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The central canal of the human spinal cord: a computerised 3-D study

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ABSTRACT

Knowledge of the structure and function of the central canal of the human spinal cord is important in understanding the pathogenesis of syringomyelia. Analysis of the morphology of the central canal is difficult using isolated histological sections. A 3-dimensional reconstruction technique using digitised histological sections was therefore developed to visualise the morphology of the central canal. The technique was used to study the canal in the conus medullaris and filum terminale of 1 sheep and 4 human spinal cords. A variety of morphological features were demonstrated including canal duplication, a terminal ventricle and openings from the canal lumen into the subarachnoid space. The findings suggest the possibility of a functionally important fluid communication in the caudal spinal cord which may have a sink function.

Key words: Syringomyelia; spinal cord.

INTRODUCTION

The structure and function of the central canal of the human spinal cord are poorly understood. Conventional dogma has been that the canal is obliterated in adults (Williams, 1969; Becker et al. 1972; Nogués, 1987; O'Rahilly & Müller, 1987; Sarnat, 1991) and therefore does not contain cerebrospinal fluid (CSF) (Sarnat, 1991; Gower et al. 1994). Recent studies have demonstrated that central canal occlusion is a progressive process (Newman et al. 1981; Milhorat et al. 1994). The canal is patent up to the 2nd decade of life with stenosis becoming more severe and involving more levels in older populations (Newman et al. 1981; Milhorat et al. 1994). Throughout most of the cord, the canal is a uniform elliptical shape, although variations in the morphology of the caudal central canal have been described, including dilatation (Choi et al. 1992) and forking and outpouchings (Lendon & Emery, 1970). Studies of various species, including some primates, have demonstrated openings from the filum terminale central canal into the subarachnoid space (Suzuki, 1959; Bradbury & Lathem, 1965; Nakayama, 1976; Ikegami & Morita, 1987; Sakata et al. 1993). There is evidence suggesting that the CSF in the central canal may facilitate nonsynaptic neural transmission (Bach-y-Rita, 1993) or have a 'sink' function with the ability to clear waste products and particulate matter from the parenchyma (Cserr et al. 1986; Milhorat et al. 1991, 1992*a*, 1993). If there is a flow of CSF in the central canal, its direction remains controversial, both caudal (Bradbury & Lathem, 1965; Nakayama, 1976; Cifuentes et al. 1994) and rostral (Milhorat et al. 1992*b*) flow having been suggested.

Study of the morphology of the central canal is difficult using only histological sections. It is almost impossible to develop an understanding of the 3-D structure of the canal from observations of isolated histological sections. Recently, modalities such as magnetic resonance imaging (MRI) have been used to examine the morphology of normal and pathological spinal cords (Wener & Perl, 1991; Beuls et al. 1993), but the central canal remains below the resolution of currently available technology. The aim of our study was to develop a technique for 3-D imaging of the central canal, and to assess its usefulness in the study of the canal in the conus medullaris and upper filum terminale with regard to its patency and the possible existence of openings into the subarachnoid space.

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MATERIALS AND METHODS

Materials

This project was approved by the Royal Adelaide Hospital Research Ethics Committee. Sheep spinal cords were used to develop the technique; 1 sheep cord and 4 human cords form the basis of this study. The human cords were taken from a 5-y-old male, a 60-y-old male, a 19-y-old female and a 38-y-old female. None of the humans studied had known spinal pathology prior to death. The sheep cord was taken from an animal used in CSF flow experiments. The spinal cords were removed at autopsy and fixed in 4% paraformaldehyde. A 40 mm block consisting of 20 mm conus medullaris and 20 mm filum terminale was removed from the fixed spinal cord. This block was then subdivided into 4 10 mm blocks that were dehydrated with graded alcohols and embedded in paraffin wax.

Spinal cord histology

The 4 separate spinal cord blocks were cut serially with a microtome into 10 μm thick sections. Every 10th section was mounted on a glass slide and stained with haematoxylin and eosin.

