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Alveoli
collateral ventilation
hemorrhagic atelectasis
neonate
pulmonary system

A New Model for Neonatal Pulmonary Hemorrhage Research

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Extract

Hemorrhagic atelectasis was successfully produced in newborn rabbits by pharmacologically narrowing airways leading to alveoli ventilated with oxygen-enriched gas. Between 48% and 62% of alveoli filled with blood cells. Areas of lung with a tendency to collapse were measured by pressure volume studies. Animals given supplemental oxygen retained 56% of total lung volume compared with 79% in the pilocarpine group, which suggested increased effectiveness of antiatelectasis factors in the latter. Less total lung gas was present in the pilocarpine group (4.0 ± 0.4 cc/g) compared with oxygen controls (5.1 ± 0.81 cc/g), which indicated more noninflatable lung. Neither surfactant deficiency nor heart failure needed to be present for pulmonary hemorrhage to occur.

Speculation

Neonates are at increased risk of pulmonary hemorrhagic atelectasis because of their incomplete pulmonary anatomic development, if their airways become obstructed while breathing high concentrations of oxygen.

Pulmonary hemorrhage in lungs of neonates at autopsy has been a common finding (1, 10, 11). One recent report considered it to be the principle cause of death in 9% of autopsies reviewed in that study (11). Diagnosis during life has been a difficult matter. Prenatal complications, such as toxemia, abruptio, and prolapsed cord, have been seen frequently but none has been specifically related (2, 5, 23). No single diagnostic laboratory test or x-ray pattern (23) has been reported. For the most part the infants have weighed less than 2,000 g but have been heavier than infants who subsequently develop respiratory distress syndrome (6, 10). The clinical diagnosis is only evident in a fraction of cases when frank airway bleeding accompanies sudden deterioration.

Some authors have suggested that pulmonary hemorrhage follows altered coagulation capacity resulting from the stress and acidosis of conditions such as hypothermia and the respiratory distress syndrome (7, 14, 20). Rowe and Avery (11, 23) have suggested that pulmonary hemorrhage may specifically follow surfactant inactivation. Others have theorized that the hemorrhage occurs because of physical changes in the lung resulting from high concentrations of therapeutic oxygen and/or left heart failure (6, 24).

Structural inadequacies might make neonatal lungs especially susceptible to pulmonary hemorrhage. Neonatal small airways are disproportionately long for their diameter and may close during tidal breathing (15, 18, 19). Peripheral airway conductance is quite low until about 5 years of age (15) and infants and children have absent or poorly developed collateral ventilation (15, 18). The term collateral ventilation describes air which passes from alveolus to alveolus without traveling along the airways.

Recently, an analysis of pulmonary interdependence suggested that the lack of adequate collateral ventilation puts infants at special risk of developing hemorrhagic atelectasis (21). Mead *et al.* (21) have theorized that if neonatal airways become obstructed while alveoli are filled with a rapidly assimilated gas such as oxygen, local alveolar pressures could fall to levels which would result in capillary rupture because of lack of pressure relief mechanisms.

We tested this hypothesis in neonatal rabbits by pharmacologically narrowing airways leading to alveoli ventilated with oxygen-enriched gas and successfully produced hemorrhagic atelectasis.

METHODS

Females from a purebred strain of New Zealand White rabbits who had successfully completed at least three prior pregnancies were mated with a single buck under direct observation. The pregnancies were carried to term and the

young allowed to deliver vaginally. Within 3 hr of birth, 24 weight-matched littermate rabbit pairs were acclimatized for 2 hr in a warm chamber at 38°, maintained at 80% oxygen concentration with 15 liters/min gas flow through the chamber. Twelve other newborn rabbits were left in room air with the doe. Miniature needle electrodes were applied to electrocardiographic and apnea monitors. Abdomens were palpated for liver size every 30 min, and the chest was radiographically studied in 10 littermate pairs. After 2 hr of exposure, each of the rabbits in room air and one neonate of each littermate pair was given 7 mg pilocarpine nitrate subcutaneously while the other member of the pair was given an equal volume of saline. Two hours later, the animals were killed and the lungs prepared for pressure-volume studies by inserting a polyethylene catheter (outside diameter 0.04 inches) into the trachea and fixing it with two silk sutures. The chest wall was left intact, and inflation and deflation were performed at room temperature by displacing air from a reservoir with saline (17). The displaced volume necessary to maintain increasing pressure was measured at 1-min intervals and related to the pressure necessary to displace that volume. Lungs were allowed to stabilize for 15 min at maximal pressure and for 3 min at intermediate pressures. The maximum volume of air that could be insufflated was related to the weight of the lung, and the percentage of volume remaining at deflation to 10 cm of water pressure was calculated. The lungs were then excised and weighed, the left upper lobes fixed immediately in 2% paraformaldehyde and later processed for light microscopy and stained with toluidine blue. Every fifth lung section was divided into quadrants. One thousand alveoli were inspected under high dry magnification and the number that were filled with blood cells were noted. The remainder of the left lung was pooled for minimal surface tension as described previously (17). The right upper lobe of each lung was taken for DNA analysis (12) and the remainder was freed of water in a lyophilizer (25) to determine dry weight. The dried lung was extracted by the technique of Bligh and Dyer (4) and phospholipid phosphorus was determined by Bartlett's assay (3). The *t* test of paired variates was applied to statistically evaluate results.

