struction in the name of "progress". It was a real pleasure to have Dr Lesley Clark, MLA, PhD, the Queensland Government Member for Barron, North Queensland, to open the SPUMS 1992 ASM. Dr Clark is not only an "intelligent greenie", with a demonstrated practical support for conservation, but she had to deputise for the task of opening our Meeting at very short notice. SPUMS appreciated her support and her opening address.

The divers' opportunity to play a key role in this drama of world conservation is in some ways unique. The wisdom of doing so needs to be made crystal clear to the diving "blind Freddies" and there are still a few of them around !

John Williamson
Convener, SPUMS ASM 1992

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ORIGINAL PAPERS

PULMONARY BAROTRAUMA: A POSSIBLE ROLE FOR SURFACTANT IN OPPOSING THE ENTRY OF AIR INTO THE CIRCULATION

Brian Hills

Abstract

The alveolar wall of sheep lungs has been studied by electronmicroscopy, employing vascular fixation, i.e. "fixation from behind", using a formulation designed to preserve any lamellated structure of surface-active phospholipid (surfactant). The electron micrographs (ems)show channels traversing epithelial cells, as reported previously, but the mouths of these channels are very close to the oligolamellar lining of surfactant which follows the alveolar surface, whether this is a fluid "pool" or the epithelium.

These findings are discussed as indicating a model whereby the relatively rigid surfactant "raft" can act as a flap-valve (non-return valve), allowing fluid to exude onto the alveolar surface under conditions which provoke oedema, while sealing the pores to prevent the entry of air if alveolar pressure exceeds capillary blood pressure.

Introduction

Pulmonary barotrauma is not a rare occurrence, and one of particular concern in view of the incidence of death or residual neurologic injury which can result.[1,2] "Burst lung" will occur if the difference between intrapulmonary and environmental pressures exceeds a threshold which has been placed at around 70 mm Hg, approximately 100 cm of sea water.[3] Hence, in the training of divers and submariners undergoing submarine escape training, much emphasis is placed upon keeping the glottis open in order to avoid any significant gradient developing between intra-alveolar and lung tissue pressures. However, even after exhaustive practice of the correct technique, cases of pulmonary barotrauma still occur in fit persons[4] and even in instructors particularly well trained and aware of the potential hazards. Cases have also been recorded[1] during the relatively slow decompression from a simulated dive performed in a pressure chamber, while even a cough or a sneeze under those conditions has been known to precipitate symptoms. In some of these instances it is difficult to believe that the pressure gradient for rupturing lung tissue had been exceeded. Such considerations raise the issue of what other means might be involved or what other pathways might exist by which air could enter the pulmonary circulation under much lower pressure gradients.

Fluid can pass from blood to air and accumulate on the epithelial surface as alveolar oedema, so some channels must exist. Moreover these must be large to enable macromolecular proteins to reach the alveolar surface from blood, the concept of "stretched pores" having been raised as early as 1934 by Landis.[5] A review of current thinking[6] states that, although the exact route by which fluid enters the alveoli from the intersitium remains controversial, there is currently general agreement that fluid enters by "bulk flow" through channels too large to permit any significant "sieving" of proteins. At the more selective capillary endothelial membrane, macromolecules of 255,000 have long

been known[7] to escape from blood; while more recent studies[6] of protein transport in the normal lung have estimated that the internal diameter of pores is about 500Å, but "strictures" reduce the effective size to 100-400Å. This applies to normal physiological conditions and much higher values have been indicated pathologically.

Channels of diameter 40 nm (400 Å) should be clearly visible by electronmicroscopy and many ems displaying gaps at intercellular junctions have been published.[8,9] Macromolecular protein markers such as haemoglobin and horseradish peroxidase can be visualized at intercellular clefts in the alveolar epithelium[10,11] While these studies clearly indicate the presence of some system of channels by which fluid and plasma proteins can traverse the blood-air barrier, the vital question is whether air is able to traverse that pathway in the opposite direction. At first sight one might dismiss this possibility on the basis that air would be excluded from entering a pore of the dimensions mentioned on account of capillarity. The penetration pressure ΔP would need to be too high on account of the very low radius of curvature (r) related to Δ by the Laplace equation, viz.

$$\Delta P = 2 g/r \quad (1)$$

where g is the surface tension at the air-liquid interface. However, according to conventional theory[12] g is "near-zero" at maximum compression of the surfactant monolayer corresponding to end-expiration. The fundamental physics have been discussed by Bangham[13] who is highly critical of the concept of near-zero surface tension. He and his co-workers have produced ems of the basic tendency for surfactant to form multi-lamellated structures *in vitro*[14], implying that "rafts" of such material occupy the fluid-air interface and so stabilise the alveoli.[15] Direct morphological evidence for an oligolamellar lining to the alveolus has been clearly demonstrated by Ueda et al.[16], but their ems display no fluid layer separating the surfactant from the epithelium.

