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Letter to the Editor

"Surface Tensions" in the Lung

A recent paper in your journal by Scarpelli and Mautone (1994) perpetuates a myth, now some 30 years old, that lung surfactants, whether "natural" or "artificial" and consisting primarily of a mixture of water insoluble phospholipids can reduce the surface tension at an air/water interface to <5 dyne/cm (Cockshutt and Possmeyer, 1991). Yet others claim to have achieved <1 dyn/cm (Schurch et al, 1976, 1992) and even 0 dyne/cm (Cochrane and Revak 1991)!

These claims are preposterous/unlikely in the context of a classical understanding of the term "surface tension" as defined, for example, by the Oxford Reference Shelf CD-ROM (Science Book) as being

"the property of a liquid that makes it behave as if its surface is enclosed in an elastic skin. The property results from intermolecular forces: a molecule in the interior of a liquid experiences a force of attraction from other molecules equally from all sides, whereas a molecule at the surface is only attracted by molecules below it in the liquid. The surface tension of water is very strong, due to the intermolecular hydrogen bonding, and is responsible for the formation of drops, bubbles, and meniscuses, as well as the rise of water in a capillary tube (capillarity), the absorption of liquids by porous substances, and the ability of liquids to wet a surface".

The surface tension at any vapor/liquid interface is an inevitable consequence of the >1000-fold difference in the density of the two phases. The surface tension of a vapor/water interface only approaches zero at the critical temperature (374.1°C) when the density of vapor molecules almost equals the density of the (very hot) water, individual molecules in the interfacial region being equally attracted to the vapor and the water phases. Any claim to have eliminated the surface tension of water (at 37°C) as a result of presence of a mixture of proteins and phospholipids implies either that the experimentalists had failed to observe the disappearance of the water or vapor phases altogether or their methodology was flawed.

So how did the mythology arise in the first place and why has it persisted for so many years? We challenged it some 15 years ago (Bangham et al., 1979) by questioning the validity of applying an equation

$$\gamma_{film} = \gamma_{water} - \pi_{film}$$

to films of saturated phospholipids spread (from organic solvent or on water >41°C and allowed to cool) on water at 37°C, at which temperature they form solid, condensed films that can be compressed to pressures in excess of 70 dyn/cm. The equation relates the surface tension of a film (γfilm) to the surface tension of water (γwater) and the surface pressure (πfilm) to which the monolayer may be subjected. But, as pointed out by Gaines (1966), this equation applies only to surface films at equilibrium. Forcing films of phospholipids to occupy areas/molecule less than observed at equilibrium and then subtracting the peak pressure (πfilm) from the surface tension of water (70 dyn/cm) to compute the surface tension of the film (γfilm) is not valid. Surface tension is not simply related to surface pressure for all values of 0–70 dyn/cm, as taught (Clements and Tierney, 1964).

Langmuir surface balances are not easy to control when surface pressures are high, and it was natural to switch to the Wilhelmy dipping plate electrobalance which, in effect, simply weighs the liquid meniscus drawn up by a high-energy metal surface (>1000 ergs/cm^2). While Wilhelmy devices are satisfactory for measuring pure liquids and surfactants up to and including equilibrium surface concentrations, they are highly unreliable if the surface of the dipping plate becomes contaminated. Now it is well known that lung surfactant preparations are rich in dipalmitoylphosphatidylcholine (DPPC) a phospholipid whose melting point (41°C) would encourage it to crystallize out from mixed melts on cooler substrates, e.g., 37°C or lower. Aggregates of DPPC, however small, would mislead the Wilhelmy balance by reducing the surface energy of the clean platinum plate from around 1000 ergs/cm^2 to that of an oily kitchen pot, e.g., 20–25 ergs/cm^2 for which water has no attraction and negative menisci. The presence of a hydrophobic, basic protein such as SP-B would, of course, greatly expedite the "dirtying" of a metal plate by adsorbing and providing basic sites to which anionic phospholipids would stick with even greater avidity (Cochrane and Revak, 1991). Thus the weight of the dipping plate in no way reports upon the true surface tension of the surface film under investigation.

Finally, I noted, belatedly (Bangham, 1992), that Pattle (1955) in his original letter to *Nature* offered two disparate descriptions as to why the bubbles emanating from traumatized lungs were unusually small and stable. In paragraph 2 line 6 of his original letter to *Nature* he wrote, "The surface tension of the lung bubbles is therefore zero", and later in the last three lines of the same paragraph "It is thus evident that the alveoli are lined with an insoluble protein which can abolish the tension of the alveolar surface". Thus the word "tension" carried two distinct meanings, the first as a manifestation of surface free energy (ergs/cm^2) and defined above, and secondly something entirely different namely, an absence of "tension" between the liquid phase of the alveolus and the air space due to the presence of solid material. Pattle imagined it to be denatured protein; Clements (1964) showed it to be phospholipid but, in my view, chose Pattle's (1955) wrong explanation as to why the bubbles failed to behave like soap bubbles. Both were in error to assign the stability of lung bubbles to the properties of surface tension (soap-bubble style). Even solid monolayers manifest surface tension (surface stress) relative to a vapor phase but too small (wax, 25 ergs/cm^2) to overcome the steric forces of the solid.

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Lung surfactant has two physiological functions, to reduce the work required to extend the area of the air/water interface at birth from a mere 1 cm^2 to some 2–3 m^2 rapidly and, even more important, that of preventing the maturing and adult lung from filling with fluid, the "antiedema" effect. I have no disagreement with von Neergard's (1929) seminal conclusion that lung surfactant facilitates the extension of the air/water interface over the greater part of a respiratory cycle and effectively, reduces the work load by 2/3. It can easily be shown that both natural and artificial lung surfactants, at surface excess and at equilibrium, can lower the surface tension of water by a requisite amount. Furthermore, experience with the pulsating bubble surfactometer (Enhorning, 1977) confirms that the Laplace equation is obeyed within such limits.

The prevailing notion, however, that low or zero "surface tension" can also account for the stability of the alveolus at full expiration cannot be accepted and for the reasons given above. The Laplace equation lacks validity for non-equilibrium systems.

In 1979 (Bangham et al., 1979) offered an alternative sequence of events based upon an observation that highly compressed films of lung surfactant became demonstrably solid on water at 37°C but not at temperatures >41°C. Our suggestion was that respiratory oscillations involving compression/relaxation of lung surfactants (natural or artificial) refines DPPC to the extent that at 37°C it crystallizes out to form solid plaques occupying an as yet unknown proportion of alveolar surface. We suggested that the plaques are normally present throughout adult life being continually eroded and replenished during each respiratory cycle. At full expiration they prevent the alveolus from collapsing in the manner of a geodesic dome where flat plates are locked together to form a stable structure. Upon inspiration, the plates move apart revealing (initially) clean water interfaces with high radii of curvature requiring (from Laplace) minimal pressure to deform and extend.

Controversy between physical and medical scientists would be resolved if the latter were to limit their reports to what they actually measure (weight of water clinging to a plate) and not what they think they are measuring (surface tension of the film away from the measuring device). Scarpelli and Mautone's (1994) paper would carry more conviction were it certain that they understood that "zero surface tension" at an air/water interface was a contradiction in terms. They are right to challenge the notion that lung stability from full inspiration to expiration is one continuous expression of surface tension as understood by physical chemists.

REFERENCES

Bangham, A. D. 1992. "Surface tension" in the lungs. *Nature.* 359:110.

Bangham, A. D., C. J. Morley, and M. C. Phillips. 1979. The physical properties of an effective lung surfactant. *Biochim. Biophys. Acta.* 573: 552–556.

Clements, J. A., and D. F. Tierney. 1964. Alveolar instability associated with altered surface tension. *Handbook of Physiology: Respiration,* Vol. 2, Section 3. American Physiology Society, Washington, D. C. 1565–1583.

Cochrane, C. G., and S. D. Revak. 1991. Pulmonary surfactant protein B (SP-B): structure-function relationships. *Science.* 254:566–568.

Cockshutt, A. M., and F. Possmeyer. 1991. Lysophosphatidylcholine sensitizes lipid extracts of pulmonary surfactant to inhibition by serum proteins. *Biochim. Biophys. Acta.* 1086:63–71.

Enhorning, G. 1977. Pulsating bubble technique for evaluating pulmonary surfactant. *J. Appl. Physiol.* 43:198–203.

Gaines, G. 1966. Insoluble Monolayers at Liquid-Gas Interfaces. Interscience Publishers, John Wiley and Sons, New York. 148.

Pattle, R. E. 1955. Properties, function and origin of the alveolar lining layer. *Nature.* 175:1125–1126.

Scarpelli, E. M., and A. J. Mautone. 1994. Surface biophysics of the surface monolayer theory is incompatible with regional lung function. *Biophys. J.* 67:1080–1089.

Schurch, S., H. Bachofen, J. Goerke, and F. Green. 1992. Surface properties of rat pulmonary surfactant studied with the captive bubble method. *Biochim. Biophys. Acta.* 1103:127–136.

Schurch, S., J. Goerke, and J. A. Clements. 1976. Direct determination of surface tension in the lung. *Proc. Natl. Acad. Sci. USA.* 73:4698–4702.

von Neergaard, K von. 1929. Neue Auffassungen uber einen Grundbegriff der atemmechanik. Die retraktionskraft der Luge, abhangig von der Oberflachenspannung in der Alveolen. *Z. ges. Exp. Med.* 66:373–394.

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Response to A. D. Bangham

Surface biophysics of the surface monolayer theory of Clements (1962) is incompatible with regional lung function. This is the inevitable conclusion of our study (Scarpelli and Mautone, 1994), which, for the first time since the theory was formulated and generally accepted by biologists, took each assumption of surfactant monolayer function as held in the theory and tested it under the conditions of lung function specified by the theory, using the in vitro methods that were used as the basis for the theory. We adhered strictly to the theory's analysis of surface film dynamics for two reasons. First and foremost was our goal to test the theory on its own terms regarding the essential interrelationship among lung function, laboratory simulation, and film dynamics that is fundamental to the theory and has been widely accepted for more than 30 years to the virtual exclusion of all other possibilities. It was clear to us that objective testing of the theory also required evaluation of the fit between experimental data and the arguments upon which the theory stands. The second reason relates to our previous experience that inclusion of alternative possibilities leads to rejection by editors and reviewers particularly of those journals that have been associated with publication of reports that are supportive of the

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theory. In any event, we found that Clements' monolayer theory was internally inconsistent and that, given its own interpretation of surfactant film dynamics, it failed to fit the conditions of lung function as described in the theory itself (Clements, 1962; Goerke and Clements, 1986). We were delighted to find a vehicle, the *Biophysical Journal*, with sufficient objectivity to publish our results.

Our paper has reaped a second dividend. It has provided a platform for Bangham to restate his quite valid objections to the concept of "zero" or "near-zero" surface tension, which is fulcral to the surface monolayer theory (Clements and Tierney, 1964; Goerke and Clements, 1986) and is widely accepted (e.g., Schurch et al., 1992). As Bangham argues (Bangham et al., 1979; Bangham, 1992), the concept as applied to the air/surfactant interface is without thermodynamic validity.

Although we applaud Bangham's critique, we must bring to the reader's attention the following enumerated problems:

1. We remind Bangham that, contrary to his opening statements, our paper does not "perpetuate" a myth that surfactant reduces surface tension at the air/water interface to <5 dyn/cm. Perpetuity is beyond its scope; the "myth" is only 30+ years old; and a careful reading of our text (last sentence of the Introduction) clearly defines our purpose, i.e., ". . . to test point by point each of the assumptions and conditions of the surface monolayer theory as given by the authors of the theory . . ." If there is mythology, it is that of the theory, which failed the tests of each of its own points including near-zero surface tension, as shown in Table 4 of our paper (Scarpelli and Mautone, 1994).
2. In paragraphs 2 through 5 of his letter, Bangham provides a quick summary of open monolayer film dynamics at the air/water interface, but not without some hitches. For example, he rightly states (paragraph 4) that film surface tension may be calculated from film pressure only when the film is at equilibrium, which is consistent with a later statement (paragraph 8) that the Laplace equation is invalid for non-equilibrium systems, but is flagrantly at odds with his championing of the pulsating bubble surfactometer method (paragraph 7). The latter is a dynamic system in which surface tension is calculated from the Laplace equation, whereas equilibrium conditions are rarely, if ever, achieved, as shown by Scarpelli et al. (1992). Another example: Bangham states that DPPC melts at 41°C and that crystallization on cooler substrates, e.g., 37°C, would "mislead the Wilhelmy balance . . ." (paragraph 4). In fact, conditions in situ are more complex than suggested by Bangham's comment. Given that the surfactant system in situ is a complex mixture of phospholipids and "surfactant-related proteins," the following observations are relevant: (a) Both aqueous and hydrophobic extracts of this system have been shown to melt below 37°C, both by calorimetry and by infrared spectroscopy (Mautone et al., 1987; Dluhy et al., 1989); and (b) film compression from equilibrium *does not* quantitatively "squeeze out" (Bangham's "refinement") unsaturated PG; it is also apparent that the proteins may not be "squeezed out" and that saturated PG moves easily between surface and hypophase when area is cycled (Rana et al., 1993; Pastrana et al., 1994).
3. In paragraph 6, Bangham discusses the discovery of surfactant by Pattle only in terms of his first short report (Pattle, 1955) and a later review. However, a consideration of Pattle's early follow-up work (Pattle, 1956, 1958, 1960, 1961a, b) would have dispelled some of Bangham's concerns and amended some of his statements: Pattle studied *normal* fetal and adult lungs as well as "traumatized" lungs; Pattle's "unusually small bubbles" in fact covered the range of *normal* alveolar diameters; Pattle quickly realized that bubble films were composed of *phospholipid* and protein; and Pattle's bubbles were quite *stable*, highly permeable to respiratory gases and the films were maximally compressed in the bubble configuration. Pattle did state that bubble film surface tension is virtually zero, and later in his life, he suggested that a similar film forms a thin skin, a "surpellic," at the alveolar level. Pattle's observations of bubble films were accurate, reliable and reproducible. However, he failed to realize that intact bubbles are the natural configuration of alveolar surfactant in vivo (see last paragraphs below). Instead, he regarded bubbles as laboratory artifacts. He erred by extrapolating closed bubble film properties to a presumed open surfactant monolayer in situ, an error which Clements (1962) compounded by further extrapolation to the Langmuir-Wilhelmy surface balance from which the surface monolayer theory was formulated and later tested directly in our study.
4. In paragraphs 7 and 8, Bangham touches on physiology. He gives two functions to surfactant. First is to reduce the work required to expand the lungs at birth. We have analyzed the fluid dynamics of this process in depth and refer the reader to our report (Scarpelli et al., 1993). We must disagree here with Bangham's allusion to von Neergaard's classical studies. The ⅔ work reduction *does not* refer to normal respiratory cycles, i.e., to tidal volume breathing (see review of Scarpelli, 1988). Second, Bangham holds that the more important role of surfactant is to prevent the lung from filling with liquid. This "antiedema" function was introduced and first explained by Pattle (1958). Bangham gives no further explanation, particularly with regard to his own theory (next paragraph).
5. In paragraph 9, the theory of Bangham et al. (1979) is outlined. We only comment briefly here. First, the "respiratory oscillations" (tidal volume) required by this theory will neither "refine" nor "crystallize out" DPPC (see Figure 3 of our paper). Second, alveolar "plaques" have not been reported in the many morphological studies of normal alveoli from birth through adulthood. Third, "revelation" of "clear water interfaces" upon inspiration would effectively open a flood gate of high surface tension, contrary to both the von Neergaard and the Pattle concepts.

For reasons given in the opening paragraph of this response, we restricted our study to the scientific idiom of the surface monolayer theory of Clements (1962; Goerke and

Clements, 1986). Our own work, only briefly alluded to in the final paragraph of the paper and our concept (Scarpelli and Mautone, 1994, Fig. 8), which we sketched over the Clements model for the same reasons, reveal an entirely unique configuration for surfactant films in vivo. We discovered by direct visual inspection that surfactant bubbles and bubble films establish normal alveolar surface architecture (Scarpelli, 1978). We have found subsequently that bubble films define intraalveolar structure from birth (Scarpelli et al., 1979, 1984) through adulthood (Scarpelli, 1988); and that the films are highly permeable to respiratory gases, contain phospholipids and so-called "surfactant-related proteins," follow normal film transitions through black film formation, are highly stable in the compressed state, and display normal viscoelastic properties. Bangham will be pleased to know that bubble films in vivo are restricted to alveoli (airways contain free gas), that the air/compressed film interface is rigid, a "surpellic" (anticollapse); and that the opposite compressed film/water (liquid) surface is near zero surface tension (antiedema). A summary of many of these points can be found in our monograph (Scarpelli, 1988). Others await the reviewers. Of further practical significance is our finding that, although intraalveolar bubbles can always be seen in fresh lungs at all volumes, the usual histopreparative methods (from intravascular fixation through quick-freezing techniques) generally lead to disruption of the bubble films.

In conclusion, theories that assume the open monolayer configuration for surfactant in situ are inherently problematic, because of the inherently false presumption. Our paper shows this clearly for the one such theory that has captured the consensus of contemporary biologists, the surface monolayer theory of Clements. Bangham's generally valid objections, when properly directed against the theory and its basic premise, strengthens our findings from a more fundamental perspective.

REFERENCES

Bangham, A. D., C. J. Morley, and M. C. Phillips. 1979. The physical properties of an effective lung surfactant. *Biochim. Biophys. Acta.* 573:552–556.

Bangham, A. D. 1992. "Surface tension" in the lungs. *Nature.* 359:110.

Clements, J. A. 1962. Surface phenomena in relation to pulmonary function. *Physiologist.* 5:11–28.

Clements, J. A., and D. F. Tierney. 1964. Alveolar instability associate with altered surface tension. *In:* Handbook of Physiology, Respiration, Vol. II, Section 3. W. O. Fenn and H. Rahn, editors. American Physiological Society, Washington, D. C. 1565–1583.

Dluhy, R. A., K. E. Reilly, R. D. Hunt, M. L. Mitchell, A. J. Mautone, and R. Mendelsohn. 1989. Infrared spectroscopic investigations of pulmonary surfactant. Surface film transitions at the air-water interface and bulk phase transition. *Biophys. J.* 56:1173–1181.

Goerke, J., and J. A. Clements. 1986. Alveolar surface tension and lung surfactant. *In:* Handbook of Physiology, Section 3: The Respiratory System, Vol. III: Mechanics of Breathing (Part 1), P. T., Macklem and J. Mead, editors. American Physiological Society, Bethesda, MD. 247–261.

Mautone, A. J., K. E. Reilly, and R. Mendelsohn. 1987. Fourier transform infrared and differential scanning calorimetric studies of a surface-active material from rabbit lung. *Biochim. Biophys. Acta.* 896:1–10.

Pastrana, B., C. R. Flach, J. Brauner, A. J. Mautone, and R. Mendelsohn. 1994. A direct test of the "squeeze out" hypothesis of lung surfactant function. External reflection FT-IR at the air/water interface. *Biochemistry* 33:5121–5127.

Pattle, R. E. 1955. Properties, function and origin of the alveolar lining layer. *Nature.* 175:1125–1126.

Pattle, R. E. 1956. A test of silicone anti-foam treatment of lung oedma in rabbits. *J. Pathol. Bacteriol.* 72:203–209.

Pattle, R. E. 1958. Properties, function and origin of the alveolar lining layer. *Proc. R.* Soc. (Lond.) B148:217–240.

Pattle, R. E. 1960. The cause of the stability of bubbles derived from the lung. *Phys. Med. Biol.* 5:11–26.

Pattle, R. E. 1961a. The formation of a lining film by fetal lung. *J. Pathol. Bacteriol.* 82:333–343.

Pattle, R. E. and L. C. Thomas. 1961b. Lipoprotein composition of the film lining the lung. *Nature.* 189:844.

Rana, F. R., Mautone, A. J. and Dluhy, R. 1993. Surface chemistry of binary mixtures of phospholipids in monolayers. Infrared studies of surface composition at varying surface pressures in a pulmonary surfactant model system. Biochemistry. 32:3169–3177.

Scarpelli, E. M. 1978. Intrapulmonary foam at birth: an adaptational phenomenon. Pediatr. Res. 12:1070–1076.

Scarpelli, E. M. 1988. Surfactants and the Lining of the Lung. The Johns Hopkins University Press, Baltimore, Maryland.

Scarpelli, E. M., M. T. Antonio-Santiago, B. C. Clutario, and A. J. Mautone. 1993. Fluid dynamics during initial aeration of mature fetal lung and effect of temperature. *Pediatr. Pulmonol.* 15:235–243.

Scarpelli, E. M., B. C. Clutario, A. J. Mautone, and J. Baum. 1984. Intrasaccular bubbles of near-zero surface tenaion stabilize neonatal lungs. *Pflugers Archiv.* 401:287–292.

Scarpelli, E. M., B. C. Clutario, and D. Traver. 1979. Failure of immature lungs to produce foam and retain air at birth. Pediatr. Res. 13:1285–1292.

Scarpelli, E. M., E. David, M. Cordova, and A. J. Mautone. 1992. Surface tension of therapeutic surfactants (Exosurf Neonatal, Infasurf and Survanta) as evaluated by standard methods and criteria. *Am. J. Perinatol.* 9:414–419.

Scarpelli, E. M. and A. J. Mautone. 1994. Surface biophysics of the surface monolayer theory is incompatible with regional lung function. *Biophys. J.* 67:1080–1089.

Schurch, S., H. Bachofen, J. Goerke, and F. Green. 1992. Surface properties of rat pulmonary surfactant studied with the captive bubble method: adsorption, hysteresis and stability. *Biochim. Biophys. Acta.* 1103:127–136.

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