Computer imaging

The histological sections were viewed with a light microscope at magnifications up to $\times 100$ to determine the location and extent of the central canal lumen and ependymal lining. They were then photographed and digitised through a light microscope at $\times 4$ magnification using a charge-coupled device camera and image processing software (Q500MC, Leica Cambridge Ltd, Cambridge, UK). Image data were transferred to a Silicon Graphics $Indigo_2$ (Silicon Graphics Inc, Mountain View, California, USA) workstation for processing. Before the digitised sections could be used to compose the 3-D image of the conus and filum, the sections had to be aligned. This was necessary because each digitised section had been rotated or translated by differing amounts during the mounting and digitisation process. A purpose-written C^{++} program was used to align and centre each image using common features between adjacent sections as alignment points. In order for the imaging software to display the central canal, preprocessing of the aligned images was necessary to enhance the ependymal cells. This image enhancement was largely achieved using purpose-written C^{++} software that identified the darker haematoxylin staining of ependymal cells (Fig. 1). Before these enhanced

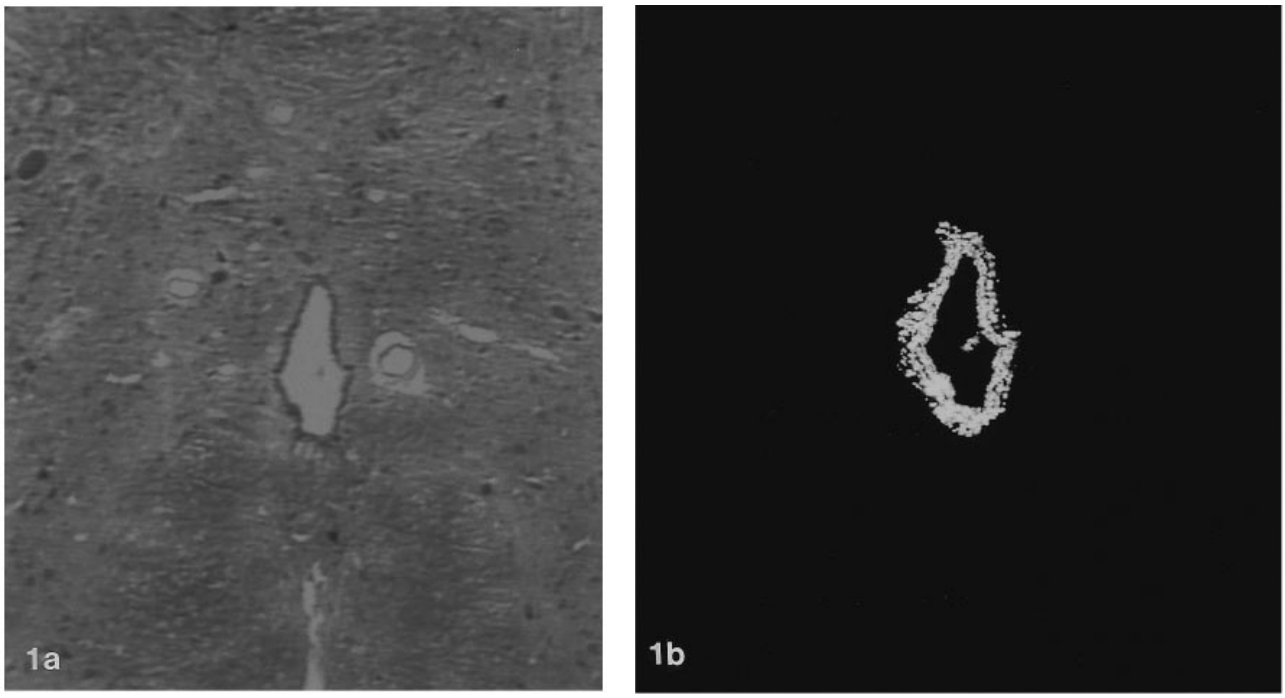


Fig. 1. Image processing. The digitised image of the spinal cord (*a*) was processed by special software to produce an outline of the central canal by enhancing the contrast of the ependymal cells to the surrounding parenchyma in an haematoxylin and eosin section (*b*).

