

## Temporal Bone Bank and Laboratory : Organization and Significance

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*Understanding of the pathologies which cause the diseases is not always easy. In disclosing the aural diseases mechanism, temporal bone pathology provides us excellent clues. Such a study is essential for progress in the diagnosis, therapy, and prevention of disorders affecting this organ. But this involves a painstaking and long run procedures including a good record keeping and patient persuasion about postmortem research on their temporal bones. One of these few centers in the world is the House Ear Institute temporal bone research laboratory. In order to bring this subject into attention of Turkish otolaryngologists we reviewed the procedures and discussed the importance of this kind of laboratory. [Journal of Turgut Özal Medical Center 1997;4(4):472-477]*

**Key Words:** Temporal bone, pathology

### Temporal kemik bankası ve araştırma laboratuvarı : organizasyon ve önemi

*Hastalıklara neden olan patolojileri anlamak her zaman kolay olmaz. Kulak hastalıklarının anlaşılmasında temporal kemik patolojisi bize mükemmel ipuçları sağlar. Böyle bir çalışma bu organı etkileyen rahatsızlıkların tanı, tedavi ve önlenmesinde esastır. Fakat bu zorlu, uzun işlemlerin yanında iyi bir takip bilgisi saklamayı ve hastaları, temporal kemikleri üzerinde post mortem çalışmayı kabule ikna etmeyi de gerektirir. Dünyadaki bu şekilde olan nadir merkezlerden birisi de House Kulak Kliniği temporal kemik araştırma laboratuvarıdır. Bu tipteki laboratuvarları Türk KBB hekimlerinin dikkatine sunarak, böyle bir laboratuvarın önemini tartıştık. [Turgut Özal Tıp Merkezi Dergisi 1997;4(4):472-477]*

**Anahtar Kelimeler:** Temporal kemik, patoloji

### INTRODUCTION

Hearing and balance disorders are among the most prevalent physical ailments and the temporal bone banks serve as an excellent source of material for research on the structures comprising the auditory and vestibular systems. Such a laboratory is essential for progress in the diagnosis, therapy, and prevention of disorders affecting this organ (1-9).

Temporal bone collections were in existence in Europe at the beginning of this century. Prosper Meniere (1861), who had been credited with first describing the vertiginous syndrome (Meniere's disease) as an inner ear disorder, was able to recognize this association based on his astute observations in temporal bone specimens. Subsequently, Hallpike (1938) and Yamakawa (1938) independently observed endolymphatic hydrops in the temporal bones of patients with Meniere's disease. During the ensuing years, temporal bone research has

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made long strides, and the knowledge emanating from the scientific study of temporal bones has provided insight into the pathologies. Now, in addition to classic light microscopy, a variety of innovative techniques such as electron microscopy, immunohistochemistry, and computer-aided imaging are in use.

The medical documentation should also accompany in detail; including, symptoms and signs, the results of audiological tests, vestibular tests, hematological tests, and imaging studies, complete drug and medication history, medical and surgical treatments provided, and follow up records.

## MATERIALS

One person, such as a technologist, researcher, or an otologist should be designated for the retrieval of bones. A portable case containing all the necessary instruments, which are periodically checked for their conditions and contents, and routinely cleaned after use, should be readily available to the person retrieving temporal bones.

The major technical objectives of temporal bone retrieval from the cadaver are proper dissection and timely fixation. There is no doubt that the time factor is critical, because deterioration of tissues begins within minutes after death, and vital research information is lost after 24 hours. Delays in procuring specimens are often unavoidable due to many factors. Fortunately, refrigeration of cadavers in a cold storage retards postmortem autolysis, so that useful bone specimens may be retrieved as long as 24 hours after death. The time involved in gaining access to the cadaver can be significantly reduced by the physician cultivating a good rapport with the patient's family during the follow-up period, by taking the appropriate legal measures in advance, by the cooperative participation on the part of the patient's family and by prompt notification of patient's death by the next of kin. Rapid mobilization for retrieval of the bones after notification of death is extremely important and can be assured by establishing certain routines and procedures before the need arises. These include, designating an individual to liaise with all parties concerned, having a technically trained person to retrieve the bone in time and having all the necessary materials available.

