



# Temporal Bone Histology and Radiology Atlas





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# Foreword

Temporal bone histopathological evaluation is simultaneously old and cutting-edge new and provides information about causality, progression, and even treatment of ear disease in pertinent ways.

Temporal bone preparation and study was initiated in Europe during the late 19th and early 20th centuries providing us with the techniques and information about common ear diseases. With the development of otomicrosurgery and sophisticated techniques of testing the audiovestibular system, it became obvious that a much-improved technique of collection and processing of temporal bones would yield pathologic information of a wide variety of ear diseases. Major institutions in the United States, such as the National Institutes of Health (NIH), the American Academy of Ophthalmology & Otolaryngology (now the American Academy of Otolaryngology-Head and Neck Surgery or AAO-HNS), the Deafness Research Foundation (now the Hearing Health Foundation), and the American Otological Society, started providing encouragement and funding during the middle of the 20th century in order to establish “Temporal Bone Banks” for acquisition of bone by pledges in many parts of the U.S. Temporal bone laboratories were established in several teaching hospitals, with 28 such laboratories in the United States in 1976, including Dr. H.K. Chandrasekhar’s lab at New York University Medical Center (Figure 0–1). However, over the ensuing decades, reduction in funding and specialized skills for this type of work resulted in closure of the majority of them.

In 1992, the National Temporal Bone Hearing and Balance Pathology Resource Registry was established by the U.S. National Institute on Deafness and Other Communication Disorders (NIDCD) of the NIH<sup>1</sup> and this was able to centralize and preserve the majority of those laboratories’ specimen collections. The Human Temporal Bone Consortium for Research Resource Enhancement was established in late 2006. It is now called the Otopathology Research Collaboration Network, has three member laboratories in Massachusetts, California, and Minnesota, and has ongoing funding from the NIDCD and NIH. A veritable treasure trove of temporal bone histopathology information can be found online at [www.otopathologynetwork.org](http://www.otopathologynetwork.org).

Continuation of funding and attention to detail in documentation, and incorporation of both the traditional Hematoxylin and Eosin staining techniques as well as newer stains and newer modalities of examination will enable practicing otolaryngologists to better understand old and new ear diseases, and target treatments and preventions toward the actual causes and not merely the symptoms of these infirmities.

There is a clear need for development and training of dedicated temporal bone histology technicians. The reader will see from the images in this book, from the collections of NYU, Massachusetts Eye and Ear Infirmary, and the University of Minnesota in Minneapolis, that a great temporal bone technician can make the story of the bone come alive.

This book began as an instructional course given annually for over two decades at the American Academy of Otolaryngology-Head and Neck Surgery Annual Meeting and as a year-long otolaryngology residents instruction course at New York University Medical School. The idea of correlating histologic anatomy with radiographic images occurred about half way along the years of the course and has been met favorably as each image seems to enhance interpretation of the other. We hope that the reader, a student of otolaryngology, radiology, or both, derives as much benefit from going through these pages as we have.

On a personal note, we are grateful to our families for understanding the hours needed away from them to pursue this project. Thank you, Sree Devi, Sumana, Sucharita, Krishnan, Keval, Kedar, Sajan, Sarika, Lakshmi and Ramanathan.

Hosakere K. Chandrasekhar, MD  
and Sujana S. Chandrasekhar, MD

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Senior laboratory technician Veronica Siverls and Dr. Hosakere K. Chandrasekhar examining a horizontal temporal bone section in the NYU laboratory, 1980s.

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# Dedication: Saumil N. Merchant, MD

My father and I dedicate this book to our friend and colleague, the late Saumil Nalin Merchant, MD, an innovator and educator who was taken from the world well before his time.