sections could be used in the 3-D image, manual editing of the image was required to remove non-ependymal features that remained after enhancement. The 3-D image of the central canal was composed using Persona 1.1 software on the Silicon Graphics workstation. The software had previously been used to generate 3-dimensional images of the skull from CT head scans (Stoodley et al. 1996) and required some modification for the purposes of this project. Two methods were available for creating a 3-D representation of the central canal and spinal cord. The first used a 'ray casting' technique to create solid models of the surface of the spinal cord and the outside of the central canal that were then superimposed to generate a 3-dimensional image. The second method converted each digitised section into a contour map consisting of one contour for the surface of the spinal cord and a second contour for the lumen of the central canal. The contour maps were processed by the software to create a polygon mesh that represented the central canal and spinal cord. This polygon mesh was displayed as a 3-D image that could be rotated and viewed at any angle, using the workstation monitor and stereoscopic glasses.

Assessment

The histological sections were used to determine the extent of the central canal and ependymal cell lining in individual sections and assess the degree of continuity of the ependymal lining. The 3-D image was used to assess certain characteristics of the central canal in the conus medullaris and the filum terminale, including morphology, patency, continuity, forking and the presence or absence of openings into the subarachnoid space.

RESULTS

The analysis of the 3-D images is summarised in the Table.

Patency and continuity

The 3-D image of the sheep central canal demonstrated that it was patent, elliptical and uniform throughout the lower 2 cm of conus and upper 2 cm of filum (Fig. 2). The central canal of the 5-y-old male was also patent throughout its length, with morphology similar to that of the sheep central canal within most of the specimen (Fig. 3). The histological sections of the 60-y-old male and the 19-y-old female demonstrated a nonpatent proliferation of ependymal cells throughout the cord segments, with the exception of an irregular lumen in the lower filum of the 60-y-old male and numerous small lumina at various levels in the 19-y-old female. The 3-dimensional analysis of these cords did not demonstrate continuity of the lumen at any level. While most of the ependymal cells were scattered irregularly within the conus and upper filum in the 38-y-old female, the 3-dimensional analysis demonstrated isolated regions of the patent lumen in these segments. The lower filum contained an irregularly patent lumen that was continuous for the majority of this segment.

Forking and duplication

The histological sections from the 5-y-old male demonstrated that the central canal separated into 2 separate canals in the lower part of the conus. The 3-D images confirmed forking of the central canal for a distance of 700 μm before the 2 lumina rejoined at the conus/filum junction. In the lower conus and upper filum of the 19-y-old female, the 3-D analysis showed that the irregular dense proliferations of ependymal cells seen in the histological sections lay in 2 distinct columns (Fig. 4). Furthermore, there was a 400 μm forking of the nonpatent central canal in the caudal segment of the conus. No forking or duplication was evident in the other specimens.

Terminal ventricle

The histological sections of the filum from the 5-y-old male exhibited irregular enlargement of the canal lumen to form the terminal ventricle. The 3-D analysis showed that the lumen became large and irregular at the conus/filum junction, remaining this way throughout the filum. The other cases did not have an apparent terminal ventricle.

Openings into the subarachnoid space

At the most caudal section of filum from the 5-y-old boy, a part of the central canal approached the pia mater, being separated from it by only a thin band of connective tissue. Although an opening was observed from the central canal into the subarachnoid space somewhat higher up in the filum, this may have been

Table. *Findings on 3-D analysis of the conus medullaris and upper filum terminale of 5 spinal cords*

Specimen	Age (y)	Patency	Continuity	Forking/ duplication	Terminal ventricle	Openings
Sheep		+	Throughout	−	−	−
Male	5	+	Throughout	+	+	?
Male	60	Irregular	−	−	−	−
Female	19	Irregular	−	+	−	−
Female	38	Irregular	Filum only	−	−	+

+, present; −, absent.

