SPINAL DECOMPRESSION SICKNESS: THE OCCURRENCE OF LAMELLAR BODIES IN SPINAL TISSUE AS POTENTIAL FOCI FOR BUBBLE FORMATION

Brian Hills

Abstract

A novel fixation method designed specifically to preserve lamellated phospholipid structures has been used to demonstrate lamellar bodies in the spinal tissue of sheep by transmission electron microscopy. The extreme surface activity long associated with these structures in the lung would make them prime agents for initiating bubble formation. Hence their widely differing incidence and distribution within sections, between sections and between subjects indicates that they could be a major factor in determining individual susceptibility to spinal decompression sickness.

Introduction

Spinal manifestations of inadequate decompression of divers are not only more likely to occur that other forms of neurological decompression sickness (DCS)[1] but are also more likely to result in residual injury. While the pathology of spinal DCS is well documented[2-4], the basic mechanisms whereby the separation of gas from solution can give rise to these potentially debilitating lesions remains a most important yet controversial issue.

The fact that only 2% of blood flow to the central nervous system goes to the spinal cord[5] has been put forward[6,7] as strong evidence against any mechanism based upon arterial embolism. At least, this assumes that embolism is synonymous with infarction, which may not be the case in view of recent evidence of the ability of arterial bubbles to pass through brain tissue[8]. Whether justified or not, the search for mechanisms beyond arterial embolism led to the theory of venous occlusion at the level of vertebral venous lakes[6]. This theory and others invoking infarction were disputed on several grounds, one being the experience that spinal symptoms are not only reversed by recompression but are repeatedly pressure-reversible[7]. Since recompression has been observed to dislodge bubbles occluding blood vessels[9], it is hard to envisage a subsequent decompression causing another shower of intravascular bubbles to lodge or form in the same sites, at least, not to the extent that the symptoms and their distribution are identical to those caused by the initial decompression. Such arguments would favour location the offending gas in extravascular sites in which fixed position the same gas could repeatedly reverse the same symptom distribution simply by compromising and restoring local blood flow as its volume changed in accordance with the decompression/recompression protocol being followed. Mechanical studies of the spinal cord[7] proved compatible with this mechanical approach to spinal DCS but still leaves open two vital questions. The first is why spinal tissue should be so prone to bubble formation upon decompression, while the second concerns why certain individuals should be more susceptible than others.

There are the obvious factors predisposing the spinal cord to bubble formation such as the higher degree of gas supersaturation which would arise upon decompression in any tissue with a relatively low blood perfusion rate. Then there is the high lipid content, especially where white matter exceeds grey. In the original studies of Haldane's group[10] their counts of bubbles in the cords of goats at different vertebrae correlated well with the white:grey ratio. However there would still appear to be some other factor which causes some spinal sites in some individuals to be so much more conducive to bubble formation than others.

Turning to basic physics, Yount[11,12] has emphasized how surfactants can stabilise bubbles and preserve macronuclei, pointing out how surface-active phospholipid (SAPL) is present in vivo. SAPL is the predominant and most active ingredient in the mixture of saturated phoshatidylcholine, other phospholidpids, unique proteins and other minor components simply known as "surfactant" in the lung.[13] In this organ, SAPL it has long been studied for its surface activity which is very high by comparison with many surfactants studied in the physical sciences.[14] Its ability to reduce the surface tension of the air-aqueous interface is not only thermodynamic but also kinetic. The alveolar Type II cell produces "surfactant" in truly remarkable "packages" known as lamellar bodies (LBs)[15] from which the highly active SAPL is instantly recruited to the interface as they "pop to the surface".[16] These lamellar bodies would thus be particularly conducive to initiating bubble formation upon decompression of a tissue or stabilising bubbles or their nuclei upon compression.

Upon decompression, LBs should pose no problem in the lung where there is never any significant supersaturation of gases due to virtual equilibration of parenchymal tissue with the environment. In other tissues, however, their presence during decompression could be much more serious. In recent studies in this laboratory of novel roles for surfactant, we have found LBs in parietal cells from which their secretion could enable SAPL could provide the gastric mucosal barrier[17,18] and in synovial fluid in which SAPL could provide the elusive load-bearing lubricant of the joints.[19,20] Moreover, in a review[14] of the morphological literature of other organs, lamellated shapes strongly resembling LBs can often be seen in electronmicrographs which the authors ignore or, occasionally in passing, describe them simply as "whorls" to which they attribute no functional significance.

This study was designed to determine whether there are any lamellar bodies in spinal tissue. A secondary reason was to search for any vascular lining of oligolamellar SAPL

which we have recently demonstrated in brain[21] where it is conceivable that it could be providing the blood-brain barrier, a barrier long known to be opened by any circulating bubbles.[22,23]

Materials and method

MATERIALS

The source of spinal cord was three healthy 4 year old sheep killed by stunning with a captive-bolt gun followed by exsanguination. Within 15 minutes of death transverse sections of the spinal cord were excised at levels T4 and L1 and placed in the primary fixative in preparation for transmission electron microscopy. These locations were selected as the two vertebrae most commonly implicated for spinal DCS in man[24], although not necessarily the most vulnerable in sheep.