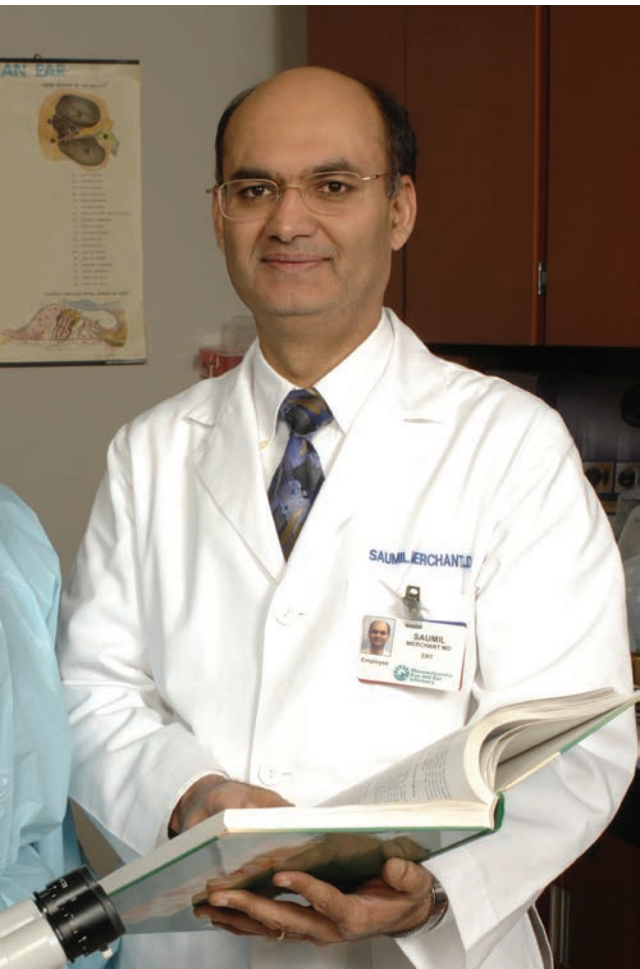
Saumil, the Indian-American son of an otolaryngologist and a gynecologist, and I shared not just a love of otology and histopathology but a similar genealogy, as I am the Indian-American daughter of an otolaryngologist and a family physician. He told me once that he had told his father he didn't need any further education after residency, as he already knew everything, but now, 20 or so years later, he was grateful that his father had pushed him to do a fellowship at Harvard

because, as a full Professor of Otology and Laryngology at Harvard Medical School, Saumil realized that he hardly knew anything at all. And so was he, the humble, brilliant, generous, consummate student.

Dr. Merchant trained in otolaryngology at the College of Physicians and Surgeons in Mumbai, India and received a master's degree in otolaryngology in 1985 at what was then called the University of Bombay. After arriving in the United States, Dr. Merchant became a research fellow in otolaryngology at Massachusetts Eye and Ear Infirmary/Harvard Medical School in 1986 and 1987. He joined Harvard's prestigious otolaryngology faculty in 1992. Believing that the best way to answer all of the unanswered questions in ear disease was through otopathology, he built on the renowned Temporal Bone Laboratory of Professors Harold Schuknecht and Joseph Nadol and used the power of the Internet to archive that institution's most illustrative educational material and make it available to students and researchers around the world. He hosted interactive discussions on otology cases and attendant temporal bone findings on the World Wide Web, with participants from around the world there in real time and able to watch the archives as well.

Dr. Saumil Merchant, with his tireless enthusiasm, inspired other young investigators, and was the architect of a true Renaissance within the field of temporal bone otopathology.<sup>1</sup> He was the inaugural speaker at NYU Otolaryngology's Hosakere K. Chandrasekhar Lectureship in March 2008, speaking eloquently on The Science and Practice of Middle Ear Surgery and correlating histopathology findings with surgical outcomes. The world lost a great physician, thinker, and educator, and we lost a great friend when Saumil passed away in 2012. We dedicate this book to his memory, and to his heroic efforts to restore the study of otopathology to the forefront where it belongs.

Sujana S. Chandrasekhar, MD



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## Dedication: Michislawa Sachs

Michislawa Sachs was employed by John F. Daly, MD, then Chair of Otolaryngology at New York University Medical Center, as senior technical assistant in the NYU Temporal Bone Laboratory during the late 1960s and all of the 1970s. By the time she arrived at NYU she had been trained in the temporal bone processing techniques under the guidance of Drs. Richard J. Bellucci and Dorothy Wolff at the Manhattan Eye and Ear Hospital. Mrs. Sachs was a meticulous, skilled technician with an excellent grasp of the temporal bone microanatomy. Her blocking of the specimens was perfectly oriented resulting in ideally sliced and stained sections in both horizontal and vertical series. She helped me in developing a course in temporal bone anatomy for our NYU residents, which has run without a break for the last four decades.

Mrs. Sachs, with her husband Jan Sachs, had to flee Nazi Germany. They were separated for more than 10 years before reuniting after the Second World War. They eventually obtained refuge in the United States. While she was with us, she always said her compulsion to be precise arose out of the extraordinarily difficult times that she had lived through.

