Pulmonary Surfactant: Evolution of Functional Concepts

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The first and clear indication that surface tension offers resistance to the initial lung aeration after birth came with von Neergaard's study in 1929 [26]. He found that lungs that were collapsed would open up more readily if the effect of surface tension was completely nullified by using liquid rather than air as the expanding medium. Following this basic study, it took considerable time until Pattle in 1955 [19] could declare that he assumed there was an agent present in the lung that had the ability to depress surface tension to extremely low values. He extruded bubbles from the sectioned lung and found that when they were surrounded by saline, so that they could be studied under a microscope, they would quickly shrink 30 % from their initial size, when their diameter was around 40 μ, but then the bubbles would persist for long periods of time (Fig. 1). Pattle reasoned that the high surface tension of water, 72 mN/m, ought to give an enormously high pressure in the tiny bubble, so that the gas inside the bubble should have been absorbed quickly by the surrounding saline solution. When this did not happen, but the bubble persisted, Pattle concluded that there must be a lining layer exerting surface pressure, almost completely counteracting the collapsing effect of water surface tension. The net surface tension according to Pattle must be very close to 0.

The Wilhelmy balance gave an exact account of how surface tension varies with surface area. It was noted that surface tension was very much reduced as the surface area was compressed to 20 % of its original size. The surface tension-area loop showed considerable hysteresis, which was felt to be a main reason for the one observed when the lungs' pressure-volume loop was studied. Clements pointed out that the change in surface tension with area would offer the lung stability [4]. The law of Laplace makes it clear that if there is no change in the value of surface tension, an alveolus would have to be surrounded by a great negative pressure when it reaches minimal size at end of expiration. With the Wilhelmy balance, Clements had demonstrated, however, that surface tension diminishes with area, a phenomenon that would protect the small alveoli from collapsing during expiration [4]. The concept that surfactant protects the small alveoli from becoming smaller or collapsing gained general acceptance. With this principle it was possible to explain that with a surfactant deficiency and the Respiratory Distress Syndrome (RDS) has developed, there are large areas of atelectasis and the alveoli remaining open tend to become overexpanded. It is also conceivable, however, that the areas of atelectasis developed already when the lungs initially became aerated. From the law of Laplace ($\Delta P = 2 \gamma/R$) it is clear that the minisci forming in the

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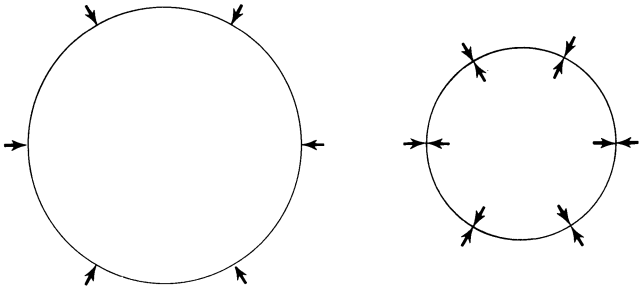

Fig. 1. Pattle (1955) noted that bubbles, extruded from the cut surface of a lung, would quickly shrink from their original size *(left)* to a smaller size *(right)* that they would maintain for a long time. He concluded that surface tension of the surrounding water would raise pressure in the bubble, but an inner layer of surfactant would offer high surface pressure when compressed to a small surface area and would counteract the shrinking effect on the surrounding water *(right)*

airways during the crucial first breath will move in the direction of the alveoli, only if the pressure difference (Δ P) is more than 2 γ/R. The premature infant with a surfactant deficiency will have a high value of γ and the value of R is likely to be small, since the airways are underdeveloped. For aeration to occur, the pressure difference created by the neonate itself or the attending neonatologist, needs to be extensive and it is probable that minisci in narrow airways will be halted and, instead, expanded alveoli and airways will become overexpanded. With such an excessive dilatation, there will be an increased leakage of serum proteins which will inhibit the surfactant and exacerbate the deficiency. Eventually, fibrinogen among the invading proteins will be converted to fibrin and form the hyaline membranes. The first notion that RDS was due to surfactant deficiency came with a publication by Avery and Mead, 1959 [2]. A report by Adams et al. offered further support [1]. They found that the lung lavage of infants succumbing from RDS had less of the phospholipids that are essential components of pulmonary surfactant. It was easy to accept that RDS could be caused by a surfactant deficiency, and since it is well known that the main component of pulmonary surfactant is dipalmitoylphosphatidylcholine (DPPC) it was felt that it might perhaps be possible to alleviate the infants' problems by supplying only this main surfactant component, DPPC. Important reasons for utilizing DPPC only were the commercial availability of the phospholipid and the fact that when its physical properties were studied with the Wilhelmy balance, they were found to be very similar to those of the whole pulmonary surfactant [3]. Nevertheless, early attempts to cure neonatal RDS by instilling DPPC into the trachea with the hope that this would replace the missing surfactant were very disappointing [3, 22, 24].