RESULTS

Treated neonates were incontinent of stool and urine and developed progressive cyanosis within 15 min after injection. Abdominal palpation revealed no significant changes in liver size over the course of the experiments. Bradycardia, defined as a heart rate of less than 80/min was prominent. The hearts were not enlarged on x-ray examination of six animals and there was no evidence of chamber overload on electrocardiogram.

Large areas of hemorrhage were seen on gross inspection of the excised lungs from the pilocarpine- and oxygen-treated animals. On light microscopy, between 48% and 62% of alveoli of all 24 treated rabbits were filled with blood cells (Fig. 1*A*) compared with none in the control animals (Fig. 1*B*) or the air and pilocarpine rabbits. From this point on, "pilocarpine treated" will refer only to the pilocarpine- and oxygen-treated animals. Initial body weights were comparable in the two groups (Table 1). The pilocarpine-treated animals developed abundant nasal and oral secretions and lost significantly more body weight than control animals ($P < 0.01$). Those lungs contained more water, but the proportion of weight attributable to water was almost identical with the control animals. The total lung DNA was slightly elevated in treated animals although the increase was not significant statistically. Liver weights in control and experimental groups were similar.

The interaction between pulmonary surface forces and surface tension lowering agents such as pulmonary surfactant is reflected in the air volumes remaining at various pressures during deflation in the course of pulmonary pressure volume studies (16). Areas of lung with a tendency to premature atelectasis can be assessed by expressing the pressure-volume data in terms of percentages of maximal air inflation volume (16). Those animals treated with supplemental oxygen alone retained a mean of 56% of total lung volume at deflation to a pressure of 10 cm of water (Table 2). The pilocarpine and oxygen group retained 79.4% at the same pressure, a finding compatible with increased effectiveness of an anti-atelectasis factor such as surfactant. An alternate explanation could be that physiologic airway closure occurred during deflation resulting in gas trapping and greater retained volume at deflation to low pressures.

If the total volumes of gas inflated at maximal pressure is expressed per gram of lung tissue, a rough measurement of noninflatable areas may be obtained (16). Less total gas volume was present in the pilocarpine group, as evidenced by the significantly lower cubic centimeter per gram, indicating a greater proportion of noninflatable lung (Table 2). There were no statistical differences between groups in minimum surface tension of lung mince or the phospholipid content of wet lung.

DISCUSSION

Pilocarpine is a parasympathomimetic alkaloid that selectively influences smooth muscles and exocrine glands innervated by postganglionic cholinergic nerves. It produces marked sweating, salivation, bronchoconstriction, and release of inclusions from granular pneumatocytes thought to contain pulmonary surfactant. The effectiveness of the drug was documented in these experiments by the abundant secretions the experimental group developed. Pilocarpine may produce bronchial blockage both by constricting the smooth muscles and by causing bronchorrhea and mucus plugging. The dose of pilocarpine used was not fatal. Another group of 40 animals were permitted to survive to adolescence. Despite an initial growth retardation they were able to "catch up" with nontreated littermates. We chose to produce airway narrowing by using pilocarpine because of its reported effects on surfactant release. Goldenberg *et al.* enhanced granular pneumocyte secretion by treating fasted rats with pilocarpine and expelling mature osmiophilic inclusions from type II alveolar cells. The intra-alveolar accumulation of osmiophilic material was maximal 2–4 hr after injection (13).

Some have suggested that pulmonary hemorrhage may follow surfactant inactivation (23). The increased deflation stability in the hemorrhage group indicates that it may have moved from storage sites. The tissue mince minimal surface tension and phospholipid phosphorus indicate that the total lung surfactant was not altered.