As an employee, she was very punctual and polite and extremely pleasant to work with. She remembered many great stories regarding technical aspects that she gained from Manhattan Eye and Ear Hospital. This helped while we worked to solve problems at the laboratory.

She and Jan quickly became close family friends. The picture here shows (from our left to right) my wife, Dr. Sree Devi Chandrasekhar, the Sachses, myself, their relative, and two of my daughters, Sumana and Sucharita, sometime in the early 1980s. I am certain that we were just about to start a day of swimming in their backyard followed by a meal featuring her delicious red beet borscht.

Many of the slides from our NYU Temporal Bone Laboratory in this Atlas are due to the work of Michislawa Sachs and her trainee, Veronica Siverls, who went on to become an accomplished temporal bone technician in her own right. We owe them a great deal of gratitude.

Hosakere K. Chandrasekhar, MD



Family photograph at the Sachs home with my daughters Sucharita and Sumana, a Sachs relative, myself (Dr. HK Chandrasekhar), Jan Sachs, Michislawa Sachs, and Dr. Sree Devi Chandrasekhar, my wife, circa 1982.



## CHAPTER 1

# Temporal Bone Preparation for Routine Histologic Analysis

*Hosakere K. Chandrasekhar*

### THE TEMPORAL BONE LABORATORY

Hospital pathology departments are not equipped to process temporal bones because of the expense, time, and effort required and because of the special interest and knowledge needed to interpret the preparations. Temporal bone study, therefore, has become a research endeavor to be supported by institutional funds and research grants. The laboratory is headed by a research-minded otologist who oversees the functional laboratory and provides pathologic interpretation, and requires a skilled technician familiar not only with bone preparation and staining techniques, but with the three-dimensional anatomy of the temporal bone.

The accumulation of knowledge of temporal bone pathology has been hampered for several reasons.

1. Complete autopsy permit including the head region is not always available.
2. Time delays in obtaining the bones result in postmortem autolysis.
3. The method of histologic preparation is quite expensive and time-consuming.
4. There are incomplete clinical histories and investigative reports, which weaken the interpretation of pathology.

These problems, having been identified, can be overcome, resulting in robust histological analysis with clinical correlation.

The laboratory needs a convenient and protected tissue slide storage: folded cardboard trays with slots for slides are available for such a purpose. As every

10th section is actually stained, protected storage of labeled, unstained specimens in alcohol in large glass jars is also vital. Those specimens can be accessed in the future for further study and use of novel techniques, as described in Chapters 2 and 9. A temporal bone register needs to be maintained with details pertaining to the medical record of the patient whose bone is being examined—including photographs, and all relevant investigative information, such as the audiogram and vestibular testing results.

### REMOVAL OF THE TEMPORAL BONE

First, properly endorsed autopsy permits (preferably to include the head region) need to be obtained. Delays in procuring specimens result in postmortem autolysis, but the practice of refrigeration of the body soon after death helps in retarding the autolysis. Thus, even 24 hours after death, useful specimens may be acquired.<sup>2</sup> Immediately after removing the bone plug by any method described below, it is placed into a fixative solution—usually 10% formalin.

#### Intracranial Method

After the deaner has sawed off the skull cap, the brain needs to be gently separated from the posterior surface of the temporal bone to identify the porus acousticus. The seventh and eighth cranial nerve trunks should be neatly cut with a scalpel. This will prevent traumatic avulsion from the internal auditory canal (IAC). The brain is then removed by the deaner, and one of the fol-