These findings raise a number of questions concerning the ability of air to enter a pore on the epithelial surface. If directly adsorbed to the alveolar membrane, the surfactant lining might seal the pore or render it sufficiently hydrophobic to encourage the entry of air. *In vitro*, an adsorbed layer of surfactant has been shown to rupture a supernatant aqueous layer[18] by a process known in the physical sciences as the "de-watering" of a surface. On the other hand, a floating "raft" of surfactant could act as a flap-valve to allow fluid to exude onto the alveolar surface without allowing air to enter if the pressure gradient is reversed. This study has been designed to try to answer some of these questions by attempting to relate the oligolamellar surfactant lining to "pores" and the epithelial topography in general.

Materials and methods

PRINCIPLES

1 All previous studies of alveolar "pores" by electron microscopy have employed the almost universal fixative glutaraldehyde introduced[19] for its ability to fix protein. However aldehydes are well known to destroy hydrophobic surfaces[20] which include other mucosal surfaces on which an adsorbed layer of surfactant has been identified. Hence most of the glutaraldehyde has been replaced in this study by tannic acid which is ideal for visualizing the lamellated structure of any surface-active phospholipid (SAPL) present[21] and has been used successfully for this purpose in the lung[16].

2 In previous studies of alveolar ultrastructure the fixative was applied via the airways, but this could float off any rafts of surfactant or SAPL in any form which has not attached to the epithelium. Hence vascular fixation, or "fixing from behind" has been adopted as introduced and described in detail by Gil and Weibel.[22]

3 Since multi-laminated structures of SAPL have been found on other mucosal surfaces where they are attributed "barrier" properties to water-soluble solutes, long (72 h) fixation times have been employed to ensure penetration by the fixative.

MATERIALS

Lungs were obtained from five healthy adult sheep killed painlessly by stunning with a captive-bolt gun followed by exsanguination, the three for vascular perfusion having been heparinised 15 minutes before death. In those cases the lungs were kept at FRC by clamping the trachea before excision. The fixative was introduced from a large syringe via the pulmonary artery until the blood emerging from the pulmonary vein was largely diluted by fixative. In the other two sheep fixative was introduced via the trachea from a large syringe which was cycled back and forth until no more bubbles could be seen emerging.

FIXATION

The initial fixation (72 hour) employed 2% glutaraldehyde plus 3% tannic acid buffered at a pH of 7.4 with 0.1 M sodium cacodylate at 4°C and rendered isotonic (320 mOs) with sodium chloride. Post-fixation was effected in excised blocks with 1% osmium tetroxide buffered at 7.4 with embedding in resin (Spurr mix "A", Probing and Structure, Kirwan, OLD) polymerized at 60°C. Very thin (<60 nm) sections were cut from each block using a very sharp diamond knife in the microtome in order to resolve any lamellated structure of the surfactant.

METHODS

Sections were cut from the six blocks of the three lungs fixed by vascular perfusion and from another six blocks of the two lungs which underwent airway fixation. Sections from the remaining eighteen blocks were not only observed for major features such as "pores" but for the number of lamellae at the mucosal surface to give the mean number of phospholipid bilayers. This was undertaken for a total linear distance of 18 μm, representing 1,000 nm per block.

Results

Examination of the sections from lungs fixed by vascular perfusion showed essentially the same features as reported previously by other investigators using more conventional fixatives. These included the "pits" and "pools" visualized so clearly by Gil et al[23] but not with quite the definition of cellular detail achieved in their studies. This could be attributed to the higher concentration of glutaraldehyde in their fixative. All the figures in this paper are from lungs fixed by vascular perfusion. However, the osmiophilic surface lining of surfactant was well defined as shown in Figure 1 where it is located at the interface between air and the fluid layer (aqueous hypophase) lining the epithelium. Another view of the air-aqueous interface is shown in Figure 2 in which a myelin figure can be seen, as reported by many previous investigators.[24] Over 70-80% of the epithelial surface, however, the surfactant followed the epithelial surface as shown in Figures 3-6. Channels permeating the epithelium reported by many previous workers[8-10] can be clearly seen in Figures 4-6.