FIXATION

Standard fixation procedures based upon glutaraldehyde[25] are the worst for preserving lamellated phospolipid since surfaces which they coat are often hydrophobic[14,17,20] and aldehydes, especially glutaraldehyde, are known to destroy hydrophobic surfaces.[26] In this study glutaraldehyde was reduced to 2% by substituting tannic acid (3%) shown to be ideal for visualizing oligolamellar phospholipid and used so effectively for demonstrating such structures in the lung.[28] The fixative was buffered to a pH of 7.4 with 0.1 M sodium cacodylate at 4 °C and rendered isotonic with CSF from the same cord by adding NaCl. Special attention was paid to isotonicity to avoid 'peeling' of any hydrophobic lining as described elsewhere.[14]

Another major departure from standard procedures was a very long (72 hours) fixation time based upon the simple reasoning[18,21] that barriers such as the blood-brain barrier are characterised by their impermeability to water-soluble solutes and this category includes fixatives. Post-fixation was effected with 1% osmium tetroxide buffered at a pH of 7.4 with embedding in resin (Spurr mix 'A'; Probing & Structure, Kirwan, Queensland) polymerised at 60 °C. Emphasis was placed upon cutting very thin (<60 nm) sections with a very sharp diamond knife in order to resolve lamellated structures. For comparison purposes two blocks were fixed from cerebral cortex of one sheep.

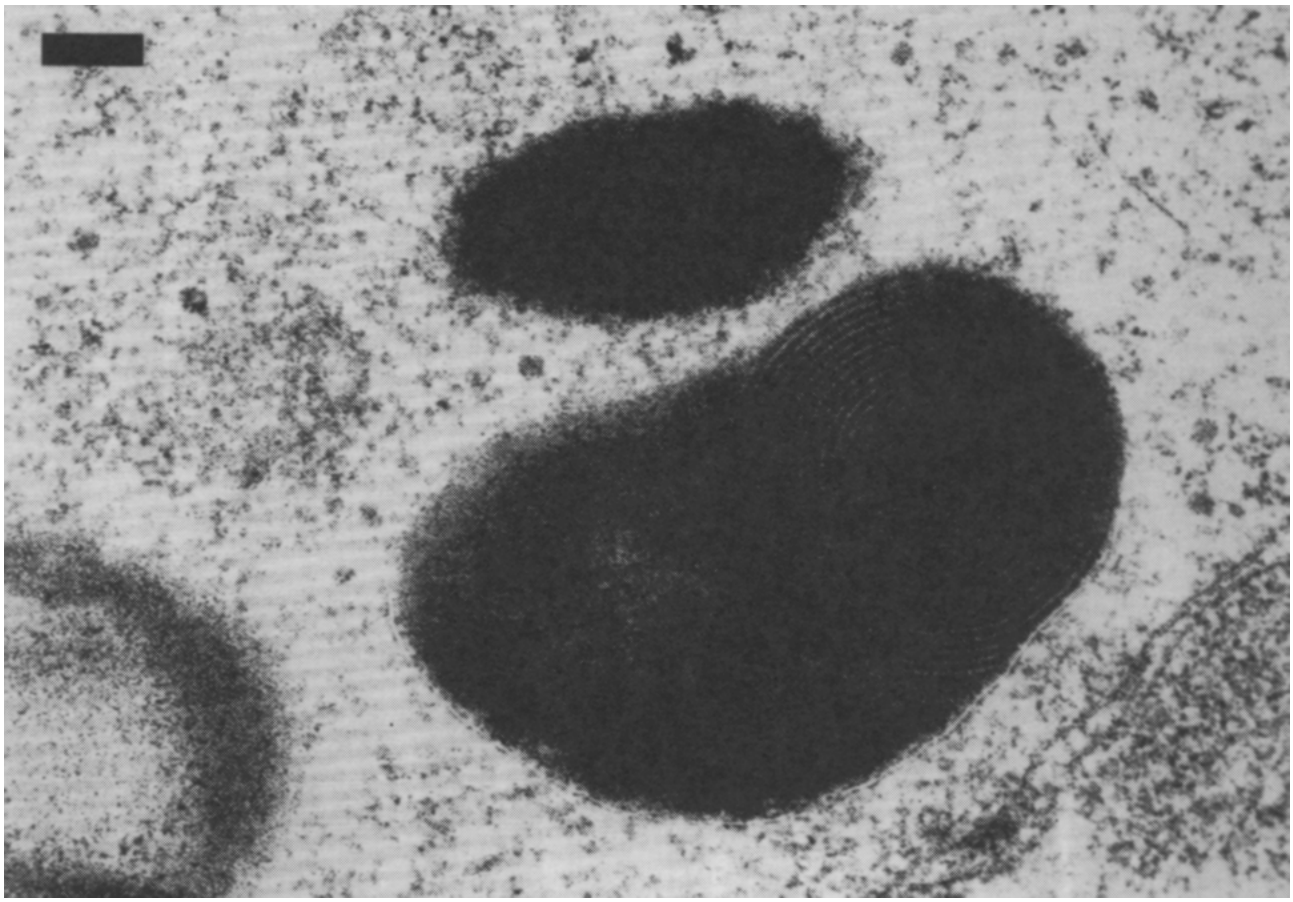

Figure 1. A transverse section of sheep spinal cord in the vicinity of an arteriole clearly depicting a lamellar body. Note the outer enveloping membrane into which the coil of phosholipid does not insert as occurs with lamellar bodies of highly active surfactant production in the lung by the alveolar Type II cell.[29] The bar represents 100nm.

