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the REGISTRY

Newsletter of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry

Inside:

The REGISTRY is published semi-annually by the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry. The Registry was established in 1992 by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health to continue and expand upon the former National Temporal Bone Banks (NTBB) Program. The Registry promotes research on hearing and balance disorders and serves as a resource for the public and the scientific community about research on the pathology of the human auditory and vestibular systems.

Application of Microslicing Techniques in Human Temporal Bone Research

Leslie Michaels, M.D.

Department of Histopathology, University College London, United Kingdom

The standard techniques of classical pathologic histology were seamlessly adapted in most organs to ultrastructural microscopy and, with the current molecular revolution in medical science, to immunohistochemical and molecular genetic tissue studies. Nevertheless, the first step in the investigation of the pathological histology of an organ must be a consideration of the gross appearances in the organ, which is usually done by cutting slices through the structure, or, in the case of hollow organs, by opening them up. Appropriate areas can thereby be selected for microscopic, ultramicroscopic and molecular studies of DNA, RNA and proteins.

The temporal bone, however, cannot easily be sliced for examination of its component structures because the latter lie at the kernel of a very hard shell of bone. The foundations of temporal bone pathology have been laid by studying histological sections prepared by decalcifying this shell of bone in strong acid and embedding and cutting the whole decalcified bone in celloidin. The histological sections so produced allow analysis of pathological changes, but ultrastructural investigations, special stains and particularly immunohistochemical and other molecular investigations are hardly possible on such material. Attempts have been made to obtain specimens for these purposes by drilling the shell of bone to enter the central kernel. This technique requires much time for the preparation of a single bone and it has been utilized to a limited degree only.

The microslicing technique was developed to surmount these difficulties[1]. The microslicing machine consists of a mounted flat, thin, round, steel blade with a round central aperture, the inner edge of which is tipped with diamond. The blade is mounted to rotate vertically in the case of the machine made by MR Semicon Inc., and horizontally in the machine made by the Leica Company. The fixed temporal bone is stuck on to a chuck by an adhesive such as dental wax and the chuck is attached to a metal arm that allows the bone to be applied to and pushed

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NEWS AND ANNOUNCEMENTS

In the news.....

NIDCD-funded Researchers Look to Nanotechnology to Build Smaller, Mightier Implantable Hearing Device

NIDCD has awarded a Small Business Innovation Research grant to NanoBioMagnetics Inc., an Oklahoma City, Okla., bioengineering firm, to investigate the use of nanotechnology in developing components for a new generation of implantable hearing devices. Hough Ear Institute, a nonprofit research, educational, and humanitarian service institute, also of Oklahoma City, will help design and test the new components. Nanotechnology is the branch of science in which materials are manipulated atom by atom and molecule by molecule to create information systems and mechanical devices of exceedingly small size. Implantable hearing devices are an alternative to hearing aids that use externally- and internally-placed electronic components to deliver sound vibrations directly to the bones of the middle ear. The use of nanotechnology for the implantable hearing device could help produce components small enough to fit inside the middle ear but more powerful than today's devices.

Did you know.....

Did you know that there is website for people with hearing loss and deafness. Jamie Berke, the guide of the Deafness/Hard of Hearing Site, runs the website on about.com. The address is http://www.deafness.about.com. They have an electronic newsletter which goes out weekly with topics such as cochlear implants, hearing aids, and otosclerosis just to name a few. You can sign up for the newsletter at their website.

ASHA Meeting

American Speech-Language-Hearing Association's 2003 Annual Convention will be November 13-15, 2003 at McCormick Place in Chicago. For more information please visit their website.
http://professional.asha.org/

American Academy of Audiology Meeting

The American Academy of Audiology will be holding their 16th Annual Convention & Expo on March 31–April 3, 2004 in Salt Lake City, Utah. For more information please visit their website.
http://www.audiology.org

International Federation of Hard of Hearing People

IFOHOH 7th World Congress
July 4-9, 2004
The 7th World Congress of the International Federation of Hard of Hearing People will be held in Finlandia Hall, Helsinki Finland.