These channels enter and leave the plane of the section, but no single channel could be traced from the alveolar surface to the basement membrane within the one section. This can be attributed to the fact that extremely thin (<60 nm) sections were needed in order to resolve the surfactant layer into an oligolamellar structure. Upon cutting serial sections, however, it was possible to obtain sections of the entrances from both the alveolar side (Figure 5) and vascular side (Figure 6).

The average number of lamellations (ñ) was calculated by weighting the number at any location (n_i) by the length of cross-sectional surface (L_i) over which that number persisted according to the following equation:

$$\tilde{n} = \frac{\Sigma n_i L_i}{\Sigma L_i} \qquad (2)$$

The result was $\tilde{n} = 5.43 \pm 1.02$. This indicates that in addition to the two lines representing the epithelial membrane *per se* there are an average of 5.43 additional bilayers, translating into about 11 additional monolayers of SAPL.

Another very interesting feature seen in all of these oligolamellar structures is the uniformity of spacing of the lines (40-50 Å) and their uniformity of intensity, which has been reported before.[16]

Discussion

An oligolamellar layer of surfactant lining alveolar epithelium has been demonstrated in all sections of the alveolar surface, of which typical examples are shown in Figures 1-6. These layers are very similar in number and overall structure to those previously visualized in human lung tissue by Ueda et al.[16] using a similar fixation procedure based upon tannic acid. The primary difference is that, in all cases, Ueda et al. showed the surfactant layer immediately apposed to the alveolar membrane whereas in this study.no more than 10-20% of these surfaces were apposed The difference might be attributable to our preference for em-

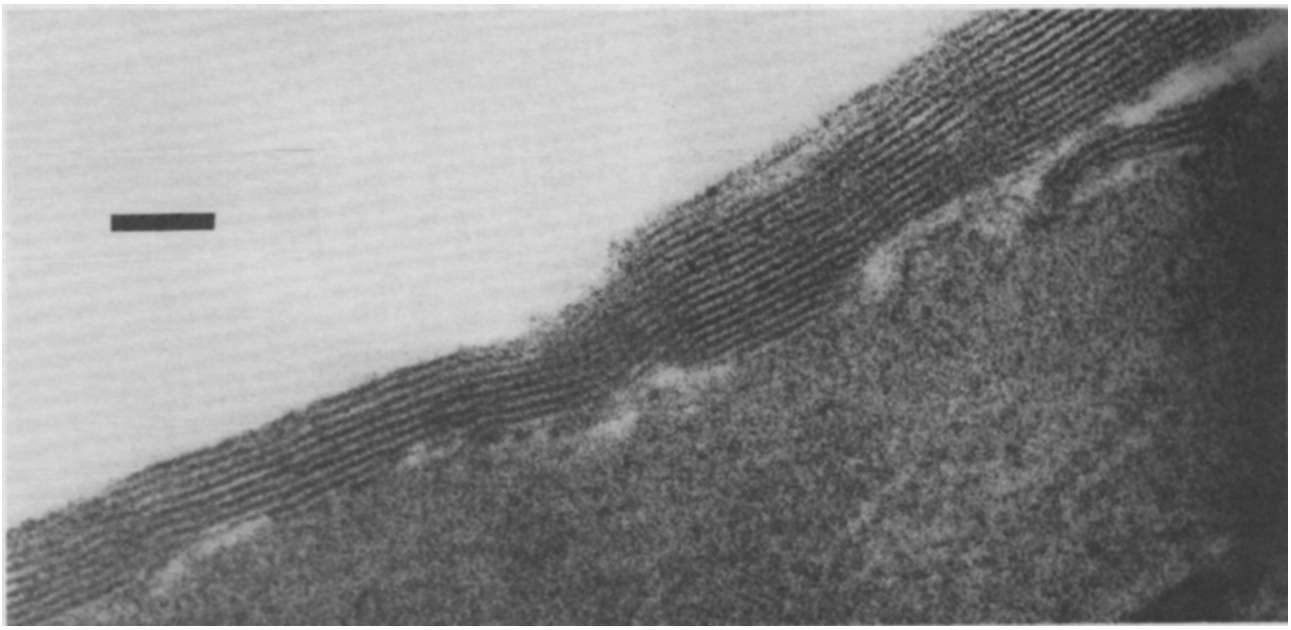

Figure 1. This and all the other figures is an electronmicrograph of the avleolar surface from a sheep lung fixed by vascular perfusion. Note the oligolamellar lining of surfactant appearing to float as a raft separatig air from the aqueous hypophase where the alveolar fluid has accumulated as a surface "pool".[22,23] The bar represents 50 nm.

