Table 2. *Specific phospholipid components of bronchoalveolar lavage surfactant**

	BAL protein	BAL phospholipid	% PC	PG	PE	PI	Sph	Res
1) Unventilated	0.24 ± 0.04	1.0 ± 0.1	82.3 ± 2.5	2.0 ± 0.6	4.1 ± 0.5	7.9 ± 0.6	1.6 ± 0.5	2.1 ± 0.5
2) CMV	0.16 ± 0.01	1.0 ± 0.1	82.4 ± 1.4	1.0 ± 0.1	3.9 ± 0.4	9.2 ± 0.7	0.9 ± 0.3	2.6 ± 0.5
3) HFJV	0.24 ± 0.05	1.2 ± 0.1	86.1 ± 0.7	0.3 ± 0.1	2.6 ± 0.3	8.7 ± 0.5	0.5 ± 0.2	1.8 ± 0.2
			$p < 0.05$ groups 2, 3	$p < 0.01$ groups 2, 3	$p < 0.05$ groups 2, 3			
				$p < 0.01$ groups 1, 3	1, 3			

* Groups of animals are unventilated ($n = 9$), CMV ($n = 9$), and HFJV ($n = 10$). BAL protein is given as mg/mL, and total BAL phosphate as μmol/mL. Phospholipid classes are in percent of total. PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol (plus phosphatidylserine); Sph, sphingomyelin; Res, residual.

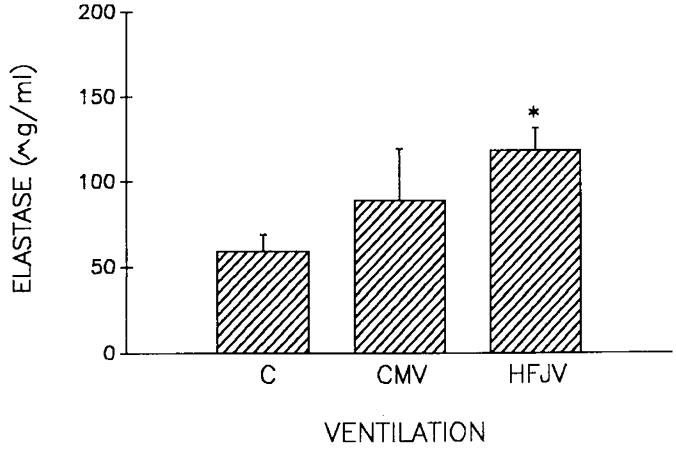

Fig. 1. Elastase concentration in tracheal aspirates of control piglets (*C*), CMV animals, and animals treated with HFJV. * $p < 0.01$ compared to controls.

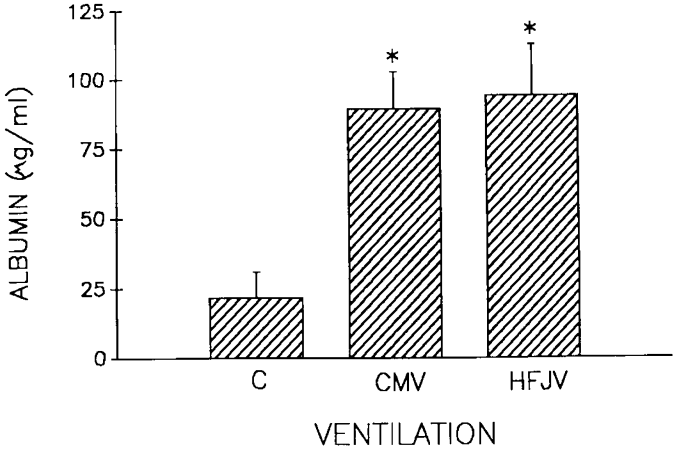

Fig. 2. Albumin concentration in tracheal aspirates of control (*C*), CMV and HFJV animals. * $p < 0.01$ compared to controls.

maintain arterial blood gases in the desired range. As is normal clinical practice, a background sigh rate of 4 breaths/min was used to prevent atelectasis. PIP on the background breaths was maintained 2–3 cm H_2O below that of the high-frequency jet ventilator to prevent overdistention and additional barotrauma (HFJV group).

