The following is the abstract of the article discussed in the subsequent letter:

Bachofen, H. U. Gerber, and S. Schürch Effects of fixatives on function of pulmonary surfactant. *J Appl Physiol* 93: 911–916, 2002; 10.1152/japplphysiol.00927.2002.—The structure of pulmonary surfactant films remains ill defined. Although plausible film fragments have been imaged by electron microscopy, questions about the significance of the findings and even about the true fixability of surfactant films by the usual fixatives glutaraldehyde (GA), osmium tetroxide (OsO₄), and uranyl acetate (UA) have not been settled. We exposed functioning natural surfactant films to fixatives within a captive bubble surfactometer and analyzed the effect of fixatives on surfactant function. The capacity of surfactant to reach near-zero minimum surface tension on film compression was barely impaired after exposure to GA or OsO₄. Although neither GA nor OsO₄ prevented the surfactant from forming a surface active film, GA increased the equilibrium surface tension to above 30 mN/m, and both GA and OsO₄ decreased film stability as seen in the slowly rising minimum surface tension from 1 to ~5 mN/m in 10 min. In contrast, the effect of UA seriously impaired surface activity in that both adsorption and minimum surface tension were substantially increased. In conclusion, the fixatives tested in this study are not suitable to fix, i.e., to solidify, surfactant films. Evidently, however, OsO₄ and UA may serve as staining agents.

**Lung surfactants: in vitro vs. in vivo**

To the Editor: The paper of Bachofen, Gerber, and Schürch, which appeared in the September 2002 issue of the *Journal* (1), is seriously misinformed and consequently substantially misleading. The aim of the reported study was to assess the effect of fixatives on structure-function relationships of “natural” surfactant films in vitro, i.e., as adsorbed films in the “captive bubble surfactometer,” in order to gain some insight into surfactant surface film fixation at the alveolar surface of the lung itself. The fixatives studied included glutaraldehyde (GA), osmium tetroxide (OS), and uranyl acetate (UA). Surfactant was obtained by separation from bovine “alveolar lavage material,” without strict chemical analysis of the product.

Among the points of misinformation, the following are enumerated.

First, the authors claim that their totally in vitro study (1) is the first to examine the effect of fixatives on function, hence structure-function, of surfactant films. This assertion is quite disingenuous, even discounting the fact that they provide no direct information about film structure and no assessment of film function as it is in vivo. As a matter of fact, the effect of fixatives on surfactant films in situ as they exist in vivo has been studied extensively (16) and reported in ample time to have been noted in the paper of Bachofen et al. (1).

Second, the authors (1) credit their own institution as “the first to convincingly demonstrate by transmission electron microscopy a thin extracellular lining layer covering the alveolar surface” in 1969 (4). This, of course, omits the equally relevant foundational work on the same topic by a number of antecedents, which defined a physicochemical structure that still requires study, e.g., Macklin in 1955 (7), Chase in 1959 (3), Groniowski and Biczyskowa in 1964 (5), and Kikkawa et al. in 1965 (6), each of whom brought a nuanced perspective to chemical composition and structure of the alveolar surface. (See historical review in Ref. 8.)

Given the disruptive effects of fixatives, as suggested by the authors (1) and given the virtually total destruction of normal alveolar surface films in situ (intact lung) by conventional processing methods, as shown in studies (16) (see below) not cited by the authors, it is clear that the historical primacy declared by Bachofen et al. (1) is as misleading as it is inaccurate.

Third, from their study with the in vitro model, which bears scant resemblance to the alveolar surface in vivo (11), the authors (1) conclude, surface jargon notwithstanding, that the fixatives GA and OS decrease surface film stability and that UA seriously impairs surface activity. They conclude further that the fixatives are not suitable to “solidify” surfactant films but that OS and UA could serve as staining agents. But what sort of confused understanding does this bring to alveolar surface biology, and how does it address certain fundamental issues? 1) Surface tension and surface structure are interdependent. Alteration of one is alteration of the other (10). Thus, when any agent changes or “impairs” the film, the altered state, even if stainable, will yield misleading results. Fixatives are but one of the problems that have cast doubt on the validity of conventional processing methods as employed over the many decades of lung research (16) (see below). This information has been ignored completely by Bachofen et al. (1). 2) The study omitted by the authors (1) is of particular importance here. It is the definitive research (16) that shows the effect of fixatives on the alveolar surface film in the lung as it is in vivo. By direct observation of the surface films, which have the form of intraacinar unit bubbles, it was shown that GA does not disrupt film integrity when the lung is fixed by immersion at any lung volume at which it was functioning in vivo immediately before fixation. The film is fixed in place over a period of ~48 h as its potential unit mobility wanes. In contrast, OS fixation rapidly produces rupture of the surface films. Thus the nominal difference between GA and OS reported from the in vitro studies of Bachofen et al. (1) is not supported by observations in situ.

