Spring/Summer 2001 VOLUME 9, Number 1

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.

Using the Microwave Oven for Decalcification of Human Temporal Bones

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The current method of temporal bone processing for histological assessment can take as long as 14 months (Schuknecht, 1993). The greatest percentage of this time is that needed to decalcify the dense temporal bone (approximately 9 months). The best agent for decalcification is ethylenediamine tetraacetic acid (EDTA) as it yields excellent morphological detail. The idea of using microwaves to decrease the time for decalcification of temporal bones was originally introduced by Hellstrom and Nilsson (1992) for rat cochleas. More recently, microwaves have been demonstrated to be useful in reducing the time needed for decalcification in EDTA of dense, primate temporal bones (Madden and Henson, 1997). The procedure was shown to maintain excellent tissue ultrastructure as viewed with the electron microscope. It has also been shown that tissue mRNA is preserved so that *in situ* hybridization assays can be employed on microwave decalcified tissue (Kaneko et al., 1999). Antigenic epitopes are also preserved during tissue heating and irradiation (Keithley et al., 2000).

In order to examine whether microwaves would decrease the time necessary for decalcification of human temporal bones and whether the tissue decalcified in this way could be used for immunohistochemical assays, we obtained human temporal bones from cadavers donated to the University of California, San Diego for teaching and research. A technique was developed for the removal of the cochlea from the petrous portion of the temporal bone

See HUMAN TEMPORAL BONES, page 4

OTOPATHOLOGY MINI-TRAVEL FELLOWSHIP PROGRAM

The NIDCD National Temporal Bone Registry is pleased to announce the availability of a mini-travel fellowship. The fellowship provides travel funds for research technicians and young investigators to visit a temporal bone laboratory for a brief educational visit, lasting approximately one week. The emphasis is on the training of research assistants, technicians and junior faculty.

The fellowship is available to:

1. Departments who aspire to start a new temporal bone laboratory.
2. Inactive temporal bone laboratories that wish to reactivate their collections.
3. Active temporal bone laboratories that wish to learn new research techniques.

Two fellowship awards will be made each year ($1,500 per fellowship). The funds may be used to defray travel and lodging expenses. Applications will be decided on merit. Those awarded the fellowship will be required to submit a brief report, which may be published in the Registry's newsletter.

Interested applicants should submit the following:

1. A 1-2 page outline of the education or training aspect of the proposed fellowship.
2. Applicant's curriculum vita.
3. Letter of support from applicant's temporal bone laboratory director or department chairman.
4. Letter from the host temporal bone laboratory, indicating willingness to receive the traveling fellow.

Applications should be sent to:

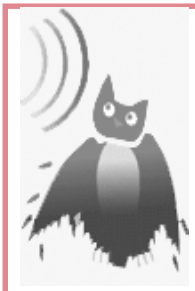
Saumil N. Merchant, M.D.
NIDCD National Temporal Bone Registry
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114

NIDCD Study on Hearing Aids

The NIDCD and the Department of Veterans Affairs Cooperative Studies Program have published a joint study in the Journal of the American Medical Association (JAMA). The study confirms that people with sensorineural hearing loss show a substantial improvement by using hearing aids. Sensorineural hearing loss effects over twenty million Americans and yet only four million of those individuals actually use hearing aids. The study was published in the October 11, 2000 issue of JAMA. For more information about the study, please contact the NIDCD Office of Health Communication and Public Liaison at (301) 496-7243.

The 16th Annual SHHH International Convention

The Self Help for Hard of Hearing People (SHHH) will be holding their annual convention in Cherry Hill, New Jersey. The convention will be held Friday, June 22 through Monday, June 25th at the Hilton Cherry Hill. Further information is available on their website at http://www.shhh.org. The Registry will be an exhibitor at this convention.



Have you heard about the NIDCD Wise Ears! Campaign?

WISE EARS! Is a national campaign to prevent noise-induced hearing loss.

Did you know that being exposed to loud noise over a period time can cause hearing loss?

When an individual is exposed to harmful sounds-sounds that are too loud or loud sounds over a long time-sensitive structures of the inner ear can be damaged causing Noise-Induced Hearing Loss (NIHL).

Click on to the Registry's website at www.tbregistry.org Wise Ears! to learn more or visit the NIDCD website at www.nidcd.nih.gov

Send us your news and announcements regarding hearing and/or balance loss or temporal bone research!

(See page 3 for contact information).

