

Breakthroughs in Bioscience

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BABIES, BUBBLES, AND BIOLOGY: THE STORY OF SURFACTANT

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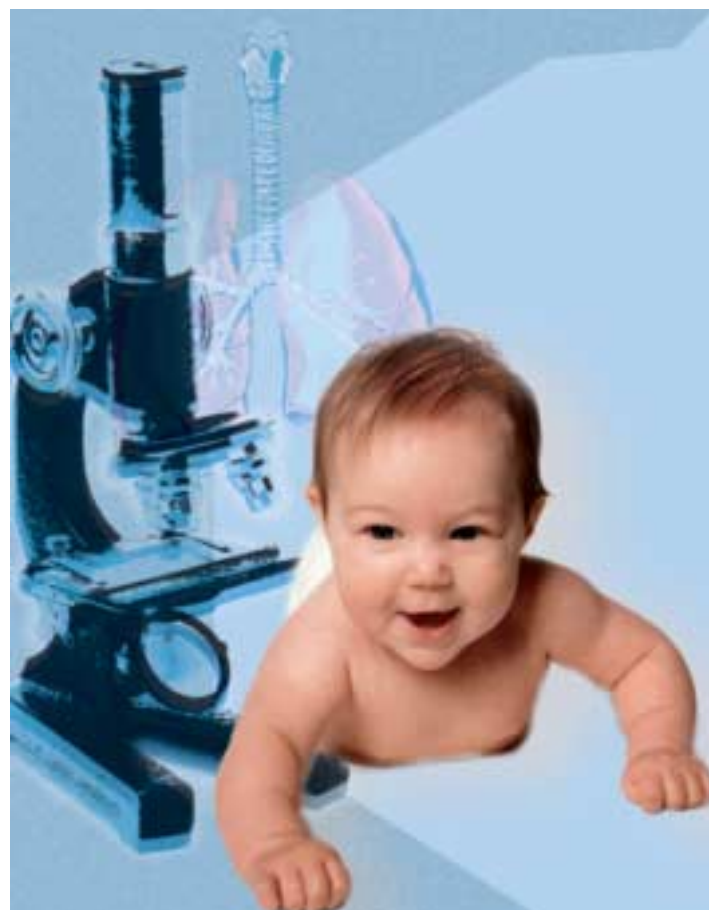
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COVER IMAGE: The discovery of surfactant began a basic and clinical research partnership that resulted in a dramatic decline in deaths of premature infants from respiratory distress syndrome. The story of surfactant is an excellent example of a problem identified in patients, elucidated in the lab through cooperation of physicians and scientists, and then brought back to the bedside for successful treatment. *Graphic design by Corporate Press.*

SURFACTANT

Bubbles, Babies and Biology: The Story of Surfactant

Second Breath: A medical mystery solved

Before scientists and clinicians, working together, discovered the existence of lung surfactant and then figured out how to overcome its absence in the lungs of premature infants, more than 10,000 newborns in the United States died each year struggling for breath. No one understood why. Another 15,000 were affected by the same disease each year but recovered, as mysteriously as the others had died. In the 1950s and 1960s, this respiratory disease, misleadingly named hyaline membrane disease, was the nation's most com-

mon cause of infant death (Figure 1). Its most visible victim was the infant son of President John and Jacquelyn Kennedy, Patrick Bouvier Kennedy, who died in August 1963, two days after he was born five and a half weeks prematurely.

As a pediatric resident at Johns Hopkins in the mid-1950s, Dr. Mary Ellen Avery had watched many newborn premature babies go through the same struggle for breath, turning blue as they strained to breathe in, making strange little grunting noises as they breathed out. If they died,

they usually did so within the first three or four days. But if they made it through those first days, the sickness appeared to vanish, as suddenly as newborns recovered from jaundice once their immature livers finally kicked in.

Most physicians at the time believed that culprits in these small babies' death were the hyaline membranes found in their lungs at autopsy. Wherever these glassy membranes came from – some speculated they were formed when babies breathed in amniotic fluid or milk – the supposition that the membranes themselves impeded breathing had given the disease its name. Dr. Avery didn't believe this. A few pathologists were beginning to point out that hyaline membranes contained fibrinogen, a protein found in the blood, which meant they originated from within the baby's body, not from the outside. Furthermore, the only babies who had them were those who had taken at least a few breaths, never stillborns, suggesting the membranes were the result of lung injury, not its cause.

Dr. Avery was more interested in the fact that babies who died of hyaline membrane disease, unlike babies who died of other causes, had no residual air in their lungs



Figure 1: Nurses with premature infant c. 1950-60. In the 1950s and -60s, very little could be done to aid premature infants who struggled to breathe. *From the National Library of Medicine, permission courtesy of the American College of Nurse-Midwives.*

at autopsy. As the babies struggled for breath over their short lives, their lungs appeared to be unable to retain air. But why? Solving this conundrum would give physicians like her badly needed clues as to how to treat, perhaps even prevent, this mysterious disease.

That's what happened. In 1959, building on a discovery by physiologist John Clements, who had himself built on years of basic scientific research, Dr. Avery and her colleague, Dr. Jere Mead, described the mechanism underlying the failure of these premature babies' lungs to expand and to retain air. Their paper in the *American Journal of Diseases of Childhood* turned the understanding of hyaline membrane disease on its head.

Hyaline membrane disease was not caused by the *presence* of something in the lungs but rather by the *absence* of something. The lungs of babies who died of hyaline membrane disease lacked a substance called surfactant, which lines the alveoli, the small air sacs at the end of the lungs' numerous, branching airways (Figure 2). The problem did not lie only with *breathing in*, as had long been assumed, but also with *breathing out*. The baby took that first breath, perhaps even a good deep breath, as any baby would. But if the newborn baby's immature lungs lacked surfactant, the alveoli tended to collapse when the baby breathed out. This meant breathing in required extra effort, as if every breath was like the first breath after birth. Not

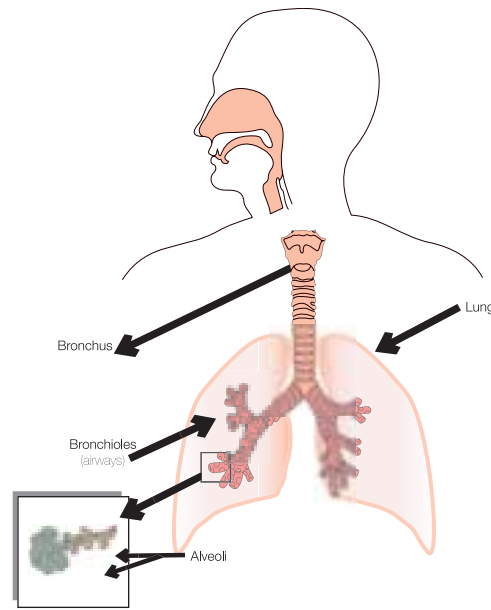


Figure 2: Anatomy of the lungs. The lungs consist of highly branched airways, or bronchial tubes, sometimes called bronchi, ending in airsacs, or alveoli (singular = alveolus). It is in the alveoli that gas exchange takes place, where oxygen enters the blood stream and carbon dioxide is removed. Surfactant is critical for keeping the alveoli inflated, which is necessary for gas exchange to take place. *Designed by Corporate Press.*

only did this extra effort tire out the newborn's diaphragm, the repeated extra force also tore the lung tissues and led to inflammation. Understanding this mechanism explained why the disease primarily affected premature babies whose lungs were too immature to produce enough surfactant. It explained why babies who survived a few days, long enough for their lungs to begin producing surfactant, often recovered completely.

