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Light and electron microscopic alterations in spinal cord myelin sheaths after decompression sickness

J. J. W. SYKES and L. J. YAFFE

Hyperbaric Medicine Program Center, Naval Medical Research Institute, Bethesda, MD 20814

Sykes JJW, Yaffe LJ. Light and electron microscopic alterations in spinal cord myelin sheaths after decompression sickness. Undersea Biomed Res 1985;12(3):251–258.—Pathological examination of spinal cords from animals subjected to experimental decompression sickness (DCS) was undertaken in an attempt to explain the disparate response to treatment observed. Eight experimental animals, four undived control animals, and two dived but untreated animals were perfusion fixed, and the spinal cords were removed. Light microscopy of toluidine blue stained, ultrathin sections from dived animals demonstrated a distinctive widened myelin sheath showing a banded pattern of myelin disruption. This pattern was confirmed by electron microscopy and showed the separation to be between abutting double layers of myelin. Artifactual changes were also present in dived and undived animals. These previously unreported changes may be caused by DCS. They are compatible with the major mechanisms proposed in the pathophysiology of spinal cord DCS and may also account for the response to treatment seen in our experimental animals. It is suggested that these findings may also explain the response to treatment seen in patients, together with the formation of late lesions described in the spinal cords of long-term survivors of DCS.

decompression sickness	myelin
evoked potentials	light microscopy
spinal cord	electron microscopy

Despite major advances in the treatment of spinal cord decompression sickness (DCS) in the last 50 yr, there remains a small, but important, group of patients that fails to respond or only responds partially to recompression treatment. Although a poor outcome of treatment can be anticipated if considerable delay occurs between the onset of symptoms and treatment (1,2), it is recognized that even under the very best of circumstances some patients do not recover fully. The reasons for this apparent failure of treatment are not well understood. Conventional views on the pathophysiology of decompression sickness account for many of the phenomena observed in patients, but do not explain adequately why some patients fail to respond to treatment.

In a series of experiments designed to study treatment regimens for spinal cord decompression sickness, we used an animal model in which spinal evoked potentials

(SEP), generated from peripheral nerve stimulation, provided a measure of spinal cord function. The changes in SEP were recorded during the initial dive, during the subsequent development of spinal cord decompression sickness, and, as an index of its efficacy, during the application of recompression therapy. Initial results from a particular series of experiments indicated that regardless of the treatment regimen applied, some animals failed to respond or only showed marginal degrees of recovery (3). In comparison, other animals demonstrated considerably better recovery of the SEP, indicating a relatively successful outcome of treatment. The similarity to circumstances involving divers led us to undertake pathological studies on the spinal cords of these animals in an attempt to detect the reason for the difference in response to treatment.

METHOD

Fourteen conditioned male mongrel dogs, 4 undived control animals and 10 dived animals, were anesthetized and prepared in an identical manner, as described by Leitch and Hallenbeck (4). The dived animals were submitted to a standard dive profile, i.e., compression to 300 fsw in 4 min, followed by 15 min of actual bottom time. They were then brought to the surface in 5.5 min. Serial SEP were recorded during the dive and the subsequent observation period. Changes that occurred in the SEP, particularly loss of amplitude, were considered to indicate the onset of decompression sickness.

Fifteen minutes after the first change in SEP was noted, 2 of the dived animals were killed and perfusion fixed. The remaining 8 animals were recompressed to treatment depth. They were then treated for a period of 2 h during which time further SEP were recorded to assess the results of treatment. In the event that spinal cord decompression sickness did not develop during the observation period, the dive profile was repeated with a reduced bottom time. This invariably resulted in spinal cord decompression sickness. The occurrence of cardiorespiratory embarrassment or shock at any stage was counteracted by a short recompression to 60 fsw and replacement of fluids as appropriate. Upon completion of the therapeutic recompression under evaluation, cardiac arrest was induced by the administration of a solution of saturated potassium chloride. All animals were immediately infused with 1 liter of 8% sucrose solution through a catheter in the left femoral artery by an infusion pump (Gilson, 100-S, Villiers de Bel, France). Blood was drained through a catheter in the right femoral vein. After the infusion of sucrose, first 1 liter of 10% Karnofsky's solution and then 1 liter of full-strength solution was infused. After perfusion fixation an autopsy was performed during which the brain and spinal cord were removed. Appropriate representative sections of tissue were prepared for light and electron microscopy.