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Fourth, three concepts employed by the authors (1) need to be addressed in light of published reports omitted from their article. They are 1) continuity, 2) solidity, and 3) preservation. Continuity of the alveolar surface liquid and gas-liquid interface has been debated (13). The citations given by Bachofen et al. (1) provide only topographically restricted appraisals that used methods of questionable reliability (see point 3 above and Ref. 16). However, direct examination of the fresh, unperturbed lung reveals that the intraacinar unit bubbles extend from the first respiratory bronchioltes to the alveolar sacs (14–16). Thus the liquid phase is continuous and the gas-liquid interface is discontinuous throughout the acinus. Solidity is discussed in terms of fixability. The term “solid” in surface chemistry describes the state of maximal compression of the surfactant molecules at the gas-liquid interface. This state is achieved spontaneously (law of Laplace) in bubbles of pulmonary surfactant in situ (10, 11). In this state, surface tension is stable near zero. Unlike true solids, the compressed film is expandable (fluidity) but returns to the solid state spontaneously when the expanding force is removed. Thus the normal fluidity of solid and expanded surface films must be taken into account when assessing the significance of “solidity.” Although the authors (1) stress faithful preservation as the fundamental goal of morphology, they fail even to consider the other conventional processing steps to which the tissue is exposed in preparation for light and electron microscopy. They omit the study (16) that shows each of the following procedures, either as isolated intervention or in series, destroys the natural alveolar surface film whether fresh or GA fixed: OS, tannic acid-formaldehyde-GA mixture, graded ethanol dehydration, chemical “clarification,” paraffin or epon embedding. This study (16) also showed that the unfortunate procedure of “degassing” ruptures all normal films, whereas cutting fresh tissue or quick freezing distorts, displaces, and ruptures normal bubbles and films.

Clearly, the authors (1) have omitted specifically and categorically research reports (14–16) and review (11) that bear directly on their paper and fundamentally change their concept (1, 2) of structure and function of the alveolar surface. The omitted research was first reported in 1978 (9) as the “foam lung” in neonatal rabbits and lambs. Its persistence through adulthood was documented and later termed “the alveolar surface network” (ASN) (10). The lungs studied were precisely those as they existed in vivo (e.g., Refs. 14–16), including lung volume, transpulmonary pressure, and normal liquid content. Bubbles of the ASN form foamy films by apposition across the acinar airways, approximately one per airway. Film thickness is <7 nm (collectively <21–112 nm, depending on airway generation), offering virtually no resistance to gas transfer. Other bubble faces form equally thin foam films at the epithelial surfaces and at pores of Kohn. The ASN is seen in the fresh lung in the fresh lung dried in air, in the GA-fixed lung (by immersion), in sections prepared for light microscopy by the new double-embedding method, and in natural bubbles prepared for electron microscopy (double-embedding method) showing well-stained bilamellar surface films. This work has been summarized in recent reviews (11, 12). The total absence of this work from the paper of Bachofen et al. (1) is as inexplicable as it is scientifically deceptive.

REFERENCES


Emile M. Scarpelli
Professor of Pediatrics and of Physiology and Biophysics (retired)
Albert Einstein College of Medicine
Adjunct Professor of Pediatrics (Perinatology)
Cornell University College of Medicine
Orangeburg, New York 10962
E-mail: embel@webtv.net

REPLY

To the Editor: Evidently, Dr. Scarpelli has written his letter “cum ira et studio.” In contrast, our reply shall stick to the facts.