## TEMPORAL BONE RETRIEVAL

Removal of temporal bone can be accomplished by one of the following three methods depending on the circumstances.

1) **Intracranial Method:** There are two techniques in this method, block technique and bone plug technique.

a) The block technique consists of making four linear bone cuts with either an osteotome or an electrically-operated oscillating saw. b) The bone plug technique, on the other hand, is performed with an electrically-operated trephine. These two intracranial techniques accomplish the removal of all ear structures that are needed for routine scientific study.

2) **Extended Intracranial Method:** This method involves five deep cuts with a saw and includes removal of the Eustachian tube and part of the palate.

3) **Extracranial Method:** This method is used under special circumstances when temporal bones have been pledged for research, but for some reason complete autopsy with brain removal has not been granted.

A special procedure is required for the retrieval of temporal bones from cochlear implant subjects and readers are urged to refer to the National temporal bone registry manual for the technical details, instrumentation, staining, etc.

## Dissection considerations

When retrieving the temporal bone, great care should be taken to avoid damage to important internal structures and to preserve anatomical landmarks. Mutilation of the external anatomy of the cadaver should be avoided at all cost except when part of the external skull is required, for example when the pathology extends to the skull or in the case of a cochlear implant subject. In any such event, the family must agree to the procedure beforehand. During retrieval, one should not remove the dura, as this would damage the endolymphatic sac, nor make the cut too close to the sella, as this would weaken the internal support of the skull to the point of collapse. Before the brain is removed, the seventh and the eighth cranial nerve trunks should be cut at the porus acusticus to avoid traumatic avulsion from the internal auditory canal (IAC).

## Fixation

Upon removal, the specimen should be immediately placed in a glass jar with a screw-on lid containing about 300 ml of fixative solution to preserve the tissue morphology. As a fixative, a 10% solution of neutral buffered formalin is recommended for temporal bones and a 15% solution for whole brain. The volume ratio of fixative to specimen should be at least three parts to one in the case of temporal bones and five parts to one in the case of brain specimen. When on site fixation is not possible, refrigerating (not freezing) the specimen helps retard deterioration. Embalmed specimens are preferable to unembalmed ones, since the embalming fluid contains formalin and other preservatives. Use of formalin is preferred because the artifacts it generates are known and standard in histology. However, one disadvantage in using formalin is its tendency to oxidize upon exposure to air, forming formic acid which is a caustic substance. Buffering the solution decreases such acidity. The final element in fixation is the duration of fixation. Temporal bones should be fixed for at least two weeks, whereas whole brain requires four weeks. The formalin solution should be changed several times, at least once a week, while the temporal bones are kept at a temperature of 4 degree C for two to three weeks. Formalin is preferred if both light and electron microscopy are planned, but, if electron microscopy only is to be done, the inner ears should be perfused with glutaraldehyde. Special care is necessary in the fixation of brain specimen to prevent disfigurement. The brain specimen should be suspended in a cradle of 12" x 20" gauze, cortex down, so that it is covered by the fixative but not resting on the bottom of the container.

## LABORATORY RECORD KEEPING

Record keeping is just as important as the preparation of temporal bone sections for research.

Temporal bone laboratory records may be categorized as 1) Procedural references, and 2) Research references. Procedural references monitor the status of a given specimen during its preparation. Research references contain descriptive and analytical information relevant to problem solving.

## Procedural references

Three types of record books are maintained: a case accession book, a procedure book and a diagnosis book.

**Accession book.** Upon receipt, each case is assigned an accession number and, thereafter, an attempt is made to refer to each case by the accession number rather than by name. This protects against confusion of same or similar names and also insures privacy for the patient's family. Since the accession number is assigned to the case rather than to the specimen, the same number refers to both right and left specimens.

**Procedure book.** The function of this book is to provide the processing history and current status of a given specimen. Each specimen in our laboratory is entered in the procedure book by accession number. Every stage of processing, from decalcification to final clearing of the embedded block, is noted by date. Any departures from routine are also noted.

**Diagnosis book.** As diagnosis is established on each case, it is assigned a line in the pathological diagnosis book by accession number. In addition to the primary diagnosis, other relevant morphological information is also recorded.

Since it is not possible to label the temporal bone itself, the containers holding the specimen are labeled with accession number, name, and the side of the specimen.