The 4-h ventilation period was chosen because previous studies have shown that significant effects on surfactant and lung morphology may begin within that time frame (12, 13). Arterial blood gases were measured three to four times by a gas-calibrated Corning 168 blood gas analyzer (Corning Glass Works, Corning, NY). Airway (mouth) pressure was continuously measured with a Pneumogard pressure monitor (Novametrix Inc., Wallingford, CT) and distal airway pressure was also measured during HFJV. At the conclusion of the ventilation period, additional studies were performed on the animals as described below.

Pulmonary physiologic status was assessed using a computerized technique to calculate a series of respiratory variables on a breath-by-breath basis (14, 15). Flow rates were measured with a pneumotachometer (Fleisch 00, OEM Medical Inc., Richmond, VA), which was inserted between the endotracheal tube and ventilator. An esophageal balloon was placed in the lower third of the esophagus to estimate pleural pressure, and transpulmonary pressure was measured with a differential pressure transducer (Validyne Engineering Corp., Northridge, CA) as airway minus esophageal pressure. Both flow and pressure signals were relayed to a computer and digitized at 75 Hz. Flow was digitally integrated to give tidal volume, and minute ventilation was calculated. Values of lung compliance and pulmonary resistance were determined by 2-factor least mean square analysis (14), and resistive work of breathing was calculated from numerical integration of pressure and volume.

of individual breaths by simultaneous display of scalar tracings, flow volume, and pressure-volume relationships. Only ventilator generated breaths that were complete and nondistorted were selected for final analysis, with average values calculated for a minimum of 10 acceptable breaths. To ensure proper comparisons of pulmonary mechanics between experimental groups, piglets in the third group had the high-frequency jet ventilator placed on standby and the background ventilator rate increased. Ten minutes of equilibration were allowed (with arterial blood gases maintained in the normal range), followed by a 1-min pulmonary function study.