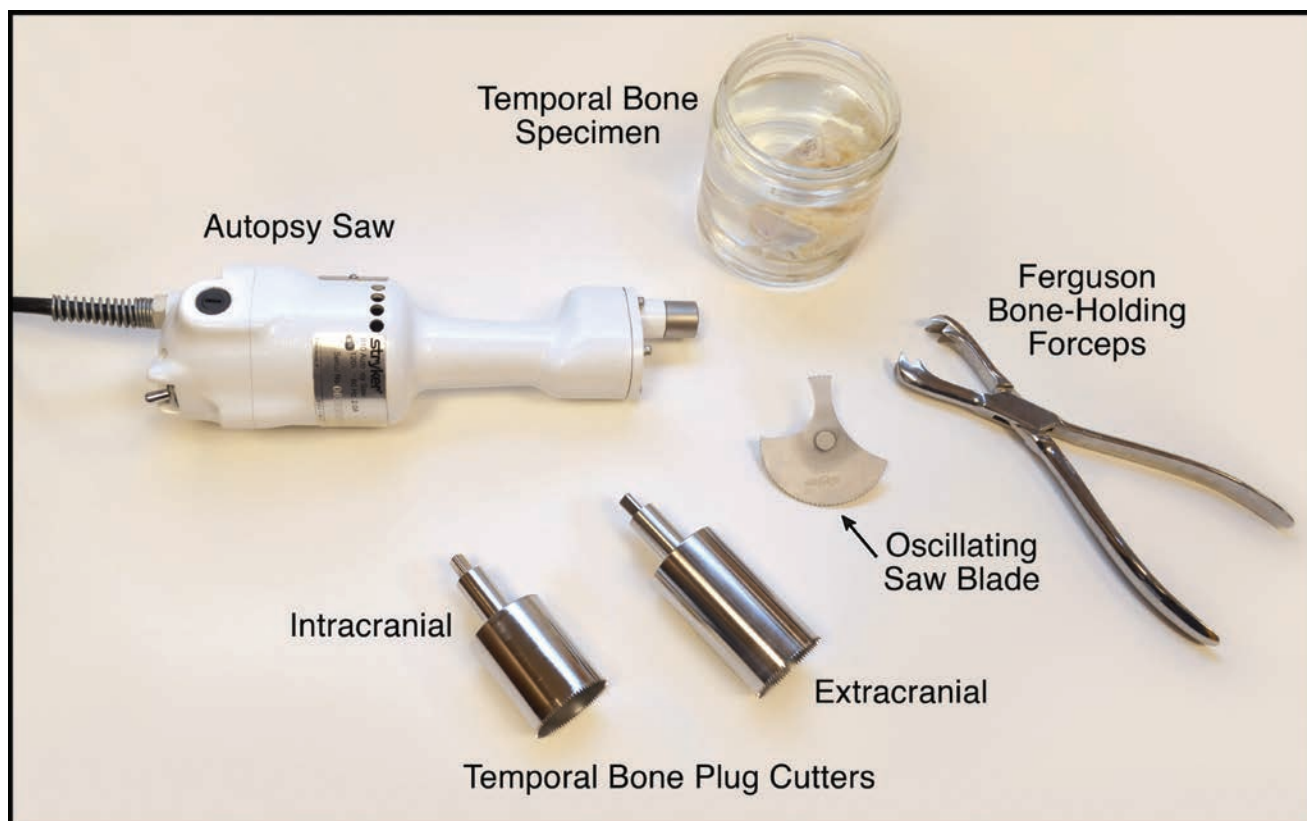


Figure 1-1. Instruments used for intracranial and extracranial removal of temporal bones for histologic study.

Following two techniques is employed to remove the temporal bone.<sup>1</sup> Instruments used for temporal bone removal are shown in Figure 1-1.

#### *a. The Block Technique*

With an electrically driven saw, four cuts are made in the cranial fossa: the first one is at the apex; the second one laterally at the squamous bone; the third one at the petrosphenoid line; and the last one along the posterior border of the temporal bone. The temporal bone is then grasped with bone forceps and rocked gently to loosen it from the skull base. Attached muscular, ligamentous, and fibrous structures are cut and the internal carotid artery is ligated at both cut ends.

#### *b. Schuknecht Bone Plug Method*

This is performed with an electrically operated oscillating trephine. For adult subjects, the trephine used is 2 inches long and 1.5 inches in diameter. For a child, the trephine diameter is 1 inch. The trephine should

be centered precisely over the arcuate eminence and directed perpendicularly to the floor of the middle cranial fossa. As the trephine progresses down, a loss of resistance indicates that the skull base has been penetrated. Using a bone forceps, the bone plug is grasped, rocked, and retracted sufficiently to permit the cutting of the soft tissue attachments and to tie off the internal carotid artery at both cut ends.

#### **Extracranial Method**

This method can be used when temporal bones have been pledged for study, but complete autopsy permit including head has not been granted. A post-auricular incision is made and the external auditory canal is transected. A special trephine 3 inches long and 1.5 inches in diameter is used to remove the bone plug. Afterward, the defect in the skull is tightly packed with gauze and the auricle is replaced.