When DPPC was studied with the bubble surfactometer [7], it did not seem the least surprising that the expected beneficial effect had been missing during the clinical trials. The surface properties of the phospholipid alone were very different from those of natural surfactant. The dominating difference was that DPPC, when suspended alone, required a very long time to form a surface

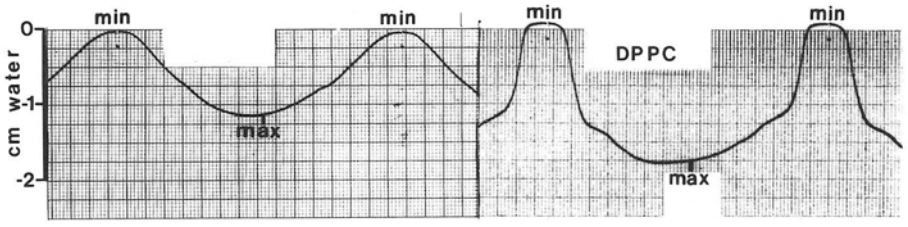

Fig. 2. Natural surfactant, or in this case, calf lung surfactant extract *(CLSE),* has a tracing characterized by a Δ P of zero at minimal bubble size *(min)* and of ~ 1 cm H_2O at maximal size *(max).* For DPPC, the Δ P at maximal bubble size is much greater, i.e., surface tension is correspondingly grater

film, i.e., adsorption rate was extremely low. Another difference was the greater change in the value of Δ P, noted with DPPC (Fig. 2). Once a film had formed, the value of Δ P, at minimal bubble size was 0, just as it would be with natural surfactant, but at maximal bubble size, the value of Δ P was much greater when the film consisted of DPPC only than when it also contained other components of natural surfactant. Perhaps those other components move into the film as it expands. When studied with a pulsating bubble surfactometer the properties of natural surfactant, obtained from lung lavage of adult rabbits, proved to be such that one could say: If they were present when the neonate takes its first breath, the aeration should be facilitated, the expansion should become even and remain stable. That was the reason Robertson and I studied the effect of instilling natural surfactant into the airways of premature rabbit neonates [8]. Later, we learned that Rüfer [23] had been working successfully on the same concept: supply the missing surfactant directly into the airways. The results obtained with our study were very promising [8]. The lung expansion was greater and improved. This was seen with pressure-volume loops of the lungs, with radiology, and with histology [8, 9]. In later studies we found that gas exchange improved, as did survival rate [10, 27]. We could not apply the principle clinically since the surfactant we used was very crude and inadequate for human use. While we were trying to develop an adequate preparation, Fujiwara et al. in Japan beat us to it by publishing how they had successfully treated ten infants with severe RDS by instilling surfactant into the airways [12]. Eventually, a surfactant preparation was prepared from the lung lavage of calves. The lipids were extracted and resuspended in 100 mM NaCl and 0.5 mM $CaCl_2$. This yielded an active preparation that tolerated autoclaving without loss of surface activity [17]. This preparation, called CLSE (i.e. calf lung surfactant extract) has been used successfully (Fig. 3) to prevent the development of RDS in preterm infants [10, 15, 25]. In several institutions, it is now used prophylactically for all infants born at a gestational age of less than 33 weeks. Since this routine was introduced, the neonatal mortality at Children's Hospital of Buffalo has been dramatically reduced (Fig. 4).