Send Us Your News!
Send us your news and annoucements regarding hearing and/or balance loss or temporal bone research!
(See page 3 for contact information.)

Look for the Registry's Exhibit at these upcoming meetings

AAO-HNSF Annual Meeting and Oto Exp will be held in Orlando, Florida from September 21-24, 2003. Please visit their website for more information.
http://www.entlink.net/index.cfm

The Association for Research in Otolaryngology 2004 MidWinter Meeting will be held in Daytona Beach, Florida on February 22-26, 2004. More information is available on their website.
http://www.aro.org/.

REGISTRY NEWSLETTER AVAILABLE ONLINE AT www.tbregistry.org

The National Temporal Bone Registry's biannual newsletter, *The Registry*, is now available for viewing on the Registry's Website. Subscribers can be notified via email about current issues and will be directed right to the newsletter by a link to the site. Please visit the Registry's website at http://www.tbregistry.org.

NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114-3096

(800) 822-1327 Toll-free Voice
(617) 573-3711 Voice
(888) 567-3277 Toll-free TTY
(617) 573-3838 Fax
e-mail: tbregistry@meei.harvard.edu
Web: www.tbregistry.org

Brochures about Temporal Bone Research and Donation Order Free-of-Charge for Your Office, Clinic or Organization

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry, which is dedicated to promoting research on hearing and balance disorders through the study of temporal bones, has published two informational brochures, which you may request for display in your office and /or waiting rooms. Both brochures encourage individuals with hearing or balance disorders to bequeath their temporal bones to scientific research.

That Others May Hear is a short form brochure which describes briefly the functions of the Registry, and answers commonly asked questions regarding the temporal bone donation process. (Dimensions: 9" x 4")

The Gift of Hearing: Learning about Temporal Bone Donation is a 16-page, full-color booklet which describes in more detail and with diagrams, the structures of the ear, types of auditory disorders, the microscopic study of the temporal bone, and the benefits of temporal bone research. It also answers commonly asked questions regarding the temporal bone donation process. (Dimensions: 7" x 10")

If you are willing to display either of these brochures, please complete the form and return it to the Registry, by fax or mail. **The brochures will be sent to you free of charge.**

ORDER FORM

Please send me (circle or fill in quantity):

The Others May Hear: 25 50 100 _____ copies (free of charge)
The Gift of Hearing: 25 50 100 _____ copies (free of charge)

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Address: _____

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(800) 822-1327 toll-free phone, (617) 573-3838 fax

Or submit your request using email:
tbregistry@meei.harvard.edu

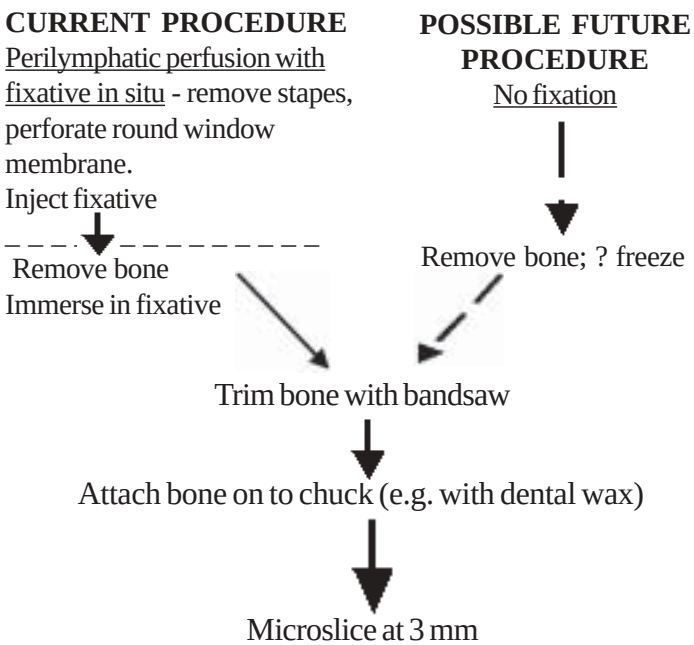
MICROSLICING TECHNIQUES, Continued from Page 1

against the interior sharp edge of the rotating blade. In this way slices of the bone of variable thickness can be prepared. We have found that a thickness of 3 mm is the most useful.

