Paparella: Volume I: Basic Sciences and Related Disciplines

Section 3: Histology and Pathology:

Part 1: Ear

Chapter 18: Histology and Pathology of the Ear (Including Temporal Bone Removal for Dissection)

Michael M. Paparella, Sherry F. Lamey, Marcos V. Goycoolea

Human temporal bones are useful for the study of anatomy, histology, and pathology and for the practice of microscopic surgical dissection. This knowledge is essential for understanding and developing rational and efficient forms of therapy, both medical and surgical.

Removal of Temporal Bone

The temporal bone must be removed from the skull for adequate study. The specimen contains the external auditory canal, the middle ear, the mastoid, the inner ear structures, and the surrounding petrous pyramid. Once a complete autopsy permit has been obtained, the temporal bones should be removed as soon as possible in order to retard postmortem degenerative changes. No external disfigurement or other complication to the cadaver should result.

The calvarium is removed in the usual way. The brain is then removed, with care being taken to cut cranial nerves VII and VIII sharply at the surface of the internal auditory meatus, so that the nerve trunks remain with the temporal bone specimens. The brain may be preserved and studied for central auditory and vestibular lesions when desired. The two recommended techniques of removal are (1) the block method and (2) the bone plug method.

Block Method

A motor-driven saw or, preferably, the commonly available Stryker saw (rocker-type oscillating saw) may be used. With the block method, four saw cuts are made as outlined.

The *first cut* is made at a right angle as close to the apex of the petrous bone as the regional anatomy will allow. With this method, the eustachian tube can be removed for study if this cut is made farther forward.

The *second cut* is made parallel to the first cut through the mastoid process as close to the lateral wall as possible. More of the mastoid process is helpful for temporal bone dissection and less is necessary for histologic study. The *third cut* joins cuts one and two and is made approximately 1 inch anterior to and parallel with the petrous ridge in the floor of the middle cranial fossa. This cut includes the bony external canal.

The *fourth and final cut* is made in the horizontal plane close to the floor of the posterior cranial fossa. This undermining cut severs the bone from its inferior attachments. The temporal bone is still not loose, and great care must be taken to avoid crushing it. A "lion-jawed" forceps is used to grasp the specimen, and the remaining bony connections are loosened by a gentle rocking motion, which frees the specimen for further dissection. A sharp chisel, knife, or scissors is used to cut remaining ligamentous fibrous and bony attachments.

Whether the temporal bone is removed by the bone plug method or the block method, the carotid artery should then be grasped and a ligature placed around this vessel. In addition, a suture may be placed in the external auditory canal to prevent any leakage of fluid.

Bone Plug Method

This method was introduced and described by Schuknecht (1968). The saw should be centered over the arcuate eminence (prominence of the superior semicircular canal on the superior surface) and directed to the floor of the middle cranial fossa. This technique requires the use of a specially designed oscillating bone plug saw attached to the conventional Stryker apparatus; the procedure is simple and provides adequate tissue for study. For the adult skull, a saw 1.5 inches in diameter, adjusted to a depth of 1.5 inches, is used, whereas a saw 1 inch in diameter, adjusted to a depth of 1 inch, is used for smaller skulls.

The head is steadied by an assistant, and a stream of water is directed at the blade for lubrication. Cutting is completed when a loss of resistance is felt, indicating penetration through the base of the skull. An improved cutting action is acquired by slight rotation of the saw. The plug is then grasped with the "lion-jawed" forceps, and the bone is rotated, permitting visualization of the internal carotid artery on its inferior surface; this is grasped with a curved hemostat and ligated. Additional attachments are cut with a knife, scissors, or osteotome.

Temporal Bone Dissection

Surgical dissection of the temporal bone is an essential prerequisite for otologic training in residency programs or for otolaryngologists who wish to practice specific techniques. Fresh temporal bones can be wrapped in water-soaked cotton, or they may be placed in Teflon bags, with all the air expelled and the bones frozen, which helps to preserve the soft tissues for later use. A dissection station for temporal bones should be arranged to simulate actual operating room conditions as closely as possible. Essential items of equipment include a proper table, a comfortable chair, an operating microscope, a motor-driven drill or other otologic drill, a suction apparatus, a complete assortment of otologic instruments, and a temporal bone holder. In general, two types of temporal bone holders can be used: one that embeds and fixes the temporal bone in a medium such as plaster of Paris, or another that secures the temporal bone specimen, allowing for study of all surfaces and relationships of the bone during dissection.

It is not within the scope of this chapter to provide a guide for surgical or anatomic dissection. In teaching or learning otologic surgery, however, it is possible to use an illustrated

step-by-step guide that allows the resident or otolaryngologist to practice most of the surgical techniques in temporal bone specimens (see the chapter on Temporal Bone Dissection in Volume II; also see Goycoolea and Paparella, *Atlas of Otologic Surgery,* 1989).

Many normal temporal bones should be used for practice dissection before any procedures are attempted in a living human being. The tympanic membrane with the attached malleus or the incus may be removed from temporal bones of donors who had been in good otologic health. The membrane should be preserved and saved for later use as a homologous transplant in the tympanoplasty surgery.

Histologic Preparation of the Temporal Bone

If the patient has had a positive otologic history, the temporal bone should be preserved for histologic study. All pertinent clinical data, especially the results of auditory or vestibular-function tests, should be recorded. Such patients may be requested to bequeath their temporal bones to science at the time of their death by arrangement with the Deafness Research Foundation. The headquarters of the National Temporal Bone Program are located in the Massachusetts Eye and Ear Infirmary, and there are centers covering the entire United States (Table 1). This is part of a national effort in which otolaryngologists in each and every location can and should contribute.