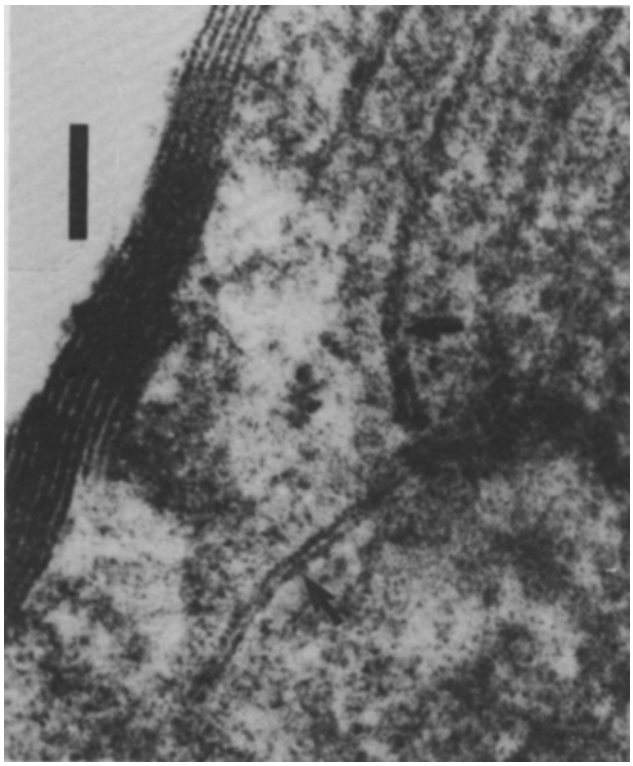

Figure 2. The oligolamellar raft of surfactant can be seen floating on the fluid in a surface "pool". The outline of a myelin figure can be seen within the aqueous hypophase as indicated by arrows. The bar represents 50 nm.

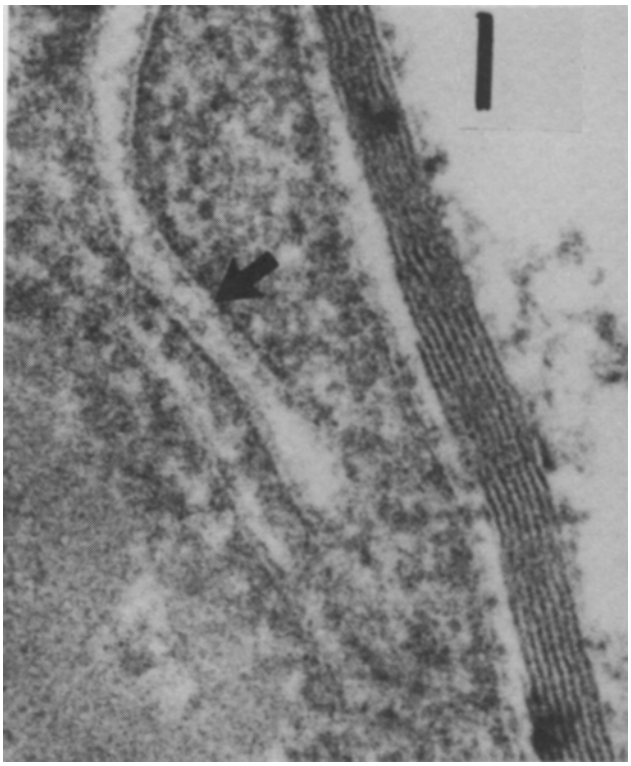

Figure 4. This also displays the oligolamellar lining of surfactant very close to the alveolar surface yet not totally apposed to the underlying epithelial cell, in which a channel is clearly visible (arrowed). The bar represents 50 nm.