This new knowledge turned current treatment on its head. For example, when doctors thought the problem of the disease was something causing resistance to breathing, it made sense to use mechanical respirators that applied pressure only at inspiration, when the baby breathed in. When it became clear that the problem also involved retaining air, mechanical respirators were

changed to provide positive pressure in the alveoli at the end of expiration, as well, when the baby breathed out.

In addition, understanding the cause of the so-called hyaline membrane disease pointed the way to two new treatments: steroid injections for pregnant women to encourage a fetus at risk for premature birth to speed up the production of natural surfactant, and development of surfactant products that could be placed in the lungs of those babies born before they were able to produce this substance on their own. The clear evidence of a previously unsuspected disease mechanism promised new hope for saving thousands of infants a year in the United States alone. There is little wonder that recognition of the importance of this discovery was immediate.

What have bubbles got to do with lungs?

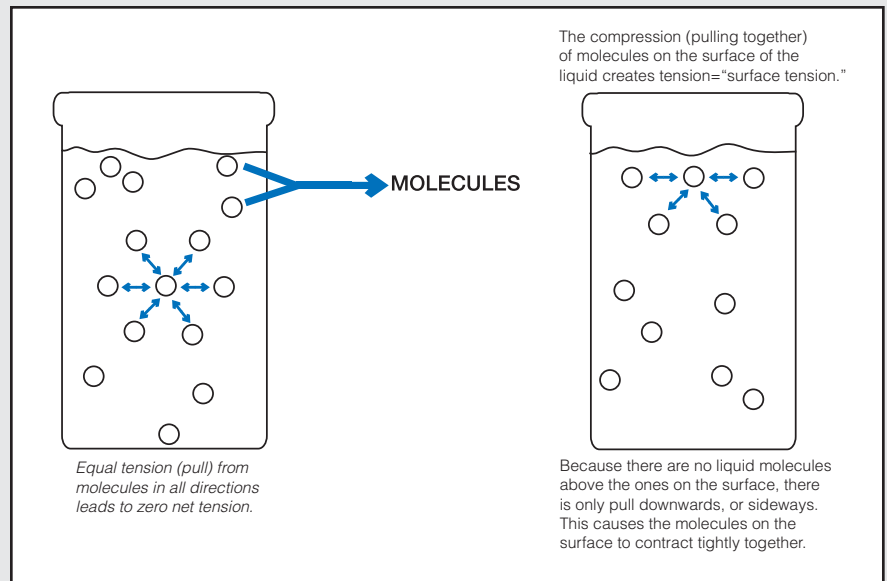
What is surface tension?

It's the virtual "membrane" that occurs at any boundary between gas and liquid. The membrane is easy to envision around the slightly concave surface of a glass of water or the drops forming from a leaky kitchen spigot, each drop rounding to the exact shape and exact size, as if it were held in an elastic skin. It's also the explanation of why insects can walk on water.

What causes it?

Molecules like to hold onto each other. In the liquid in the middle of a glass of water, the forces exerted on molecules

of water by all other molecules average the same in all directions. But at the upper layer of liquid, at the boundary between gas and liquid, the water molecules below the layer exert a stronger pull than do the gas molecules above the layer. As a result, the molecules in the upper layer of the water tend to leave the surface for the bulk, and this tendency makes the surface shrink to the smallest permitted area.

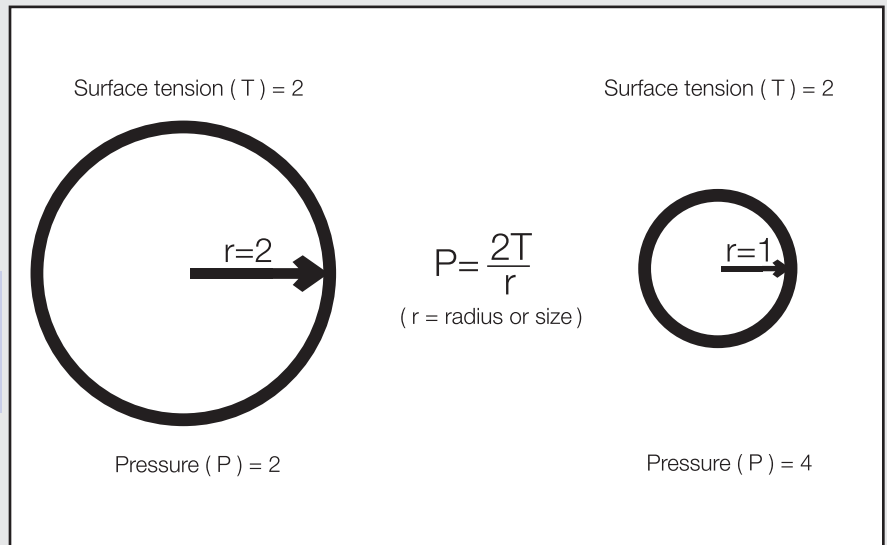


What does size have to do with surface tension?

In 1805, Thomas Young, an English physician, and in 1806, Pierre Simon Laplace, a French mathematician, physicist and astronomer, independently derived an equation still known as the **Young-Laplace Law**.

$$\text{Pressure} = \frac{2 \times \text{surface tension}}{\text{radius of the surface}}$$