Surfactant inactivation follows exposure to blood elements (2). If surfactant inactivation occurred in association with complete atelectasis it would not be reflected by a change in deflation stability since the completely atelectatic alveoli by definition would not be inflatable. This condition would decrease the amount of maximal air per unit lung tissue. There were more noninflatable areas as demonstrated by the significantly smaller cubic centimeter per gram measurements. Since these areas were not available for our pressure-volume studies we could not determine whether surfactant inactivation occurred in those areas. If surfactant inactivation occurred in the hemorrhaged areas we suggest that it followed rather than preceded the bleeding. A study of human infants dying of massive pulmonary hemorrhage reported that extractable surface tension of lung extracts and lung pressure volume studies were normal (22). The authors concluded that deficient pulmonary surfactant seemed unlikely to be a predisposing factor.

The lungs of our pilocarpine-treated animals were heavier. Part of the increase in weight was due to water, part attributable to increased dry material. Since the proportion of water was not changed the increase in weight could be due to

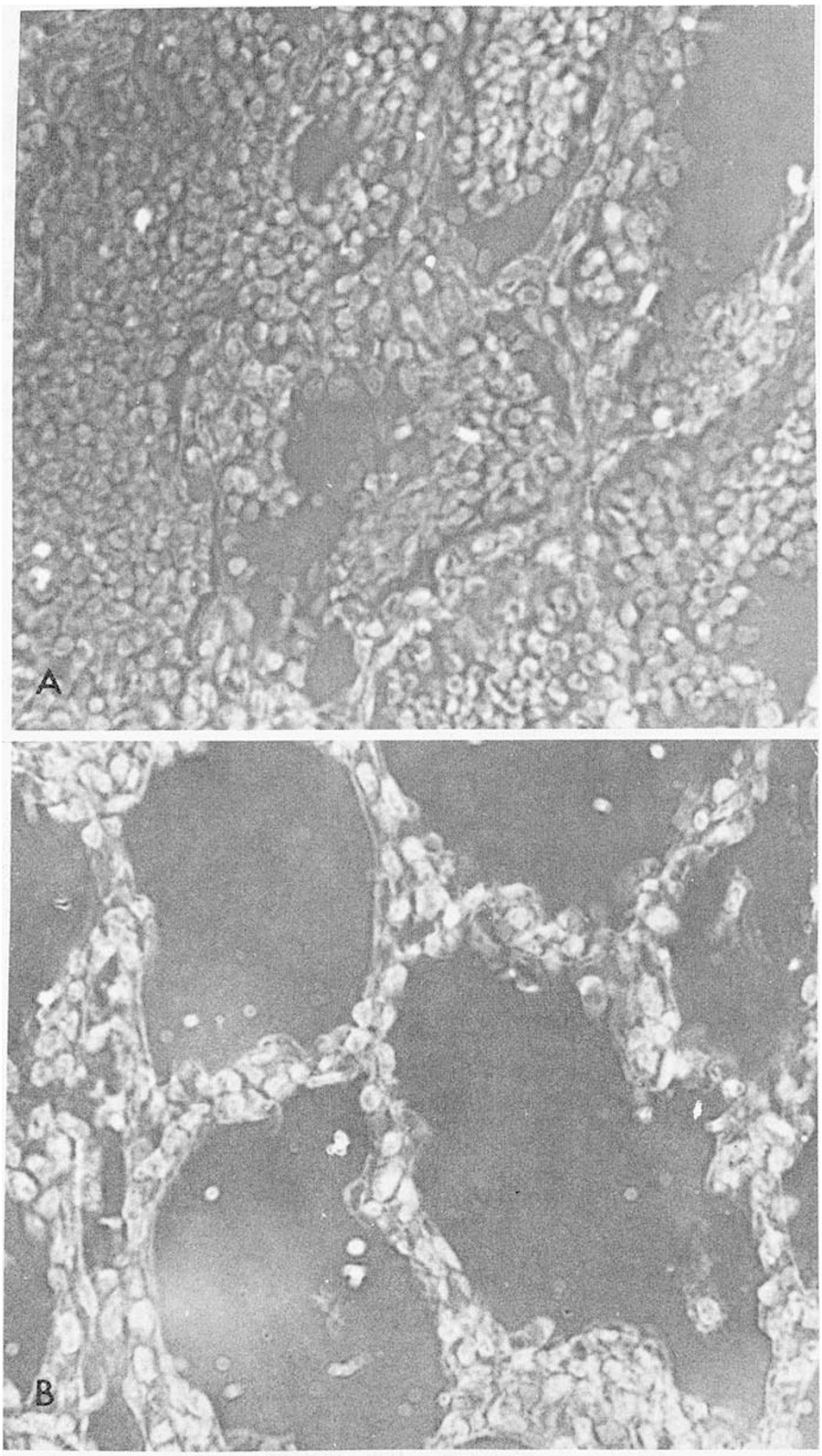

Fig. 1. Phase contrast light microscopy of lung of newborn rabbits receiving injections of pilocarpine (*A*) and control (*B*) newborn rabbits, 4-μm sections. × 400.