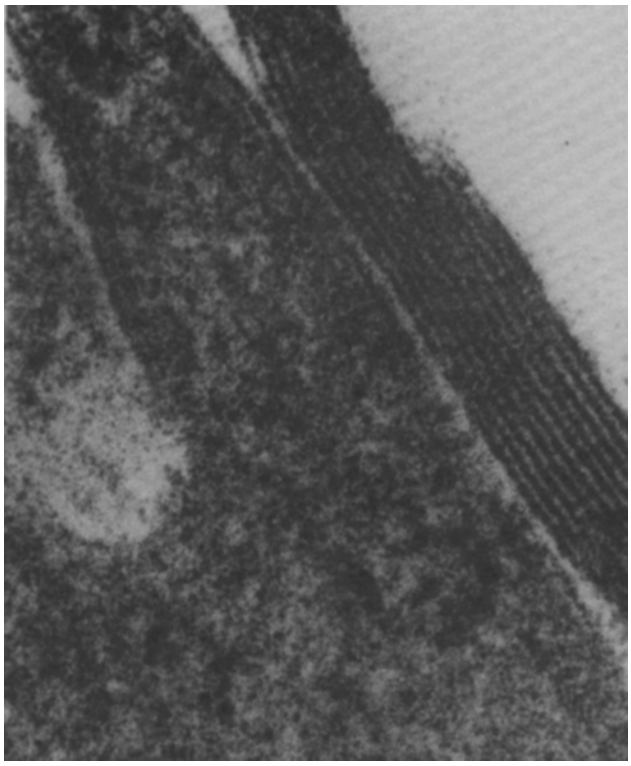

Figure 3. Note the oligolamellar lining of surfactant adjacent to the epithelial surface but still separated from it by a very thin fluid layer of varying thickness.

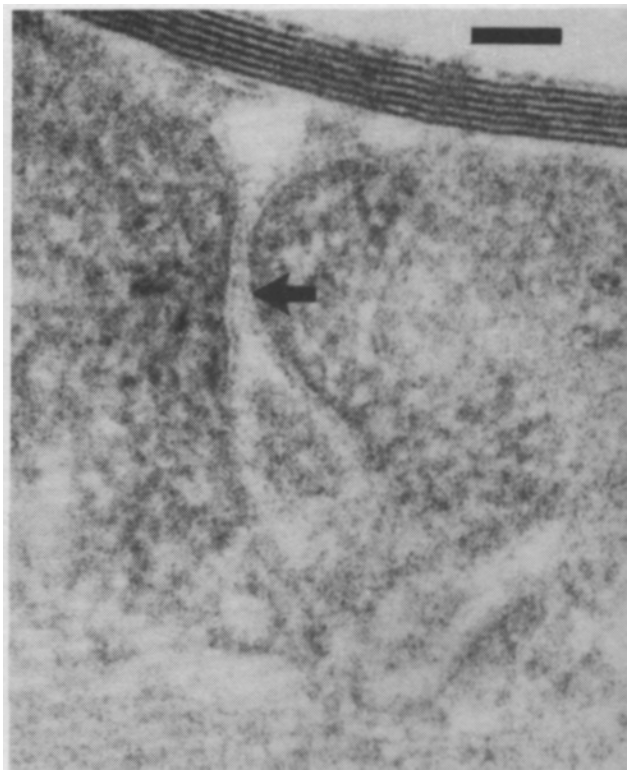

Figure 5. Note the "pore" (arrowed) in the epithelial cell and its location relative to the oligolamellar "raft" of surfactant lining the alveolar surface. The bar represents 50 nm.

