Hydrogen Peroxide Head Preparation: Enabling Cuttings and Anatomic Studies of Skull Base Dura Mater and Arachnoid Without Use of Drilling

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■ INTRODUCTION: **Anatomic pieces that are preserved using formaldehyde allow us to undertake high-quality skull base studies. However, extensive drilling is often necessary, which can lead to damages to the dura mater and thus arachnoid. Formaldehyde and hydrogen peroxide can soften the bone, which, in turn, can be easily cut with a scalpel or removed with a curette. After having discovered this technique by chance, our aim was to establish a study protocol of the skull base dura mater without the use of the drill.**

■ METHODS: **Ten heads were set with a 10% formalin solution and then injected with colored latex. Five heads were then subsequently bleached with 20% hydrogen peroxide solution (HPS). The following were studied weekly: 1) macroscopic modification of the bone, dura mater, arachnoid and brain; 2) histology; 3) computed tomography scans; and 4) calcium concentration screenings were studied weekly.**

■ RESULTS: **After several weeks (mean 6.1, range 5–8 weeks), all HPS specimens were flexible, similar to rubber in consistence. Geometrical bone cuts could be made while preserving all the surrounding anatomic structure (cranial nerves, dura mater, and vascular elements). Histologically, the dural and bone structure are preserved. The HPS cadavers appear to be radiologically demineralized. We note a significant calcium concentration augmentation in HPS solution after 1 month, 6 weeks, and 2 months compared with day 0.**

■ CONCLUSIONS: **The softening of the bone, probably caused by decalcification from the use of corrosive chemicals present in hydrogen peroxide solution, can ease the cutting of the skull base geometrically, which is useful for anatomic and workshop studies.**

INTRODUCTION

Skull base dissection is an important method for acquiring thorough neuroanatomic knowledge for surgical practice. Previous studies have definitely improved our understanding of skull base anatomy.[1-5] The cadavers or fresh anatomic pieces preserved with formaldehyde allow for high-quality neuroanatomic studies. However, because of the hardness of the bone, it is difficult to simultaneously study the skull base and structures that pass through it, especially the basal cistern arachnoid and cranial nerves. Indeed, for skull base dura mater and arachnoid studies, extensive drilling is often necessary, which can lead to dura mater and arachnoid damages. Moreover, drilling-induced bone powder, which sticks to the arachnoid, can be released.

Bone softening helps to avoid these pitfalls while allowing easier cutting without damaging the soft tissues. In anatomopathology, decalcification is usually employed to cut the bone. Bones are exposed to a corrosive agent—ethylenediaminetetraacetic acid (EDTA), nitric acid, formic acid, or acetic acid—for a short period of less than a week.[6,7] The main concern is soft tissue decomposition, such as the brain, in the presence of corrosive agents.[8] Therefore the anatomopathologic piece has to be set first.[6,7,9] We have discovered fortuitously that formaldehyde and hydrogen peroxide can soften and bleach the bone, which can then be easily cut geometrically, using a scalpel or being removed using a curette. This method has not yet been documented in the

Key words
- Head embalming
- Histology
- Hydrogen peroxide
- Neuroanatomy
- Skull base

Abbreviations and Acronyms
CT: Computed tomography
EDTA: Ethylenediaminetetraacetic acid
FPC: Formaldehyde preserved cadaver
HPS: Hydrogen peroxide solution
HU: Hounsfield unity

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Citation: World Neurosurg. (2018) 119:325-330.
https://doi.org/10.1016/j.wneu.2018.08.041

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

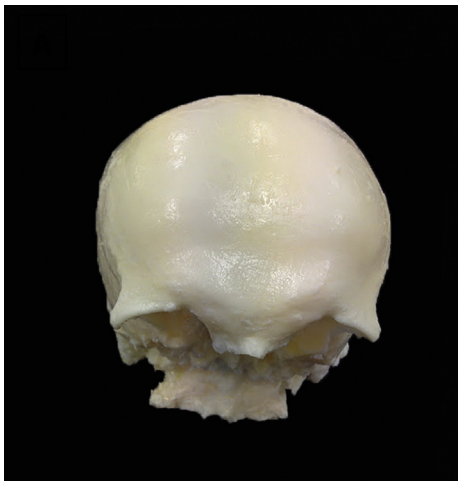

Figure 1. Decalcified head, frontal view.

literature and remains unexplained. With this study, our aim is to establish a new study protocol of the skull base dura mater without recourse to drilling and understand its impact.