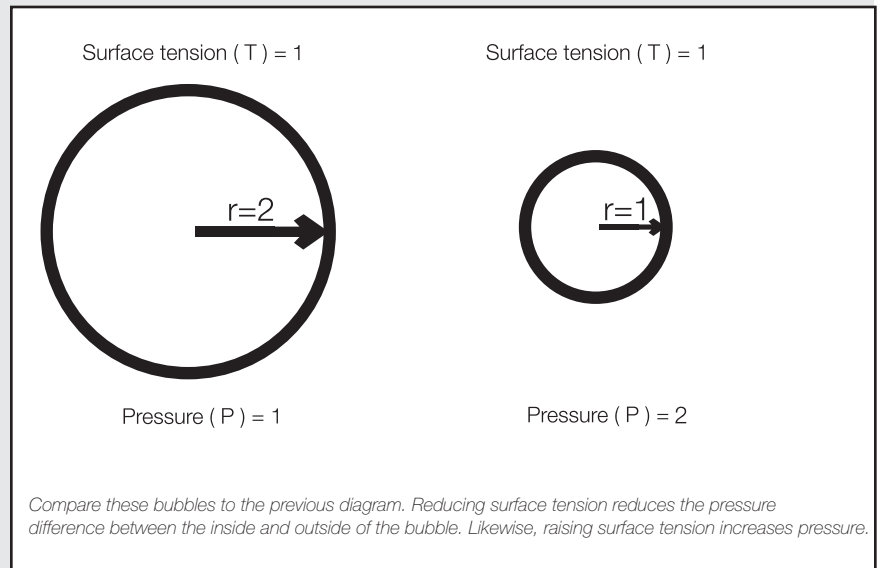
Pressure, in this case, refers to the difference in the pressure inside a liquid droplet and the pressure outside the droplet. This pressure difference is dependent upon two factors: size of the droplet and surface tension. In other words as seen in the diagram to the right, the smaller the droplet, the higher the pressure.



A surface tension primer.

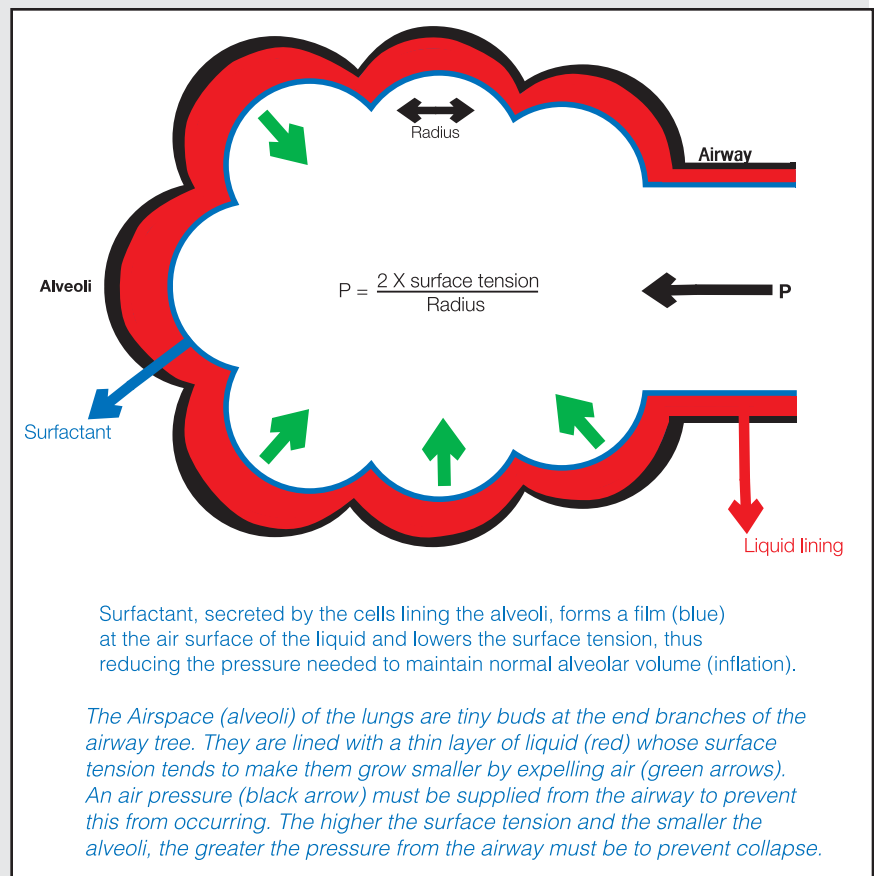
Why do soap bubbles last longer and why do they burst?

Because the soap in the water solution reduces the surface tension, and a relationship similar to the Young-Laplace law applies to bubbles. The reduction in surface tension by the soap reduces the pressure difference between the inside and outside of the bubble, keeping it in equilibrium, at least until it begins to dry and the water film gets thin enough to break. If the surface tension was high, the pressure difference between the inside and outside of the bubble would be so great the bubble would be unable to maintain its spherical shape and it would collapse.



And why is this important in terms of lungs?

Because of what they tell us about the behavior of the alveoli, themselves like small bubbles surrounded by wet tissue. The Young-Laplace Law means that if the alveoli were subject to a normal pressure and the surface tension was high, they would collapse. That does not happen in normal, mature lungs, suggesting that some substance in the lungs must be reducing surface tension, as the soap does to the surface of the bubble. That is what John Clements' studies of surfactant showed. But this collapse does happen in newborns with lungs too premature to produce the surface tension reducing substance, as Mary Ellen Avery and Jere Mead suggested.



As findings with a clear medical implication often do, the Avery and Mead paper turned the spotlight on a small group of basic scientists, separated by time, geography and discipline, whose research on lung physiology had largely been known only to each other. Without their work, this important clinical discovery would have been impossible. These scientists were going against the view, widely held in the 1950s, of the lungs as being little more than bellows, or at least mere bags for gas exchange. The elasticity of lung tissue was assumed adequate to explain the lung's unique ability to expand and contract. No one outside of this small confederation of scientists credited the lung with having an active metabolic life that would include production of something like surfactant.

Dr. Avery's much admired colleague at Johns Hopkins University, pathologist Peter Gruenwald, was one of the rare scientists in this group. So was her co-author on the 1959 paper, Dr. Jere Mead, head of a respiratory physiology laboratory at the Harvard School of Public Health. But the scientist who actually proved that surfactant existed and precisely measured how it performed was Dr. John Clements, a physiologist then working at the United States Army Chemical Center in Edgewood, Maryland.

When Dr. Avery heard that Dr. Clements had identified surfactant, she instinctively knew it was the missing piece of the hyaline

membrane disease puzzle. During her Christmas vacation, Dr. Avery drove from Boston to Maryland to meet with Dr. Clements. "The gift I gave her," Dr. Clements later wrote, "was a demonstration of my homemade ... balance [for measuring the effect of the hitherto only suspected surfactant material] and an exposition of everything I knew about lung physiology."

The following Christmas, Drs. Avery and Mead – an old colleague of Dr. Clements – gifted him in return. Publication of Avery and Mead's widely heralded article abruptly ended what Dr. Clements has called the "monastic era" of lung surface tension and surfactant research. No longer were he and other scientists working in the shadows, their research of interest only to students of lung mechanics. What had seemed theoretical, esoteric research – perhaps even useless research – now had been shown by Drs. Avery and Mead to have immediate, powerful clinical applications.

Surfactant research became respectable, with an influx of grant money, especially from the rapidly growing National Heart, Lung and Blood Institute at the National Institutes of Health (NIH), as well as charities like the March of Dimes. Young scientists from a wide variety of disciplines flocked to the field, and publications increased exponentially. Dr. Clements' seminal paper, initially rejected by the premiere journal *Science* and vir-

tually ignored at the time of its publication in a less well-known journal, quickly became one of the most widely cited papers in the medical literature.

