ORIGINAL PAPERS

SURFACTANT UPDATE

Brian Hills

In 1983 it was a great pleasure to be the invited speaker at the Annual Scientific Meeting of SPUMS held in Fiji. One of my lectures was entitled "Surfactant", a topic which I have pursued vigorously since returning to Australia.

In medical textbooks surfactant is mentioned only in connection with the lung and then only as acting at the liquid-air interface of a continuous fluid lining assumed to coat the alveolar surface. Although I have challenged this assumption in the normal adult lung (Fig.1), it is much more likely to hold in the neonate where, at birth, the liquid-air interface needs to expand from about 2 cm^2 to 30,000 cm^2 within a few minutes. Hence the field tends to be dominated by paediatrics in which "surfactant rescue", i.e. the application of exogenous surfactant for the respiratory distress syndrome (RDS), has reduced mortality at birth by the order of 40-60%. The major research thrust over the last decade has been directed at better formulations for this exogenous surfactant. The recent recipes include not only the highly surface-active dipalmitoyl phosphatidylcholine (DPPC) which actually reduces surface tension but also the associated proteins which enable those DPPC molecules to be recruited so rapidly to enable expansion of the interface to occur. Two of these proteins are so hydrophobic as to be "co-extractable" from lung surfactant with DPPC by typical lipid solvents. These are termed "hydrophobic surfactant proteins". Although never referenced in the respiratory literature, these hydrophobic proteins have a remarkable resemblance to proteolipids discovered in the CNS in 1953 and subsequently found known to have a major role in the myelination process, the predominant component of myelin (and the electrical insulator) for the axons being DPPC and similar saturated phospholipids.

Having emphasised the highly desirable role of DPPC and proteolipid in the rapid *expansion* of the liquid-air interface in the newborn, this combination would be highly undesirable in the tissues of a deep-sea diver where the gas phase is the last thing one would wish to create or to have its growth promoted. It was therefore particularly interesting last year to find this same combination of DPPC and proteolipid when I looked at the CNS of sheep and, moreover, to find even more in spinal tissue.[1] Maybe this is the vital entity which renders the spinal cord so vulnerable to decompression injury compared with the brain. Our studies of spinal tissue on the electron microscope with our special "surfactant fixative" have also revealed more lamellar bodies (LBs) in spinal tissue,[2] these being

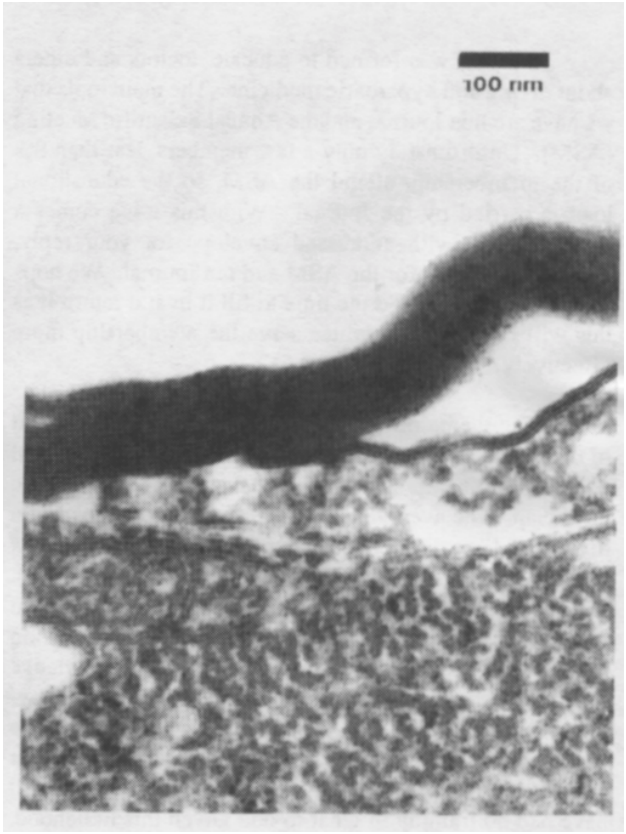

Figure 1. The alveolar lining showing an oligolamellar layer of surfactant both attached to the epithelial wall and detaching from it, although one extra lamellation remains adsorbed to the membrane. Note the traditional "tramlines" of membranes in deeper tissue structures.

the "packages" in which the body stores surfactant (and its proteins) in such a highly surface-active state, especially in the lung. In these "packages" surfactant is instantly available for recruitment to a bubble surface.