RESULTS

Grossly, the spinal cords were generally firm and well fixed, although the cords from dived animals had lumbar and occasionally cervical segments that were less well fixed and somewhat softer than surrounding cord tissue. Otherwise, the cords appeared normal. In addition, the dived animals revealed petechial hemorrhages in

the pericardium, perinephric fat, and retroperitoneal regions. The lungs in several animals showed some evidence of peribronchial edema. No gas bubbles were observed.

Light microscopy

Spinal cord sections from undived and untreated control animals showed no abnormality except artifactual disruption of some myelin sheaths (Fig. 1*B*) that appeared as a wavy, partial disruption of myelin interspersed with tight, circular, and homogeneously stained sheaths. In contrast, sections from dived animals, both treated and untreated, showed scattered microscopic hemorrhage located principally in the white matter throughout the length of the cord. Ultrathin, 1-μm sections stained with toluidine blue showed a widened appearance to the myelin sheath with a banded pattern of disruption involving scattered and small groups of axons (Fig. 1*A*). In addition to these dive-specific changes the artifactual changes noted in undived animals were present. Thrombi, gas distended vessels, and necrosis were not evident in spinal cord sections from dived animals.

Examination by light microscopy of representative sections from the remainder of tissues of dived animals revealed only microscopic hemorrhages in pericardium and retroperitoneal fat. Mild to moderate perivascular and peribronchial edema was seen in the lungs of some dived animals.

Electron microscopy

Examination by transmission electron microscopy of spinal cord sections from dived animals treated and untreated revealed dramatic changes in the ultrastructural appearance of the myelin sheaths of many axons. These changes were in the form of an apparently uniform separation of the myelin layers alternating with short zones of contact (Fig. 1*C,D*). The separations appeared to occur in the region between abutting outer leaflets of myelin sheath membrane rather than the internal, fused membrane surfaces. We believe that this alternating pattern of separation and contact between the myelin layers accounts for the banded appearance seen by light microscopy. This distinctive alternating pattern was not observed in spinal cord sections of undived animals, although the artifactual pattern of irregular, wavy myelin disruption was seen in the spinal cords of both control and dived animals. The striking difference between artifact and dive-related myelin disruption is clearly visible in Fig. 1*C,D*.

DISCUSSION

The gross changes that occurred in our dived animals and the more general appearance of spinal cord sections by light microscopy agrees with findings reported previously that showed the upper lumbar region was predominantly affected, with the cervical region involved less frequently (5). The occurrence of hemorrhage within the spinal cord has also been reported, although when specified, the location has generally been restricted to the white matter (6–9). This appearance has been used as evidence for both arterial (6) and venous (7) embolization models. Nevertheless, some infarction does occur in grey matter, and the difference in appearance of these lesions from those in white matter has been commented on by Heller et al. (5),