NIDCD National Temporal Bone,
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Web: http://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 below 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 quantity or fill in other quantity):

That Others May Hear: 25 50 100 _____ copies (free of charge)

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

Name: _____

Address: _____

Address: _____

City, State, Zip: _____

Telephone: _____

Mail or fax this form to the Registry at:

NIDCD National Temporal Bone,
Hearing And Balance Pathology Resource Registry
Massachusetts Eye & Ear Infirmary,
243 Charles Street, Boston, MA 02114-3096
(800) 822-1327 toll-free phone, (617) 573-3838 fax

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

HUMAN TEMPORAL BONES, continued from page 1

that removes less bone than the classic Schuknecht technique. The external features of the head were not disturbed, except for the craniotomy. The dissection included removal of the brain by the usual procedure. The internal auditory meatus was identified and using a Stryker saw, 2 cuts were made, one medial and one lateral to the meatus. The cuts were angled to meet at a point almost 2 cm below the meatus so that a wedge of the petrous ridge could be removed. This piece of bone contains the cochlea, part of the vestibule and part of the middle ear. It is however, very much smaller than the classic "temporal bone plug" that includes the middle ear, epitympanum and hypotympanum, and a good part of the external ear canal. Immersion fixation of the tissue was done as soon as possible (4% paraformaldehyde in 0.1M phosphate buffer). This wedge of bone was further trimmed with a hack saw, using a vice to stabilize it, to include exclusively the cochlea (1.5-2 cm^3). A total of 11 individual bones were prepared histologically.

The microwave decalcification protocol described by Maddon and Henson (1997) was used to decalcify all tissue. This method uses a microprocessor controlled microwave oven that is now available commercially (Model 3450 Laboratory Microwave System, Ted Pella, Inc., Redding, CA). The tissue was immersed in a beaker containing 300 ml of 10% EDTA with 2% paraformaldehyde (pH 6.8). The beaker and tissue were then placed in a predetermined "cool" spot in the microwave oven. A temperature probe that regulates the delivery of radiation was placed in the beaker to assure that the tissue temperature did not rise above a set limit. Another beaker, filled with distilled water was also placed in the oven. This beaker is connected via tubing to a water pump and refrigeration unit that sits on top of the oven and is used to cool the oven air temperature (temperature set at 20°C). The EDTA and paraformaldehyde solution was changed every 6 hours. In practice, 2 runs/day were made, one in the morning and the second beginning just before the end of the day. The tissue was left in the microwave even when the oven was off (12 hours/day). This time was not used to calculate the decalcification time. The tissue was trimmed with a razor blade to test the softness, but was also x-rayed to assure complete decalcification prior to embedding.

Following decalcification, the tissue was embedded in paraffin and immunohistochemistry was performed using standard protocols. The following primary antibodies were tested: anti-cytochrome c oxidase, subunit I (anti-COX, clone 1D6-F1-A8, RDI, Flanders, NJ), monoclonal anti-neurofilament (clone NE-14 against neurofilament purified from human brain, Biogenex, Inc., San Ramon, CA) and anti-peripherin (Chemicon International, Inc., Temecula, CA). ABC-peroxidase labeling kits (Vector Laboratories, Inc., Burlingame, CA) were used to detect the bound antibody. Background label of the secondary antibody was evaluated by incubating sections with PBS instead of primary antibody.

Initial experiments using guinea pig tibias demonstrated that the higher the temperature, the faster the decalcification. The temperature that resulted in good histochemical staining and minimal evaporation of solution during the 6 hour runs in the microwave was 45°C. While higher temperatures resulted in shorter decalcification times, they also resulted in increased evaporation of solution with potential damage to the tissue, oven and the temperature probe. Lower temperatures resulted in much longer decalcification times. Using the 45°C temperature setting, human temporal bone plugs (approximately 1-2 cm^3) containing the cochlea required 190 - 400 hours to decalcify. The number of hours reflected the size of the plug, with the smaller pieces requiring much less time. The classic

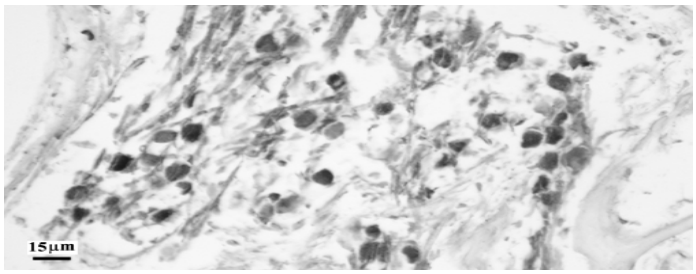

Figure 1: Photomicrograph of spiral ganglion neurons in a human cochlear section immunolabeled with antibody against peripherin, an intermediate filament. The antibody labeled most, but not all spiral ganglion neurons and their processes.

larger plugs will take much longer.