## Research references

**Medical Record:** When a specimen is received, the medical record is sent to the laboratory from the clinic and it remains in the laboratory thereafter. Each chart is labeled with the case name and accession number. In addition to the medical history, each chart contains the final autopsy report as well as such legal documents as the autopsy consent form and death certificate.

A good computer database management system for documenting the information for temporal bone research has been implemented (8).

## **PREPARATION OF THE RETRIEVED TEMPORAL BONE**

This is a rather laborious process involving several steps of lengthy procedures ranging from decalcification to clearing the embedded block.

### **Decalcification**

Undecalcified bone cannot be sectioned under common microtomy conditions because calcium is a metal which will destroy the cutting edges of the steel knives. Calcium must be removed from the bone prior to sectioning without destroying cellular morphology. Most chemicals used for the decalcification process are acids which are sufficiently powerful to cause serious tissue damage. In our laboratory, we use Ethylene-Diamine-Tetra-Acetate (EDTA), because we find it less destructive than other commonly used agents. However, the time required to complete decalcification is lengthier with EDTA (16 weeks) than with other agents (6-8 weeks with nitric acid and 4-6 weeks with trichloroacetic acid). Preferences differ regarding the use of decalcifying agents, because various agents produce different staining effects. EDTA is available in stock liquid form as "Versene 100" which is corrosive to metal and from which a working 1.0 molar solution is prepared for decalcification purposes. The pH of the solution is crucial and should be maintained at an optimal pH of 7.4 (acceptable range 7.2-7.8). If the pH is too high, decalcification will take an inordinately long time, but on the other hand if the pH is too low, calcium versinate crystals will form on the surface of the bone. The bones are washed to remove excess fixative before placing in EDTA, which is changed twice per week for 16 weeks (adult human bones) or 8 weeks (infants and small animals). Bones are incubated in the solution at 37-40 degree C and washed in water for 24 hours. As a final step, bones are X-rayed to rule out the presence of residual calcium which will appear as dense white areas in the negative.

Following decalcification, the temporal bones need to be trimmed to 1) promote penetration of the embedding medium, 2) to enhance the ease of sectioning and 3) to reduce the sections to a size compatible with a 3" x 1" microslide. The superior semicircular canal is cut open at the same time to release any entrapped air in the bone. One should take great care to avoid desiccation of the specimen during

dissection. The cerumen should be removed from the external ear canal without damaging the tympanic membrane.

### **Embedment**

Embedment of the decalcified temporal bone is the next most important step in the process. A variety of materials are available for this purpose. In our laboratory, we use Celloidin for embedment, because it provides several advantages. Celloidin is a concentrated solution of pyroxylin dissolved in equal parts of ethyl ether and absolute ethyl alcohol. It forms blocks that are more supportive to large specimens, it penetrates without producing a thermal reaction and, because it hardens by evaporation rather than polymerization, celloidin circumvents the problems of tissue shrinkage and distortion. There are many factors that make the temporal bone especially difficult to impregnate with the embedding medium. The bone possesses the delicate membranes of the auditory and vestibular organs encased in the densest bony tissue in the body, the Otic capsule, and contains numerous air spaces which must be evacuated for the embedding medium to penetrate the tissues completely. This lengthens the embedding time. A disadvantage with the use of celloidin is that it is expensive and its hardening property by evaporation requires large quantities of celloidin to compensate for shrinkage. The embedding schedule that we use in our laboratory is a modification of the procedure followed by the Otopathology laboratory of Massachusetts Eye and Ear Institute. The whole process takes approximately five months. At first the specimen is dehydrated in increasing concentrations of ethyl alcohol (70%-100%) and finally in a solution containing equal parts of ether and alcohol (50/50). After this, the specimen is embedded in 2% celloidin for the first 4 weeks, 6% celloidin for the next 4 weeks and 12% celloidin for the final 4 weeks. Subsequently, the specimen is desiccated with chloroform fumes for 2 weeks and poured chloroform for 2 days. Before storage, the block is cleared with cedarwood oil once daily for 3 days.