Biochemical and cellular studies. Tracheobronchial aspirates

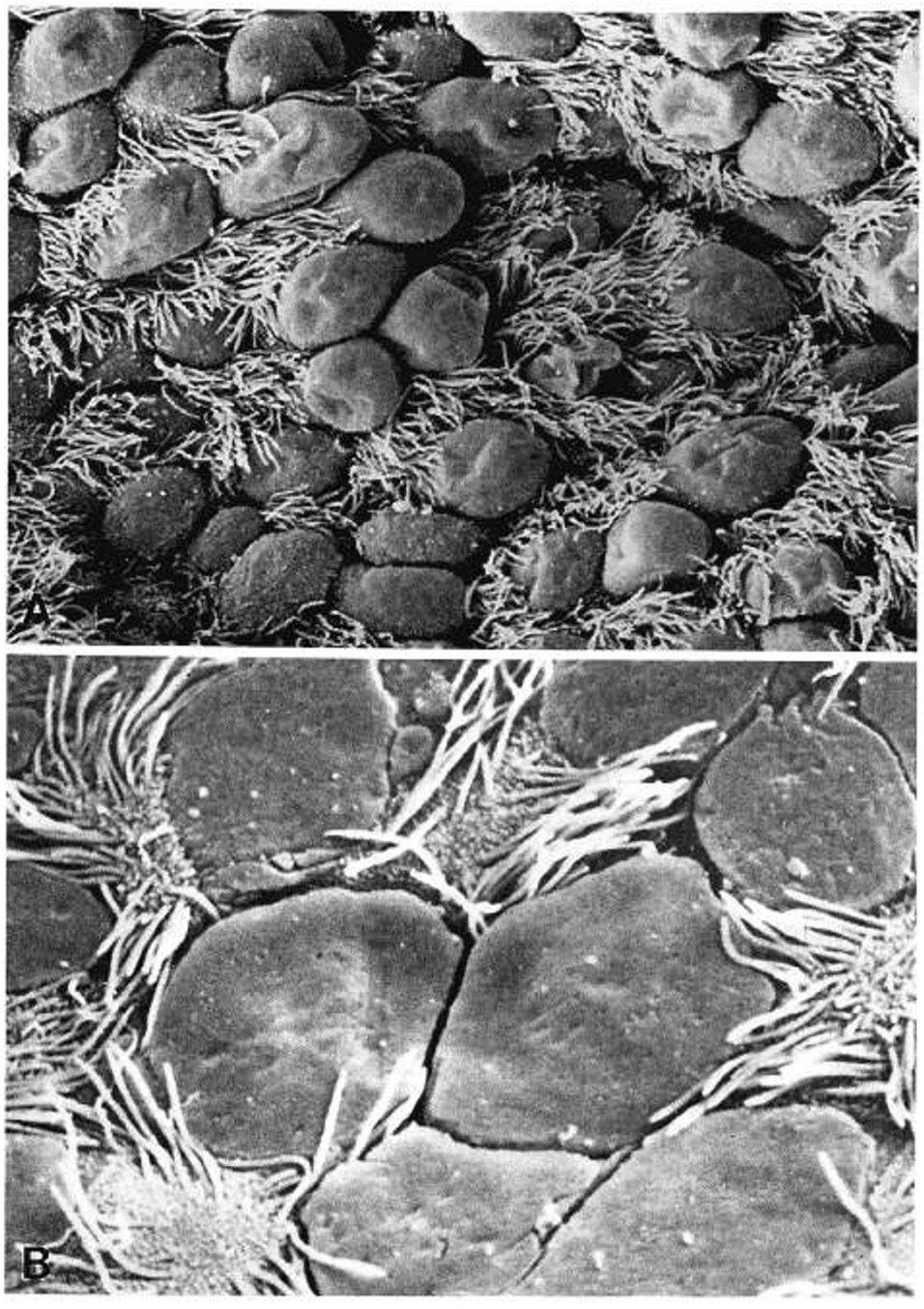

Fig. 3. *A*, scanning electron micrograph (×1000) showing cuboidal, domed Clara cells in the terminal bronchiole of a control animal. Ciliated cells are interspersed with the Clara cells. *B*, scanning electron micrograph (×4400) showing flattening of the Clara cells in an animal treated with 4 h of HFJV. Note how the cilia now extend well above the tops of the Clara cells.

were obtained from control and experimental animals at the end of the 4-h ventilation period for cell analysis and protein measurements. One and one-half mL saline was instilled, suction catheters were inserted 1–2 cm beyond the tip of the endotracheal tube, and secretions were suctioned into a Leuken's trap. An additional 1.5 mL normal saline was used to rinse the catheter. Aspirates were then centrifuged at 1200 rpm (approximately 150 × *g*) for 6 min, and the supernatant frozen at −70°C for later biochemical analysis. The cell pellet was resuspended in Hanks' balanced salt solution, and total cell counts were obtained using a hemocytometer after staining with 2% trypan blue. Cell differentials were also obtained after cytocentrifugation (1200 rpm for 5 min) and Wright staining.

Aspirates were analyzed for total protein, albumin and α-1-proteinase inhibitor concentrations, and elastase activity. Total protein in pulmonary effluent was analyzed by the Pierce bicinchoninic acid technique which is a modification of the Lowry's analysis (16). The albumin concentration of pulmonary aspirates was measured by ELISA (17). A 1:1000 dilution of suctioned effluent was incubated with 100 μL of a 1:100 solution of rabbit antiswine albumin (primary antibody). This reaction was blocked with Tween, and 100 μL of a 1:1000 solution of biotinylated goat antirabbit antibody (secondary antibody) was added. After incubation, blocking, and washing, vectastain avidin-biotin complex alkaline phosphatase reagent was added followed by paranitrophenyl phosphate; the resultant color formation was then read on an ELISA microplate reader to quantify albumin. α-1-Proteinase inhibitor concentration was determined by rate nephelometry (18).