Another worrisome aspect of our recent spinal studies is our finding that bubble formation by decompression not only liberates DPPC from spinal tissue[1] but also a proteolipid which is known to be encephalitogenic, i.e. it promotes demyelination and is used to produce experimental animal encephalomyelitis (EAE). EAE is the animal model widely used for studying multiple sclerosis. Hence it is tempting to speculate that this finding might provide the link with multiple sclerosis (MS) in retired divers proposed by James[3] although, apart from one very impressive case known to me personally, published case histories are difficult to find.

To return to more positive aspects of surfactant, it has always amazed me that so little of the vast wealth of knowledge of industrial surfactants has been applied to

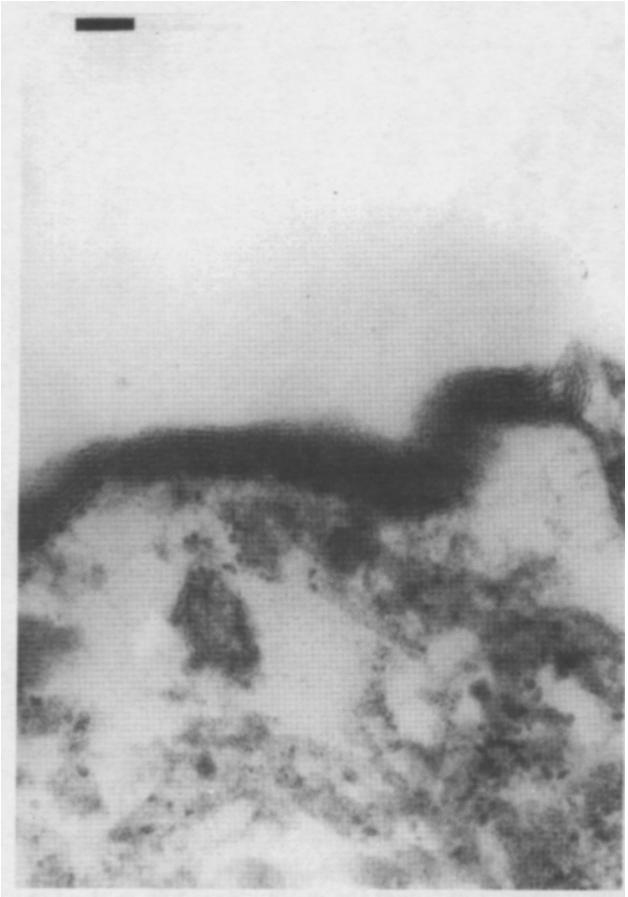

Figure 2. Gastric surfactant found at the interface between the gastric mucosa and gastric contents of the stomach. This layer appears to reform if gastric mucus is rinsed away leaving the stomach wall equally hydrophobic. The bar represents 50 nm.