Successive slices through the whole temporal bone can be examined and both normal structures and pathological changes identified. Parts of slices can be removed for histological section or whole slices embedded after decalcification.

To date, only fixed temporal bones have been used for microslicing. In studies on the membranous labyrinth, early fixation after death is important because autolysis is very rapid. The best way to achieve this is by perilymphatic perfusion of fixative as soon as possible after death. This is performed by carrying out a tympanotomy, removing the stapes with footplate and making an incision in the round window membrane. Fixative solution, e.g. formaldehyde or paraformaldehyde solution can now be perfused repeatedly through one opening in the perilymphatic space so that it exits through the other opening, in this way perfusing the whole perilymphatic space and by diffusion fixing the endolymphatic structures. After perilymphatic perfusion the temporal bone is removed and the whole bone fixed before microslicing (Table 1, left arm). It is possible that in the future, depending on the requirements of the molecular methods to be used, it may be necessary to microslice unfixed temporal bones. For this purpose, it may be useful to freeze the bones to a low temperature before microslicing (Table 1, right arm).

Table 1. Handling of Temporal Bone Before Microslicing



There is ample published evidence that temporal bones microsliced after perilymphatic perfusion are well enough preserved to enable refined studies of the membranous labyrinth to be made by light and ultrastructural microscopy. Studies of large numbers of fixed temporal bones which have not been perilymphatically perfused have also been made for purposes to determine the incidence of otosclerosis in the bony labyrinth. The following paragraphs describe some publications which present these attributes of the microslicing method.

Surface Preparation Study of Prebycusis after Microslicing[2]

A clinical, audiological and electrophysiological study was carried out in a geriatric unit to determine the changes in presbycusis and the cochleae of those that died were subjected to perilymphatic perfusion and microslicing. Surface preparations were made from the microsliced cochleas. All geriatric patients had a hearing loss, mean value of approx. 60 dB HL. There was a moderate loss at most frequencies and a severe loss at the highest. Brain stem evoked responses showed diminished amplitudes. Cochlear microphonics obtained by extratympanic electrocochleography were reduced in proportion to the age of the patients. Perilymphatic perfusion was carried out in all temporal bones. Stained surface preparations of the organ of Corti revealed marked changes in all of the elderly cochleas in comparison with cochleas from younger subjects treated in the same way. There was complete atrophy of all hair cells at the end of the basal coil and severe outer hair cell loss in all three coils, accompanied by severe giant stereociliary degeneration in some surviving cells, in all cases.

Evaluation of Electron Microscopy after Microslicing[3]

After perilymphatic perfusion two adult temporal bones were fixed and then sent to our department in London for microslicing. They were then returned to their home countries for scanning and transmission electron micrscopy. The microsliced bone subjected to transmission electron microscopy was found to show satifactory cellular resolution in the organ of Corti. The bone subjected to scanning EM showed mechanical distortion and detachment from bone.

Scanning Electron Microscopy Study After Microslicing[4]

In contrast to the previous report, this one illustrates that good preservation of perfused, microsliced tem-

poral bones is possible when the bones are observed by scanning electron microscopy. Perilymphatic perfusion was carried out on the two temporal bones of a 26 gestational week old infant who had died of respiratory distress syndrome and four temporal bones from four preterm infants between 26 gestational weeks and near term. Scanning electron microscopy was performed on one organ of Corti in each of the others. Unlike the unsatisfactory preparation at scanning electron microscopy in the study mentioned above,[3] all five temporal bones yielded satisfactory results. In one of the temporal bones of the first infant an almost complete loss of inner hair cells was found. Normal hair cells only were seen in all of the other bones (Fig. 1). Surface preparations in all contralateral bones showed all hair cell rows to be normal.