The principle of surfactant supplementation to prevent or treat neonatal RDS is becoming generally accepted [11, 13–16, 18, 21, 25]. However, several unanswered questions remain: Which preparation is optimal? Is a very

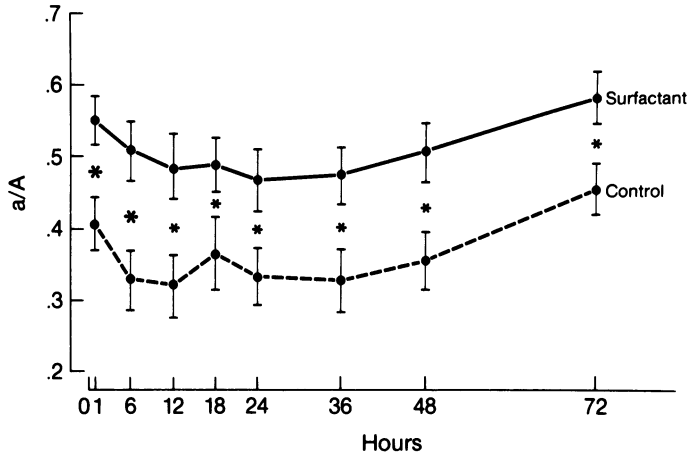

Fig. 3. In a randomized clinical trial [11] it was shown that calf lung surfactant extract (CLSE) can be used successfully to prevent neonatal RDS. The ratio of $_2$ in artery and alveolus is significantly higher among the infants treated with surfactant (CLSE) prior to the first breath. *Large asterisk* indicates $P < 0.005$; *small asterisk* indicates $P < 0.05$. (From Enhorning et al. [11])

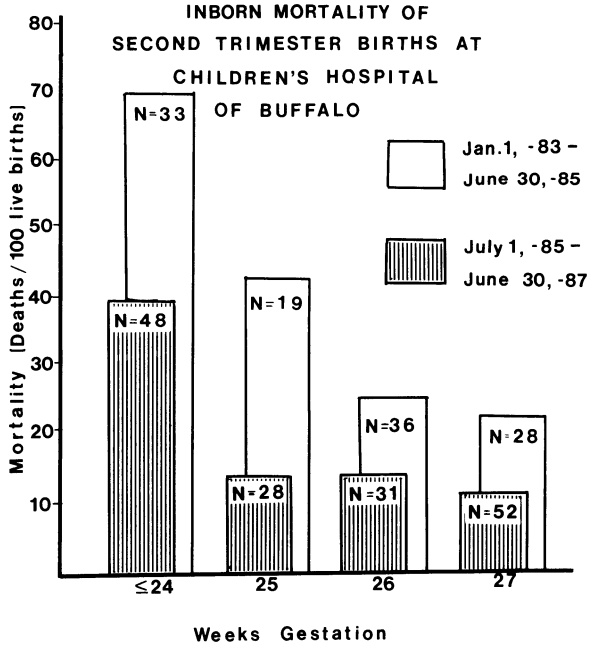

Fig. 4. Prophylactic surfactant treatment was introduced in Buffalo in 1985. In extremely prematurely born infants, mortality has been dramatically reduced. (Data kindly supplied by E. A. Egan)

hydrophobic, low molecular weight protein (5,000–18,000 daltons) the only one needed? Which phospholipids are needed and which neutral lipids have to be present? Should the surfactant of necessity be able to form myelin figures? Should the surfactant be given to prevent RDS, or to treat the manifest condition? Are there any toxic side effects? If used to prevent RDS, should it be given prior to the first breath and can it be given into the pharynx? Some of these questions will be discussed in this book.

Neonatal RDS is a fairly simple condition of surfactant deficiency that well lends itself to treatment by supplementation. If the infant is born before alveolar cells type II have synthesized and released an adequate amount of surfactant, then the principle is to give the infant that which is missing by instilling surfactant into the upper airways, prior to the first breath. The infant's lung maturity is thereby upgraded instantaneously and the first breath can be taken without the difficulty characterizing a surfactant deficiency, and the secondary changes, that of injury to the lungs, can be avoided. Lung injury may allow proteins to leak into the airways and those proteins may further inhibit the surfactant, which was already inadequate. A vicious circle may thus be started, which could be prevented if the surfactant is supplied at an early stage, preferably prior to the first breath. The question then arises: Are there pathological conditions other than neonatal RDS that might be due to a surfactant deficiency, albeit not as pure as in the neonatal period?