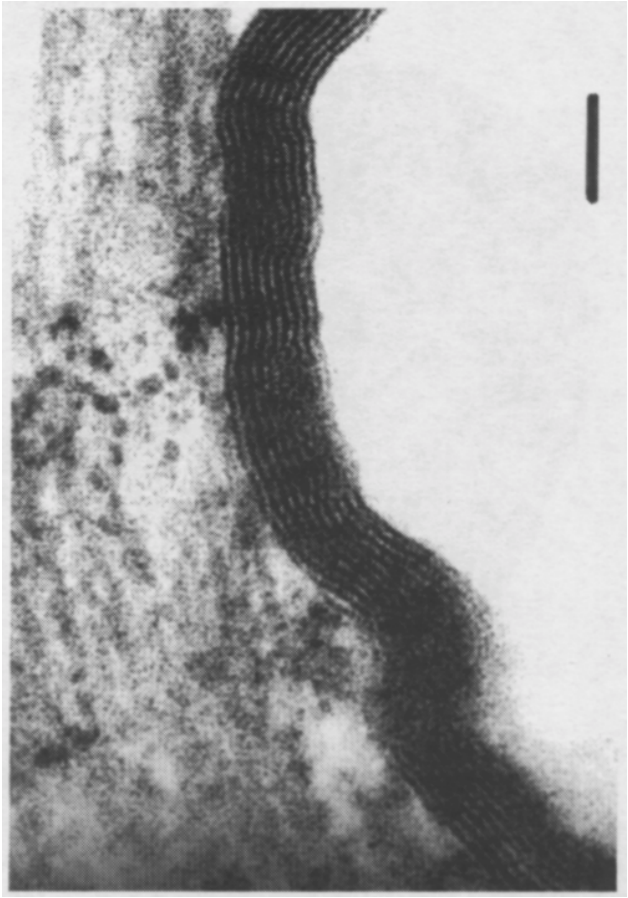

Figure 3. An oligolamellar lining of surfactant coating the epithelial surface of an oxyntic duct through which hydrochloric acid, pepsinogen, etc. at pH = 1 is secreted into the lumen of the stomach. The bar is 50 nm.

Physiology and Medicine and that what has been applied has been limited to the liquid-air interface. The major commercial application of surfactants nowadays is to solid surfaces, to which surfactants can bind reversibly by adsorption and, in so doing, impart many highly desirable properties. One of these is the inhibition of corrosion as typified by the monolayer which is the active ingredient in the underseal applied to motor cars to prevent rusting. Since the early days when we had identified a layer of surface-active phospholipid (SAPL) on the gastric mucosa as a characteristically hydrophobic lining[4] and its elimination by the “barrier breakers”, e.g. bile salts, NSAIDs, etc., there have been several new developments.

Since arriving at the University of New England my major effort has been devoted to the morphology of the adsorbed lining. Three decades ago Davenport[5] had proposed a “gastric mucosal barrier" of unknown composition to the back diffusion of acid, but this term has faded from the literature for lack of ultrastructural evidence. Our approach was to argue that the universal fixative (glutaraldehyde) used in a multitude of previous ultrastructural studies would never reveal any adsorbed SAPL because it is well known to destroy hydrophobic surfaces. Substituting tannic acid we were able to demonstrated a lining of SAPL over the gastric mucosa (Fig. 2). However, it was not a monolayer, as theory might predict, but an oligolamellar layer and also coated the oxyntic ducts, through which acid produced by the parietal cells (at pH = 1) pass into the lumen of the stomach (Fig. 3). Moreover, when we investigated the parietal cells and mucus-neck cells we found lamellar bodies virtually identical to those found in the lung (Fig. 4).

These findings led us (and others) to administer exogenous surfactant as an anti-ulcer remedy with moderate success. My next move was to look for a natural source of lamellar bodies, ideally a vegetable source, and this we found in the ripe banana. This, in turn, has led to a banana-based product which is currently undergoing clinical trials as a very inexpensive anti-ulcer product which avoids suppressing acid and the associated side-effects. To date, the results are most encouraging.

