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Surface Cryoplaning

A Technique for Clinical Anatomical Correlations

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During the past decade, rapid advances in the clinical disciplines and milestone achievements in diagnostic imaging such as x-ray computed tomography, magnetic resonance imaging and ultrasonography, have aroused renewed interest in gross anatomy and detailed segmental anatomical correlations.In orthopaedic surgery, the development of new techniques for the treatment of disorders of the joints and the spine such as alloplastic joint replacement, ligament reconstruction, arthroscopic surgery, decompressive surgery for spinal stenosis or improved management of spinal fractures all call for thorough knowledge of functional anatomy and biomechanics.

In a clinical-anatomical investigation of popliteal cysts and their relationship to the gastrocnemio-semimembranosus bursa (4), knee specimens were frozen in various postures and then serially sectioned on a large, heavy-duty cryomicrotome, devised by Ullberg (7,8) for autoradiographic studies in undecalcified experimental animals up to the size of monkeys. Specific requirement inherent in studies of musculoskeletal anatomy prompted modifications of the original cryosectioning technique. In this article, a description of the cryoplaning technique is supplemented with illustrative examples of spine anatomy.

TECHNIQUE

The acquisition of fresh cadaveric specimens is the prime prerequisite for good anatomical studies. Since the procurement of such materials from willed body donor programs is limited with respect to number and the usually high age of the deceased, the major ity of specimens is obtained from departments of pathology and forensic medicine during routine autopsies.

To prevent the deformation of the topographic relationships between the bone and the soft tissues as well as drainage of blood and emptying of fluid-filled cavities, freezing of the specimen in situ is essential. Arteries and veins are more readily distinguished if they are filled with colored latex or dye prior to freezing. While a whole cadaver, properly positioned and supported,

may be frozen in toto, in situ freezing of a joint or a spine segment is difficult and time-consuming. The specimen is prepared by division of adjacent soft tissues and insulated against the skin and organs with cellulose. Crushed dry ice is packed into these spaces and liquid nitrogen is repeatedly poured over the ice to lower the temperature of the cryogenic.

Dependent on the size of the specimen, it takes two to four hours before it is frozen solid and can be cut out with an oscillating electric saw. For direct correlations with computed tomographic scans, the specimen may be embedded in orthogonal styrofoam boxes filled with precooled carboxymethyl cellulose gel (CMC)which is frozen to ice. Scanning may be performed in optional planes prior to sectioning (5).

Previously embedded specimens are transferred to the microtome stage. Other specimens may be embedded directly on the stage by filling the box and surrounding metal frame with CMC gel and freezing at -70^{o} C.

The anatomical images are not obtained from sections collected on adhesive tape, but by repeatedly photographing the specimen surface (4,5).

Modern scanners "zoom" through an orthogonal block of tissue in the patient at millimeter intervals. After tests with cinematographic equipment had shown that the microtome sledge stops exactly in the same position after each stroke, the following provisions were made for sequential image registration: A stable camerastand with a fixture for the vertically moving knife holder also carries a horizontal bar to which an adjustable hinged ruler with a millimeter scale is attached. The ruler is aligned along the lower baseline of the image frame and the camera then focused on the plane specimen surface. As the knife is fed down during sectioning, the camera and scale are lowered, rendering identical alignment and magnification of the images throughout the specimen.

In still cameras,the image frame is not accurately related to the transport sprocket holes of the film. A 35 mm single lens reflex camera was therefore rebuilt for exact pin-registration. Photographic images are taken on 25 ASA color reversal film with flat field lenses and extension tubes allowing for various magnifications. Automatic electronic flashes are used for illumination. In addition to in-register overview images, closeups are taken with a second, hand-held camera in areas of particular interest.

Depending on the anatomical region, the cutting height intervals vary. Specimens containing larger proportions of hard or brittle bone (such as the temporal bone) are cryoplaned at a few microns' thickness whereas larger,soft tissue specimens can be sectioned at 30-40 microns' thickness. Photographs are taken at intervals ranging from .1 mm to 1.0 mm.

The microtome is programmed to cut a certain number of sections of the selected thickness and stops automatically in the position for photography.

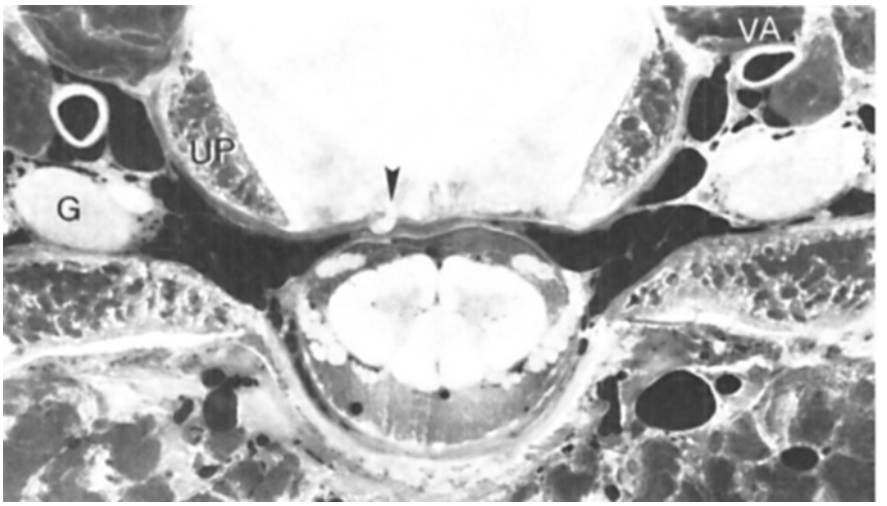

Fig.1. Axial anatomy in the lower cervical spine (C6-C7 disc).The specimen was frozen in flexion. Therefore the upper articular processes of C7 lack contact with the inferior articular processes of C6. Also the epidural veins and the venous sinuses in the foramina are engorged.The arrow points at a small paramedian disc herniation.
G= dorsal root ganglion UP= uncinate process VA= vertebral artery

