

The Origin of the Spinal Subdural Space: Ultrastructure Findings

Miguel Angel Reina, MD*, Oscar De Leon Casasola, MD†, Andrés López, MD*, José Antonio De Andrés, MD‡, Miguel Mora, MD§, and Agustín Fernández, MD||

*Department of Anesthesiology and Critical Care, Hospital General de Móstoles, Hospital de Madrid Montepríncipe, Spain; †Department of Anesthesiology and Critical Care Medicine, Roswell Park Cancer Institute, Buffalo, New York; ‡Department of Anesthesiology and Critical Care, Hospital General Universitario, Valencia, Spain; §Department of Urology, Hospital de Móstoles, Madrid, Spain; ||Electron Microscopy Center, Complutense University, Madrid, Spain

Previous studies of samples from cranial meninges have created doubts about the existence of a virtual subdural space. We examined the ultrastructure of spinal meninges from three human cadavers immediately after death to see whether there is a virtual subdural space at this level. The arachnoid mater had two portions: a compact laminar portion covering the dural sac internal surface and a trabecular portion extending like a spider web around the pia mater. There was a cellular interface between the laminar arachnoid and the internal layer of the dura that we called the dura-arachnoid interface.

There was no subdural space in those specimens where the dura mater was macroscopically in continuity with the arachnoid trabecules. In the specimens where the dura mater was separated from the arachnoid, we found fissures in between the neurothelial cells that extended throughout the interface. We hypothesize that the subdural space would have its origin within the dura-arachnoid interface when the neurothelial cells break up, creating in this way a real subdural space.

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The subdural space is described by textbooks as “a potential cavity between the dura and arachnoid mater” (1-3); it has been visualized by contrast injection, and epidural catheters have been introduced using radiological techniques (4-8). However, when Blomberg (9) attempted to evaluate the subdural space by endoscopic methods in cadavers, he could not visualize it in all cases.

During the last decades, different authors (10-16) have studied the ultrastructure of the cranial dura-arachnoid interface in animal and human meninges and have doubted the existence of a subdural space. Vandenebeele et al. (17) studied samples of the spinal meninges, and they could not find it. In all these studies, the dura-arachnoid interface has been identified by different names: the medial border of the spinal dura mater (10), dural border cell layer (18), subdural mesothelium (13), or subdural compartment (14). We observed the ultrastructure of the dura-arachnoid interface under transmission and scanning

electron microscopy from samples of spinal meninges to evaluate the presence or absence of a subdural space.

Methods

The dural sac and its neural content at the lumbar level were removed from three donors immediately after their death. Approval from the research ethics committee and family consent for the donation of the organs and the procedures included in this research was obtained. The subjects were 48, 55, and 60 yr of age and had been admitted to the intensive care unit because of cerebral hemorrhage and subsequently diagnosed with brain death. The laminectomy and dissection were performed with the help of an orthopedic surgeon. The dural sac was removed in a block from T11-L5 section. Extracted specimens containing the dural sac were studied without further manipulation by scanning and transmission electron microscopy. After subsequent dissection, they were also studied by surgical optical microscopy. Care was exercised to observe specimens where the dura mater was in continuity with the trabeculated portion of the arachnoid as well as specimens where the laminar arachnoid was

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Address correspondence and reprint requests to Miguel Angel Reina Perticone, MD, Valmojado, 95 1st B, 28047, Madrid, Spain. Address e-mail to miguelangel.rei@terra.es.

macroscopically separated from the dura mater as a result of the dissection.

Preparation of Samples

Transmission Electron Microscope. The specimens were fixed for 4 h in a solution of glutaraldehyde 2.5% and a buffer phosphate solution to a pH value of 7.2–7.3. They were later fixated with a solution of 1% osmium tetroxide for 1 h. The specimens were dehydrated with acetone and were soaked in resin epoxy Epon 812. Control group slides were dyed with Richardson's methylene blue dye. Ultra thin slides of 70 nm of thickness were made with an ultramicrotome and treated with 2% acetate of uranilo solution and with Reynolds lead citrate solution. The specimens were observed under a Zeitz transmission electron microscope (EM 902; Carl Zeitz, Oberkochen, Germany).