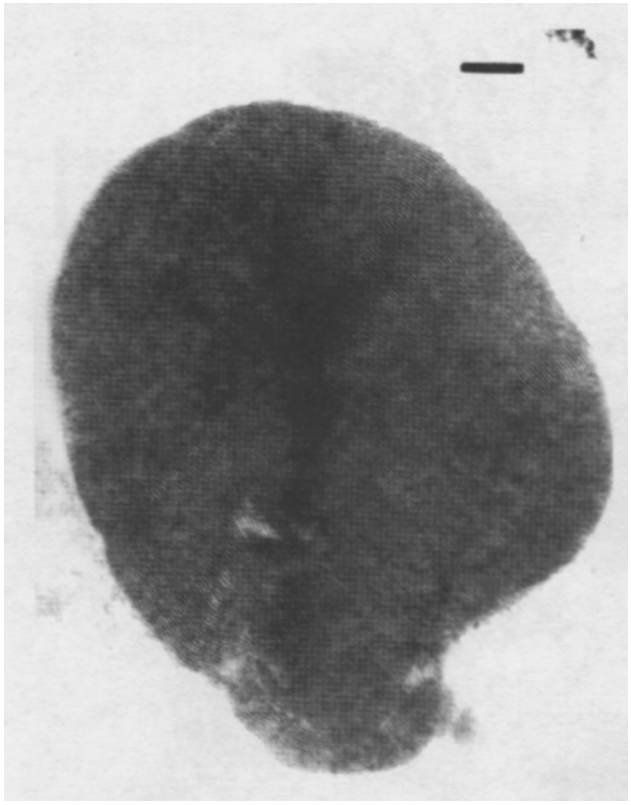

Figure 4. A lamellar body found within an oxyntic duct similar to those found within parietal cells, i.e. the cells which produce hydrochloric acid. The bar represents 100 nm.

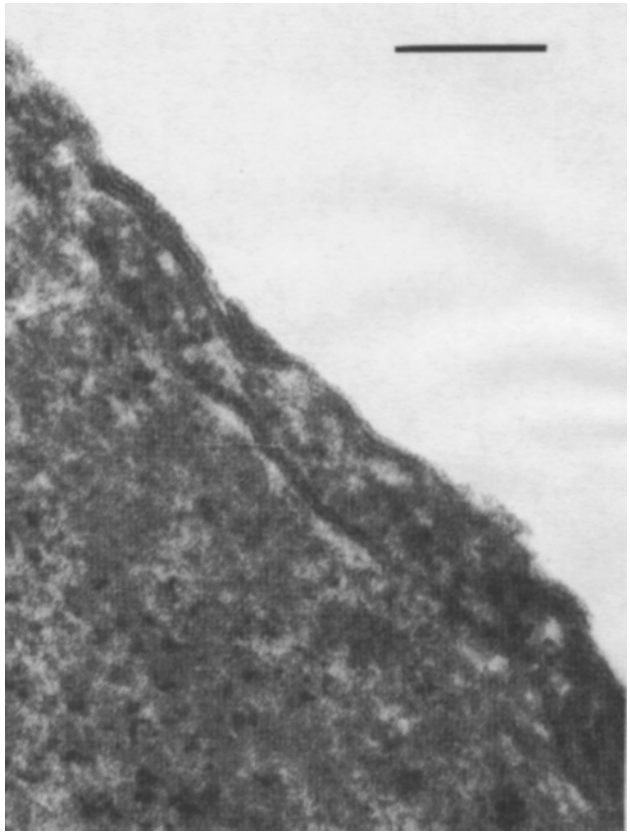

Figure 5. Lamellated surfactant coating the ocular surface and, presumably, adsorbed to corneal epithelium. This lining appears to render the eye hydrophobic causing the tear film to break up, even in saturated air, unless one blinks every 20 seconds or so. The bar represents 100 nm.