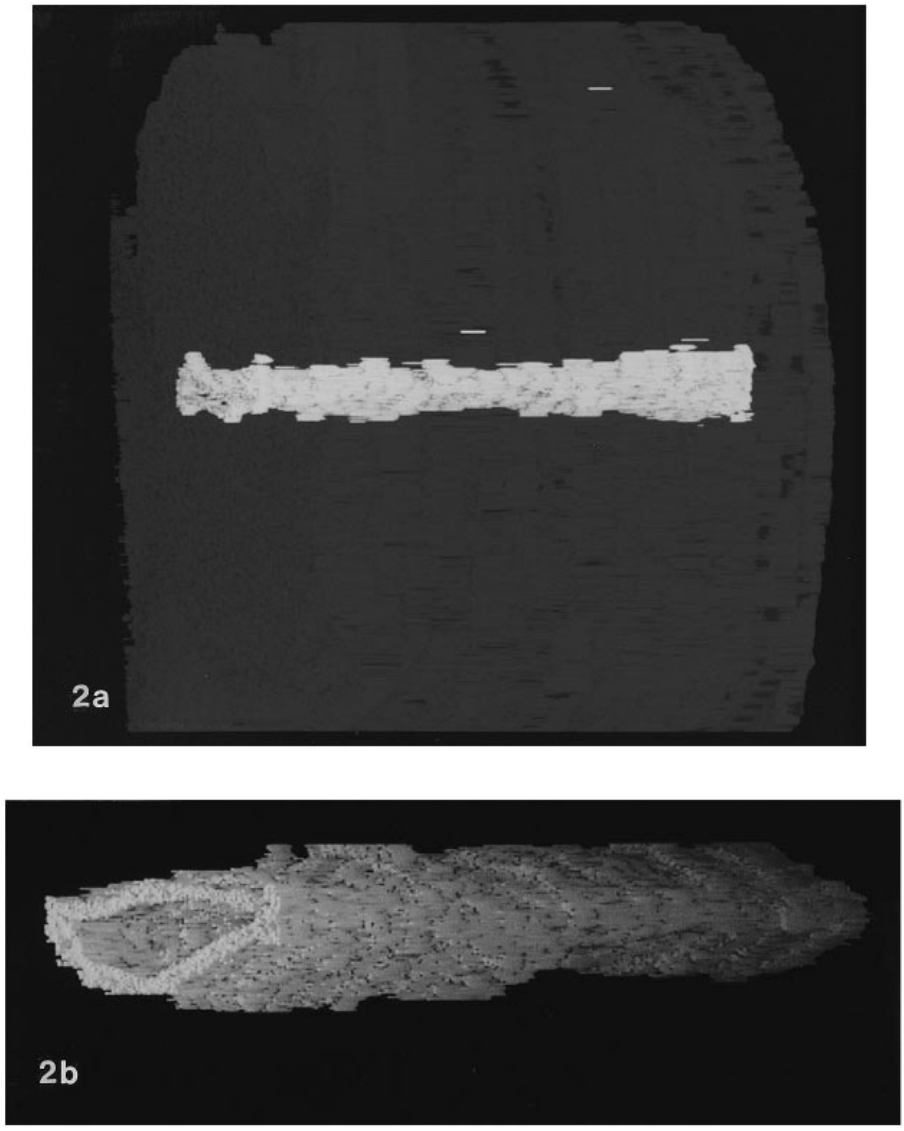
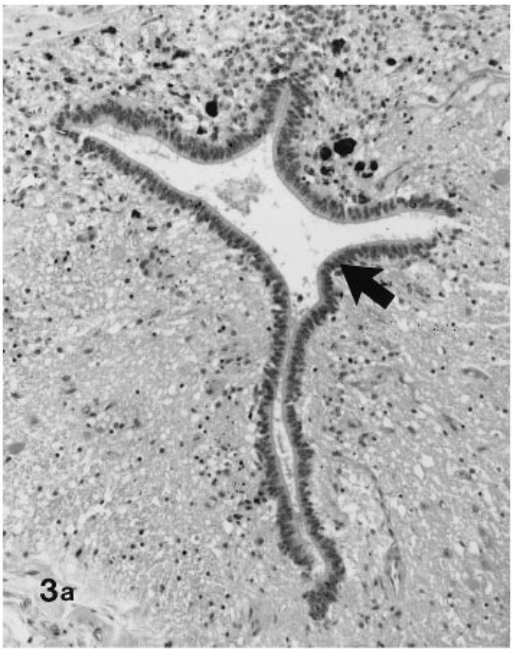
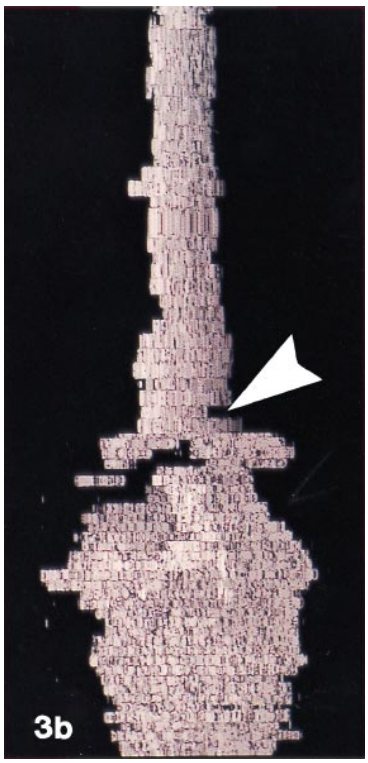


Fig. 2. Three-dimensional ray casting reconstruction of sheep conus medullaris. (*a*) Pial surface of conus medullaris (grey) and ependymal outline (white). The rostral surface is on the left. (*b*) Ependymal outline of central canal from the same segment of cord demonstrating the patency of the lumen. The caudal surface is on the left.

an artifact since a torn piece of filum parenchyma was seen adjacent to the opening. The 3-D analysis demonstrated an elongated extension of the central canal lying close to the pial surface for a distance of 1500 μm within the lower filum.

Histological sections of the caudal filum from the





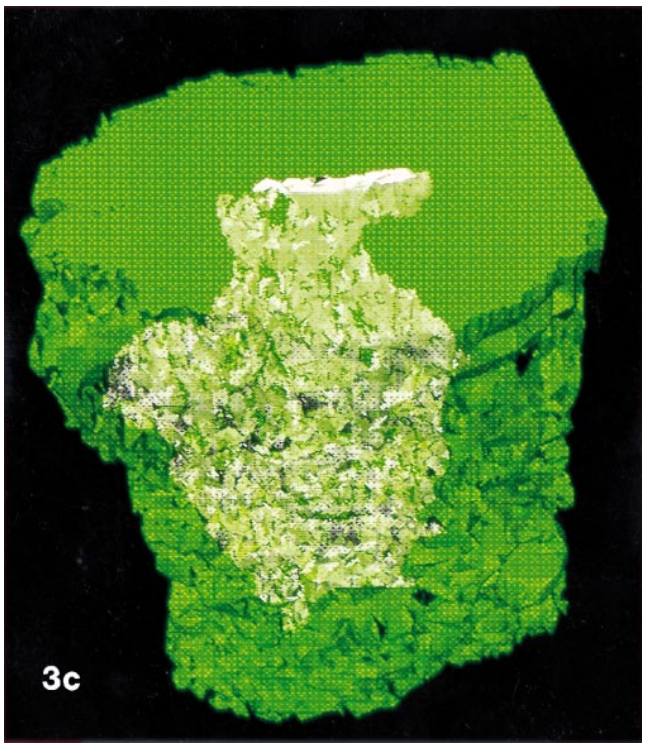
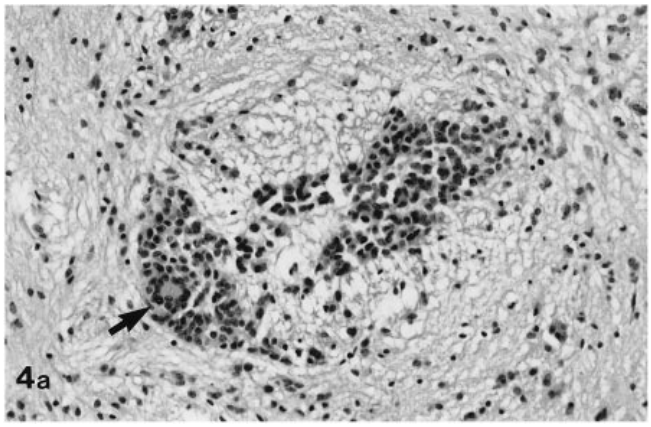


Fig. 3. (*a*) Photomicrograph of spinal cord section from the 5-y-old male. The central canal has enlarged to form the terminal ventricle (arrow) in the caudal end of the conus medullaris. (*b*) Ray casting reconstruction demonstrating forking of the canal (arrow) below which the canal enlarges to form the terminal ventricle. The region below the arrow has been reconstructed as a polygon mesh (*c*) with the terminal ventricle (white) superimposed on the surface of the spinal cord (green). Viewing the images on the computer screen with stereoscopic glasses confirmed that the central canal abutted the surface of the cord in this region. *a*, Haematoxylin and eosin, $\times 33$.

38-y-old female demonstrated openings from the irregular central canal into the subarachnoid space at 2 separate levels. At other levels, the ependymal cells lining the central canal were adjacent to the pial surface of the spinal cord. The 3-D images demonstrated the close proximity of the ependymal lining of the central canal to the surface of the filum throughout its length (Fig. 5).



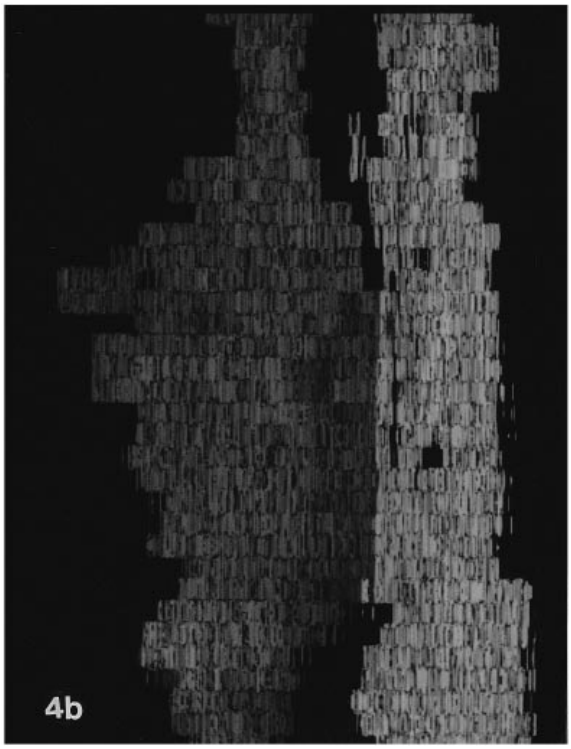


Fig. 4. (*a*) Photomicrograph of a section of conus medullaris from the 19-y-old female. There are clumps of ependymal cells with rosette formation (arrow). (*b*) Three-dimensional ray casting reconstruction of the ependymal cells in the conus medullaris of the 19-y-old female. The cells are arranged in 2 discrete columns, the left column corresponding to the group of ependymal cells nearest the arrow in *a*. No lumen is identifiable. *a*, Haematoxylin and eosin, ×66.

DISCUSSION

A technique for studying the 3-D morphology of the central canal has been developed. The technique is time consuming and utilises expensive equipment. Rendering and reconstructing hundreds of images requires expensive and powerful computer hardware and software. Detailed 3-D images have been produced that facilitate the study of central canal morphology. Much information can be gained from studying 2-D photographs of the 3-D reconstructions, but an even greater appreciation of the spinal cord and central canal structure can be gleaned from the use of stereoscopic glasses to view the images with appropriate depth cues. These 3-D images can be manipulated on the screen to provide an even greater understanding of the morphology.