But what, exactly, had he discovered and how?

The Winding Road: Understanding the role of surface tension

To get a sense of the reason surface tension is important in lung function, it would help to spend a few minutes following Mary Ellen Avery over the early months of 1957 as she completed her pediatric residency at Johns Hopkins and moved to Boston. She was on a special two-year fellowship from the NIH, to join Dr. Jere Mead's laboratory at the Harvard School of Public Health. Her goal was to gain the background in pulmonary physiology to help her solve the mystery of hyaline membrane disease.

During the day, she studied respiratory physiology with Dr. Mead, who was researching lung mechanics. In the early mornings and evenings, she crossed the street from Harvard to the Boston Lying-In Hospital and observed newborns with Dr. Clement Smith, who was taking precise measurements of their respiration. At night, having been asked by Dr. Mead to help him understand more about bubbles that formed in the lungs during the pulmonary edema caused by poison gases, she went to the Massachusetts Institute of Technology library to

check out books unavailable in the medical libraries, on surface tension. Amusingly, this included a nineteenth century children's book called, "Soap Bubbles, Their Colours and the Forces Which Mould Them." Dr. Avery has said that this clear explanation of surface tension, along with its kitchen sink experiments aimed at young students, was invaluable as she tried to master this difficult new concept. By the time she first heard of Dr. Clements' surfactant finding, she had been through a crash course in surface tension that allowed her to appreciate it.

Surface tension is the virtual "membrane" that occurs at any boundary between air and liquid, such as the slightly concave surface of a glass of water or the watery film of a bubble. (See - "What do bubbles have to do with lungs?") By the beginning of the 19th century, Thomas Young an English physicist / physician, and Marquis Pierre



Simon de Laplace, a French mathematician, had independently worked out the equation that describes the relationship between the radius of this curved surface and the pressure necessary to maintain the curve (Figure 3). The Young-Laplace Law was quickly embraced by engineers, while biological scientists were slower, and far fewer, to appreciate its application to the body.

But the Young-Laplace Law has a direct implication for what happens in the bubble-like alveoli, where the moist lung tissue meets air during breathing. Because the liquid molecules on the outside of the alveoli exert a stronger pull on each other than they do on the air molecules which fill up the center of the alveoli, this should – according to Young and Laplace – create a high surface tension whenever the alveoli are filled with air, as they are after each breath. Under these circumstances (alveoli filled with air, surface



Figure 3: Young and Laplace. Thomas Young (1773-1829, left) and the Marquis Pierre Simon de Laplace (1749-1827, right) independently developed the formula used to describe the relationship between the pressure gradient across a liquid film sphere (such as a bubble) and the tension in the film membrane (surface tension). *Young photo courtesy of the National Library of Medicine; Laplace portrait by Jean-Loup Charmet/ Science Photo Library.*

tension high), the outside of the alveoli would put so much pressure on the inside of the alveoli that the alveoli should collapse. Since that does not happen in normal lungs, some substance in the lungs must be reducing surface tension. In the 125 years since Young and Laplace formulated this law, a handful of scientists, working in isolation, had come tantalizingly close to recognizing there had to be a tension reducing substance in the lungs – and that the absence of this substance would explain why the lungs of premature babies collapsed.

First was Dr. Kurt von Neergaard, a Swiss physiologist well educated in physics, whose classic study in 1929 showed that more pressure was required to inflate lungs with air than with aqueous solutions like water. Using the Young-Laplace Law, he argued that surface tension at the boundary of the moist tissue of the lung and the air was the reason for the difference in pressure needed for the lungs to expand. Otherwise, the lungs would require high pressures to inflate. Indeed, when he measured the surface tension of lung extracts – the first scientist to do so – he found it was indeed lower than that of serum and extracts of several other tissues. Von Neergaard suggested that some other researcher should investigate whether surface tension was a force impeding the first breath of the newly born. But he himself did no more work on lung mechanics, and his insights led nowhere.

In 1947, long before computers much less the ability to search the Internet for previous research in databases like Medline, Dr. Avery's colleague, Dr. Peter Gruenwald, appeared to have no knowledge of von Neergaard's work. Investigating the lungs of newborns who had died of various causes, he independently repeated Dr. von Neergaard's elegant experiments. As had been true for von Neergaard, Dr. Gruenwald found more pressure was required to fill lungs with air than with saline (salt water) and attributed this to the laws of surface tension. Then he went a step further, adding an agent to the lungs which lowered surface tension. Again, as with Dr. von Neergaard, no one followed through, perhaps because of the continuing conviction that hyaline membranes deserved all the research focus. Without easy access to the work of respiratory physiologists and other scientific colleagues, and with no clear direction as to how to make his ideas clinically applicable, Dr. Gruenwald was unable to take his inquiry much further.

Then in the early 1950s, as so often happens in science, a flurry of targeted research – aimed at understanding, treating and preventing the disastrous effects of war gases on lung tissue - began to produce results which would serve seemingly unrelated purposes. The chemical warfare laboratories of England and the United States supported virtually all of the mid-century pioneers

along the road to the discovery of surfactant, including Dr. Mead in Boston and Dr. Clements in Maryland. A volunteer for military service during the Korean War, Dr. Clements himself had had no particular interest in the lungs until he was assigned the task of figuring out how nerve gases affected them. With the war over and funding still in place, discoveries related to lung mechanics came more and more rapidly, each enhancing the next. Rather than focusing on the entire lung, or on lung extracts, some of these militarily funded scientists focused on bubbles. This was because bubbles appeared in the airways of the lungs when a person was exposed to certain poison gases.

Dr. Richard Pattle, a physicist-physiologist working full time for the British Chemical Defence Experimental Establishment in England, had gained a reputation as a bubble expert because of his ability to prevent bubbles from forming in cultures to which air had been added. When he was asked to help a fellow researcher dispel the foam that welled into the airways of goats experiencing pulmonary edema, he assumed he could. He began working with rabbits, a smaller animal model, to see which of several known antifoams would work best for this accumulation of excess watery fluid in the lungs.

These antifoams made short work of any bubbles when added to edema fluid from other parts of the body or to blood. But to Dr.

Pattle's surprise, the foam from lung airways remained stubbornly sturdy no matter what he did. He surmised that the air bubbles of foam originating in the alveoli must be covered with a unique substance from the lining layers of alveolar surface and that this material was conferring increased stability on the bubbles. "If the surface tension were that of an ordinary liquid, enough suction would be exerted to fill the alveoli with a transudate from the capillaries [fluid that would move through the membrane of the capillary wall, because of an imbalance in pressure]. Means for keeping the surface tension low must therefore be part of the design of the lung," he wrote in 1955, in a brief note describing his findings in the scientific journal, *Nature*.