In ancillary gastrointestinal studies, I have identified surfactant in lesser quantities in the lower oesophagus, colon and in the duodenum, but only of patients with coeliac disease. Hence we have speculated that coeliac disease could result from unwanted surfactant effectively extending the gastric mucosal barrier into the duodenum where it now impairs absorption.[6] In a recent study we have found morphological evidence of surfactant lining the duodenal epithelium of these patients. Since the discovery in Perth, Western Australia, of *Helicobacter pylori* thriving in the highly corrosive environment of the stomach, and their association with peptic ulcer,[7] these bacteria have become a major, (and controversial) topic in the gastroenterological literature. Hence it was most interesting when our ultrastructural studies displayed how *H. pylori* derive protection against digestion in the stomach by adopting the same defence as the gastric mucosa, i.e. an oligolamellar layer of surfactant. Another surprise was to find essentially the same coating on Barber's pole worms. These parasites thrive in the highly acidic conditions of the abomasum of some ruminants making them the scourge of the Australian sheep farmer.

Another very hydrophobic surface is the articular surface on which we demonstrated adsorbed SAPL and the remarkable lubricating properties of this material.[8] It could reduce the coefficient of kinetic friction (μ) to 0.001 at high load (13 kg/cm^2) and low velocity which is phenomenal when compared with the best man-made boundary lubricants, e.g. Teflon for which $\mu = 0.04$. When applying our novel fixation procedure for electron microscopy, it was fascinating to see the same oligolamellar layers, highly reminiscent of graphite or molybdenum disulphide, which are used widely in many industrial lubricants. We have now found this same graphite-like layer on other sliding surfaces *in vivo*, such as the pleura and cornea (Fig. 5). This has obvious clinical applications to the irrigation of arthritic joints and better artificial tears, both of these applications are being pursued in clinical trials.

Another possible application of lubrication by SAPL concerns premature rupture of the membranes (PROM) which, when it occurs before term, is often termed the enigma of the obstetrician. Amniotic surfactant is an excellent load-bearing lubricant which also deposits a graphite-like layer on the chorioamnionic sac.[9] Hence it was interesting to find a very good correlation between pre-term PROM and lack of lubricity for placentas of the same gestational age. When surfactants impart lubricity to

a surface, they also reduce sticking, acting as release agents, and this could apply to any potential for adhesion of the foetus or maternal viscera to the membrane to cause the local mechanical stress required to initiate rupture.

Returning to diving, there is an interesting application of surfactant in aural barotrauma since we have demonstrated it lining the Eustachian tube where it is a very good release agent.[10] This is also relevant to serous otitis which can occur if the inner ear is not ventilated every 20 minutes or so. Hence the application of exogenous SAPL offers a challenge to the otolaryngologist.

Another surprising finding with implications to underwater medicine is our discovery of the same oligolamellar layer lining cerebral endothelium, rendering it hydrophobic.[11] This has several implications for the diver during decompression. Firstly, it offers a hydrophobic surface which is more conducive to bubble formation as demonstrated so simply and elegantly in the 1950s when Harvey[12] produced profuse bubbling upon plunging a candle into soda water. The second implication is much more speculative in so far as this SAPL lining might provide the elusive blood-brain barrier (BBB).[11] A liquid-air interface in the form of a circulating bubble could be expected to compete much more successfully than endothelium for its SAPL coating in which case, it would open the BBB. This very serious action of circulating bubbles has been known since the work of Broman[13] and, more recently, emphasised by Gorman[14] to explain the neuropathology they cause. It is my contention from early studies of air embolism[15] that small isolated bubbles transverse the cerebral circulation, but clusters of bubbles coalescence to cause the infarction postulated since the work of Paul Bert in the 1840s.[16]

Returning to the lung, and pulmonary barotrauma in particular, it was interesting to find surfactant not as the monolayer located at the surface of the liquid lining but as rafts of solid surfactant either floating at that interface or adjacent to alveolar epithelium. In either case they appeared capable of sealing small pores connecting the air space to deeper structures and even to the vascular space.[17] This could shed some light upon the question asked over a century ago by Ewart and Kobert:[18] "ist die Lunge luftdricht?" for which his answer was "nein". The rafts of surfactant might act as flap valves to raise the overpressure before air could enter the pulmonary vasculature.