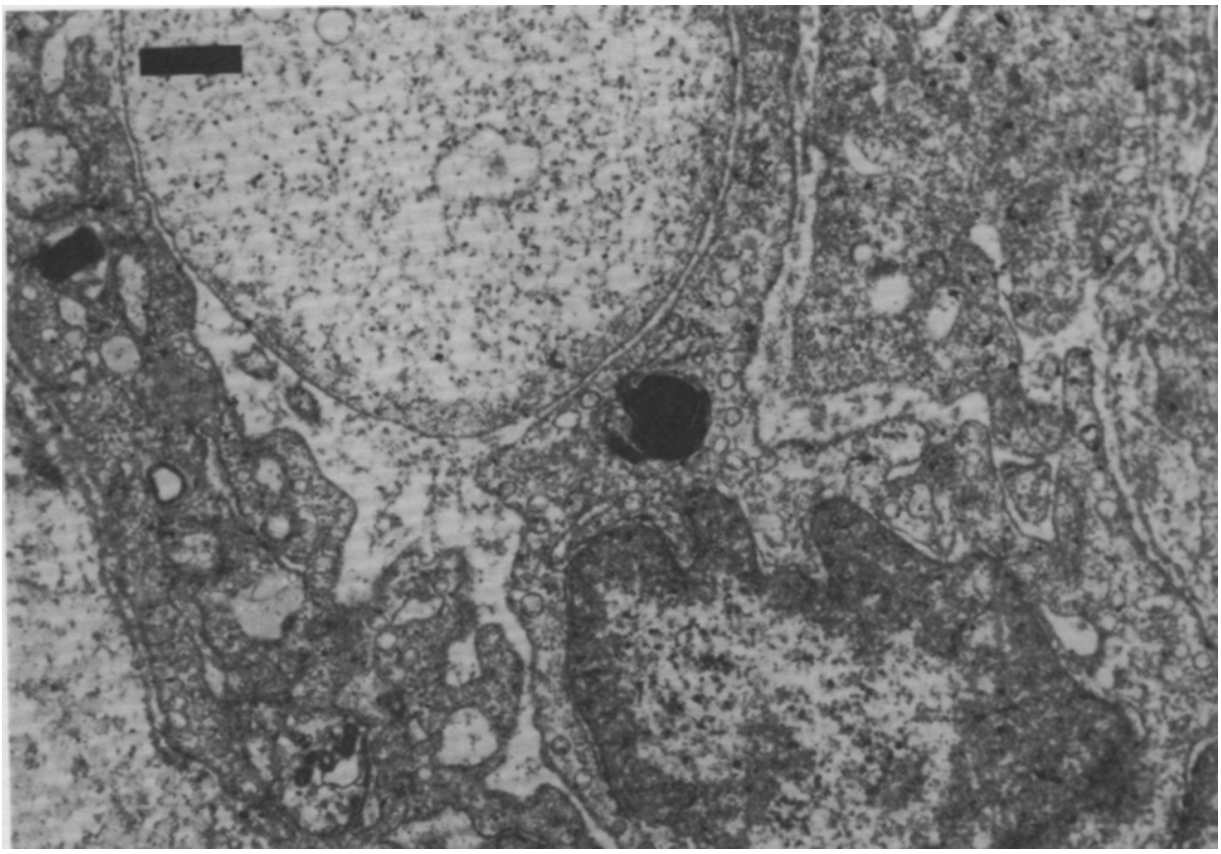

Figure 2. A transverse section of sheep spinal cord showing a lamellar body at lower magnification. The bar represents 500nm.