Table 1. Histologic Preparation of Temporal Bone Specimens

Step	Time	Solution	Technique
Fixation	1st and 2nd weeks or 48 hours	10% buffered formalin or Heidenhain Susa	Fixation in refrigerator 1st week, at room temp. 2nd week or Fixation in refrigerator
Decalcification	3rd to 9th week	5% trichloroacetic acid	Solution is changed every day for first week, three times weekly after that. Test for calcium with 5% ammonium oxalate in fourth week. Test every other day until three negative reactions are recorded.

Neutralization
1 day
5% sodium sulfate
Place in solution

Rinse
1 day
Water
Rinse in running tap water

Dehydration
10th and 11th weeks
Concentrations of alcohol
Change daily through concentrations of: 35%, 50%, 70%, 80%; two changes of 95%; three changes of absolute alcohol

Clearing
2 days
1/2 Ether, 1/2 absolute alcohol
Change daily

Embedding
12th to 25th week
Concentrations of celloidin
Place in 1% celloidin for 2 weeks, then in 3% celloidin for 3 weeks, then in 6% celloidin for 4 weeks, then in 12% celloidin for 4 weeks

Hardening
26th to 30th week
12% celloidin
Ether and alcohol permitted to evaporate slowly to prevent formation of air bubbles

Mounting
After hardening (1 to 2 hours)
Surface of block is softened with ether-alcohol solution, pressed on a mounting block layered with 12% celloidin, and coated over to 12% and hardened in chloroform. Blocked specimen may be stored in 80% alcohol until time for sectioning

Cutting
After mounting (2 to 3 hours)
Block is sectioned on a sliding microtome. Sections are cut in a horizontal plane with modiolus of cochlea at a thickness of 20 microns. Sections can

also be cut in a vertical plane parallel to axis of bony modiolus.

Staining

After cutting (6 to 8 hours)

Routine hematoxylin and eosin staining; other histologic stains can also be used.&

Autolysis can be retarded and more rapid fixation achieved by injecting formalin solution through the tympanic membrane to fill the middle ear promptly after death. When delay in removal of the specimen is unavoidable, the body should be refrigerated to reduce autolytic changes; the temporal bones should be removed within 24 hours.

Excess soft tissues and bone are trimmed from temporal bone block specimens by use of scissors and rongeur. The specimen removed by the bone plug method is smaller and can usually be placed in the fixative solution with little or not trimming.

After the bones have been removed, they should immediately be placed in 400 mL of 10 per cent formalin (one part commercial formalin to four parts of distilled water) or in Heidenhain's Susa fixative solution. The jar containing the fixative and the specimen is placed in the refrigerator for a period of 48 hours. It is desirable to shake the jar once or twice a day to improve penetration of the fixative. After the bones have remained in the fixative for 2 to 3 days or more, they may be shipped to a laboratory properly equipped to examine temporal bones. They should be placed in a plastic or glass jar that has been filled to the top with the fixative (formalin or Heidenhain's Susa) and properly sealed with tape or paraffin. The jar is packed in a carton with sufficient padding to protect it from breakage and mailed by either first class or air mail.

The preparation of temporal bones for light microscopic study is a somewhat complex and expensive process requiring an average of 7 to 9 months for human specimens and a shorter time for animal temporal bone specimens. One must decalcify one of the hardest bones of the body (petrous bone) while at the same time preserving all the detailed and delicate structures of the membranous labyrinth. Proper histologic slides for interpretation are dependent on: (1) reduction of postmortem autolysis, as suggested previously, and (2) elimination of preparation artifacts. Specially trained technical personnel working in a properly equipped temporal bone laboratory are necessary. It is desirable for the temporal bone laboratory to function in conjunction with the general pathology laboratory of a hospital, which will help ensure the cooperative interest of the pathologist.

An age-old problem in the study of temporal bone has been its cost, and the difficulty of preparation, especially that related to decalcification and sectioning of the bone while preserving the cellular components of the membranous labyrinth. Large blocks of celloidin-embedded bones are sectioned using a sliding microtome. The long (250-mm) knives used in most laboratories are difficult to sharpen. Each knife allows the specialist to perform an average of 100 sections. Since 350 to 450 sections are needed for each human temporal bone, three to five large knives have to be used. Lamey (1985) recently adapted a system in which a clamp holder for a commercially available 80-mm disposable razor blade was designed. The clamp is strong enough to maintain

rigidity of the small blade and long enough to fit the sliding microtome. The cost of disposable blades (one dollar each) is better by far than the purchase price and costs for sharpening long knives. Moreover, this system enables sectioning down to 15 microns and is more consistent.

Table 1 provides an outline for the procedures in histologic preparation. More detailed instructions should be obtained from the literature (Schuknecht, 1968).

Normal Histology of the Human Temporal Bone

The temporal bone sections shown are selected to demonstrate anatomic relationships. The temporal bone seen here was sectioned from the superior surface, in a plane horizontal to the midmodiolus of the cochlea. An insert shown with each section to illustrate the approximate plane of sectioning. Normally, the temporal bone is sectioned at 20 micron thickness, and every 10th section is stained with hematoxylin and eosin for microscopic examination. In this chapter, approximately every 20th section was photographed and labeled for study. An enlarged view of the midmodiolar section of the cochlea is also shown.

Temporal Bone Pathology

Examples of types of pathology found in temporal bones can be seen. Since a comprehensive description of pathology is not possible in this chapter, the descriptions are brief. Further discussion of otologic pathology will be found in the appropriate chapters of Volume II of this series. More elaborate discussion of pathology may be found in texts by Schuknecht (1974) and Friedmann (1974).