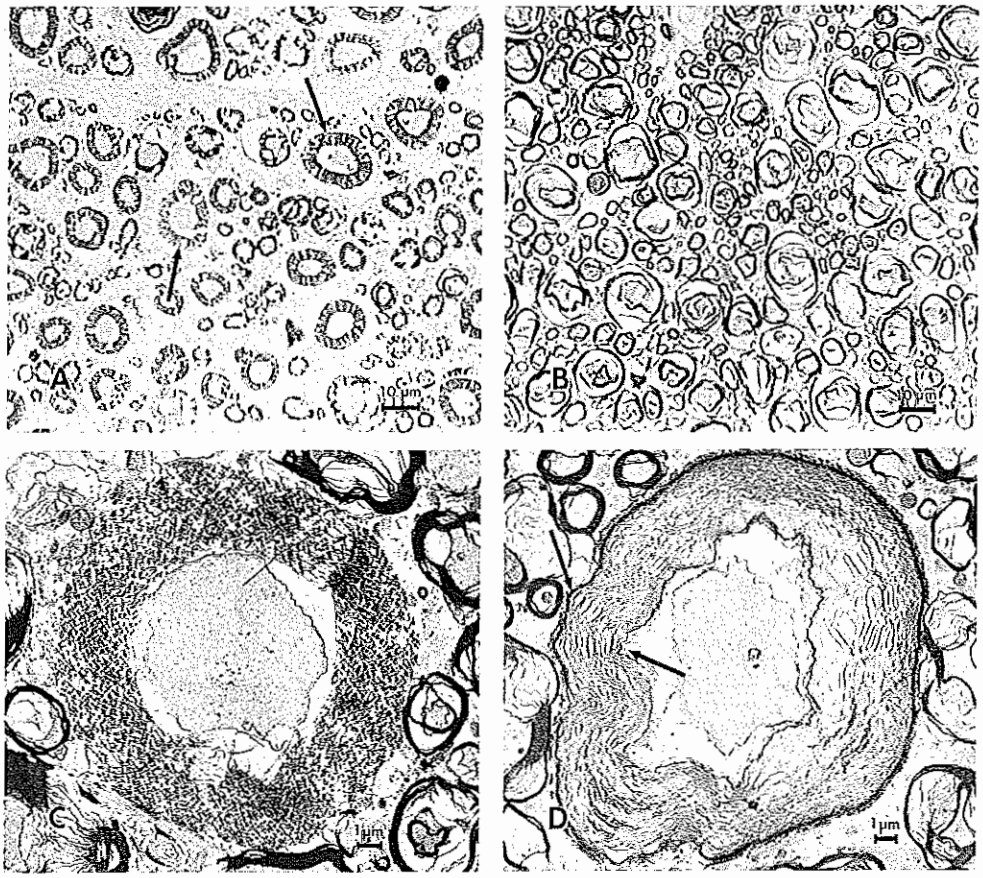

Fig. 1. Photomicrographs of lumbar spinal cord regions. *A)* Ultrathin 1-μm section of spinal cord from dived animal showing widened myelin sheaths with banded pattern of disruption (→) and considerable interaxonal edema; *B)* ultrathin 1-μm section of control animal spinal cord showing only partial wavy artifactual disruption of myelin sheaths and absence of edema; *C)* photomicrograph of spinal cord section from dived animal showing dramatic, uniform separation of myelin layers and adjacent axons with only mild to moderate sheath separations as noted in control animals; *D)* photomicrograph of cord from dived dog showing axon with typically widened, banded pattern (→).

Catchpole and Gersh (9), and Palmer et al. (10). The difference in appearance between lesions in the white and grey matter in DCS has been used to ascribe a vascular etiology for delayed radiation damage to the spinal cord (11) where similar changes have been noted. The changes to the myelin sheath that we describe, however, have not been reported previously, as the result of either a decompression insult or other disease of the nervous system. Other changes to the myelin have been described. Axon swelling has been reported by Hayashi et al. (12) and Haymaker (8), and the presence of disrupted myelin sheaths of peripheral nerves has been reported by Catchpole and Gersh (9), although the morphological details of the myelin alterations were not described in detail.

Examination by electron microscopy of nervous tissue after decompression sickness has rarely been performed and existing descriptions refer primarily to possible intracellular gas bubbles (13), which we did not observe. Sections of myelin sheath are shown in these published photomicrographs depicting what we believe to be simply artifactual changes since the alterations appear similar to the wavy, incomplete pattern of disruption we observed in sections from both control and dived animals.

The uniform separation of myelin layers, particularly along the external surfaces of concentric layers, is entirely compatible with decompression sickness, given the known high solubility of nitrogen gas in fatty tissue and the association of spinal cord damage in decompression sickness. The release of nitrogen from the myelin layers as the result of decompression would result in the formation of a gaseous phase if the injury was severe enough. Once present, this gas phase would tend to disrupt the normal structure of tissue. In this case, the natural plane of cleavage would be expected to be the space between double layers of myelin rather than the internal fused membrane surfaces, because the myelin sheath is formed by the concentric deposition of a double rather than a single layer (14).

The relationship of the changes reported to the generally accepted etiology of decompression sickness theory, namely, arterial, venous, or autochthonous bubble formation, is not clear. It is conceivable that these changes arise de novo within the myelin sheath of axons and are responsible for the loss of function by interruption of normal nervous transmission. Alternatively, the changes may arise as a secondary phenomenon to interrupted blood flow resulting from arterial or venous blockade. Although Boycott (6) generally supported the concept of arterial embolization, he described bubbles of gas within the substance of the spinal cord and suggested these were formed by the release and accretion of dissolved gas from myelin. Bubbles of gas within the substances of the cord were also reported by Clay (15). Richter et al. (16) reported on the appearance by electron microscopy of bubbles in various tissues after DCS, but in view of the medical histories of the patients involved and the dive profile, arterial gas embolism, as a result of pulmonary barotrauma, is a more likely mechanism for their findings. Cessation of blood flow would result in the accumulation of nitrogen molecules in the tissue because their safe elimination by the blood to the lungs would cease. If the partial pressure of nitrogen in the tissue exceeded the so-called critical pressure, a gas phase would result. The presence of such gas released from the myelin layer may give rise to the appearance seen in Fig. 1*C,D*.