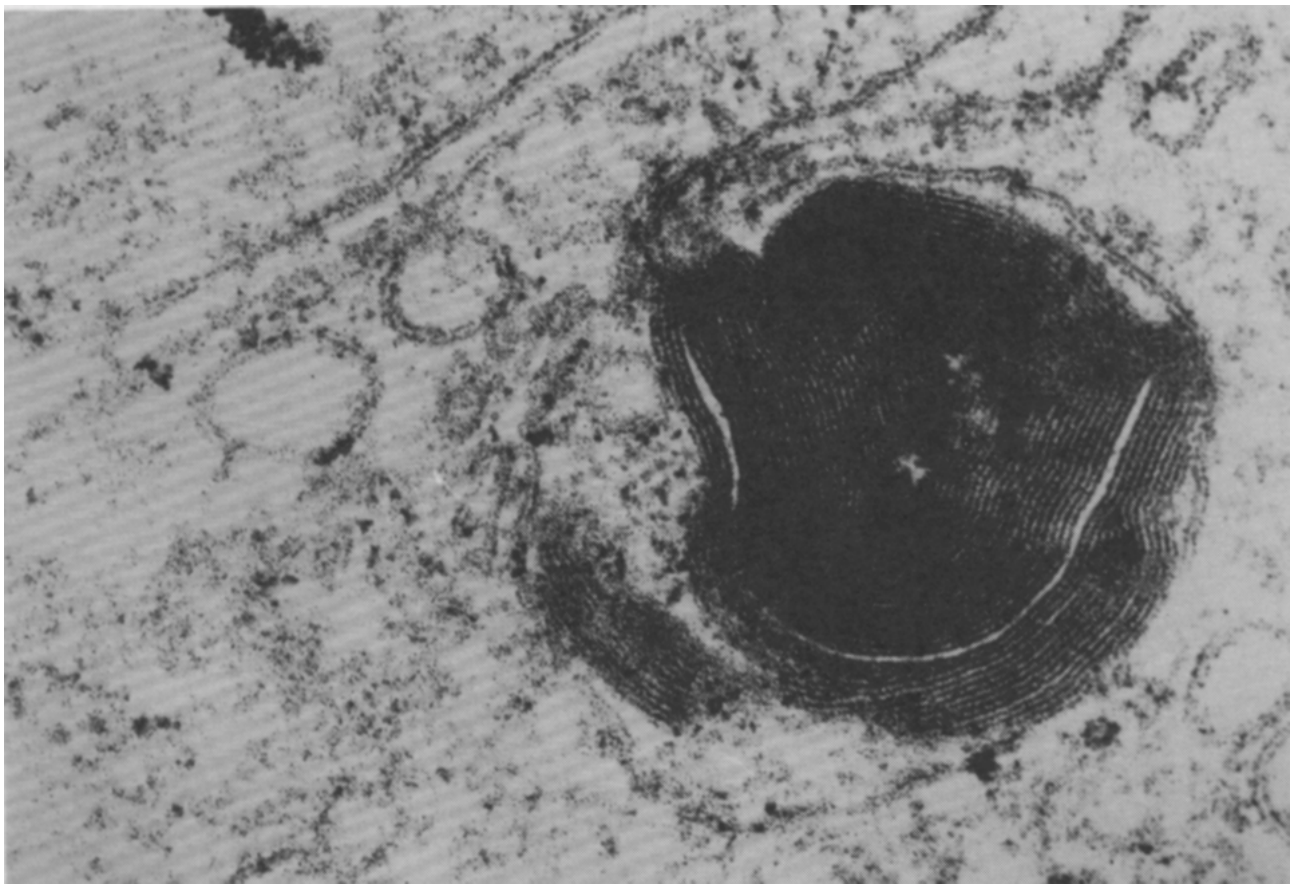

Figure 3. The same lamellar body shown in Figure 2 shown at higher magnification. Note the mutliple foci which, if found in the lung, would be termed a multilamellar body to which greater surface activity is attribute.[29]

Results

In two out of three animals lamellar bodies were clearly discernible in spinal tissue as shown in Figure 1. There were also a number with multiple foci known in the lung as "multi-lamellar bodies".[29] At lower magnification, these lamellar bodies can be seen in several tissue locations, including perikaryon, endothelial cells and peri-vascular glia (Figure 2), the lamellated structure and multiple foci being clearly discernible at higher magnification (Figure 3).

These lamellar bodies have a solid core which distinguishes them from vesicles such as lysosomes (Figure 4) seen in comparable numbers in perikaryon and as conglomerates of both (Figure 5) reported in perikaryal cytoplasm of rat cortex as "lipofusin granules".[30] At higher magnification the laminated nature of this complex is clearly discernible (Figure 6). Occasionally these hybrid granules can bee seen clustered quite close together (Figure 5).

Focus upon the vascular lining was frustrating, demonstrating a quite strongly osmiophilic vascular lining to endothelial cells (Figure 7). Upon higher magnification, however, this could not be resolved to reveal an oligolamellar phospholipid lining of the form recently discovered in sheep cerebral cortex.[21]

Discussion

Figures 1-3 leave no doubt that lamellar bodies are present in spinal tissue with a high proportion being multi-lamellar bodies. This is particularly interesting since, in the lung[29], these are even more surface active than regular LBs and should therefore be even more conducive to initiating bubbles upon decompression. The extreme surface activity of LBs and the unique form of this "packaging" can be appreciated from the ability to simulate "dry" surfactant as opposed to the "wet" form discussed by Bangham and co-workers.[31] The "dry" form has been credited with the ability to reduce the surface tension of water rapidly from 72 dynes/cm (mN/m) to "near zero"[32] or, even if such values are artifactually low[33], to less than 8 dynes/cm for a condensed monolayer[34].