The information obtained using this 3-D technique could theoretically be achieved by studying each histological section, but it would be extremely difficult. The 3-D images allow rapid and clear appreciation of the central canal morphology and its relationship to the spinal cord surface. Using histological sections alone, it may be difficult to determine whether an apparent opening is real or artifactual. By using the 3-D image to assess the morphology of the central canal lumen, an overall impression of the shape can be

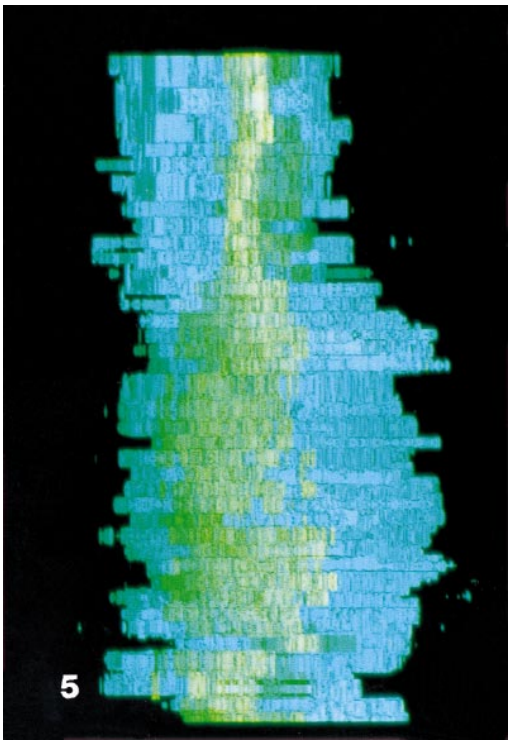


Fig. 5. Three-dimensional ray casting reconstruction of filum terminale from the 38-y-old female. The pial surface (blue) and ependymal outline (green) are shown. The rostral end is uppermost in this image. The intensity of the colour of the ependymal outline indicates the distance of the cells from the surface of the filum. The bright green areas represent those cells that are within 25 μm from the pial surface. The central canal lies close to the pial surface throughout most of this section of the filum.

appreciated—an artifactual opening would appear inconsistent with the morphology of the central canal near that point whereas a true opening would following the overall shape of the canal.

In the specimen from the 5-y-old boy, there was forking of the central canal within the lower conus, closely coinciding with the location of the terminal ventricle. Forking began as a minor dorsal duplication of the main canal at the caudal end of the conus, which progressively increased in size in the ventricular region. This pattern is consistent with the findings of Lendon & Emery (1970). Outpouchings of each fork of the central canal were also observed in the filum. Although the lumen of the central canal was not in continuity with the subarachnoid space, ependymal cells extending from the lumen did contact the pial surface. The pia and ependymal cells have gap junctions rather than tight junctions allowing water and molecules up to 1600 Da to cross the pia readily (Fishman, 1992) and molecules as large as 5000 Da to pass between the ependymal cells (Fishman, 1992). This arrangement may represent a functional communication between the central canal and the subarachnoid space similar to those described in other species. It is likely that the central canal is patent throughout the rest of the cord given the findings of Newman et al. (1981) and Milhorat et al. (1994).

The ependymal lining of the central canal in the specimen from the 38-y-old female was in close proximity to the pial surface of the filum for most of its length and had openings into the subarachnoid space at 2 levels within the caudal filum. This morphology is suggestive of a functionally important fluid communication. Such a communication may play a role in the sink function of the canal. While the canal was patent at this level, the age of the patient would indicate that there are levels of stenosis rostrally (Newman et al. 1981; Milhorat et al. 1994).

Other potential uses for this technique include studying the 3-D morphology of syrinxes and their relationship to the spinal cord surface and central canal. The technique could also be used to study central canal and spinal cord morphology in congenital spinal cord abnormalities such as myelomeningocele and diastematomyelia. Study of a large number of human cords would allow a better understanding of age-related central canal changes and of the normal arrangement of the central canal in the conus medullaris and filum terminale.

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