Paraffin-embedded cochlear sections were used for all immunohistochemical assays as these sections give superior immunohistochemical labeling and are much easier to handle than celloidin sections (Tian et al., 1999). All antibody labeling was the same in the microwave-decalcified cochleas as in cochleas decalcified at room temperature or at 4°C. Antibody to neurofilaments labeled the spiral ganglion cell bodies in Rosenthal's canal as well as their peripheral and central processes. Antibody to peripherin, an intermediate filament, labeled most spiral ganglion cell bodies, as well as their central and peripheral processes in our adult human cochleas. As with the neurofilament antibody, among the labeled cells, some labeled more darkly than others (Fig. 1). Antibody to cytochrome c oxidase (subunit I) labeled most darkly the cell bodies of the spiral ganglion neurons, Scarpa's ganglion neurons and the cells of the stria vascularis.

The idea of using microwaves to reduce the time required for decalcification and to improve other histological procedures was introduced many years ago (Boon and Kok, 1988; Ng and Ng, 1992; Helstrom and Nilsson, 1992; Kovacs et al., 1996; Leong and Milios, 1990), but seems very slow to become incorporated in routine histological procedures. Microwave decalcification of human temporal bones can greatly reduce the time required for histological preparations, from as long as 14 months to as short as 2-6 weeks depending on the size of the tissue. Paraffin embedding of the tissue yields acceptable histology provided that the initial fixation is good. The greatest advantage of the technique is that it will greatly facilitate the procedures for immunohistochemistry and *in situ* hybridization (Kaneko et al., 1999). Although the superior histological quality of the celloidin processing technique is lost by the paraffin technique, microwave decalcified bones can certainly be embedded in celloidin and the same morphological quality will be achieved. The reduction in time will still be appreciable, from 9 months to 5-6 weeks. While we trimmed our blocks to include only the cochlea, the techniques will work equally well on the traditional temporal bone block that includes the vestibular system and the middle ear.

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Acknowledgments

This work was supported by the NIH, DC RO-1 003395, and the Medical Research Service, Department of Veterans Affairs.

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 IN HRADEC KRÁLOVÉ

by Viktor Chrobok, M.D., Ph.D. and Eva Simakova, M.D.*

**Director, Temporal Bone Laboratory Department of Otorhinolaryngology, General Hospital Pardubice, Department of Pathology, Charles University, Faculty of Medicine, Hradec Králové, the Czech Republic*

The Temporal Bone Laboratory in Hradec Králové is located in the city of Hradec Králové, which has a population of over 100,000. The city is situated in East Bohemia, about 60 miles from Prague, the capital of the Czech Republic. The local Faculty of Medicine is part of the Charles University in Prague.

Beginning in 1990, the Department of Pathology in Hradec Králové began collecting and processing temporal bones from fetuses, newborns and infants. The idea of a temporal bone laboratory where there would be histological examinations of temporal bones was supported by Professor Ivo Steiner, who was the head of the Department of Pathology and the Dean of the Faculty of Medicine in Hradec Králové. With the support of Professor Steiner, Dr. Viktor Chrobok was able to establish the Temporal Bone Laboratory of Hradec Králové in 1991.

Dr. Chrobok finished his medical studies in Hradec Králové in 1988 and later became assistant professor in the Department of Otolaryngology in Hradec Králové. In 1998, he changed his position to assistant professor in the Department of Otolaryngology in Pardubice. Dr. Simakova, who is the co-director of the lab, worked in the Department of Pathology in Hradec Králové after finishing her medical studies. Other lab members include Dr. Eva Juttnerova (the head of the Department of Genetics), and Bruno Jezek and Karel Antos, who are both from the Purkyne Military Medical Academy in Hradec Králové. Bruno Jezek and Karel Antos help with 3-D reconstructions, which are computer generated.

Viktor Chrobok, M.D., Ph.D., Director and Eva Simakova, M.D., Co-director

The lab is the only active lab currently processing human temporal bones in the Czech Republic. The temporal bone collection has over four hundred specimens. All of these are encased in paraffin blocks, cut and stained. Clinical and pathological data are available from each case such as: gestational age or age, sex, cause of death, time between death and fixation, clinical history, autopsy diagnosis and histopathological findings in the temporal bones. The lab currently processes twenty to thirty temporal bones annually.

The main topics of research for the lab are 3D reconstructions, genetic defects, chromosomal disorders involving the ear, and histopathology of the middle ear cleft in fetuses and newborns. Histological studies of fetal and neonatal bones deal with presence of mesenchymal tissue, amniotic fluid, and epidermoid formations.

The 3D reconstructions are accomplished in several stages. First, particular histological sections are digitized. The digitized sections are then put into a common orthogonal space, which means that data is transformed so that the reference points in all sections correspond. This transformation usually requires rotation and shift, or a process called registration. For the registration part of the project, Bruno Jezek and

Charles University in Hradec Králové started its educational activities on November 25, 1945 as the first university faculty in East Bohemia. For its entire 55 years existence, the Faculty of Medicine in Hradec Králové has been among the top Czech university institutions.