### **Sectioning**

A sliding microtome is used for sectioning the celloidin block containing the temporal bone. The

block is mounted on the sectioning platform with the middle fossa side up. Under dripping 80% ethyl alcohol, 400-600 thin sections, each about 20-25 microns thick, are cut. Each section is mounted on a sequentially numbered onionskin paper and stored along with other sections of the same specimen in 80% alcohol. This is done to identify each individual section and establish its relationship to the whole specimen. Every 10th section is stained with hematoxylin and eosin (H&E) for histopathology and mounted on a microslide. Each microslide receives a double label. On the top side, a paper label contains the name, accession number, side of the head and section number. On the back are the accession number, laterality and the section number.

### **SAFETY PRECAUTIONS IN THE TEMPORAL BONE LABORATORY**

Every laboratory is a hazardous place and the temporal bone laboratory is no exception to these hazards. Two main categories of hazards exist in the temporal bone laboratory, namely, biological and chemical. Biological hazards exist due to the potential presence of pathogens in human tissue specimens. Chemical hazards exist because various procedures during the preparation of the temporal bone for sectioning require the use of toxic and combustible substances. It is vital that anyone planning to operate a temporal bone laboratory be educated as to the types of hazards present and how to deal with them. The laboratory personnel should be encouraged to attend occupational safety seminars.

#### **Biological hazards**

Because most specimens are fixed at the time of acquisition, biological hazards are fortunately few, since the fixation process destroys most pathogens. Some pathogenic organisms (particularly the viruses), however, are not destroyed by fixation. Therefore, safety precautions should be taken while handling all tissue specimens including the fixed tissue. The laboratory should be fitted with a fume hood for airborne pathogens and a sink with a mesh strainer for catching small scraps of biological material which should be discarded into a special container. All food and drink should be prohibited from the dissection area. Gloves should be worn to handle all tissues.

Other important measures include, stringent cleaning procedures of the work area and proper disposal of dissected materials, autoclaving of tools and other materials and quarantine of biologically contaminated materials.

#### **Chemical hazards**

Chemical hazards are much more serious than biological hazards in a histopathology laboratory. Numerous chemicals are caustic e.g., HCl, Xylene; others are toxic e.g., Chloroform; some are carcinogenic e.g., Diaminobenzedene, Formaldehyde, Xylene; and others are flammable e.g., Versene, Alcohol; whereas Ethyl ether is explosive. Every person working in the laboratory should receive instructions regarding the early detection, avoidance, treatment and notification of hazards. Numerous legal regulations govern the use of equipment, as well as handling, storage and disposal of dangerous substances. The local building code authorities should be consulted regarding ventilation, electrical installations and other construction problems relating to the maintenance of an explosion-free environment.

### **SIGNIFICANCE OF A TEMPORAL BONE BANK AND LABORATORY**

Temporal bone banks serve as a unique source of material for conducting research in the field of auditory and vestibular pathophysiology. All the temporal bone banks in the nation can join to form a National Temporal Bone Registry, so that the information from every other temporal bone bank in the country becomes available to researchers through a computerized data bank. The laboratory provides an environment for innovative research to understand the physiology and pathophysiology; and to trace the etiology of many hearing and balance disorders through the analysis of tissues of the temporal bone. The value of the temporal bone bank and laboratory is that they provide the researchers a ready access to clinical records charting the disease, enabling them 1) to match their histology findings of temporal bone sections to what is known of the patient's condition prior to death, 2) to compare diseases in different specimens and 3) to share the wealth of information through the National Temporal Bone Bank Registry. Using Immunohistochemistry, it is possible to stain

specific structures and molecules in the temporal bone sections. In our laboratory , we are currently using this technique to demonstrate, a) the substances that may be involved in the pathogenesis of Meniere's disease, b) the inflammatory cells that will be helpful in the understanding of autoimmune diseases of the inner ear and c) the enzymes and/or the chemical factors that may cause bone destruction in otosclerosis, cholesteatoma and other skeletal diseases. Computer technology has greatly enhanced the bone bank's ability to collect information on the structures comprising the auditory and vestibular systems. Thus, with the help of the bone bank data base, we are able to create a computer-aided three dimensional (3D) reconstruction of microscopic anatomic structures such as the endolymphatic sac and measure the fluid in the inner ear. The above facts highlight the pivotal role of the temporal bone bank and laboratory in accomplishing research in the field of auditory and vestibular pathophysiology and thereby enabling the researchers to get to the root of debilitating diseases and to develop effective treatments.

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