In a related paper in the *Proceedings of the Royal Society of London* (1958), he even noted in passing that the absence of a lung lining substance may "sometimes be one of the difficulties with which a premature baby has to contend" and may "possibly play a role in causing some cases of atelectasis neonatorum" (failure of the lungs to expand at birth). Someone, he added, needed to do research on this issue.

In 1953, Harvard's Dr. Edward Radford wondered how the effects of surface tension in lungs might help him estimate alveolar surface areas. To answer this question, he made pressure-volume measurements on lung extracts, much like von Neergaard had done. To cal-

culate area from these data, he assumed that lung surface tension was near that of serum. Radford briefly considered the possibility that the surface tension in the lungs might be lower, but rejected it based on von Neergaard's extract measurements. Even though his calculations proved wrong, it was nonetheless a valuable stop on the road to the discovery of surfactant, since it brought to the attention of other physiologists the effects of surface tension in the lungs. Dr. Radford's discussions of these results with Dr. Clements stimulated the latter's interest in this question.

Unlike some of the scientists before him, Dr. Clements had little training in mathematics or physics (Figure 4). A friend had taught him the rudiments of calculus when they were medical students together; he taught himself physics and physical chemistry. He also benefited from open and enthusiastic exchanges of information among his fellow scientists across the country.

Expanding upon the work of the earlier researchers, Dr. Clements decided it was time to take precise, quantitative measures of surface tension in lung extracts. Most importantly, he decided not to use methods that provided a single, static value of surface tension as others had done. Instead, he used a dynamic method that would enable him to see how surface tension changed as he altered the surface area of the lung tissue. His homemade sur-

face balance was a fairly crude contraption that one medical historian described as made from sealing wax, chewing gum, string and other odds and ends. But it worked. Dr. Clements placed extracts of minced whole lungs in a shallow trough; a moveable barrier allowed him to alternatively compress and expand the surface layer while he measured the surface tension.

The results were stunning. Dr. Clements confirmed that surface tension of the tissue extracts containing the lining of the lung is low. What was new was the fact that surface tension changed as the surface layer expanded or contracted – evidence that the fluid from the lung linings contained a substance, capable of affecting surface tension, a substance he would later call *pulmonary surfactant*. When the surface layer of the lung extracts expanded, as if a person were taking a deep breath inward, the surface tension rose. In an actual working lung, the higher surface tension would keep the lung from over-expanding and help it return to its normal size. But when the surface layer contracted and compressed, as would happen when a person exhaled, the surface tension fell to as little as a tenth of the higher value. Again, in a working lung, this lower surface tension would allow the alveoli to stay open at normal pressure – instead of failing to expand, a condition called atelectasis. That's why Dr. Clements first referred to lung surfactant as the "anti-atelectasis factor." It was



Figure 4: Dr. John A. Clements and Dr. Mary Ellen Avery. Dr. Clements, professor of pediatrics, University of California, San Francisco, is credited with the discovery of surfactant, a basic research breakthrough in the treatment of neonatal respiratory distress. Dr. Mary Ellen Avery, professor of pediatrics, Harvard Medical School, discovered how to apply Dr. Clements' discovery for treating premature infants suffering from RDS. Photos courtesy of Dr. John Clements and Dr. Mary Ellen Avery.

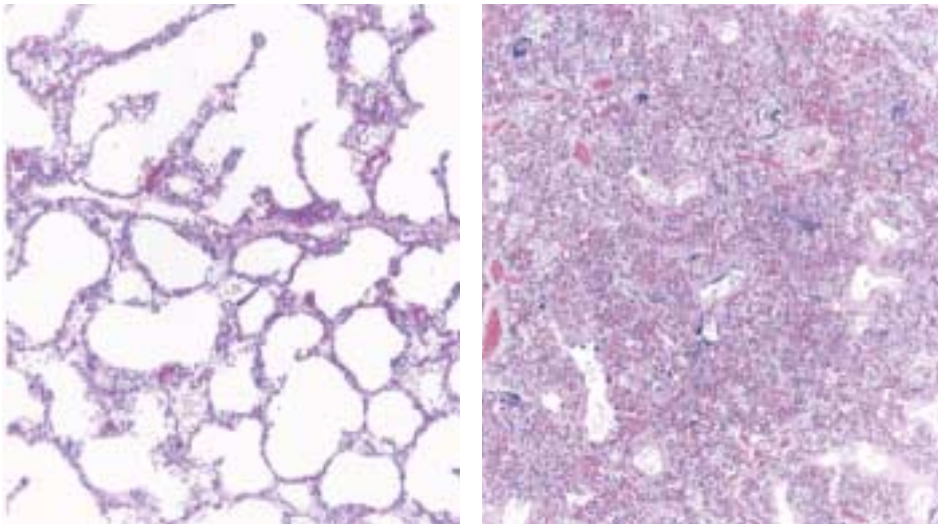


Figure 5: Photomicrograph of normal alveoli compared to an infant who died of RDS. The image to the left shows the normal microscopic structure of the lung of a newborn infant. The clear areas that make up the majority of the image are the air-containing expanded alveoli. The colored structures that form a honeycomb lattice are the walls that line the alveolar space. The alveolar walls contain tiny blood vessels that absorb oxygen from the inspired air and release carbon dioxide into the air to be expired. The image on the right shows the microscopic structure of the lung from a premature infant who died from RDS. The normal honeycomb lattice is collapsed (atelectasis), the alveolar walls are adherent to each other, and the lung is almost airless. Those air-containing spaces (clear areas) that do remain are lined by a pink-staining layer of inflammatory protein termed the Hyaline Membrane. *Photos courtesy of Dr. Richard Lynch.*

surfactant that was causing the lower surface tension during exhalation, maintaining inflation of the alveoli.

Dr. Avery (Figure 4) interpreted Dr. Clements' findings in reverse. As he explained how pulmonary surfactant allowed the lung to expand, contract, and expand again, keeping the alveoli from collapsing, she substituted in her mind what would happen if there were no pulmonary surfactant. It described precisely what happened to her baby patients who struggled for breath, only to die with airless, foamless lungs.

She returned to Boston, where she and Dr. Mead set about having their own balance made. Because she was working at the Boston Lying-In Hospital, she had rapid access to the lungs of

babies who had recently died of hyaline membrane disease (which she now thought of as *respiratory distress syndrome* or RDS).

Working quickly, before the lung cells had a chance to deteriorate, she was able to make extracts of these babies' lungs and spread them in her new balance. For comparison, she did the same with tissue from the lungs of babies, children and adults who had died of other causes.

When Dr. Avery measured surface tension in the lung extracts of those babies without RDS (normal infant lungs), she saw the same picture as had Dr. Clements. The surface layer expanded and surface tension rose. The surface layer compressed, and surface tension fell. These infants, as well as children and adults, would

have had the capability to exhale and, thanks to the presence of surfactant in their lungs, inhale again with ease.