Haymaker (8) also supports the idea of arterial bubbles, but he concedes the possibility of a venous effect as the result of stagnation in the venous complex of Bateson. This concept has been expanded by Hallenbeck et al. (7) to include the products of blood-bubble interactions within the epidural vertebral venous system as

complicating factors, a view supported by experimental and pathological evidence. Venous obstruction to the outflow of blood from the spinal cord would result in the formation of a gas phase within the myelin sheaths of spinal cord axons in a similar way to that proposed for arterial obstruction.

Our findings can also explain the mechanism proposed recently by Hills and James (17) who suggested that autochthonous bubbles within the cord, confined by the relatively rigid membrane of the cord, would result in an increase in the internal pressure of the cord that may be sufficient to exceed perfusion pressure and result in ischemic injury. They believed that "watershed" areas may be especially prone to this mechanism. We believe that our findings represent a type of autochthonous bubble formation but acknowledge the difference between it and more "conventional" forms of these bubbles. If a sufficient number of axons are affected and expand as we describe, the mechanism of Hills and James will be fulfilled.

Finally, we believe that our findings may explain the phenomenon of incomplete recovery evident both in divers and in our experimental animals. Clearly, whatever mechanism of spinal cord decompression sickness one supports, ischemia must play a large part in the development of symptoms and signs of decompression sickness. Under the right circumstances of treatment this ischemia can be reversed with subsequent recovery of function. The resistance of the spinal cord to prolonged ischemia, compared to the brain, is now recognized and accounts for the expectation of a better outcome of treatment when delay is minimal. In this animal model the lesion develops and is treated within a period of time in which the permanent ravages of ischemia would not be expected to be extensive (18), accounting for those animals for whom treatment was considered successful judged by the return of the SEP. To account for a poor outcome of treatment, we need to identify a mechanism that induces permanent damage to the spinal cord, rendering its axons incapable of transmitting normal inpulses, but is not affected by ischemia. The changes to the myelin sheaths of spinal cord axons we describe appear to meet this requirement. The attending hypothesis we intend to investigate is that there is a relationship between the relative number of affected axons and the response to treatment measured by the return of SEP in our experimental model.

Lichtenstein and Zeitlin (19), and more recently Palmer et al. (20), described pathological damage to the spinal cord following DCS that appears to be much more substantial than clinical examination would suggest. It has been postulated that the process of plasticity within the cord results in this dichotomy. It would not be unreasonable to suggest that the changes we describe may represent the initial lesion to spinal cord axons that develops into the extensive changes seen, predominantly in the white matter by those authors.

In summary, we describe changes to the myelin of spinal cord axons as the result of decompression sickness. The appearance of these changes is compatible with the major etiological mechanisms proposed for spinal cord DCS. It is not known, however, whether the changes arise de novo or are secondary to a primarily vascular mechanism. In addition, these changes account for the incomplete recovery from DCS seen in our experimental animals and, by inference, in divers, and as such they may be responsible for the extensive lesions of white matter seen in patients who are long-term survivors of DCS.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Pub. No. (NIH)78-23.

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Sykes JJW, Yaffe LJ. Altérations sous microscopie optique et électronique dans les couches de myéline de la moëlle épinière après la maladie de décompression. Undersea Biomed Res 1985; 12(3): 251–258.—L'examen pathologique des moëlles épinières d'animaux soumis à une maladie de décompression (DCS) expérimentale fut entrepris afin d'expliquer l'observation des réponses disparates au traitement. Après fixation sous perfusion, les moëlles épinières furent prélevées chez 8 animaux expérimentaux, 4 animaux témoins non soumis à la plongée, et 2 animaux non traités mais soumis à la plongée. La microscopie optique de sections ultra minces, colorées au bleu de toluidine, d'animaux soumis à la plongée démontra une couche de myéline distinctivement élargie avec une destruction de la myéline en forme de bandes. Cette forme fut confirmée par la microscopie électronique, laquelle montra la séparation qui devait être entre deux couches attenantes de myéline. Des changements artéfactuels étaient également présents chez les animaux soumis et ceux non-soumis à la plongée. Ces changements non-rapportés auparavant peuvent être causés par la DCS. Ils sont compatibles avec les mécanismes majeurs proposés dans la patho-physiologie de la DCS de la moëlle épinière et peuvent aussi être responsables de la réponse au traitement notée dans les animaux expérimentaux. Il est suggéré que ces découvertes peuvent aussi expliquer la réponse au traitement observée chez les patients, de même que la formation des lésions tardives décrites dans les moëlles épinières de survivants à long terme de la DCS.

maladie de décompression
potentiels évoqués
moëlle épinière
myéline
microscopie optique
microscopie électronique

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