Lysosomes would be expected to have the relatively low surface activity of other hollow-core vesicles such as liposomes of SAPL which typify "wet" surfactant.[31] It is a moot point whether conglomerates, seen in Figure 6, of lamellated phospholipid with multiple hollow and multiple solid cores, i.e. lipofusin granules, would be as surface active as LBs alone. Some indication might be afforded by the presence of very similar conglomerates in parietal cells from which their secretion to afford acid-protection by

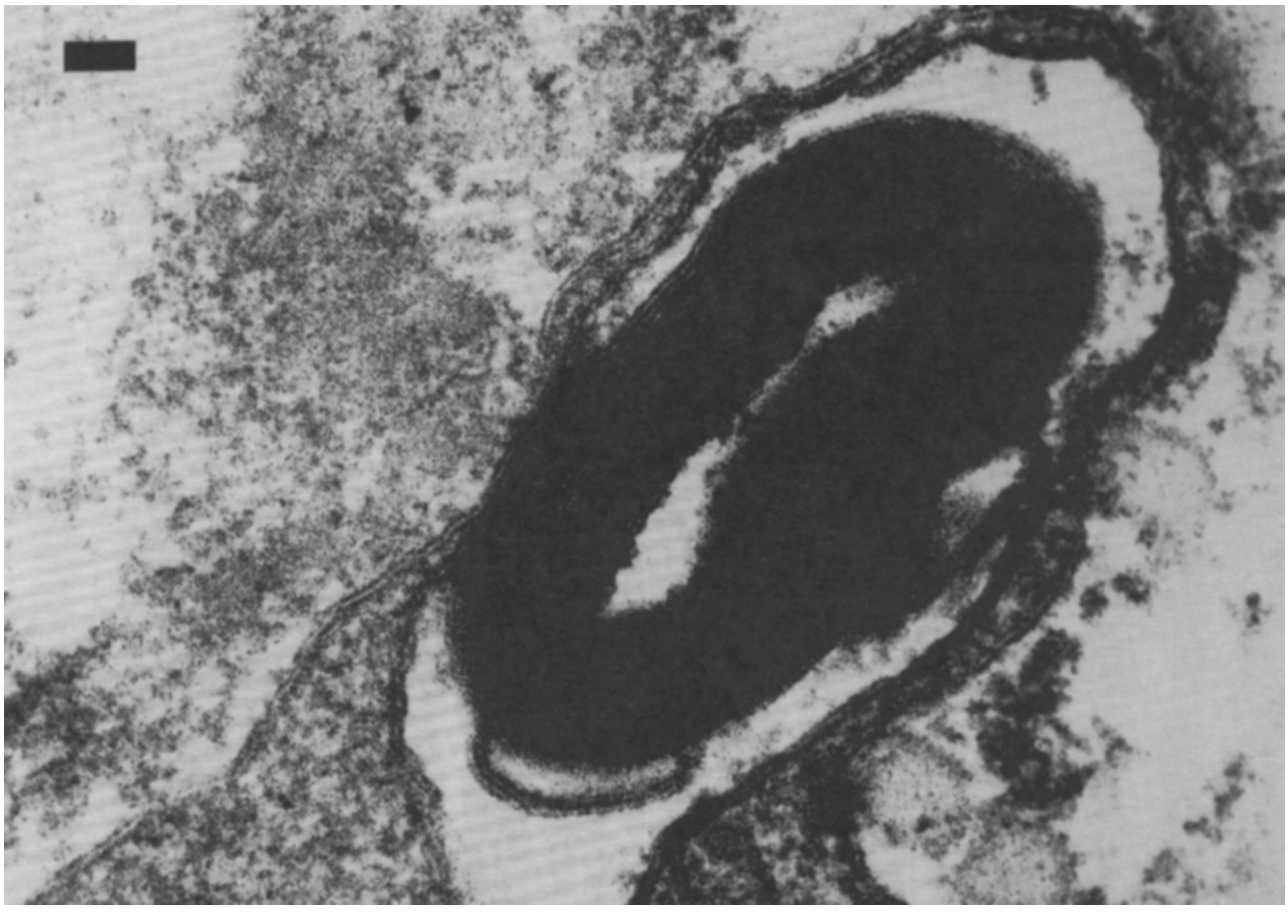

Figure 4. A transverse section of sheep spinal cord displaying a vesicle differing from lamellar bodies by posessing a hollow core. The bar represents 50nm.