Elastase activity in aspirates was determined by the elastin-

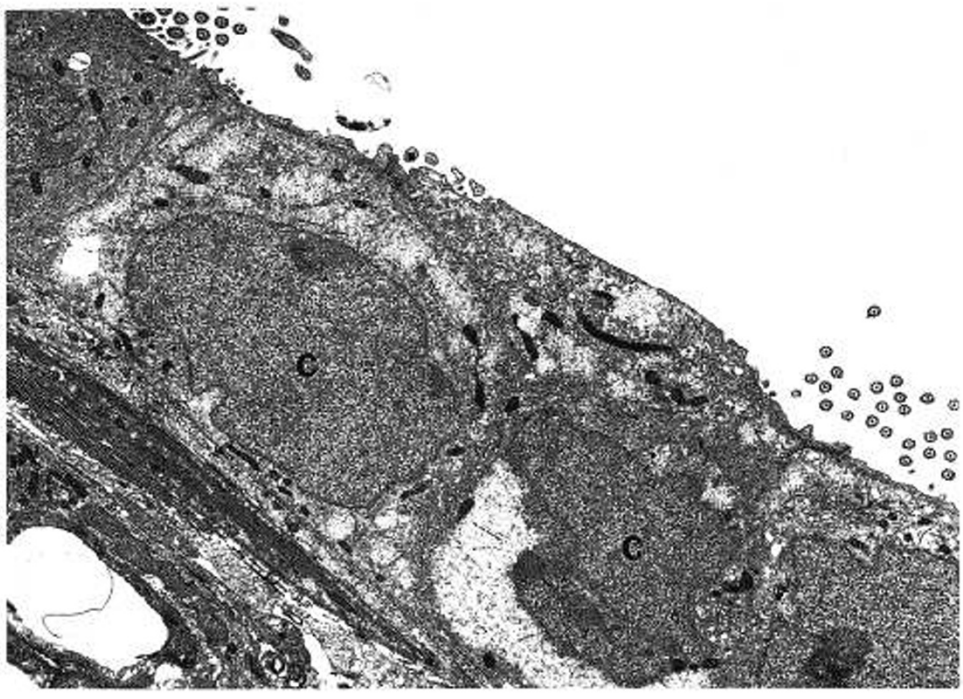

Fig. 4. Transmission electron micrograph (×8860) of a portion of a terminal bronchial wall. Clara cells (*c*) are flattened and do not protrude beyond the level of adjacent ciliated cells.

agar plate method (19) and by cleavage of a peptide substrate according to the technique of Yasutake and Powers (20). Twenty μL of aspirate fluid were reacted with 0.3 mM methoxysuccinyl-L-(alanyl)$_2$-prolyl-valyl-p-nitroanalide peptide substrate, 0.2 M Tris, and 1 mg/mL of BSA. After a 15-min incubation period, the reaction was stopped with 1 N acetic acid and the change in OD was measured at 410 nm using a Beckman Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA).

Microscopy studies. At the conclusion of the study period, animals were killed with 100 mg/kg of pentobarbital. In half the animals in each group, the chest was opened and a catheter inserted into the right ventricle and secured in the main pulmonary artery (21). The left atrium was opened to allow for escape of fixative. While the lungs were maintained at a constant inflating pressure of 20 cm H_2O, 60 mL of normal saline were infused to wash blood out of the pulmonary circulation. Phosphate-buffered formaldehyde-glutaraldehyde was then perfused through the vascular system for fixation over 15 min at a fluid pressure of 25 cm H_2O. After fixation, the lungs were removed and tissue blocks obtained from each lobe for light microscopy and EM. The location of the blocks within each lobe was varied among animals in an attempt to minimize sampling error. Light microscopic specimens were embedded in paraplast, sectioned, and stained with hematoxylin and eosin. Blocks obtained for transmission EM were dehydrated in ethanol, embedded, sectioned using an LKB NOVA (LKB-Produkter AB, Bromma, Sweden) ultramicrotome, and stained with lead citrate and uranyl acetate. Sections were then examined in a transmission (Zeiss 10-A and 10-CR, Zeiss Inc., New York, NY) electron microscope. For scanning EM, specimens were fixed and dehydrated as described above, then critical point dried, coated with gold-palladium, and examined in a JEOL-35CF (JEOL USA, Peabody, MA) scanning electron microscope. The pathologists were blinded as to treatment group.