Adult RDS

Adult RDS might be such a condition when, for 'various reasons' proteins have invaded the airways and inhibit the surfactant action. Alveoli may then collapse so that the exchange of blood gases is disrupted, allowing hypoxia and acidosis to develop. A vicious circle has started that often will end with death. If surfactant treatment is to be considered, it probably would have to be preceded by a cleansing lavage of the airways with saline, perhaps in one lobe at a time, followed by instillation of a massive quantity of surfactant (see chapter on ARDS).

Pulmonary Edema

Pattle was the first to suggest that pulmonary surfactant is needed to prevent the development of pulmonary edema [20] and Clements published an article on this subject [5]. The line of thought was that if surface tension was excessive, a greater negative pressure would be required in the space surrounding the air-liquid interface of the alveolus and liquid could thereby be sucked into that space. There is probably a surfactant deficiency in most cases of pulmonary edema, but it would be difficult to prove that the deficiency caused the edema and not vice versa.

Edema develops when the hydrostatic pressure in the capillaries exceeds the transcapillary colloidal osmotic pressure. Normally, the hydrostatic capillary

pressure is far from being in excess and the direction of flow is from alveoli to capillaries. Primarily a change in surface tension will not have an effect on this direction of flow. If surface tension increases, so that a greater negative pressure is required on the convex side of the interface, inspiratory muscles would have to supply that pressure change. A fall in the total intrathoracic pressure will have no influence on the difference in pressure between capillary and alveolar hypophase but it might result in a dilatation of pulmonary capillaries. This, in turn, could lead to a less obstructed passage of proteins and alter the transcapillary colloid osmotic pressure, allowing edema to develop. The proteins invading the alveolar space might also inhibit the surface activity of phospholipids at the air-liquid interface.

Pneumonia

Pneumonia, caused by virus or bacteria, might also be a condition of surfactant deficiency. Not because the total amount of phospholipids is subnormal, but because phospholipases have interfered with their action. For instance, an excess of lysophosphatidylcholine, that acts as a detergent, may have formed. Furthermore, there may be an excess of inhibiting proteins. Therapy might include an attempt to correct the surfactant deficiency as for Adult RDS.

Asthma

When discussing the effect surfactant might have on the etiology of asthma I am truly out on thin ice. One might start with a generally accepted concept. When surfactant is deficient so that surface tension remains at a fairly high value during compression of the surface area, i.e., at end of expiration, small alveoli are considered to be at risk of collapsing and emptying their air into larger alveoli (Fig. 5). I am not denying the existence of that risk, but judged from a review of the lungs' structure and dimensions, the risk of collapse would seem greater in small cylindrical airways. The law of Laplace is $\Delta P = 2\gamma/R$ and $\Delta P = \gamma/R$ for spherical and cylindrical surfaces, respectively. That means that if surface tension (γ) is the same at the two surfaces the pressure difference (ΔP) required to counteract collapse would have to be greater in the cylinder than in the sphere if the radius of the cylinder (R_c) is less than half that in the sphere (R_s). Very often the radius of the cylinder is less than half that in the sphere and, furthermore, it is quite conceivable that during expiration the surface film will be more compressed, reaching a lower value in the alveolus than in the small cylindrical airway. Collapse of the latter, resulting in air trapping, is therefore threatening and has been observed with photography when there was a deficiency of surfactant, and it disappeared when the missing surfactant had been replaced [6].