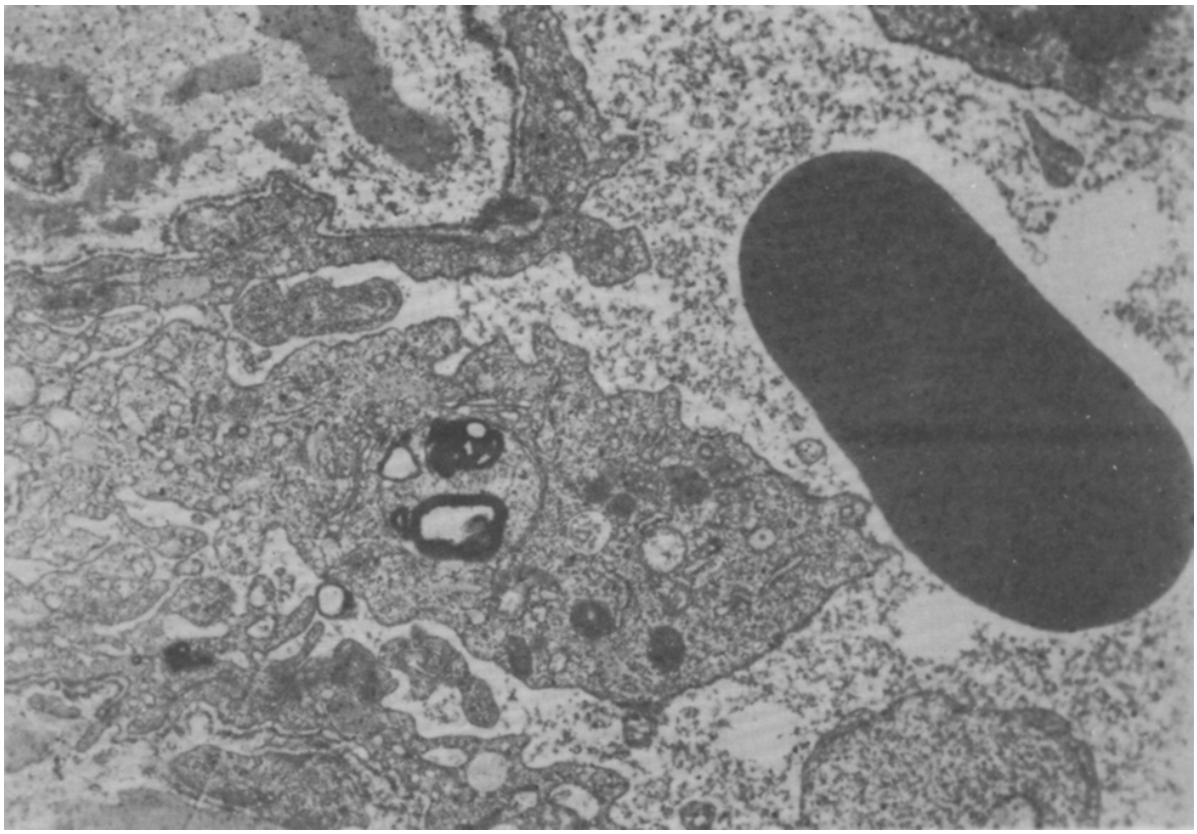

Figure 5. A transverse section of sheep spinal cord displaying an array of visicles clustered quite closely together in some areas and absent in others. Some of these vesicles resemble "lipofusin granules" previously reported in the brain.[30]