METHODS

This study was conducted in the anatomy laboratories in Tours and Angers, France.

Experimental Protocol

We studied 10 consecutive adult cadaveric heads obtained from our body donations programs. The following steps were taken:

1. Vascular injection on fresh cadavers: We injected red latex in the blood vessels for the internal carotid and vertebral arteries and blue latex for the jugular veins.
2. Exocranial base exposition: A large exposure of the exocranial skull base and convexity was undertaken. Next, we removed all the soft tissue (face, orbit, muscles, periosteum), the mandibula, and maxilla and made a large anterior opening of the sphenoid sinus to expose the bone to the corrosive agent (Figure 1).

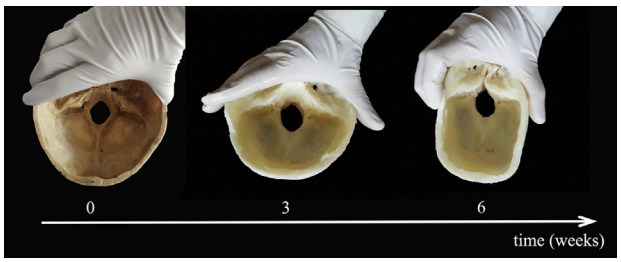

Figure 2. Head before (left), after 3 weeks (center), and 6 weeks (right) of formaldehyde hydrogen peroxide (HPS) 20%. After 3 weeks, the HPS solution bleached the bone. After 6 weeks the HPS solution softened the bone. The skull could be bent easily without deformation.

3. All specimens were set with a 10% formalin solution: 3-cm diameter craniotomies with dural opening were performed on the coronal suture, to expose the brain to a 10% formalin solution for 1 week.
4. Exposition to hydrogen peroxide solution (HPS): Among these cadaveric 10 heads, 5 were then subsequently bleached with 20% hydrogen peroxide solution (HPS) added to the 10% formalin solution.
5. Baths were renewed weekly.

Macroscopic Study

All samples were observed weekly. The softness of the bone was evaluated using a scalpel. When the bone could be easily cut, we analyzed the macroscopic preservation of the bone, dura, brain, arachnoid, colored latex, and decomposition. Therefore we performed coronal and axial dissections with geometrical cuts. As an example of a poorly exposed skull base area, a superior approach to the jugular foramen and an anterior approach to the brainstem were performed. Silver halide photographs were obtained with a Hasselblad camera and lenses (Victor Hasselblad AB, Sweden), and Velvia 50 ISO roll-film (Agfa-Gevaert S.A., Germany).

Histologic Study

After 2 months, 2-cm^2 bone and dura biopsies were taken in the midline from the upper clivus between the posterior clinoid processes. All HPS- and formaldehyde-preserved cadavers (FPCs) were biopsied. Sections were stained using hematoxylin, phloxine, and safran. This coloration made possible the visualization of collagen, cells, and tissue morphology. The sample sections were observed under the optical microscope at different magnifications ($\times$20 and $\times$40).

Radiologic Study

All sphenoidal bones were computed tomography (CT) scanned weekly in order to study bone demineralization using Housfield unity (HU) measurements. CT scans were performed using standard techniques on a multislice computed tomography scanner (Philips Healthcare, Amsterdam, The Netherlands). An average HU value of CT attenuation was calculated by placing a 2-cm^3 single circular click-and-drag region of interest confined to the upper clivus between the posterior clinoid processes. For each measurement, an oval region of interest was placed over an area, excluding the cortical margins.

Biochemical Study

In each specimen, calcium concentration in HPS and FPC baths was determined with a relative error of 0.025 by means of an atomic absorption spectrophotometer at day 0, 2 weeks, 1 month, 6 weeks, and 2 months.

Statistical Analysis

All variables are presented as the mean $\pm$ standard deviation. Statistical analyses were performed using commercially available SPSS software, version 17.0 (SPSS Inc., Chicago, Illinois, USA). Statistical analysis was carried out using Welch's t-test with statistical significance set at $P < 0.05$.

This study has been approved by our body donation program.