If we assume that the patient with asthma has a surfactant deficiency leading to air trapping, how can that cause an airflow obstruction which is dominating during expiration? During normal breathing, the airflow is due to

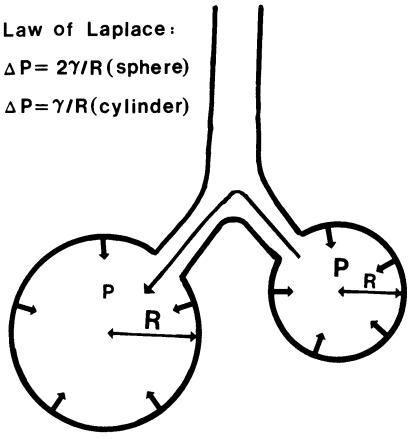

Fig. 5. According to a widely accepted concept, one risk of surfactant deficiency is that the small alveolus will build up a larger pressure than the large alveolus and empty into the latter. This will be prevented, though, if there is a surfactant film building up a high surface pressure which counteracts the surface tension of water. However, according to the law of Laplace, the small cylindrical airway is at greater risk of collapsing if its radius is less than half the size of the radius in the spherical alveolus

the difference in pressure between alveoli and atmosphere. Resistance to that flow is mainly in the larger airways that have cartilage rings preventing them from changing their width when transmural pressure is altered. Since the main resistance is in the larger airways, only a moderate pressure difference will build up across the walls of alveoli and small airways, even when pleural pressure undergoes considerable changes such as during vigorous breathing. If, however, there is collapse of the smallest cylindrical airways due to a surfactant deficiency with air trapping in the alveoli, then the main resistance will shift from airways with cartilage rings to the smallest cylindrical airways, now filled

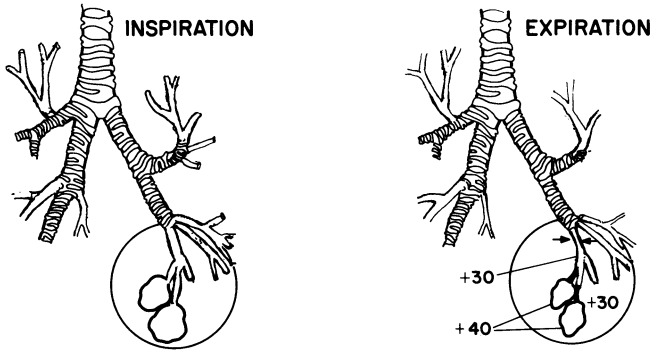

Fig. 6. The main resistance to airflow is normally in larger airways with cartilage rings. The more distant airways are thereby protected from developing a transmural pressure that is dilating during inspiration and collapsing during expiration. Normally, during expiration, pressure is higher in the alveoli than in the surrounding parenchyma due to alveolar recoil. During vigorous expiration the pressures may be 40 and 30 cm water, respectively. Pressure in airways, too small to have cartilage rings, is also higher than in the surrounding parenchyma. With a surfactant deficiency, causing collapse of small cylindrical airways, the main resistance has shifted to those airways from the more centrally located. Pressure in the larger airways, without cartilage rings, may then fall to that of surrounding parenchyma and the airway will be compressed

with fluid. Under these circumstances, a greater pressure difference will develop across the walls of larger airways, but since they do not have cartilage rings that will prevent them from changing their width, they will dilate during inspiration and become compressed during expiration (Fig. 6). They will thereby offer considerable resistance to expiration and perhaps cause the wheezing of asthma. When salbutamol, terbutaline, or other β-adrenergic agonists are used during an asthma attack, these drugs are said to relax smooth muscles of the bronchi and thereby dilate them. It is well known, however, that the drugs will also cause a release of surfactant into the alveolar space. Perhaps such a release will prevent the previous collapsing of the smallest cylindrical airways. Air trapping would no longer occur, and probably there would be not further need for the asthma patient to exert so excessive an effort during expiration. If a surfactant deficiency is indeed causing an asthma attack in the way described above, the surfactant deficiency should obviously be prevented. Perhaps this could be achieved by continuously supplying surfactant to the airways or by stimulating synthesis of surfactant, or inhibiting its catabolism.

Summary

Pulmonary surfactant is needed for normal lung function, and its role is becoming increasingly clearer. In the lungs of preterm infants there may be a lack of surfactant, because release from alveolar cells type II was still inadequate; the infant may then develop RDS. This can be avoided, however, if the missing surfactant is instilled into the airways, optimally prior to the first breath, before the lungs have been damaged.

Later in life there are probably other conditions of surfactant deficiency that may affect the child or the adult. These conditions are less straightforward, since other injurious and complicating factors may be involved. Adult RDS, pulmonary edema, pneumonia, and asthma have been discussed.

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