Karel Antos designed their own software called Pix, which enabled manual registration of sections. The next step is segmentation, which focuses on marking selected structures in the sections. This is accomplished with Analyze, v.6.3 software. Two methods of segmentation are then employed – a manual drawing of the edge of the structure, and an automatic thresholding method based on the different intensities of gray dependent on the properties of the structures. During segmentation, different colors are assigned to the structures of interest for better resolution. The final step is rendering: this visualizes the data prepared in the previous steps. Rendering is performed with Medicus software. With rendering, 3D objects can be viewed from different positions, and different structures can also be chosen for viewing.

Dr. Chrobok has visited several temporal bone laboratories around the world. He has visited Professor Leslie Michaels and Dr. Jianning Liang at the Institute of Laryngology and Otology in London, England. He has also visited Dr. Joseph B. Nadol, Jr. and Dr. Saumil M. Merchant at the Massachusetts Eye and Ear Infirmary Temporal Bone Lab in Boston, Massachusetts and Dr. Anita Pollak at the Department of Otolaryngology in Zurich, Switzerland.

Dr. Chrobok also has had researchers from abroad visit his lab. Rindy Northrop (chromosomal trisomy 18 and 21), Dr. Anita Pollak (development of the anterior mallear ligament) and Dr. Jianning Liang (epidermoid formation) have all visited the lab.

The Temporal Bone Lab at Hradec Králové has published ten papers on the histopathology of temporal bones (six papers were published in English and 4 papers were published in Czech). The paper "Hemorrhage in the Inner ear of Newborn Infants" was awarded the prize of the "Czech young ENT Doctors Award" in 1993. The presentation of the poster entitled "The autosomal trisomy syndromes and their influence with histopathological changes of temporal bones of newborn infants" was supported by the Ethicon Scholarship of the 9th British Academic Conference in Otolaryngology in 1995. The lab's research has been supported by two grants of the Czech Ministry of Health (IGA MZ 3682/3 and IGA MZ 6189/3).

Although the history of the lab is very short, the staff hopes the research will continue developing and progressing well into the future.

2001 Meeting Schedule

for the National Temporal Bone Registry

Self -Help for Hard of Hearing (SHHH), June 22-25, Cherry Hill, NJ

American Academy of Otolaryngology-Head and Neck Surgery Foundation, September 9-12, Denver, CO

Molecular Biology of Hearing and Deafness, October 4-7, Bethesda, MD

REGISTRY NEWSLETTER AVAILABLE ONLINE AT

www.tbregistry.org

The National Temporal Bone Registry's bi-annual 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 to the newsletter by a link to the site. Please contact us through our website or call (800) 822-1327 to be added to our email list.
Please visit the Registry's website at **http://www.tbregistry.org.**

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Address Correction Requested

PLEASE! Notify us of your change of address before you move. Each undelivered newsletter is returned to the Registry office at a cost of $.50. Our loss is over $1.00 per unit. *Thank you*!

Questions From Our Donors

Q. Why do we need to complete update forms and medical history forms?

A. The scientific value of your temporal bone donation is greatly enhanced if it is accompanied by up-to-date medical records. Results of hearing tests (audiograms) that you may have had are extremely valuable. Researchers need your medical records so that they can correlate and link your ear disorder(s) to the changes they observe in your donated temporal bones.

Q. Will there be any costs associated with the donation of my temporal bones?

A. No. The medical professionals who remove the temporal bones donate their time or are paid by the laboratory receiving them. The funeral and burial expenses will remain the responsibility of the family.

Q. How can I order books such as Keys to Living with Hearing Loss by Marcia B. Dugan?

A. That book and other titles are available through the SHHH or Self Help for Hard of Hearing People, Inc. Their address is: 7910 Woodmont Ave., Suite 1200, Bethesda, MD 20814. Contact information: 301-657-2248 voice, 301-657-2249 TTY , 301-913-9413 Fax, email-National@shhh.org, or web-www.shhh.org

Q. Will my family be able to have open casket after temporal bone donation?

A. Yes. The removal of the temporal bones does not change the appearance of the donor's head. Funeral arrangements can be made however the family would like.

Q. What information about my temporal bone donation should I give to my lawyer for my living will?

A. Your lawyer needs to list the Registry contact information, which includes the Registry's toll free number (800) 822-1327. The hospital should keep this on file so they can contact the Registry 24 hours a day, seven days a week if something should happen.

Q. Will I be able to donate my body to science and my temporal bones to hearing research?

A. The policies of each donor program vary. Discuss this with your body donor program to determine if it would be best to donate to only one program.

Please contact us at (800) 822-1327 if you have any questions you would like us to answer.