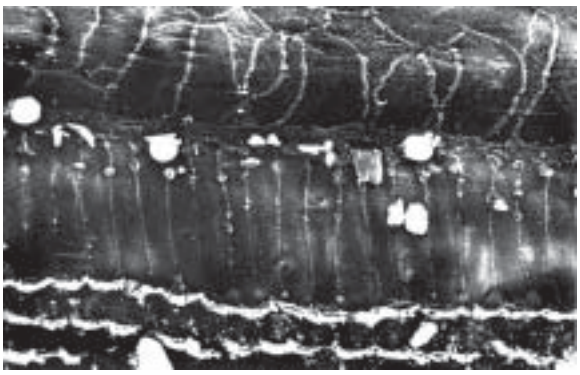
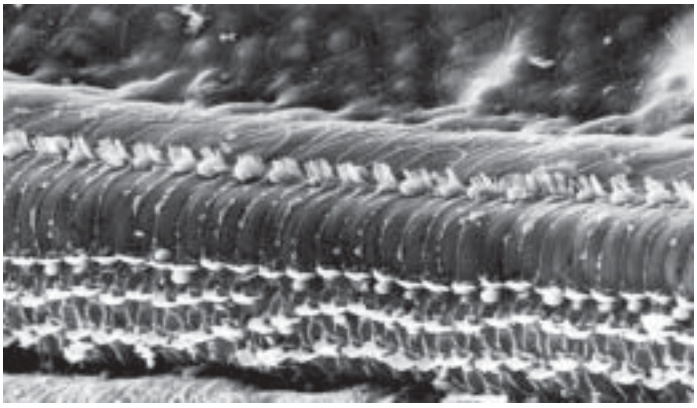
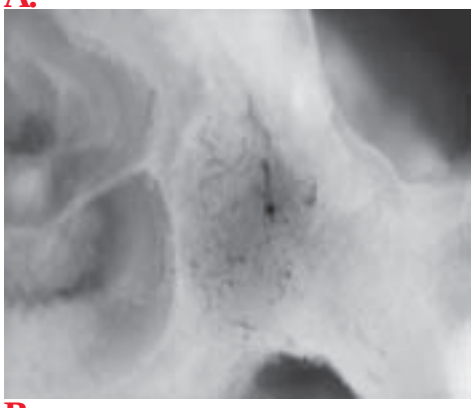


Figure 1. Scanning electron microscopy of cochleae of preterm infants. A. Complete loss of inner hair cells and normal outer hair cells in this cochlea. B. Normal inner and outer hair cells in another preterm infant. A and B were both from microsliced temporal bones. Reproduced from Michaels and Hellquist 2001.[8]



This finding of complete loss of the inner hair cells in one infant was a precursor of an important recent light microscopic histological study of 15 infants, which showed severe inner row hair cell loss in three of them.[5]



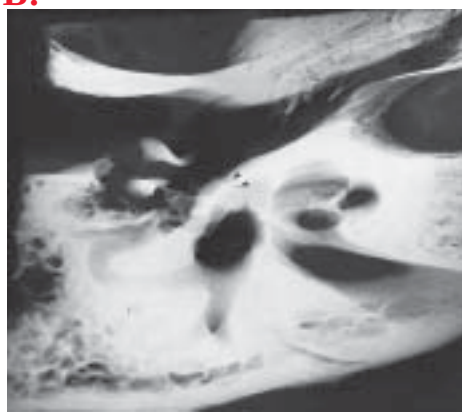


Figure 2. Otosclerosis in microsliced temporal bones. A. The otosclerosis appears as a mottled well-demarcated area, reddish in the original, situated between the cochlea and the footplate of the stapes. B. Radiograph of temporal bone with a patch of otosclerosis. The latter is seen as a well-defined area of mottled translucency between the cochlea and the footplate of the stapes. Note the fissula ante fenestram (arrowheads) near the otosclerotic plaque. Reproduced from Michaels and Hellquist 2001.[8]