Figure 1-2 demonstrates the two types of intracranial temporal bone removal techniques.

# Two Methods of Temporal Bone Removal

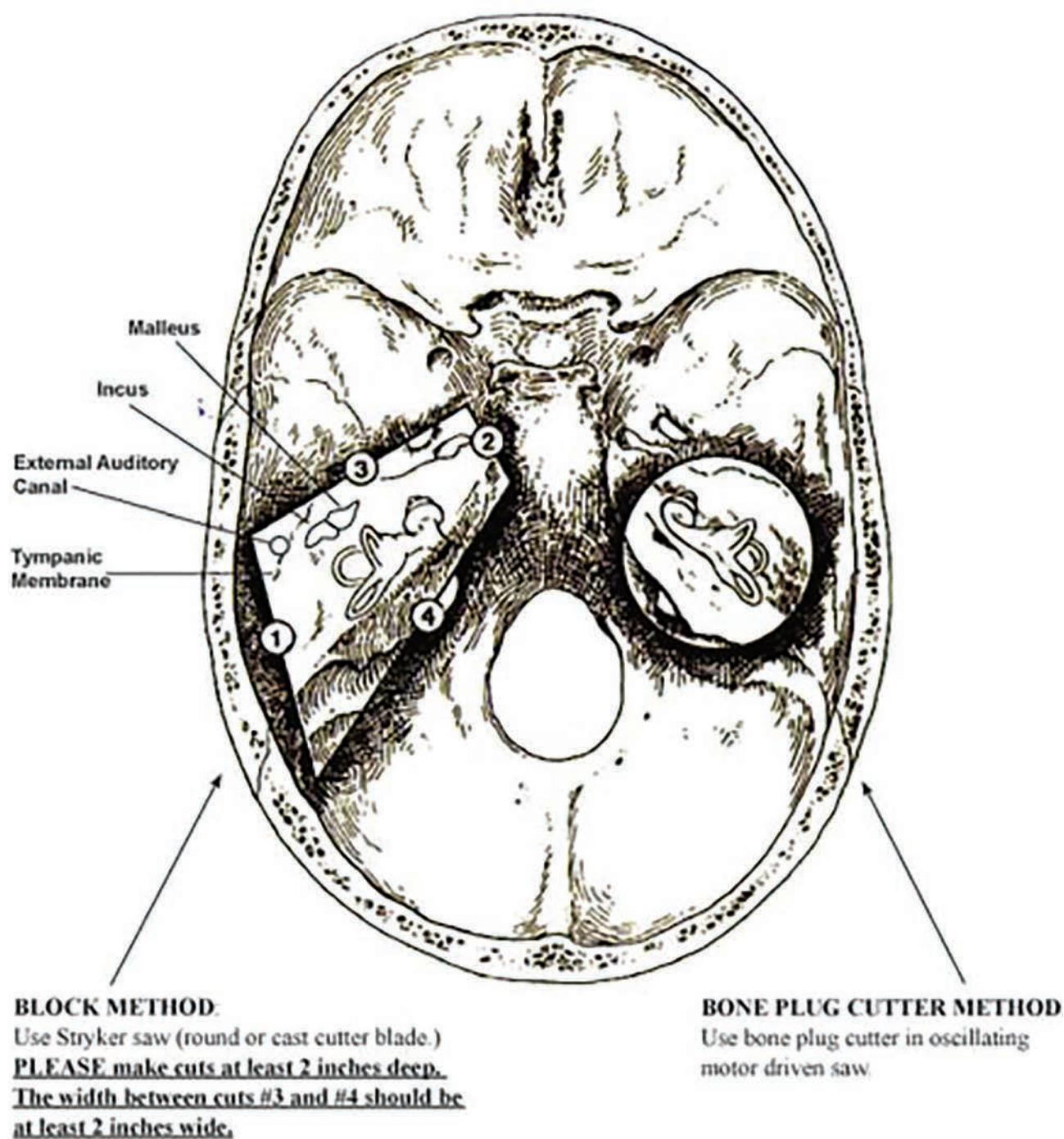


Figure 1-2. Two methods of intracranial removal of the temporal bone for histological processing. (With permission, from: <http://otopathologynetwork.org/tech-resources/techniques/>.)