When she measured surface tension in the lung extracts of those infants who had died of RDS, however, she found the reverse image she had expected. When the surface layer expanded, surface tension rose as in normal infants, but to much higher levels compared to babies without RDS. And, without exception, in the lungs of these babies with RDS, the surface tension remained much higher even when the surface layer was compressed (Figure 5). This would make it harder for the alveoli to re-expand for a second breath. To a somewhat lesser degree, this also occurred in the lungs of very small, very premature babies without RDS.

There could be no clearer illustration that the absence or delayed appearance of surfactant was the mechanism underlying RDS. After Avery and Mead's article was published, no one thought of this disease in the same way again.

Overcoming Surfactant Deficiency

The Kennedy baby obituaries, written in 1963, four years after the Avery-Mead article, bemoaned the fact that so little was known about treatments for this devastating disease, which was suddenly front of mind for the American public. But under-

standing the mechanism of RDS in these premature babies had given clinicians and scientists a clear vision of the points where they might attack the problem, and work was proceeding along on at least three major fronts: respiration, steroid treatment, and – the holy grail – creation of a surfactant replacement.

Ventilator therapy:

As the specialty of neonatology and the concept of neonatal intensive care emerged in the 1950s and 1960s, clinicians tried hard to help premature babies through those critical first days of respiratory distress. The obvious answer seemed to be respiratory machines that would help the distressed baby breathe.

Avery and Mead's paper answered the question as to why ventilators had been generally unsuccessful. Mechanical ventilation at the time was nonspecific, directed toward symptoms rather than the mechanisms of a specific disease. Consequently, ventilators supplied pressure only during inhalation. While lifesaving for babies with other problems, this approach did not do enough to prevent the collapse of the alveoli during expiration in babies with RDS.

In 1968, desperate to save a dying baby, Dr. George Gregory, an anesthesiologist at the University of California School of Medicine, first used a breathing aid with continuous positive airway pressure (CPAP) for treating RDS. In some ways, CPAP

worked like the missing surfactant in the babies' lungs. When pressure was maintained sufficiently as the babies breathed out, their unstable alveoli were less likely to collapse. In 1971, Dr. Gregory reported that use of CPAP reduced mortality from RDS from the 80 percent seen in the general population to 20 percent. These results were so compelling that the use of CPAP was never subjected to a randomized clinical trial.

Steroid therapy:

Since the early 1950s, scientists had known that steroids affected maturation. However, it was not until 1968 that Dr. Sue Buckingham and her colleagues, based on studies of fetal rabbits exposed to steroids, speculated that they might cause lung maturation. The following year, trying to ascertain whether glucocorticoids (a type of steroid hormone) given to pregnant ewes would hasten delivery, Dr. Graham Liggins

unintentionally discovered that steroid treatment also accelerated lung development of the lamb fetuses (Figure 6). His lambs were born a month early, ordinarily a guarantee of a quick death from respiratory distress. But lambs treated with steroids as fetuses were able to breathe better than expected.

An obstetrician, Dr. Liggins wanted to see if such steroid treatment would hasten lung maturation in human babies at risk of being born prematurely. He carried out a controlled trial in which 213 women in spontaneous premature labor were given either a steroid called betamethasone or a placebo. When steroids were administered at least 24 hours before delivery, RDS occurred in only 9 percent of babies from treated mothers compared with 25.8 percent of untreated ones. Early neonatal mortality from all causes was 3.2 percent in the treated group compared with 15 percent in the untreated. No



Figure 6: Sheep and lambs prove important in RDS research. Pregnant ewes and premature lambs served as crucial animal models in early studies of using steroid treatment to prevent RDS. Animal models often play an invaluable role on the path of discovery towards understanding and treating diseases. *Photo by Françoise Sauze / Science Photo Library.*

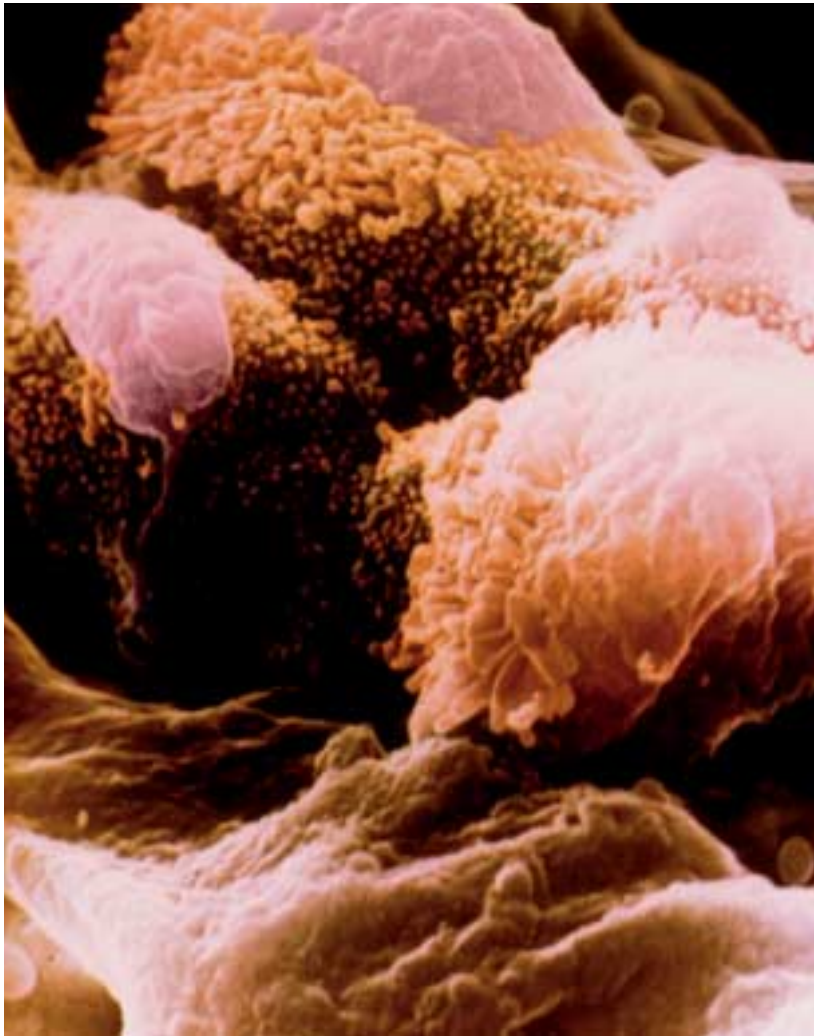


Figure 7: Lung alveolar cells. Colored Scanning Electron Micrograph (SEM) of epithelial cells lining an *alveolus* (air sac) of the human lung. At lower frame are smooth *alveolar* cells type I which cover 90% of the air sac surface, and function in gas exchange. Oxygen and carbon dioxide pass through these cells, to and from the bloodstream. At center left & top are two *alveolar* cells type II. They are covered in fine microvilli and secrete surfactant, a substance that reduces surface tension in the air sac and prevents it from collapsing. At center right is a brush cell with thick microvilli, whose function is unknown. Magnification: x5,100 at 6x4.5cm size. Photo by Prof. Arnold Brody / Science Photo Library.

babies from treated mothers died of neonatal RDS.