Surfactant studies. To assess any acute effects of ventilation on pulmonary surfactant, studies were done on the composition and activity of material obtained by BAL. In the 14 animals with lungs not sectioned for microscopy, BAL was performed using 100 mL (in four 25-mL aliquots) of normal saline; overall recovery was always ≥80%. The lavage fluid was immediately centrifuged (300 × *g* for 10 min) to remove cells and debris, and the surfactant phospholipid in the supernatant was examined by thin-layer chromatography with the solvent system of Touchstone *et al.* (22). Aliquots of cell-free BAL were then concentrated by evaporation under nitrogen to a uniform concentration of 1 mg phospholipids/mL, and were examined for surfactant biophysical activity with a pulsating bubble surfactometer at 37°C (23, 24).

Statistical analyses. Data were analyzed by unpaired, two-tailed *t* tests that compared variables among the three animal groups (unventilated, CMV, HFJV).

RESULTS

The results in Table 1 demonstrate that HFJV provided ventilation at a lower PIP than needed for CMV (8.6 ± 0.3 *versus* 13.8 ± 1.3 cm H_2O, $p < 0.01$). Mouth pressure did not differ significantly from distal airway pressure during HFJV. The mean airway pressure required by the two groups was not significantly different (4.0 ± 0.2 *versus* 4.1 ± 0.3 cm H_2O). There were no significant differences found between the three groups in terms of minute ventilation, dynamic compliance, total flow resistance, and resistive work. There was a small difference measured for tidal volume between the CMV and HFJV groups, but this was consistent with the lower inspiratory pressures in HFJV animals, and was not reflected in any decrease in minute ventilation.

The BAL samples were found to be equivalent in total phospholipid and protein contents among unventilated, CMV, and HFJV animals (Table 2). Some statistically significant compositional differences did emerge between groups when surfactant analyses were done with thin-layer chromatography; however, these differences did not affect activity. Surfactant samples for all animals were able to reach minimum surface tensions below 1 dyne/cm.

Biochemical analyses on tracheobronchial aspirates demonstrated several differences among the three groups of piglets studied. As shown in Figure 1, elastase activity was significantly greater in the HFJV group (118.5 ± 14.1 μg/mL) compared to unventilated animals (57.7 ± 8.4 μg/mL; $p < 0.01$), whereas the CMV animals (89.1 ± 30.2 μg/mL) did not differ significantly.

Albumin concentration (Fig. 2) was significantly higher in both the HFJV (94.2 ± 18.7 μg/mL) and CMV (89.4 ± 13.4 μg/mL) groups compared to unventilated animals (23.2 ± 6.5 μg/mL; $p < 0.01$). Total cell counts were higher in the HFJV group (33.2 ± 13.4 × 10^5) compared to the CMV group (5.9 ± 1.1 × 10^5) or unventilated animals (9.3 ± 2.1 × 10^5), although not statistically significant due to large variability. There were no significant differences in white blood cell populations in the aspirates among the three groups, and this was also the case for total protein and α-1-antiproteinase concentration.

Light microscopic evaluation of the lungs of animals from all three groups showed normal architecture without any striking variations. Light microscopy of the upper airway from two animals in the CMV group and three animals in the HFJV group revealed focal, mild inflammation. The location of the inflammation was in the upper trachea and was milder in the HFJV animals than in the CMV group. The inflammation was thought to be related to mild trauma associated with intubation. There was no correlation found between degree and location of airway abnormalities and biochemical changes in any of the animals studied. Differences also emerged on EM examination, particularly with regard to Clara cells. Figure 3*A* shows a representative scanning EM micrograph from the lungs of a control animal, and demonstrates plump, cuboidal Clara cells in the terminal bronchioles interspersed with ciliated cells. In contrast, scanning EM of lung sections from HFJV animals shows that the Clara cells are completely flattened (Fig. 3*B*). This was a common finding in all areas of both lungs that were examined. Clara cells are slightly flattened in micrographs from CMV animals, but the effect was much less pronounced than in the HFJV group.