**SLIDE PREPARATION TECHNIQUE FOR  
STANDARD LIGHT MICROSCOPY**

A short account of a histologic preparation technique for standard light microscopy follows. (For a more detailed description, the reader is referred to standard texts.) This technique was devised more than a century ago and the basic formula has, over time, been refined by employing purer chemicals, better knives and microtomes, and atraumatic handling of tissues.

**Step 1: Fixation; 2 Days**

The tissue block is trimmed to size and immediately placed in 10% formalin solution. Alternatively, Heidenhain Susa solution—a mixture of mercuric chloride, sodium chloride, trichloroacetic acid, and glacial acetic acid dissolved in water—can be used. With the advent of better immunostaining techniques, newer fixation protocols use a mixture of acetic acid and formaldehyde solution, as that improves the ability to use immunologic stains, as shown by O'Malley et al. in 2009.<sup>2</sup>

**Step 2: Decalcification, 30 to 40 Days  
in Adults; 7 to 14 Days in Infants**

The block is then washed in tap water and placed in 300 cc of 5% trichloroacetic acid solution—which is renewed three times per week for 6 weeks. The average time required for decalcification for adult bones is 30 to 40 days. It can be longer in a larger specimen. The temporal bone of an infant may completely decalcify in only a week or two.

The endpoint of decalcification may be determined either by x-ray test or by a chemical test. A mixture of 1 mL of 5% ammonium hydroxide and 1 mL of 5% ammonium oxalate is added to 2 mL of trichloroacetic acid from the specimen. If a precipitation forms, decalcification is not complete. The test is repeated until the result is clear.

Alternatively, ethylene diamine tetra acetate (EDTA) may be used in place of trichloroacetic acid.

**Step 3: Neutralization, 2 Days**

In order to neutralize the acid in the specimen, it is washed in running water for 24 hours and then placed in 300 mL of sodium sulfate solution for 24 hours.

**Step 4: Dehydration, 10 Days**

Dehydration is accomplished over a period of 10 days. During this time, the specimen is placed in a series of jars containing 300 mL quantities of alcohol of increasing concentration. Fifty percent alcohol is used for 24 hours on the first day, followed by 70% on the second day, 80% on the third, 95% on the fourth, 95% on the fifth, 100% on the sixth and seventh, and on the eighth and ninth days, the specimen is placed in ether alcohol.

**Step 5: Embedment, 12 Weeks**

This is accomplished over a 12-week period by placing the specimen in sequence in 300 ml quantities of celloidin (Parlodion®) according to the following schedule:

1. 1.5% celloidin for 1 week
2. 3% celloidin for 3 weeks
3. 6% celloidin for 3 to 4 weeks
4. 12% celloidin for 3 to 4 weeks

**Step 6: Hardening, 2 to 4 Weeks**

The specimen is placed in a desiccator filled with fresh chloroform, tightly sealed to prevent evaporation. The process takes 2 to 4 weeks to complete, and then the specimen is placed in cedarwood oil for another week before the block can be cut.

**Step 7: Blocking**

This is the term applied to fixing and orienting the celloidin embedded specimen on the microtome to achieve the desired plane of cutting. The principal landmarks are the internal and external auditory canals (which should be on the same plane) and the arcuate eminence.

**Step 8: Sectioning**

Sectioning is done on a sliding microtome. The specimen is blocked or oriented for sectioning on the microtome. To section the temporal bone in the horizontal plane, the internal auditory canal and external auditory canal should be approximately in the same plane