Regarding point 1: We did not claim to examine the effects of fixatives on the structure-function relationship of surfactant films. Numerous investigations (the reference list of our paper contains a fragmentary selection only) that have examined different preparations with different techniques have not yet adequately
explained the complex relationship between structure and function of pulmonary surfactant films. What we have done (for the first time) is examine the effects of some fixatives on the surface activity of surfactant films adsorbed to the surface of a captive bubble, with the aim to improve the interpretation of the unsatisfactory film morphology observed in lungs fixed under physiologically well-controlled conditions. Scarpelli gives us a good scolding for not having cited one of his papers (4). Because Scarpelli and co-workers neither measured the surface activity of the foam films nor convincingly demonstrated the ultrastructure of these films (the quality of the only electron micrograph shown does not allow an unequivocal interpretation), we could not see a sufficient coherence between their and our findings to be discussed in our paper. (In retrospect, the continuous increase of surface tension of compressed films observed in our experiments could tentatively explain the instability of Scarpelli’s surfactant foam.)

Regarding point 2: First, we want to point out that the institution of E. R. Weibel is not the institution of the defendants. Second, as to our statement, we told the truth and nothing but the truth. In their paper (3, 6), Weibel and Gil have honestly cited and discussed the work of previous authors. However, we did not consider our concise paper to be an appropriate vehicle for a historical review. Scarpelli’s statement that we had declared historical priority on surfactant film fixation is absurd. Regarding point 3: In this paragraph, Scarpelli addresses numerous and complex issues. He correctly grasped our results, that glutaraldehyde and OsO₄ impair the stability of surfactant films adsorbed to a captive bubble, after their compression to near-zero minimum surface tension. This instability is reflected in a spontaneous, continuous increase in surface tension (2). This behavior does not prove a rupture of the film. Equally important is the observation that the surface activity of the films was essentially preserved in that near-zero minimum surface tension on film compression could be obtained. This implies that the molecules of the film are still free to rearrange on an aqueous hypophase and hence that the film is not sufficiently stabilized for processing for electron microscopy (cutting and embedding). As we have mentioned in our paper, continuous surfactant films can be visualized if the film together with its hypophase is solidified (5). 1) The statement that “surface tension and surface structure are interdependent” is inaccurate and an oversimplification. Surfactant films differing in composition and hence in structure can yield surface tensions close to zero on compression. Indeed, it is the aim of the ongoing research to define the structural arrangement of films to explain their extraordinary functional properties. Many investigators have conducted an immense amount of research pertaining to the “facts and artifacts” of fixation of biological material, and, as we have stated earlier, it is a misconception to ask that morphology be a complete presentation of the “true” state (1, 7). 2) Doubts are justified regarding whether the observation of surfactant foam is the “definitive research” to elucidate the structure and function of surfactant films. The structural information provided so far is less than convincing.

Regarding point 4: Space does not allow us to discuss all the manifold ideas and objections outlined in this section. Some of them are immaterial, such as the problem of lung degassing (the last experiments with degassed lungs that we have carried out was 25 years ago). Some are wrong, such as the concept that the state of maximal compression of the surfactant molecules is achieved spontaneously according to the law of Laplace. This idea has originated from Scarpelli’s erroneous interpretation of some of Pattle’s work. A simple calculation shows that the “Laplace pressure” would be orders of magnitude too low to compress bubbles lined with surfactant to achieve near-zero surface tension from the equilibrium surface tension of ~25 mN/m. The main controversial question is whether alveoli are lined with a surfactant film and form an open air space system or whether alveolar structures, i.e., the entire fine lung parenchyma, serve as scaffold to accommodate surfactant foam. In contrast to Scarpelli, we and others have not been successful to observe foam-filled alveoli in healthy adult lungs, regardless of whether they were native or fixed (besides, the physiology of gas exchange and lung mechanics has to be completely rewritten if lungs were foam containers). Dr. Scarpelli is free to uphold his belief in the foam model. However, at the occasion of his recent and friendly visit to Berne, he must have realized that Berne is an infertile ground for making proselytes.

REFERENCES


Samuel Schürch
University of Calgary
Calgary, Alberta, Canada T2N 4N1
E-mail: schurch@ucalgary.ca

Hans Bachofen
Division of Pneumology
University Hospital
3010 Berne, Switzerland