From the moment he first discovered the impact of steroids on fetal lambs, Dr. Liggins was eager to share his findings with laboratories better equipped than his for the necessary biochemical, biophysical and electron microscopic studies. In 1976, the National Heart, Lung and Blood Institute sponsored a multimillion dollar

trial that established the value of steroids to prevent respiratory distress in premature newborns. Nevertheless, it was not until a consensus conference on this topic, held in 1993 by NIH, that steroid treatment for RDS became widespread.

But how to determine which babies would need help? Amniocentesis (in which fluid from the sac surrounding the fetus

is withdrawn for diagnostic testing) had been around for decades when, in a series of well-designed animal studies, Dr. Liggins helped prove that amniotic fluid also contained fluid from the fetal lungs. The next step was to find measures that gave some indication of fetal lung maturation. Dr. Louis Gluck and colleagues showed that proportions of certain phospholipids (fat-like molecules) produced by the lung changed as fetal development proceeded – and that these proportions could be measured in the amniotic fluid. Although contemporary tests utilize more sophisticated analyses than those of the early 1970s, the principle remains the same, allowing clinicians to “read” the amniotic fluid to determine whether the lungs are producing enough surfactant to enable the fetus to breathe if born prematurely, or whether the mother should be given steroids.

Making Surfactant for Babies Without it.

Once scientists understood what surfactant *did*, they set about trying to understand what it was, where it came from, how it was regulated – and how it could be replicated or synthesized.

At first, some scientists remained dubious that the lung was biochemically active enough to produce surfactant, but increasingly powerful electron microscopes made it possible to actually track down and see where surfactant was made, stored and released (Figure 7). Surfactant is made in a

specific type of cell found in the epithelium (lining) of the alveoli, called type II alveolar epithelial cells. As the lungs matured, unusual lamellar or stacked structures formed within these cells. And as the lungs matured further, these lamellar bodies could be seen releasing surfactant onto the inner surface of the alveoli. Unraveling the composition of surfactant and the functions of its many components proved daunting, because surfactant turned out to be extremely complex. It was a step-by-step process that continues today, almost fifty years after its discovery.

The first finding, in the early 1960s, was that surfactant is built somewhat like a cell membrane, containing proteins and phospholipids. The most abundant component, the saturated lipid dipalmitoyl phosphatidylcholine (DPPC), stabilizes a thin film at the interface of liquid and air in the alveoli. This alveolar surface film appeared to control surface tension, stretching as the lung expanded, causing surface tension to rise, then packing in molecules more tightly as the lung contracted, lowering surface tension. Based on this information, several teams gave babies with neonatal respiratory distress aerosolized DPPC. The failure of this approach to treat RDS suggested that other components of surfactant were also necessary.

The focus turned to the four constituent proteins in natural surfactant, which had been given the highly pragmatic names SP

(for Surfactant Protein) A, B, C, and D. Knowing the job of each protein would be crucial in the effort to create surfactant replacements that would imitate the stabilizing effects of the body's own surfactant. The first proteins to be described, SP-B and SP-C, proved to be hydrophobic (they avoid water) proteins that bind to lipids. Without these proteins, the surfactant lipid DPPC could not move rapidly enough from the water-phase where it is secreted, (produced and released), to get up to the air-liquid layer in the alveolus in order to control surface tension. The absence of SP-B and SP-C was a major reason why the early trials of pure DPPC hadn't worked. Furthermore, the lack of SP-B, shown using "knock-out" mice (in which specific genes are absent, allowing scientists to see what these genes, and the proteins for which they encode, do), turned out to be sufficient to cause fatal respiratory failure in the newborn.

SP-A and SP-D were even more challenging to explain, but advances in molecular biology made it possible to determine what these proteins did by understanding how they were structured at the molecular level. Large databases had been developed by scientists around the world with information on the molecular structure and function of thousands of proteins. Once the genes for SP-A and SP-D were found and their structure determined, it was possible to use powerful computers to search through these databases and see how these proteins compared to others. SP-A

and SP-D were similar to a family of proteins called Collectins that help the immune system.

This finding seemed to suggest that surfactant played a role in stimulating immune responses in the lungs. Perhaps these proteins serve as part of the innate immune system: the first line of defense that recognizes and kills invading microbes. Although the two proteins work in different places in the lungs (and SP-D is present on epithelial surfaces – those thin layers of cells covering almost all body surfaces, internal as well as external), both may help protect against the lung infections to which premature infants are vulnerable. But this gets ahead of the surfactant replacement story. Although new discoveries about the proteins were advancing rapidly, no one wanted to wait for science to come up with the perfect formula when thousands of babies continued to die every year.

While the roles of surfactant proteins were still being teased out, Japan's Dr. Tetsuro Fujiwara created a bovine surfactant replacement that he hoped would contain all the necessary ingredients – even if they were not yet fully understood – to tide babies over until they began producing their own surfactant. He had been encouraged by the success of Drs. Goran Enhörning and Bengt Robertson who had instilled surfactant from adult rabbits into the trachea of immature rabbits. After animal studies of his own, he washed out material from cows' lungs and added surface-active phospholipids. The mixture would

be delivered as a liquid, into the lungs of babies with RDS, via a tube placed directly into the windpipe, or trachea (intratracheal injection). The ten infants who received the surfactant replacement therapy in 1980 did well, stimulating Dr. Fujiwara's group and others to begin prospective, controlled, clinical trials. Dr. Avery himself later visited his lab and returned home to set up a clinical trial with the Fujiwara surfactant. What was especially remarkable about Dr. Fujiwara's success was that not only was this the first time surfactant replacement had been accomplished, but the delivery route, intratracheal injection, was also fairly new. Today, this is a common method of drug delivery.

At the University of California at San Francisco, where Dr. Clements was now working, doctors turned to their resident expert on lung surfactants for advice on starting their own clinical trial. Dr. Clements didn't feel comfortable with the idea of putting cow lung extract into premature babies – he was worried about how their immune systems might respond. He volunteered to design a surfactant that used only synthetic materials. Using his physical chemistry background, he designed a mixture of pure lipids. And since the roles of SP-B and SP-C were well known by then, he added a detergent to make up for the absence of these proteins and to facilitate spreading.