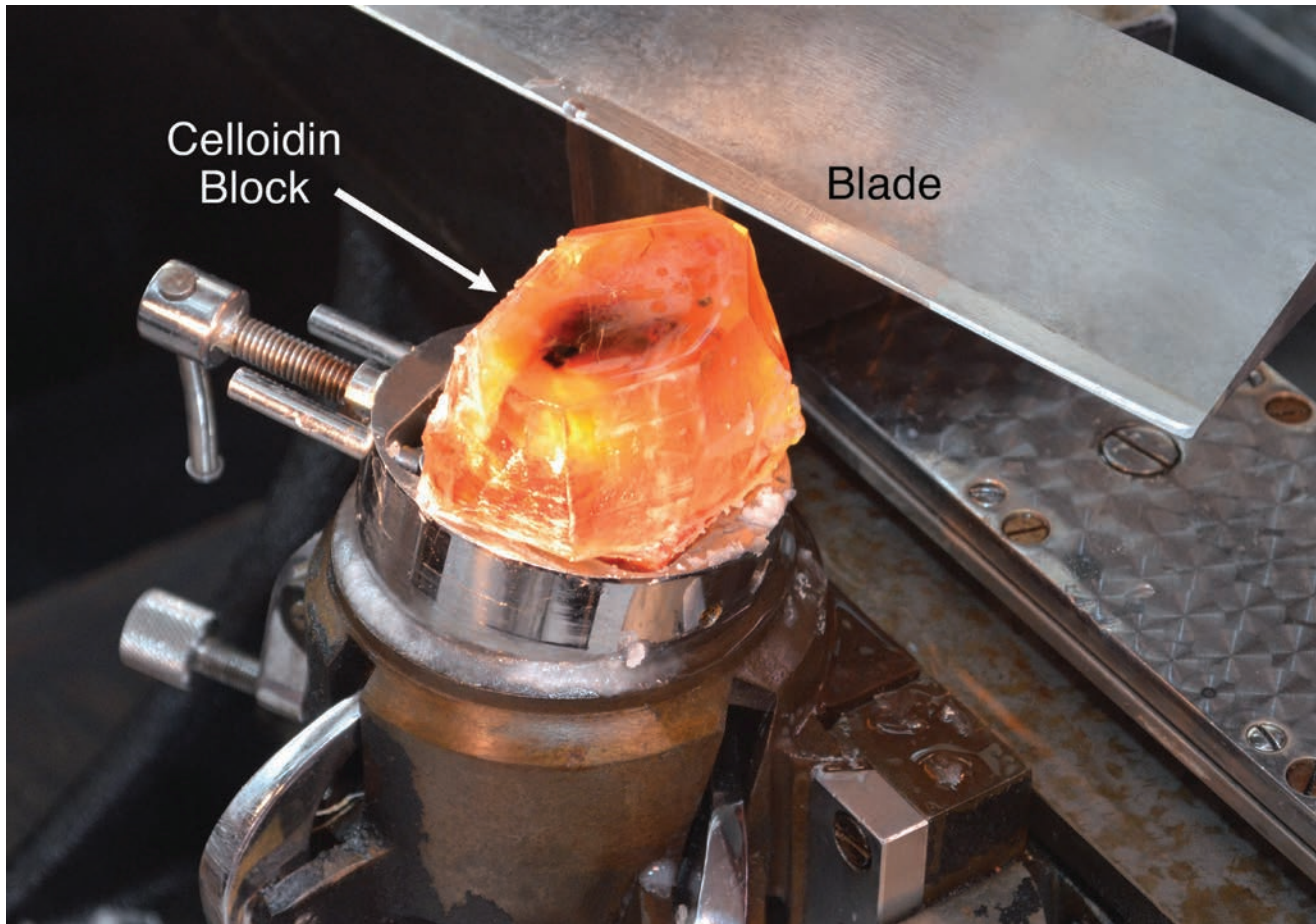


Figure 1-3. Celloidin block ready for horizontal sectioning.

with the superior canal ampulla, facial genu, with the superior surface of the malleus and incus appearing in the same section (Figure 1-3). Vertical sections are performed in the plane of the superior semicircular canal and correlate to sagittal imaging—both CT scan and MR images. The sections are cut at a thickness of 20  $\mu\text{m}$ . Cutting at this thickness usually results in about 400 to 500 sections in the horizontal plane, and 800 to 1000 sections in the vertical plane. All sections are placed on numbered pieces of onion skin paper. Every horizontal section is labeled from superior to inferior and every vertical section from anterior to posterior. Every 10th section is kept separately in 80% alcohol for tracer slides.

The remaining sections are wrapped in gauze, labeled, and stored in large jars with 80% alcohol.

### Step 9: Staining

Sections saved for tracer slides are then stained with Haematoxylin and Eosin (H&E). The staining solutions are prepared freshly from stock solutions and the process is elaborate and meticulous. A good technician is a major asset. Similarly, special stains such as Toluidine blue, Gomori's trichrome, or Luxol fast blue can also be employed satisfactorily.



### Step 10: Mounting

The stained sections are mounted on 1 × 3 inch glass slides, coverslips are placed, and lead weights are used to compress the coverslips onto the slides. These are arranged in sequential order in cardboard folders.

The slides are now ready for microscopic examination.

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