Transmission EM findings demonstrated that the flattening of Clara cells was associated with decreased glycogen granules and vesicles in the domes of these cells (Fig. 4). Transmission EM also revealed increased surfactant secretion in alveoli and PMN infiltration in the alveoli, interstitial space, and capillaries of some of the animals in the HFJV group.

DISCUSSION

The results of this study show that despite lower PIP, HFJV can cause subtle biochemical and morphologic changes in normal newborn piglet lungs after 4 h compared with unventilated animals. The newborn piglet was used in this study since it is large enough at birth for relevant physiologic and biochemical studies and its lungs are morphologically similar to that of the newborn infants (5). Hyperoxia and barotrauma have been shown to significantly damage the lung of the neonatal piglet as well. Animals with normal lungs were studied to eliminate variables associated with an underlying disease process, so the direct effects of CMV and HFJV could be specifically evaluated. Biochemical analyses of tracheobronchial aspirates displayed increased elastase activity and increased albumin concentrations in HFJV animals over unventilated animals, with the latter also significantly elevated in animals studied with CMV (Figs. 1, 2). These biochemical changes appear consistent with a number of other studies. The effects of barotrauma on the lungs of newborn infants has resulted in the production of inflammatory mediators, neutrophils and elastase (3, 4). These factors lead to increased permeability to albumin and other proteins. Man *et al.* (25) demonstrated significant alterations in ^{99m}Tc-labeled diethylenetriamine pentaacetate clearance in adult dogs after 4 h of high-frequency oscillatory ventilation, suggesting that this mode of ventilation can increase pulmonary epithelial permeability, possibly through damage to intercellular tight junctions.

The biochemical abnormalities found in our study occurred in the absence of any major morphologic or physiologic alterations. The existence of early biochemical effects agrees with the results of Davis *et al.* (5) and De Los Santos *et al.* (6), who detected such abnormalities before the development of significant morphologic or physiologic deficits in experimental animals with lung injury secondary to prolonged hyperoxia and/or hyperventilation.

In terms of cellular differences in aspirates, the animals in the HFJV group had increased total cell counts, although this was not statistically significant. A linear relation did not exist between cell counts, elastase activity, and albumin concentration on correlation analysis over the entire group. However, the animals with the highest cell counts (in the HFJV group) also had the highest elastase activity and albumin concentration. There was variability between animals and data should not be overinterpreted, but data did reach statistical significance, suggesting that some animals might have been more susceptible to lung injury than others. Increased elastase activity is usually associated with increased concentrations of PMN. However, elastase activity was significantly higher in HFJV animals without significant increases in PMN. Although our study was not designed to specifically evaluate functional changes in elastase activity, it is possible that increased elastase activity in the HFJV group resulted from a decrease in elastase inhibition. α-1-Proteinase inhibitor concentrations were not different among the groups, but activity was not measured. It is also possible that although the concentrations of PMN were not increased, PMN that were in the lung were more activated and produced more elastase. This phenomenon warrants further investigation.

Morphologically, Clara cells were found to be flattened in all areas of the lungs of HFJV animals, although overall lung structure was normal. Clara cells tend to be located in small airways, and their precise functions are still largely unknown. They do have limited stem cell capacity and are rich in cytochrome P450, suggesting that they have active metabolic and detoxifying capabilities (26, 27). In addition, morphologic studies have indicated that they may be secretory cells because they contain electron-dense cytoplasmic granules that may contain surfactant apoproteins or other products. The absence of granules in the Clara cells of the HFJV animals suggest that they had secreted their products in response to the challenges of this ventilation modality.

In conclusion, short periods of HFJV and CMV were not found to cause significant ultrastructural or mechanical alterations in the lungs of newborn piglets. Short periods of HFJV produced evidence of inflammation and increased albumin permeability, accompanied by a flattening of Clara cells on EM. This may indicate the early, subtle onset of lung injury in the HFJV group, and in the CMV group to a lesser degree, when compared to controls. Further long term studies are indicated to fully evaluate the effects of HFJV on the neonatal lung and to determine if these early changes may precede significant lung injury.

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