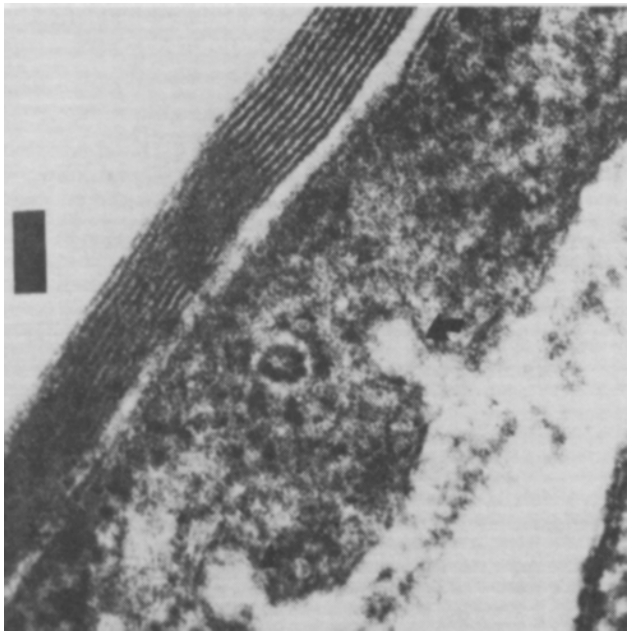

Figure 6. This displays a slight separation of the oligolamellar surfactant lining from the epithelial membrane. Invagniations of the endothelial cell can be seen at its interface with the basement membrane (arrowed).

ploying vascular fixation with an isotonic fixative, although the published information was insufficient to ascertain the tonicity adopted by Ueda et al. The oligolamellar lining covered both the fluid in the "pools" and "pits" (Figures 1 &2) and followed the epithelial membrane as described earlier by Weibel[26] but, even then, a very thin intervening fluid film was apparent (Figures 3-6).

Another feature of the electron micrographs is the system of channels within epithelial cells (Figures 4-6) and their connections to the aqueous hypophase on the alveolar surface (Figure 5) and to the basement membrane (Figure 6); although there is no certainty that they present a direct connection. Such channels have been demonstrated in many previous ultrastructural studies employing aldehyde fixatives.[8-10] The calibre of these channels is just about that predicted for the "bulk flow" of alveolar oedema as described earlier.

The feature of particular interest in Figure 5 is the relation of the mouth of the "pore" to the surfactant lining. On could easily envisage fluid exuding from the "pore" onto the epithelial surface beneath the oligolamellar layers, allowing alveolar oedema to accumulate by enlarging the aqueous hypophase as explained by conventional theory.[12] The question arises to what would occur if the pressure gradient were reversed. A small excess of alveolar over vascular pressure would tend to revers the flow of oedema. This is exploited in the clinical use of positive end-expiratory pressure (PEEP) with ventilators to control alveolar flooding.[27] If the gradient is larger, however, then one could envisage the higher pressure of alveolar air forcing the surfactant lining against the mouth of the pore, effectively sealing it against penetration by air. The surfactant could be playing an important role as a flap-valve, preventing air traversing the blood-air barrier and causing air embolism, in addition to its traditionally accepted role of reducing surface tension.[12]

Unless the pressure gradient is excessive, the floating "raft" of surfactant is unlikely to be deformed sufficiently to admit air to the "pore" it is sealing. Although SAPL in the form of lipid-bilayer membranes is very flexible, this property is generally attributed to cholesterol which occurs in much lesser amounts in lung surfactant. In any case the major component (DPPC) is recognised as a "rigidifying agent".[28]

If this hypothesis of the oligolamellar raft of SAPL acting as a flap-valve (non-return valve) to exclude air is correct, then any agent likely to compromise the surfactant lining is also likely to increase the risk of air embolism. A major deficiency in surfactant, as occurs in premature lambs, can not only greatly increase the permeability of the blood-air barrier to protein but does so bidirecitonally.[29] In humans the adult respiratory distress syndrome (ARDS), associated with SAPL deficiency, is characterised by increased permeability and leakage of macromolecular protein onto the alveolar surface.[30] It is reported that ARDS is often associated with embolism.[28] Leakage of proteins onto the epithelial surface could have an adverse effect upon the integrity of the surfactant lining, the surface activity of which has been shown to be compromised by albumin.[31]

Another factor to consider in maintaining the viability of the surfactant 'flap-valve' is sepsis, since septic or traumatic shock can greatly increase permeability.[32] In acute inflammation, the formation of exudate has been shown to depend upon the presence of gaps of 0.1 to 1 μm in diameter, i.e. 100-1,000 nm, indicating a severe test of the competence of the surfactant flap-valve in excluding air if the pressure gradient were reversed.

This discussion strongly supports the current practice of excluding from submarine escape training anyone with the least indication of respiratory tract infection or lung disease. It should also be borne in mind for considering fitness to dive or participate in any operation involving change of pressure. The concept of a surfactant flap-valve is also relevant to the selection of the pressure range for ventilatory support of patients and the risk of embolisation in ARDS, which can ensue if the wrong choice is made.

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