Scanning Electron Microscope. The samples were fixed by immersion for 4 h in 2.5% glutaraldehyde and phosphate solution buffered to a pH value of 7.2–7.3. They were later dehydrated in acetone and pressurized under CO₂ to reach the critical point. A carbon layer was then deposited on the samples to a thickness of <200 Å and covered with a gold microfilm. The samples were studied with a JEOL JSM 6400 scanning electron microscope (JEOL, Tokyo, Japan).

Results

Observations During the Dissection. We observed a translucent membrane that detached from the internal coating of the dural sac when light pressure or light friction was applied on its internal surface. This membrane wrapped around all the nerve roots at their exit from the medullary cone and the spinal cord, joining the internal surface of the dura mater only in the areas where the nerve roots come out from the subarachnoid space to cross the dural sleeves and in some isolated randomly located points within the dural sac (Fig. 1). As a result, this membrane formed a cylinder with lateral short sleeves and edges that joined the internal surface of the dural sleeves.

Electron Microscopy. The results of the findings obtained by transmission electron microscopy (TEM) or scanning electron microscopy (SEM), referring to the same structure, will be described by indicating the method in parentheses. The arachnoid membrane was seen as a compact laminar portion covering the dural sac in its inner surface and a trabeculated portion that extended like a spider web to the pia mater of the spinal cord and the medullary roots (SEM). Between the laminar arachnoid portion and the most internal layer of the dura mater, there was a cellular interface (TEM) that we called the dura-arachnoid interface (Fig. 2).

The subdural space, as classically described, was not present (TEM) in the specimens where the dural sac had its entire thickness and the dura mater was macroscopically in continuity with the arachnoid trabeculae. The dura-arachnoid interface was occupied by neurothelial cells, and there was not a real space between the arachnoid and dura mater membranes (TEM, SEM; Fig. 2). These cells were arranged concentrically below the dura mater, and their thickness varied between 5 and 7 μm, distributed in 4–8 parallel cellular planes (TEM). The length of each neurothelial cell was longer than 100 μm, and their width varied between 0.5 and 1 μm (TEM, SEM; Fig. 3). They were oriented in different directions, and their morphology presented numerous ramifications (SEM; Fig. 3). The union of elongated and flat cells formed each plane with multiple intercellular "digitlike" cytoplasmic prolongations (TEM). Depending on the cells studied, an intercellular space or cells joined by scarce desmosomes or other specialized bridging were found. When the intercellular space was present, its thickness varied. Sometimes, this space resembled lacunar spaces occupied by amorphous material where the collagen or elastic fibers were almost absent (TEM; Fig. 2).

The lack of fibers between these cells makes them different from adjacent cellular planes in the dura mater and the arachnoid membrane (TEM). The neurothelial cells presented a nucleus with disperse chromatin, few mitochondria, a poorly developed endoplasmic reticulum, and pinocytotic vesicles (TEM). In those areas with fissures, the tearing extended mainly through the amorphous material. In larger planes, a fissure was generated and expanded towards the lateral zones where a significant amount of amorphous material was usually present (TEM). Once the epicenter of the fissure is established, it enlarges towards areas of low mechanical resistance where the amorphous substance is located between the neurothelial cells. There were lacunar zones with large amounts of amorphous substance. In these zones, the coherence and mechanical resistance appeared to be less because the fissure developed easily. When the tips of a new fissure hit a more resistant zone, like joints between neurothelial cells, its advancement causes one of these cells to break or, if the generated forces are too weak, the fissure expands in another direction (SEM). This explains the presence of folded cellular fragments at the surface of the generated subdural fissure (SEM).

In those specimens where the dura mater was macroscopically separated from the arachnoid, we could observe two laminae with a thickness of 300 and 40 μm, respectively (SEM; Fig. 4). Both laminae were separated by a space that corresponds to the subdural space (SEM), and both surfaces in contact with the subdural space were formed by neurothelial cells (Fig.

Figure 1. Dissection of the dural sac at the level of cauda equina. The arachnoid mater appears as a translucent membrane. This has been separated from the internal surface of the dura mater. Caudal nerve roots and epidural fat are also seen.

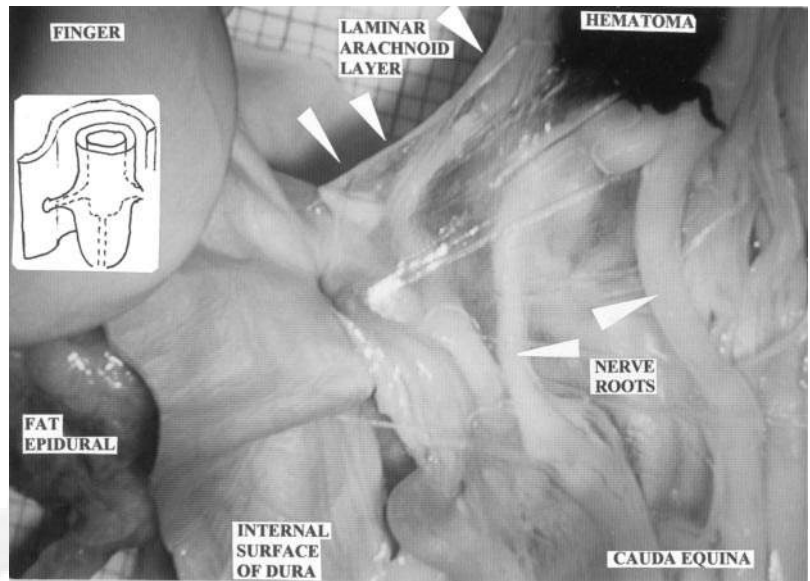
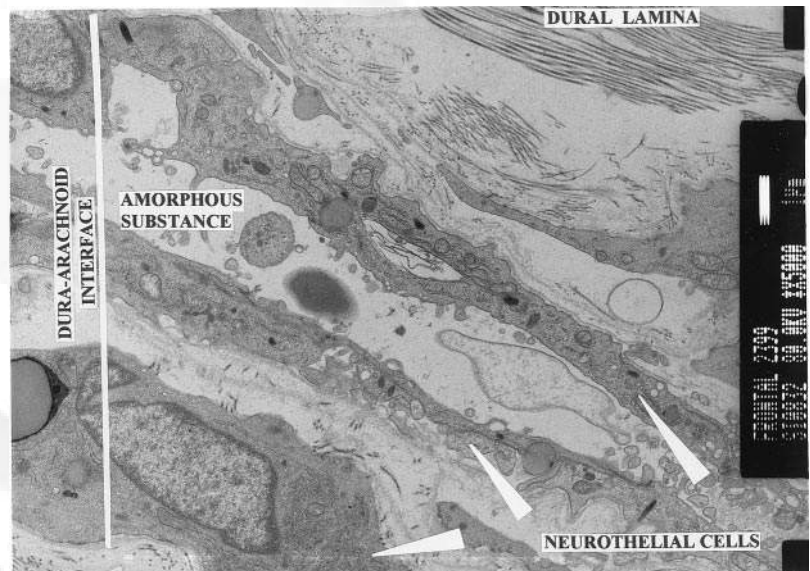


Figure 2. Dura-arachnoid interface is seen below the dural lamina (most internal aspect of the dura). The dura-arachnoid interface is filled with neurothelial cells and amorphous material (transmission electron microscopy [TEM], magnification $\times 5000$, bar = $1 \mu\text{m}$).



4). Parallel to the subdural space, and inside the dura-arachnoid interface thickness, we encountered other subdural fissures (two or three small fissures parallel to the longitudinal axis) (SEM; Fig. 4). We called these spaces the secondary subdural spaces. The surfaces adjacent to the subdural space appeared smooth, shiny, and had some cytoplasmic fragments originated from broken neurothelial cells (SEM). The shiny appearance observed via electron microscopy was produced by the intercellular amorphous substance (SEM).

Discussion

We could not find the subdural space in those samples of human spinal meninges where surgical manipulation was avoided. The subdural space was not seen

under transmission electron microscopy. Instead, we found a compartment between the dura and arachnoid mater filled with neurothelial cells that we named the dura-arachnoid interface.

Although the number of subjects studied was very small, the absence of a subdural space in all of them creates doubts about the existence of a virtual subdural space, believed until now to be present in all individuals at birth. The use of transmission electron microscopy allowed us to identify the ultrastructure of the dura-arachnoid interface. The presence of neurothelial cells filling up the dura-arachnoid interface helped us to consider that the subdural space would not be comparable to other virtual spaces such as the interpleural space. We did not observe within the interface other structures such as collagen fibers; there were few intercellular joints that made this cellular

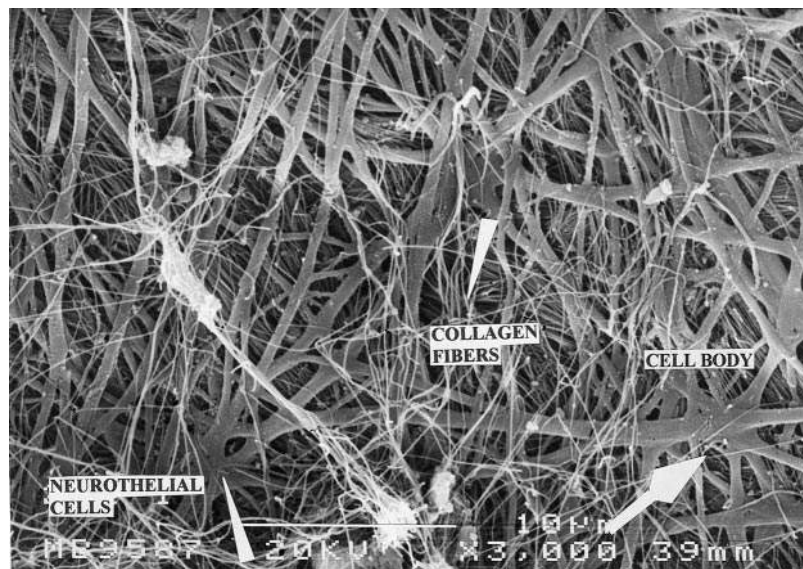


Figure 3. Dura-arachnoid interface. Neurothelial cells have a syncytial structure and show ramifications. They present an irregular diameter (approximately $1 \mu\text{m}$) and a smooth surface. Collagen fibers can also be seen, and they belong to the laminar portion of the dura mater. However, they are about $10\times$ thinner, and they do not have ramifications. Between the neurothelial cells, collagen fibers may be seen from the last dural lamina of the dura mater (scanning electron microscopy [SEM], magnification $\times 3000$, bar = $10 \mu\text{m}$).

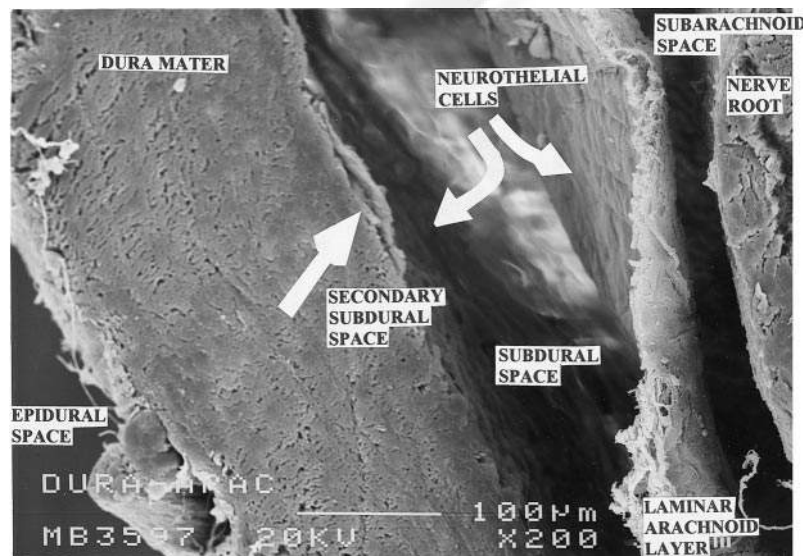


Figure 4. Subdural space seen by scanning electron microscopy, limited by dura mater (thickness of $300 \mu\text{m}$) and laminar arachnoid (thickness of $40 \mu\text{m}$). The internal surface of both membranes is covered with neurothelial cells. Inside the dura-arachnoid interface and parallel to the subdural space, there are other secondary subdural fissures (magnification $\times 200$, bar = $100 \mu\text{m}$).

plane softer with regard to the dura mater and arachnoid (neighboring cellular planes). There were large intercellular lacunar spaces separated by thin bridges of cytoplasm. These spaces were filled with an amorphous material of low resistance and unknown composition. Therefore, the cohesion forces at the level of the interface would be significantly lower. Using scanning electron microscopy, we observed the neurothelial cells tridimensional structure (Fig. 3).

A subdural space can appear if neurothelial cells break up because of pressure exerted by mechanical forces, air or fluid injection, creating fissures within the interface. Fissures could grow larger towards weaker areas, giving up a subdural space that could expand according to the pressure exerted.

We saw a number of parallel fissures under scanning electron microscopy (Fig. 5). These fissures can be produced within the dura-arachnoid interface in relation to specific tissue characteristics, depending on the distribution of the applied forces. Some fissures are produced in an incomplete form, whereas others expand to a greater extent, creating a real subdural space.

We took samples from the cadavers immediately after death and avoided surgical manipulation of a known number of specimens to prevent the results from being altered by artifacts, for these could be responsible for the appearance of a false subdural space. In our study, we applied light pressure over a number of samples, and it was possible to observe how the arachnoid layer separated from the dural sac, creating an extensive subdural space.

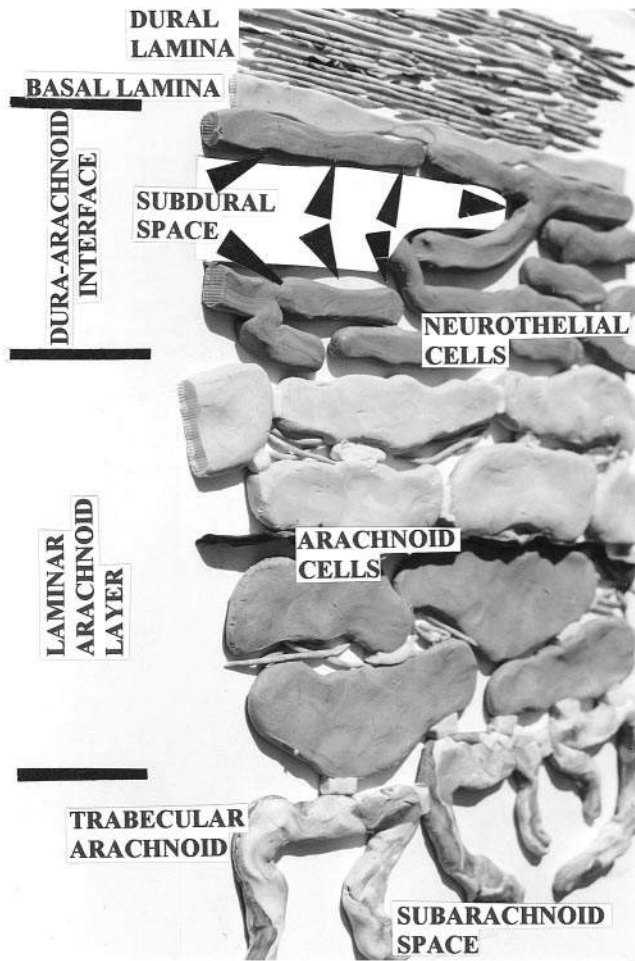


Figure 5. Dura-arachnoid interface model. From top downwards there is the dural lamina (dura mater's most internal portion) followed by the dura-arachnoid interface; the interface seems filled up with neurothelial cells, showing the formation of the subdural space. Below are the laminar and trabecular portions of the arachnoid mater.

These findings confirm the view of other authors, in particular, the results of Vandenabeele et al. 17, who obtained human specimens of the dura mater and arachnoid at spinal level during surgical procedures on the spine. In contrast to Vandenabeele et al., we studied the complete lumbar segment of the dural sac, avoiding surgical manipulation of the samples. This new concept may have clinical implications because it could better explain the variability of the onset and extension of the neural blockade caused by a nonintentional subdural injection.

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