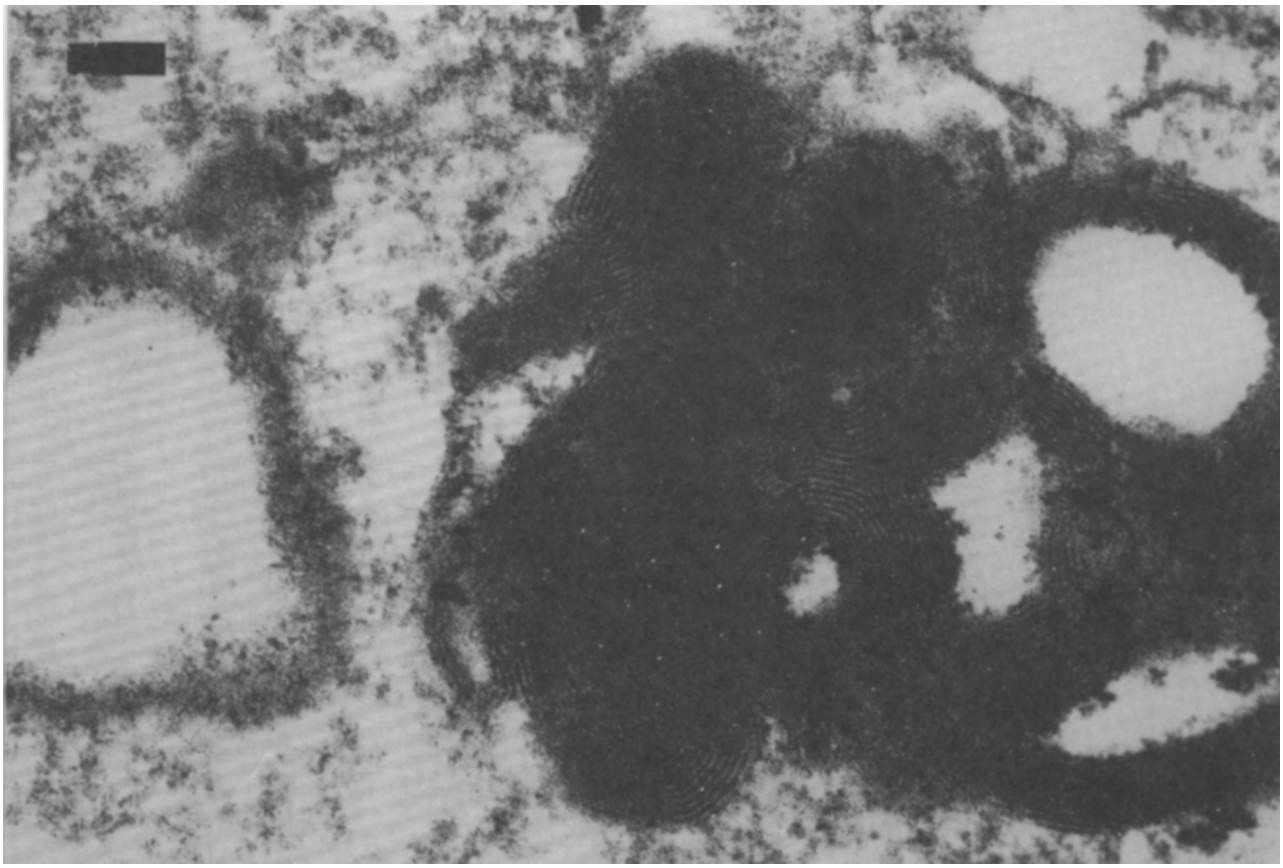

Figure 6. A "lipofusin granule" from Figure 5 shown at higher magnification when it can be seen to be a conglomerate of both solid- and hollow-core vesicles with phospholipid lamellations entwined one to another. The bar represents 50nm.

adsorption of SAPL to the apical surfaces of gastric epithelial cells has been attributed to their surface activity.[18] The same surface activity which renders SAPL or other surfactants so effective in reducing the surface tension of air-water interfaces is also effective at solid surfaces, functioning by adsorption to the surface.[14] The tendency for lipofusin granules to occur in clusters (Figure 5) or to be absent from a sequence of serial sections demonstrates a very variable incidence and distribution. It is also interesting that, in rats, these granules have been reported[30] to increase in number with age.

The propensity for bubbles to form in lamellated phospholipid is reflected in the fenestration of myelin seen upon autopsy of divers[2] and the observation in many decompressed animal tissues that bubbling occurred almost exclusively in the myelin sheaths.[35]

The similar variability in the incidence and distribution of lamellar bodies (Figures 2 and 5), and their potential for extreme surface activity, indicates that this could be a major factor in determining the distribution of bubbles for a given degree of tissue supersaturation by gas and could have a major influence in determining individual susceptibility. Other factors such as the white:grey matter ratio and random features of the microcirculation, such as intermittent perfusion[36], must also be important in determining the local level of supersaturation and, hence, separation of gas from solution. However, these factors would not appear as variable as the incidence and distribution of LBs and their conglomerates.

If LBs are a major factor in determining individual susceptibility, it could explain one very puzzling observation. This is the propensity for neurological DCS to occur upon surface decompression as discovered using goats in carefully titrated decompressions[37] and subsequently confirmed by much field experience. It was found that any "upward excursion" at the start of decompression predicted to induce supersaturation for however short a period, e.g. 1 minute[37], caused the presenting symptoms upon titration of the decompression to be neurological rather peripheral, i.e. Type II rather than Type I DCS. Some mechanism must be "triggered" during an upward excursion or surface interval and it is tempting to suggest that the LBs in the CNS are "activated" into bubble formation.

The tantalising question arising from this electron microscopy is what normal physiological function could be attributed to lamellar bodies in spinal tissue. A major search of the literature has revealed LBs in conventional electron microscope studies of many tissues, including spinal tis-