Polymerase Chain Reaction for Herpes Simplex Virus after Microslicing[6]

Microslicing was carried out on the temporal bone of a patient who had had the symptoms of Bell's palsy shortly before death. The bone was then fixed and then embedded in paraffin wax. Microslices of the temporal bone on the affected side showed congestion of the geniculate ganglion and facial nerve and histological examination indicated severe ganglionitis and inflammation of the geniculate ganglion/facial nerve. Herpes simplex virus type 1 was demonstrated in the genomic DNA extracted from paraffin sections of the affected geniculate ganglion region by carrying out PCR followed by electrophoresis on agarose gel.

Incidence of Otosclerosis: a Study Using Microsliced Temporal Bones[7]

One hundred and eighteen pairs of temporal bones derived from consecutive adult deaths in a single hospital were screened for otosclerosis by gross observation of microslices combined with microradiography and positive cases (Fig. 2) were confirmed by

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LABORATORY SPOTLIGHT

Scientific study of the human temporal bone and related brain tissue is a time-consuming process performed in highly specialized otopathology laboratories by researchers who are dedicated to enhancing our understanding of the pathology underlying disorders of hearing and balance. "Laboratory Spotlight" is a continuing series of articles offering a glimpse inside the laboratories in the United States and abroad conducting temporal bone research.

TEMPORAL BONE LABORATORY OF THE UNIVERSITY OF CALIFORNIA, SAN DIEGO

Elizabeth M. Keithley, Ph.D., Director, Jeffrey P. Harris, M.D., Ph.D., Co-Director

The Otologic Laboratories at UCSD have been active in animal research for the last 20 years. Research issues have centered on inner ear immunopathology and mechanisms associated with normal aging in the cochlea. We have pioneered the study of immunologic responses in the inner and middle ear. We are relatively new to human temporal bone research, but in collaboration with the House Ear Institute Temporal Bone Laboratory and Drs. Fred Linthicum and Julia Tian, we have developed methods for immunohistochemical staining of celloidin embedded temporal bone sections.

We have a small collection of hematoxylin and eosin stained celloidin sections from temporal bones with various otologic pathologies that were a gift of Dr. Schuknecht. These sections are intervening sections from bones in the collection at the Massachusetts Eye and Ear Infirmary. We also obtained through the Registry, a collection of celloidin sections made by Dr. Brunner from temporal bones collected in the pre-antibiotic era. Dr. Chole and Dr. Benitez in the Department work with residents to teach them temporal bone histopathology and 3-D reconstruction of temporal bone structures.

Our current research project is headed by Dr. Cecilia Canto, an otolaryngologist and visiting scholar from Chile. In collaboration with The California Neuro-AIDS Tissue Network (CNTN), made possible through a 5-year, $5 million grant from the National Institute of Mental Health to the University of California, San Diego, we have been able to collect normal temporal bones and temporal bones from individuals who died of AIDS. Much is known about the effects of this infectious disease on many organ systems. At the University of California, San Diego investigators are studying the effects on the brain and eyes. Very little is known, however, about the effects on the inner ear. This collaboration is the first to provide a large sample of temporal bones from AIDS patients. We are evaluating temporal bone sections for the presence of opportunistic infections using immunohistochemistry in the cochleas and auditory nerves of HIV infected patients. At this time, we have found a significantly higher incidence of inflammation and abnormal structure in the inner ears of patients who died of AIDS, when compared with non-HIV-infected subjects. We concluded from our study that HIV-infection also affects the inner ear and auditory nerve in many individuals. The project is partially funded by the Academy of Otolaryngology/Head and Neck Surgery through a grant from the Trilogical Society.

The Temporal Bone Laboratory is located in the Surgery Research Laboratory at the UCSD Medical Center in Hillcrest.