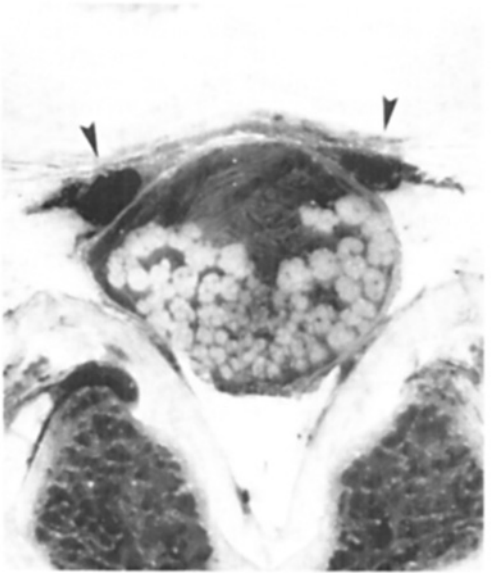

Fig.2. Axial anatomy of the lumbar spinal canal at L3-L4. The thecal sac containing the cauda equina roots is surrounded by epidural fat and firmly affixed to the concave posterior disc margin. The ventral internal veins are designated by arrows.

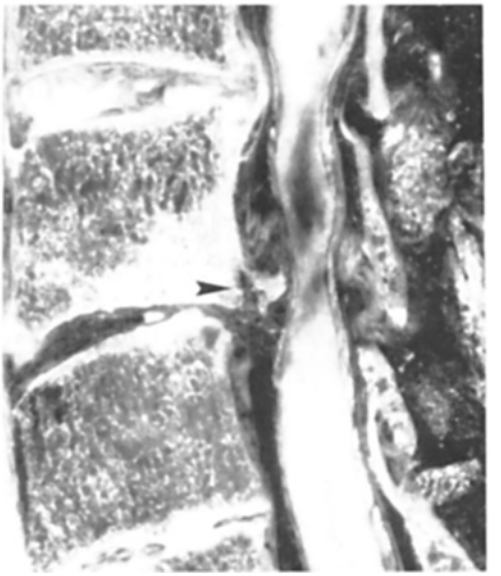

Fig.3. Sagittal section through the cervical spine of a 63 y.o. male with a fracture dislocation at the C5-C6 level. Note the complete disruption of the disc, rupture of the ligamentum flavum and retropulsion of fractured spondylophytic ridge into the vertebral canal (arrow)

Exacting care must be taken in the preparation of the specimen surface for photography. Especially at higher magnification, the ice crystals on the freshly cut specimen surface are disturbing. Compressed air of room temperature is used to remove debries and to thaw the specimen surface. Fraying of soft tissues bordering pneumatic cavities (e.g. in the skull) is avoided by filling them with water and allowing it to freeze to ice. To prevent recrystallization, the specimen surface is gently wiped with a warm cloth pad slightly soaked in ethylen glykol.

APPLICATIONS IN RESEARCH AND TEACHING

The great number of atlases on segmental correlative anatomy published in recent years and the rapid advances in diagnostic imaging underscore the need for improved reference material for the interpretation of computed tomograms. These tomographic scans are taken not only in the axial, sagittal and coronal plane, but increasingly in oblique, nonorthogonal planes. Familiarity with the topographic anatomy in these unconventional planes is essential for a correct diagnosis.

A systematic assessment of the multiplanar human anatomy in series of closely spaced sections should also encompass the variations of the normal anatomy with respect to shape, dimensions and intrinsic relationships, define borderline cases and abnormalities and should also demonstrate significant pathoanatomical changes typical for each region.

Cryoplaning is a powerful tool in basic research, notably for quantifying macroscopic pathoanatomical changes in experimental animal models (3). It also is used to supplement biomechanical studies, when specimens are frozen under a specific load. Subsequent cryoplaning then renders a quantitative assessment of the magnitude of dislocation and deformation and morphometric data (1).

The technique of surface cryoplaning allows to study the normal and pathological anatomy in specimens containing large amounts of undecalcified bone. The undistorted and also the functional relationships of the skeleton to contiguous soft tissues is maintained as well as the natural colors. Sequences of detailed images taken at submillimeter intervals, high magnification and in exact registration allow to "zoom" through a block of tissue in a movie mode.

The irrefutable need for an improved knowledge of multiplanar anatomy for better diagnosis and treatment may entail the necessity of incorporating it in undergraduate anatomy curricula. Conventional atlases and textbooks can only accommodate a limited number of illustrations (6). New electronic media with the capacity of storing vast numbers of images (2) and facilitating computer aided interactive use would seem to have a greater potential for teaching of complex three dimensional anatomy.

CONCLUSION

The Uppsala technique for surface cryoplaning of frozen specimens containing large proportions of undecalcified bone, renders series of accurately registered photographic images which in high detail and natural colors depict the undistorted relationships between skeletal elements and contiguous soft tissues. It is used to clarify complicated topographic and functional anatomical relationships in joint and spine specimens and for diagnostic correlation with computed tomographic scans. Apart from its high impact on teaching and enhancing diagnostic acumen, cryoplaning facilitates morphometric studies of pathological conditions induced in experimental animal models and a quantitative assessment of pathological conditions in the musculoskeletal system, which also includes multiplanar reformatting and three-dimensional modeling.

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