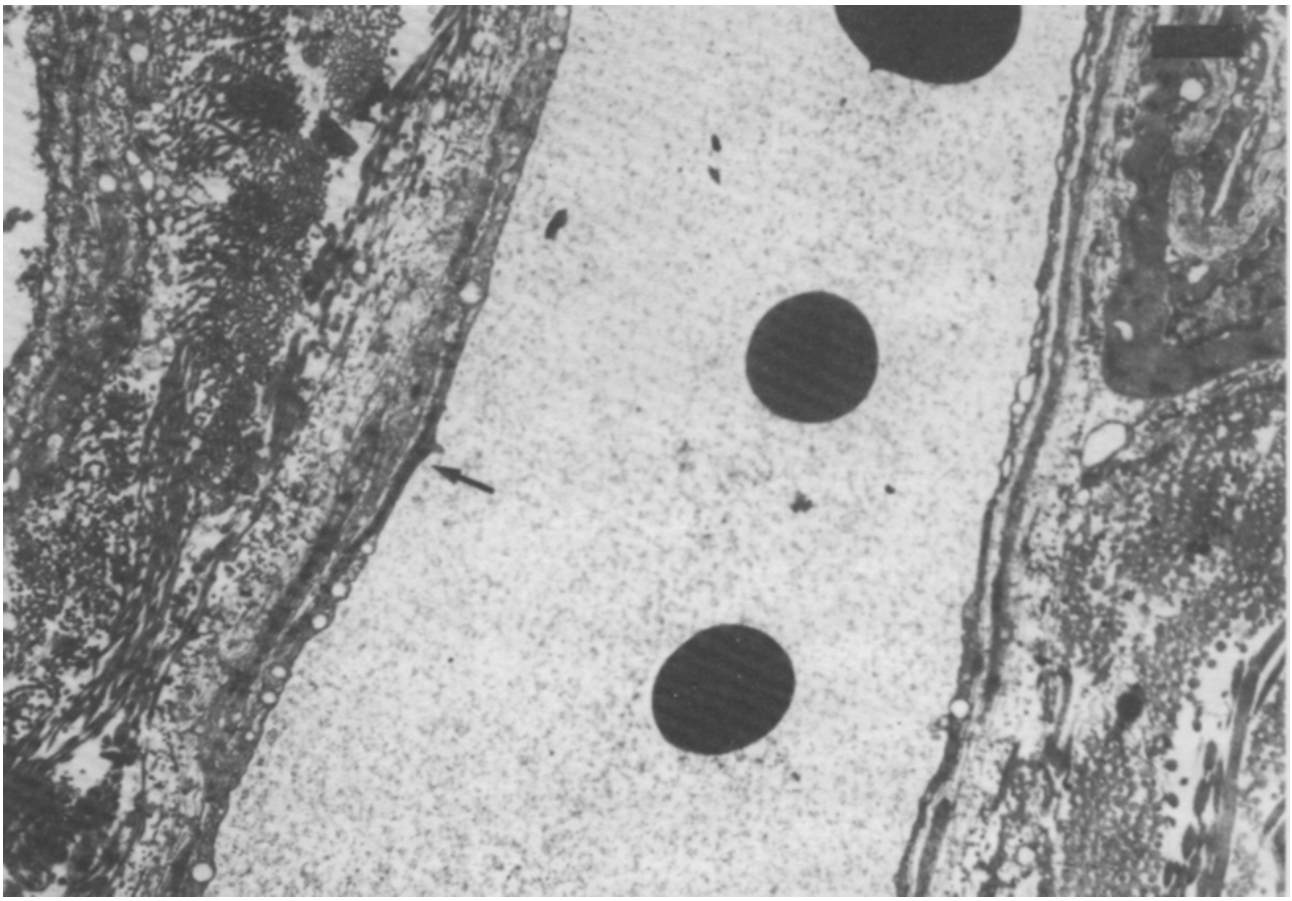

Figure 7. A longitudinal section of a venule in sheep spinal cord. Note the osmiophilic nature of the endothelium indicated by the arrow. The bar represents 2 μm.

sue[30], but they are usually ignored or dismissed by some morphologists as the membranous remains of dead cells. This explanation is difficult to accept even for lipofusin granules within non-phagocytic cells, but the remarkable similarity to LBs in the alveolar Type II cell indicates that they have been produced for a purpose. One possible function reflecting their propensity for peri-vascular sites is to provide or enhance an endothelial lining, a high incidence of LBs having been reported in aortic endothelial cells.[38] Such a lining has been implicated by factors influencing blood pressure[33] and by electron microscopy of cerebral cortex.[21] It was therefore tantalising to demonstrated an osmiophilic luminal lining to the endothelium of spinal tissue (Figure 7) and yet not to the able to resolve it with higher magnification into the oligolamellar structure seen in cerebral cortex. On the other hand this difference might explain why spinal tissue is more susceptible to circulating bubbles known to open the blood-brain barrier[22,23], despite receiving only 2% of those entering the CNS on the basis of blood flow distribution.[5]

In conclusion, it was surprising to find lamellar bodies in spinal tissue, but their widely differing incidence and distribution might prove a useful lead in explaining the variation in individual susceptibility to spinal DCS. If nothing more, this study provides morphological evidence of surfactant in a highly surface-active state for the type of bubble, and nucleus - stabilization long emphasized by Yount.[11,12]

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COMPUTER RECREATION

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All diving computers continually sample pressure and time, either using an algorithm, or a look-up table, to determine approximate nitrogen saturation from a theoretical model of the human body. Dissolved nitrogen is estimated in a series of tissue compartments ranging from one to many (there is an infinite continuum in which nitrogen may dissolve) which for mathematical simplicity are usually limited to about 6 to 12.[1,2] The concept of tissue compartments and the mathematical model of nitrogen uptake and elimination were derived by J.S.Haldane.[3] Some dive computers provides a record of the diver's depth-time profile and this can later be used to review the dive profile, and for comparatively accurate estimations of air consumption. These results in turn allow detailed dive planning.

Computer models of breathing gas usage and nitrogen gradients are a useful means of presenting graphically what may happen during a dive. They are idealised and hence cannot be used as an accurate representation of physiological reality.

This paper grew from electronic doodling using a spreadsheet (Microsoft Works) with an accompanying charting program. It is easy to calculate variations associated with nitrogen uptake and air consumption. I have used an IBM compatible with only 1 megabyte of random access memory, so my models have been limited to 5 tissue compartments with half-times of 2.5, 5, 10, 20 and 40 minutes, but these are the ones relevant to sports dives of less than 60 minutes duration and not suitable for repetitive dive calculations.

Using a computer to estimate air consumption

Obviously air consumption is dependent on the amount of physical work the diver does and his or her breathing rate. The latter is dependant on the pH of the CSF (which depends on blood CO_2 levels), the partial pressure of oxygen in the arterial blood (both are affected by exercise) and the psychological state. Lippmann[4] gives a method of calculating air consumption in his book that is limited in accuracy by the approximation to a trapezoidal dive profile.

Using a computer that replays a depth-time profile will give 3 minute samples of depth that allows the derivation of a weighted average of the depth (or an estimate of the integral of the depth/time curve). Table 1 provides such a profile.

In this table the respiratory minute volume (RMV) has been calculated (it is directly reproduced from the spreadsheet). The method of calculation is: