controversies in physiology

Opposing views on the alveolar surface, alveolar models, and the role of surfactant

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Emile M. Scarpelli: There is a body of work that relates directly and specifically to the review article of Dr. Hills, “An alternative view of the role(s) of surfactant and the alveolar model,” which appeared in the November 1999 issue of the Journal of Applied Physiology (5). In August 1998, another review article, “The alveolar surface network: a new anatomy and its physiological significance,” was published in The Anatomical Record (17). It defined the configuration and limits of the alveolar surface as an infrastructural agglomeration of bubbles, i.e., a foam, that fills the acinar air space from respiratory bronchioles to alveolar sacs. The unit bubbles are complete bubbles, the surfactant-containing films of which surround (incorporate) units of alveolar gas. They are neither bubble segments nor “one-sided bubbles” as described by Dr. Hills (5). Discrete portions of a unit bubble’s film form discrete “foam films” by apposition to adjacent portions of other bubble films (namely, at the alveolar entrance, at pores of Kohn, and across the alveolar duct) and of nonbubble surfaces (namely, the epithelial cell surface and the liquid surface of the terminal conducting airways) (Fig. 1). (Conducting airways from trachea to terminal airways are themselves bubble free.) Foam films occupy virtually all of the surface area of the unit bubbles, except for their reflections at Plateau borders and cell surface niches. Their location, both individually and collectively, and their extraordinary thinness (~7 nm) afford a substantially smaller barrier to gas diffusion than that assumed (1, 2) for the traditional models (5). Collectively, the foam films form a continuous channel for alveolar surface liquid, which permits movement both in series and in parallel (Fig. 1). In addition, the lamellar arrangement of interfacial surfactants of the films provides both infrastructural support to stabilize aerated alveoli and near-zero surface tension to virtually eliminate the tendency of the bubbles to collapse. Near-zero surface tension was first reported and validated by Pattle (8–10) from studies of bubbles expressed from the lung. It was the cornerstone of his discovery of lung surfactant (14, 15). It is applicable directly to the alveolar surface network (17) but not to the “one-sided” bubble and “morphological” models, which are the topic of Dr. Hills’ review (5). Discovery of the “foam lung” architecture (11, 12), first applied to the neonatal lung and then to all lungs through adulthood as the “alveolar surface network” (13), was advanced by investigations reported over the years in original research papers (3, 6, 7, 12, 18–21, 23–28), other scientific reports (e.g., Refs. 11, 16, 17, 22), and a monograph (13). Remarkably, Dr. Hills’ review (5) is totally devoid of any reference (direct, indirect, or even dismissive) to this body of research. He does, however, cite one in vitro study (24) to support his argument against the “one-sided” bubble model but ignores the paper’s conclusion (24) that only a discrete unit bubble can satisfy the surface dynamics of normal breathing in vivo. All these omissions might be reason enough to disqualify Dr. Hills’ review (5) as incomplete and inaccurate, but there are more serious problems that ultimately are dispositive.

The first unfortunate consequence of Dr. Hills’ omissions is failure to recognize and address the scores of photomicrographs, published over the last quarter century, of fresh, unprocessed lungs as they occur in vivo (6, 7, 12, 13, 16–20, 23, 25–27). When the lung is examined by stereomicroscopy immediately after the thorax is opened (either in thorax or excised and with in vivo lung volume unperturbed), an agglomeration of unit bubbles in all aerated air spaces from respiratory bronchioles to alveolar sacs is revealed. Removal of unit bubble(s) renders the site(s) airless, unless adjoining bubble(s) move in. No free gas is observed, as would be the case in all models discussed by Dr. Hills (5). Consistently, all lungs in vivo, at all ages and at all lung volumes, are aerated by unit bubbles that form, collectively, the alveolar surface network (17). The criteria for optimal examination have been summarized and explained (17, 25–27). They are logical, easy to follow, and require that the lung be otherwise unperturbed from its immediately preceding condition in vivo.
Ultimately, the irreconcilable flaw in Dr. Hills’ review (5) is his need to establish and validate models of alveolar surface structure on information from published light and electron photomicrographs. Indeed, this turns out to be the flaw in all studies that look to conventionally processed lung tissue as the paradigm. The reason is that processing and other common methods of tissue “preparation” destroy the natural relationships in and among the alveolar surfaces, a problem that has concerned some investigators (e.g., Ref. 4). This problem, in fact, has been the principal obstacle to general recognition of the normal alveolar surface. Thus it is now clear (17, 26) that virtually each and every step, either individually or in sequence, of tissue preparation for light and electron microscopy dislocates, distorts, and disrupts the unit bubbles, including 1) osmium tetroxide and tannic acid fixation, 2) chemical dehydration (ethanol) and clarification (xylene; acetone), 3) both paraffin and epoxy embedding, and 4) transection and dicing, which accelerate bubble egress from and reagent-bubble contact within the air spaces. Other “preparative” processes are less common but also destructive (17, 26): 1) lung freezing for morphological studies distorts the surface and produces artifacts, and 2) lung degassing before volume-pressure studies destroys all natural bubbles. Clearly, the paradigm (above) is not valid, and the models of Dr. Hills’ review (5) are not supportable.

Albeit many in the physiological community have not recognized the natural alveolar surface network
and its constituent unit bubbles, these structures are readily defined by 1) direct examination of the intact, fresh lung immediately from the in vivo state, 2) aldehyde immersion fixation without subsequent processing, 3) drying the intact lung in air, and 4) processing for light and electron microscopy after preembedding in agar (“double-embedding”) (17, 26). These approaches permit virtually limitless studies of the network (e.g., Refs. 17, 25–27), such as 1) analysis of surface structure, conformation, and chemistry; 2) evaluation of surface liquid balance; 3) analysis of infrastructural dynamics of the network and its films; and 4) integrated surface fluid mechanics (gas and films) as determinants of volume-pressure dynamics. Such studies can target the network from the first breath of extrauterine life through adulthood, i.e., the lifespan of both the organism and the network. It should not be ignored by pulmonary physiologists (see introduction in Ref. 27). Dr. Hills’ review (5) may only be the latest example of this neglect.

REFERENCES


Brian A. Hills: My omission of Dr. Scarpelli’s model (12), the “dry” model of Colaciccio (5), and studies of black films (6) was due in part to the lack of interest shown by previous authors and myself in his foam concept of the lung. Another reason was the strict word limit imposed by the Journal. However, in his communication above, Dr. Scarpelli makes no mention of the major theme of my review, which is the ability of surfactants to adsorb to solid surfaces and the highly desirable properties, which such adsorption can impart.

Let us then review the model of Dr. Scarpelli (12) in which he proposes that there is “no free gas” in the alveoli or terminal lung units in the adult lung, a foam filling these units to “impart infrastructural stability.” As shown in Fig. 5, for example, in his paper in The Anatomical Record (12), the photomicrographs taken across the pleural surface do indeed show a number of adjacent, largely spherical units, with an ensemble that closely resembles what could well be a foam. However, the diameters of these units (commonly 30–160 μm) (12), on checking the scale, encompass the mean diameter of the rabbit alveolus of 75 μm (2). Hence, these and other photomicrographs could simply be nice pictures of alveolar structure with no indication of the menisci needed to subdivide larger structures into the foam that is claimed to fill them. It would hardly be an “irreconcilable flaw,” as Dr. Scarpelli
Dr. Scarpelli’s “foam” model is based on Pattle’s original observation (11) that foam expressed from an incision in an excised lung is very stable and the bubbles are unusually small. This is undoubtedly true. However, Dr. Scarpelli is totally incorrect when he claims that this proves “near-zero surface tension,” as originally suggested by Pattle. Near-zero surface tension is an “absurd” concept physically as explained by Bangham (3), who goes on to express his disappointment as to how this concept “has become cemented in American concrete” (personal communication). Surface tensions of less than 6 mN/m (dynes/cm) cannot be achieved by any procedure without going far outside of physiological conditions, especially surface compressions, as described in detail in my review (10). These and other shortcomings in the surface physics underlying Dr. Scarpelli’s model have been pursued in detail by Bangham (4).

The whole field of physiological research into surfactant is dominated by the medical focus on respiratory distress syndrome (RDS), as reinforced by the funding that its studies attract. It would seem quite reasonable to expect foam to be formed in the terminal lung units of neonates with RDS or even in normal neonates during switch over to air breathing. However, it is dangerous to extrapolate from neonatology and the foam model to normal air-breathing adults, including humans (which were the focus of my review), and even more speculative to do so on the basis of studies that predominantly used rabbits. Unlike humans or most mammals, rabbits pant at 200 breaths/min and have a resting heart rate that exceeds 200 beats/min (1); in addition, it is our experience that they form pulmonary edema very readily, even by handling, and this is conducive to foam production.

Although Dr. Scarpelli produces gas diffusion calculations to the contrary (10), it is very difficult to envisage his stable foam not compromising gas exchange. A foam sufficiently rigid to mechanically stabilize terminal lung units must surely compromise any convective gas transfer within the respiratory zone of the lung; in addition, how could one ever explain the well-established phenomenon of collateral ventilation (8, 13)?

Dr. Scarpelli (12) emphasizes how the foam is so stable that it remains unchanged if expelled into the bronchi and other conducting airways. Hence, if a balloononist or an aviator in a nonpressurized aircraft were to ascend to an altitude exceeding 14,000 feet, the expansion of the foam (initially 60% of lung volume) should, according to Boyle’s law, cause frothing at the mouth or, at least, rales should be heard on auscultation. Similarly, a diver who has spent many hours equilibrating at 33 feet of sea water should be frothing at the mouth when he or she returns to the surface from such a bends-free decompression (9). Neither frothing nor rales have ever been reported in such decompressions either in divers or aviators, except in extremely rare cases of pulmonary barotrauma as confirmed by blood streaks in the foam.

Therefore, there would appear to be no reason to include Dr. Scarpelli’s foam model of the alveolus and terminal lung units as a viable alternative for the normal air-filled lung either in adults or infants. It is also very important in neonatology to know the end state, which one is trying to attain in treating cases of RDS. Quite different therapies would be indicated if one accepted foam as normal compared with the culturally embedded model of the alveolus as a single one-sided bubble (7) or the “morphological model” (10), in which any liquid lining is discontinuous in its normal physiological state. By avoiding bubbles altogether, the lung then avoids the instability problems introduced by the conventional model of interconnected one-sided bubbles. However this model (7) becomes relevant as a pathological state in the edematous lung when the “pools” and “pits” of surface fluid link up (Fig. 8 (B→A) in Ref. 10) to form a continuous fluid lining, thus introducing instability as manifest by atelectasis.

REFERENCES


REBUTTALS

Emile M. Scarpelli: Because of space limitations, a brief commentary is made on each of the seven paragraphs in order of Dr. Hills’ response above.

1) Dr. Hills’ “major theme” rests on his assumption of an open alveolar surface, which my research shows is not anatomically correct (3). Hence, his obligation to address it. I critically reviewed Dr. Hills’ and the other models in 1988 (2) and have found no new biological data to support them (3). Interestingly, my work with Exerowa (6) shows that lung surfactants rapidly form stable black foam films, of the kind sketched in Fig. 1 above, under conditions expected in the acinus in vivo.
2) Dr. Hills misrepresents the following: when microrinised, alveolar gas exits as bubble(s), while conducting airway gas exits in a stream. Thus I have adopted the terms “unit” and “free” gas, respectively. The ultrathin bubble films (Ref. 3; also see my comments above) have no more and probably much less effect on gas diffusion than that assumed for the open surface models. Dr. Hills may have misunderstood the “irreconcilable flaw” discussed in my critique above. The “flaw” is universal reliance on preparative methods that actually destroy the alveolar surface conformation (3). Dr. Hills objects as follows: My photomicrographs closely resemble a foam, but there are no indications of “menisci” and so they are simply “nice pictures.” However, they should not fall either to a simplistic interpretation or to the failure to note the full spectrum of criteria (from bubble mobility in situ to foam film preservation in tissue) used to identify and define the alveolar surface network as normal anatomy, not a “model” (3).

3) The network was discovered by direct inspection; it is not based on Pattle’s observations. Regarding near-zero surface tension, the hard line of Bangham and Hills applies to their open surface model, not to bubble films, as explained to Bangham (4) after he wrote the letter (1) that was cited by Dr. Hills above.

4) On discovery of intrapulmonary foam at birth, I presumed the phenomenon was essential to that period (“adaptational”). Subsequent research, however, revealed the alveolar surface network at all ages! There was no “extrapolation” (3). Adult animals had normal pulmonary liquid content, with no signs whatsoever of excessive liquid (5). Also, rabbit respiratory rate could, arguably, reduce bubble formation. Bubbles and network have been seen in adult mice, rats, cats and pigs, the species examined thus far.

5) I have not calculated gas diffusion. The collective thickness of all foam films spanning the acinar air spaces varies with air space generation. At maximum, it is less than that assumed by others for open alveolar surfaces; at minimum, it is minuscule. It is the latter at pores of Kohn.

6) Conducting airways contain no foam, no bubbles, and no network. “Decompression” is a straw man, not taking into account 1) all gas laws, 2) so-called “explosive” vs. “rapid” vs. “slow” decompression, and 3) “trapped gas” vs. “loculated gas” vs. bubbles. Suffice it to say here that bubble films rupture with overexpansion, and Dr. Hills need not worry about “fothing at the mouth” (see his communication above). (Foam is not froth.)

7) Surfactants delivered either as aerosol or as liquid suspension to surfactant-deficient lungs effectively generate formation of the alveolar surface network (3). Also, the fundamental effect of pulmonary edema is alveolar flooding not atelectasis. Bubbles in edema are displaced from the network; edema liquid itself does not form stable bubbles.

One either repeats my studies or develops others that equally protect the alveolar surface from iatrogenic damage. There are no reports (save one) to my knowledge in which this may have been done. I regret Dr. Hills’ failure to address our methodology and the “irreconcilable flaw” in others’, both of which invalidate the open surface models.

REFERENCES


Brian A. Hills: As stated in my first response to Dr. Scarpelli, it is perfectly reasonable to expect some foam in the neonatal lung at the switch-over from fluid to air breathing. Dr. Scarpelli states in his second communication, “there was no extrapolation” to adult animals and presumably to the normal air-filled lung. It should not, however, be allowed to cloud the major purpose of my review (4), which was to address much more fundamental issues as embodied in some myths of surfactant physiology. These have become culturally embedded in respirology in isolation from the real world of surfactants in general.

These myths include 1) surfactant is not unique to the lung (3, 4), as it was once believed (2); 2) surfactant proteins are also not unique to the lung (1), as once believed (2); 3) surfactant proteins by mesentery cells in rat and man. Biochem 87: 1567–1583, 1999.

REFERENCES