After a small feasibility study, Dr. Clements' surfactant replacement moved quickly through clinical trials and was the first replacement to be approved by the Food and Drug Administration (FDA) for clinical use. There would be others. By 1990, an estimated 30,000 infants in 500 hospitals in North America, Europe, and Japan had been enrolled in clinical trials of different surfactant replacements, many of which also gained FDA approval.

Although new information and technology have enabled modern surfactant replacements to become closer and closer to naturally occurring surfactant, the original categories remain. Six of the nine surfactant replacements that are commercially available today are *natural* surfactants, like the one originally created by Dr. Fujiwara, derived from cow or pig lungs by extracting the DPPC-rich lipid with care to preserve essential proteins. Three contemporary surfactant replacements are *synthetic* surfactants. Like the original synthetic surfactant created by Dr. Clements, these have no animal proteins but usually have synthetic proteins or, more recently, a synthetic peptide (a relatively short chain of amino acids) modeled after the structural patterns of the surfactant proteins.

The Road Ahead

Today, thanks to an armamentarium of methods made possible by the discovery of surfactant, including surfactant replacement, respiratory distress syndrome is

an uncommon cause of death for babies in developed nations. Annual deaths from respiratory distress syndrome in the United States decreased from between 10 to 15 thousand babies annually in the 1950s and 1960s to fewer than one thousand per year in 2002.

And that success – the hundreds of thousands of babies for whom surfactant made it possible to take a second and third and fourth breath and grow up to live good lives and have children of their own – is where our breakthrough story must end.

Of course, as with all important scientific discoveries, the real story of surfactant continues, each new answer bringing to light a dozen new questions. For example, despite all the tremendous advances in understanding and treating surfactant deficiency, why do several hundred babies in the United States continue to die from respiratory distress each year? Why are some of them full-term babies whose lungs, by all ordinary reckoning, should be producing sufficient surfactant? Why do some of them actually show sufficient surfactant being produced while their bodies act as if it weren't there? Using new genetic tools such as “knock-out” mice, scientists have been able to determine which mutations in the surfactant protein genes cause breathing problems and which could be used as markers, or signs, of susceptibility to pulmonary disease.

The modern day surfactant story has taken a strange new twist away from the surfactant proteins themselves into an entirely new arena – that of transport proteins, which move proteins and other molecules from one part of the body or cell to another. It turns out that some of these sick babies’ genes for surfactant proteins are just fine. However, by comparing genes of these babies to each other and to the gene sequence provided by the human genome project, scientists are pinpointing problems linked to genes for the proteins that *transport* surfactant so that the lungs can use it. As the surfactant story itself illustrates so strikingly, understanding the mechanism of a disease is the first step to finding the therapy – and sometimes the therapy will be broadly applicable to patients suffering from other diseases. For instance, many diseases, including cystic fibrosis, involve abnormal transport proteins. Therefore, understanding problems that originate in the genes encoding for these transport proteins may point the way to interventions for these diseases, just as understanding of surfactant led to treatments for RDS.

Other questions now under study focus on how to make a good thing better – and expand the population of people who might benefit from it. A new generation of scientists is drawing from molecular biology and chemistry to create a new generation of synthetic surfactant

replacements, ones that work more like the body’s own surfactant, with less risk from infection or risk of the body’s immune system responding negatively to a substance from another living creature. While newborn babies’ less mature immune systems are unlikely to have such a negative response, lowering or eliminating this risk will become increasingly important as new uses for surfactant are found in older children and adults. In fact, how much and in what ways surfactant replacement can help older patients is an area of active exploration.

Surfactant given at the time of lung transplantation improves outcomes in some adults, and scientists are asking whether surfactant replacement might help in lung injury or acute respiratory distress in adults.

The story of the discovery of surfactant began with a small group of men and women intrigued by challenging questions and driven by an abiding trust that the answers they found would change lives, even when – as with the early work by Dr. Clements and others – they were not yet sure exactly how. Working across disciplines, they learned to speak each other’s languages, shared their findings and created new partnerships between clinicians and scientists, government, academia and industry.

Today, with an astonishing array of new scientific disciplines and tools, and with increased commitment of support from the federal government and other

partners, research still comes down to scientists and clinicians, working together, intrigued by the unknown, ever aware of the pain and suffering caused by diseases not yet fully understood, and building on and encouraged by the successes of those who went before.

And there is one other thing. “Those dying babies were a powerful motivation,” recalls Dr. Avery. “But figuring out what it all meant was so much fun.”

Biographies

Sylvia Wrobel, Ph.D. has headed public relations and communications for Emory University's Robert W. Woodruff Health Sciences Center for more than 20 years. She writes frequently about science and medicine and was the author of the first *Breakthroughs in Bioscience* article in the *FASEB Journal*.

John A. Clements, M.D. MACP is a professor of pediatrics at the University of California, San Francisco medical school, where his research focuses on pulmonary physiology, lung lipid metabolism, surfactant and interfacial phenomena in biology. A member of the National Academy of Sciences, Dr. Clements has been the recipient of the Lasker Award, Gairdner Award, Christopher Columbus Discovery Award and Warren Alpert Foundation Prize, among other prestigious honors for his breakthrough discovery of surfactant and subsequent development of the first artificial surfactant therapeutic, Exosurf.

Selected Publications

J. H. Comroe, Jr. "Premature Science and Immature Lungs," in *Retrospectroscope: Insights into medical discovery*. Menlo Park, California: Von Gehr Press, 1977, pp. 140-182. *The three detailed, readable chapters devoted to the path leading to the discovery of surfactant offer insights into what happens when discoveries are "premature," that is, not easily connected to the current canon of knowledge, and how a multidisciplinary attack on immature lungs, made by the scientists you meet in this Breakthroughs article, finally won the day.*


J.A. Clements and M.E. Avery. (1998) Lung Surfactant and Neonatal Respiratory Distress Syndrome. *American Journal Respiratory Critical Care Medicine*. 156: 559-566. *Two of the main characters in the surfactant story outline the steps in the hyaline membrane story, beginning with the initial description of the disease in 1903.*

Dr. Clements' "Lung Surfactant: A Personal Perspective" (1997) *is just that, giving readers a glimpse of the painful disappointments and doubts that can precede the triumphs of discovery. Annual Review of Physiology*: 59:1-21

C. V. Boys. "Soap bubbles, their colours and the forces which mould them", originally printed in London in 1890, was reprinted by Dover Publications in 1911. *Although she also plodded through the most dense textbooks, Dr. Mary Ellen Avery says this clear explanation of surface tension, along with kitchen sink experiments intended for young students, was an invaluable source of information as she tried to master this difficult new concept. It still holds its appeal.*

M.E. Avery and J Mead. (1959) Surface Properties in Relation to Atelectasis and Hyaline Membrane Disease. *American Journal of Diseases of Childhood*. 97:517-523. *And finally, written in the most medically cautious style, the article that changed how the respiratory disease that killed so many babies each year was viewed and treated.*

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