increased blood elements in the airways and/or vessels of the lung. Part of the increase can be explained by the finding of red cells in the alveoli on light microscopy. When the maximal lung volumes were expressed in terms of milliliters of gas per whole lung, the treated animals contained about 8.3% less gas. We assume that 8.3% of the lung volume formerly occupied by gas was now occupied by blood. Using Enesco and LeBlond's formula (8, 9), we estimated that the O_2 alone group had a mean of 546 million nucleated lung cells compared with 600 million nucleated cells in the experimental group. Including the data concerning dry weight per cell in controls, we calculated that the pilocarpine group had at least an additional 27.2 million more non-nucleated cells per lung if a non-nucleated cell can be assumed to have the same dry

Table 1. *Measurements on neonatal rabbits*[1]

	O_2	PC + O_2	*P*
n	24	24	
Initial BW, g	51.8 ± 8.13	51.2 ± 7.84	N.S.
Final BW, g	51.4 ± 7.69	50.1 ± 7.33	N.S.
Weight loss, % BW	0.8	2.1	<0.01
LDW, mg	143.9 ± 19.5	165.3 ± 33.3	<0.01
LDW/BW × 1,000	2.8 ± 0.03	3.3 ± 0.33	<0.001
Lung water, %	83.0 ± 0.8	83.3 ± 1.6	N.S.
Lung water, mg	846.5 ± 11.55	989.8 ± 26.4	<0.001
DNA, mg/lung	8.82 ± 1.62	9.68 ± 1.81	N.S.
Liver wt, g	2.14 ± 0.26	2.07 ± 0.3	N.S.

[1] n: number of animals; LDW: lung dry weight; BW: body weight; N.S.: not significant; O_2: oxygen treated; PC + O_2: oxygen and pilocarpine treated. All values are means ± 1 SD.

Table 2. *Further measurements on neonatal rabbits*[1]

	O_2	PC + O_2	P *P*
V_{10}%	56 ± 18	79.4 ± 6.55	<0.001
cc/g	5.1 ± 0.81	4.0 ± 0.4	<0.001
γ min, dynes	7.17 ± 5.58	7.8 ± 4.31	N.S.
mg PL/g wet tissue	16.4 ± 3.01	16.7 ± 2.67	N.S.

[1] V_{10}%: percent of total lung volume remaining on deflation to 10 cm water pressure; cc/g: cubic centimeters of gas at maximal pressure (35 cm water) per gram of wet lung; γ min: minimal surface tension; PL: phospholipid; N.S.: not significant. All values are means ± 1 SD.

weight as a nucleated cell. It is more likely that non-nucleated blood cells are lighter than nucleated ones, in which case more of the increase in lung dry weight could be due to non-nucleated cells.

Coie *et al.* (6) analyzed 12 infants with documented pulmonary hemorrhage and concluded that coagulation disorder "probably served to exacerbate the condition but not to initiate it." Histologic examination of brains, kidneys, livers, and body cavity linings of our experimental animals revealed no sites of hemorrhage. Under the conditions of the experiment, hemorrhage was restricted to the lung.

Cole *et al.* (6) concluded that acute left ventricular failure due to asphyxia "was the most important precipitating factor." We do not believe that our animals were in left ventricular failure. Liver size is a sensitive index of congestive heart failure in neonates. Our experimental animal liver weights were not significantly heavier than the control rabbits. Radiographic studies revealed no increase in heart size nor evidence of pulmonary edema in the treated animals. Neither did we find electrocardiographic evidence of cardiac strain. The absence of heart failure was supported clinically by the finding of bradycardia, lack of liver enlargement, and loss of body weight.