Staff of the Temporal Bone Lab (left to right): Peter Billings, Ph.D., Cecilia Canto, M.D., Elizabeth Keithley, Ph.D., Shighehisa Hashimoto, M.D. and Xiaobo Wang, M.D.

MICROSLICING TECHNIQUES, Continued from page 5

histology. Histologic otosclerosis was found in 3.4% of the patients by these methods.

Discussion

These reported studies confirm that microslicing of temporal bones is a valuable method in the investigation of inner ear pathology in post-mortem temporal bones. Two other techniques enhance the usefulness of the microslicing technique. Perilymphatic perfusion as soon as possible after death is an important adjunct to ensure rapid fixation of the membranous labyrinth. Radiography of the temporal bone slices provides information that may not be obtained by gross examination alone.

After microslicing, inner ear structures are sufficiently well-preserved, not only when observed in standard histological sections, but also with refined light and ultramicroscopic methods. Surface preparations of organ of Corti displayed marked pathology in elderly cochleas compared with normal.[2] Both transmission and scanning electron microscopy techniques usually showed fine structures well.[3,4] Important findings were made with scanning electron microscopy in regard to such a delicate alteration as inner row hair cell loss in an infant.[4] Few molecular studies have so far been carried out in microsliced temporal bones. PCR of the geniculate ganglion region in the microsliced temporal bone of a patient with Bell's palsy demonstrated herpes simplex virus type 1 and confirms that microslicing has potential for future molecular investigations on the inner ear.[6]

Microslicing combined with radiography of the slices is a useful screening method for surveying large numbers of temporal bones for otosclerosis.[7] The otoclerotic areas so displayed appear well-preserved and the technique is promising for future molecular research in otoclerosis.

The technique of microslicing does have drawbacks, however. There is, inevitably, a loss of tissue from the cutting of the diamond-edged blade. This must correspond to the thickness of the blade, together with some tissue disruption underlying each cut surface. The amount of lost tissue has been calculated to be as much as 1 mm on each side of the slice.[3] Whatever the actual loss, it is clear that microslicing has limited value when measurements of the distribution of a change have to be applied throughout the length of a structure, e.g. in attempting to produce a strictly accurate cochleogram. Bone dust also presents a problem as it is inevitably deposited on cut surfaces and may obscure important histologic areas. The amount of this contaminant can be considerably reduced, however, by constantly washing the bone by water during microslicing. Each of the two commercially available microslice machines provide good facilities for this washing process.

Acknowledgements

It is a pleasure for me to acknowledge the important contributions of three people to the microslicing technique. The late Tony Frohlich set up the microslicing method in our department. Sava Soucek MD, PhD displayed the value of perilymphatic perfusion in studies of the membranous labyrinth by carrying out this procedure in large numbers of cases prior to microslicing. Jianning Liang MD, PhD perfected the technique of microslicing in recent years.

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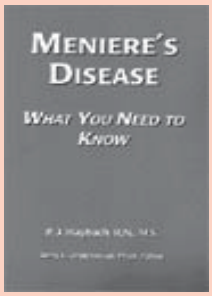
If you or anyone you know suffers from a balance disorder, these books are good resources. They cover everything from medical care to insurance coverage. Each title is informative and thorough. They offer valuable information for patients, healthcare providers, family and friends of people afflicted with balance disorders.



Meniere's Disease: What You Need to Know by P.J. Haybach, R.N., M.S., 336 pages, illustrated, indexed. Comprehensive book that includes the symptoms of Meniere's disease, hearing anatomy, different methods of testing, treatments options currently available, diet, drugs, research, insurance, surgery, other information sources, support groups.

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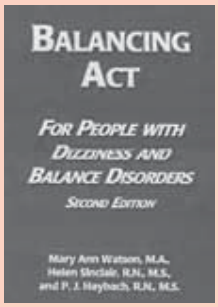
These titles can all be purchased through the Vestibular Disorders Association.



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