Our latest study of the lung has pursued the hypothesis that there is an additional role for surfactant in the lung apart from those traditionally attributed to it. This is attributed to the large hysteresis in surface tension (γ) versus surface area (A) during the respiratory cycle and, hence, a large hysteresis in the collapsing pressure acting upon stretch receptors in the lung as its volume changes. Hence there is a hysteresis in the afferent neural input to the brainstem vital for normal respiration which would enable the respiratory pattern generator to distinguish between inspiration and expiration at the same lung volume. Hence it was particularly exciting to find that surfactant samples obtain from SIDS victims or from infants experiencing recurrent cyanotic episodes had γ:A loops cycling *anticlockwise* rather than in the normal *clockwise* direction.[18] Thus the brainstem would receive a highly confusing neural feedback from the lungs and might even mistake expiration for inspiration, which would fit the clinical findings. Even if this hypothesis proves incorrect the reversal of γ:A hysteresis offers a simple test of those infants at risk of SIDS, enabling them to be monitored carefully until they have outgrown the problem. This test would be particularly useful for detecting the risk *at birth* by using nasopharyngeal aspirates routinely obtained by the standard clinical practice of suctioning neonates during delivery. These aspirates are currently flushed down the drain but they actually provide a very good sample of lung surfactant and one routinely available just at the age when it is needed. I have joined the staff of the Mater Children's Hospital in Brisbane to pursue this very exciting avenue in more detail.

In conclusion, the very wide range of diseases discussed above emphasises the ubiquity of surfactant, while there are other locations in the body in which we have identified it such as the kidney and the cochlea. The area of immediate clinical application, however, is the ubiquitous barrier it offers to potentially corrosive and abrasive agents.

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UNINTENTIONAL CARBON MONOXIDE POISONING FROM CHARCOAL BARBECUES

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Summary

In two weeks in the winter of 1993 eight members oftwo families suffered paoisoning with carbon monoxide produced by charcoal barbecues used indoors to provide heating. Following these incidents a review of the literature was carried out. This paper presents clinical details and reviews the literature. Recommendations are made for public health measures to combat this unintentional form of CO poisoning.

Introduction

Carbon monoxide (CO) is an agent frequently used for deliberate self-harm. Poisoning with CO is the commonest cause of death by completed suicide in industrialised nations.[1-3] Unintentional CO poisoning is also common although the incidence is more difficult to determine. Many cases are probably unrecognised.[4-6] Dolan suggests that it is the protean nature of symptoms of CO poisoning which causes its true incidence to be grossly underestimated.[7] It is likely that many cases are also missed at autopsy because the pathological lesions are non-specific.[8] Common mechanisms include leaky motor vehicle exhausts, faulty home heaters, and inadequate ventilation around appliances producing CO.[1,9-11]

This paper describes two non-English speaking families who presented to the Emergency Department of Fremantle Hospital approximately two weeks apart in the middle of winter 1993. Eight people, six in one family and two in the other, suffered CO poisoning following indoor use of a charcoal barbecue for heating. There have been occasional reports of CO poisoning in similar circumstances in the medical literature. In January 1994, a large series of 79 patients was reported from Washington.[12] CO poisoning from charcoal barbecues has not previously been described in Australia.

The cases reported here illustrate the ease with which accidental CO poisoning can occur. This leads to a review of the epidemiology and effects of unintentional CO poisoning with particular emphasis on chronic exposure which has thus far not been discussed in detail in the literature.

Case reports

FAMILY A

This family of six (mother 24 years old, father 37 years old, two daughters 11 and 9 years old, and two sons, 10 and 6 years old) presented to the Emergency Department at Fremantle Hospital at 0400 hours on 30 June 1993. They had taken a charcoal barbecue indoors to use as a heater overnight after cooking the evening meal. In the early hours of the morning, the mother lost consciousness on the way to the toilet. On regaining consciousness after an estimated period of four minutes, she alerted the rest of the household. Her 11 year old daughter was roused but lost consciousness twice for a total of 15 minutes and vomited once while being taken outside the house. The six