The study of stress distribution in lungs by Mead *et al.* (21) indicated that regions within a lung that are restricted in their expansion are subjected to greater expanding stresses than unrestricted regions. Regions subjected to abnormally high expanding stresses risked increased capillary transmural pressure. Alveolar pressure within the obstructed region would fall as the contained gas passed into the blood until it equalled the pressure required to produce transudation from alveolar capillaries. If gas uptake were rapid, alveolar pressure could fall to levels which could cause capillary rupture, and the obstructed alveoli would fill with fluid. The authors further speculated that infants should be at risk of hemorrhagic atelectasis if they developed small airway obstruction since the lungs of infants have poorer collateral ventilation than the lungs of adults. Mansell *et al.* (19) found that younger children began closing small airways at higher lung volumes than older children. They extrapolated that small airways in the immature lung may be unstable enough to close within the tidal volume resulting in trapped gas. If the trapped gas is primarily oxygen it theoretically could be absorbed so completely that alveolar-capillary pressure gradients become excessive and rupture of blood vessels results, spilling blood elements into the gas space.

Coagulation disorders, surfactant deficiency, inspired gas toxicity, and/or heart failure are no doubt involved in the causality of clinical pulmonary hemorrhage. We would like to emphasize that animals breathing oxygen-enriched air do not develop hemorrhage in pulmonary parenchyma unless pilocarpine is also given. By producing airway obstruction with pilocarpine in neonatal animals breathing oxygen-enriched air, we were able to experimentally produce pulmonary hemorrhage because the neonate lacks a compensatory ventilation mechanism for airway obstruction.

There has not been an adequate animal model of pulmonary hemorrhage prior to these experiments. All publications have been retrospective and concentrated on finding common characteristics in involved infants. Until recently, no one has considered that pulmonary anatomical inadequacies in combination with a therapeutic agent such as oxygen might predispose to intra-alveolar bleeding. The pilocarpine-oxygen animal model promises to be useful in studies on pathogenesis and prevention of pulmonary hemorrhage.

SUMMARY

Pulmonary hemorrhage was successfully produced in newborn rabbits by pharmacologically narrowing airways leading to alveoli ventilated with oxygen-enriched gas. Neonatal airways are disproportionately long for their diameter and susceptible to obstruction. Since neonates lack pathways alternate to the airways for ventilation, local alveolar pressures can fall to levels resulting in vessel rupture if airways become obstructed while breathing high concentrations of oxygen. Neither surfactant deficiency nor heart failure need be present for pulmonary hemorrhage to occur.

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Fetus
glycerol
lung
newborn rabbit
pulmonary phosphatidylcholine

The Significance of Circulating Glycerol as a Precursor of Pulmonary Phosphatidylcholine in the Developing Mammalian Lung

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Extract

There is scant information regarding the contribution made by circulating precursors to pulmonary phosphatidylcholine synthesis in the developing mammalian lung. *In situ* pulmonary artery perfusions were performed in term New Zealand newborn rabbits with physiologic buffer containing either 3.6 mM or 10.8 mM glycerol. There was a twofold increase in nanomoles of glycerol-phosphatidylcholine synthesized at 30 min when the higher concentration of glycerol was used. Continuing with the higher concentration, a near three-fold increase was observed between the 30-min and 60-min perfusions. This data indicates that the *de novo* synthesis of pulmonary phosphatidylcholine is influenced by the concentration of glycerol in the perfusate as well as the duration of perfusion.

Speculation

The observation that the concentration of circulating glycerol can influence the *de novo* synthesis of pulmonary phosphatidylcholine suggests that glycerol may also play a role in providing precursor for pulmonary surfactant synthesis. The biochemical similarity of lipid metabolism at birth between human newborn infants and the newborn rabbit encourages extrapolation of this data to humans. The question is raised as to the influence that intravenous glycerol at physiologic concentration would have on pulmonary phosphatidylcholine synthesis in the infant with hyaline membrane disease.

Scant information is available regarding circulating precursors used for pulmonary surfactant synthesis in the developing mammalian lung. Naimark (6) reports that the normal lung's *de novo* lipid synthesis is not limited by availability of circulating substrate. Godinez *et al.* (2) observed, however, increased pulmonary incorporation of [1-^{14}C]palmitate with increased medium palmitate concentration. They reported decreased [1-^{14}C]palmitate incorporation when oleate was added to the medium, suggesting that fatty acid incorporation into phospholipid was related to the total fatty acid concentration in the medium. Scholz and Rhodes (7) reported that rats decreased the *in vitro* utilization of glucose for pulmonary phospholipid fatty acid synthesis during starvation as compared with the fed state. This suggested that during starvation, pulmonary phospholipid fatty acid synthesis relied on circulating fatty acid rather than *de novo* synthesis.

Recent *in vitro* and morphologic data (4, 5), however, strongly suggests that circulating glycerol could be used for the