A NEW METHOD FOR IMPROVED FIXATION OF THE CHICK'S INNER EAR

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Abstract: *Our method for fixation of the chick's inner ear involves puncturing the soft temporal bone with a needle and directly flushing the membranous labyrinth with cold glutaraldehyde. The temporal bone is trimmed and immersed in the same cold, aerated fixative. This improved method eliminates the need to perfuse through the oval window and preserves better than vascular perfusion. The method is easy to perform and with practice can be completed in 45-60 sec.*

In recent years, the chick has become an important animal model for inner ear research (Cohen and Fermin, 1978; Hirokawa, 1978; Takasaka and Smith, 1971; Tanaka and Smith, 1978). In investigations of avian ototoxicity (Fermin and Cohen, 1982; Fermin et al. 1980; Park and Cohen, 1982; Park et al., 1982), we placed special emphasis on achieving optimal cellular preservation. We empirically determined the optimal fixative composition and thereby eliminated the possibility of confusing fixation artifacts with drug damage. However, a second problem remained. The avian inner ear, along with its mammalian counterpart, is encapsulated by the temporal bone. The temporal bone prevents the fixative from reaching the inner ear embedded within it. Thus, the temporal bone must be quickly opened to flush the inner ear with fixative before the onset of degradative changes. We outline our method for ensuring rapid penetration of the fixative. By using this method, we have quickly and reliably preserved the various cell types of the chick's inner ear.

Materials and Methods—White Leghorn chicks between 6 and 30 da old were used. They were obtained from Mussel-White Hatchery, Maitland, Florida. The inner ears were fixed in 3.5% glutaraldehyde buffered with 0.1 mM cacodylate containing 5 mM Ca^{2+} and 2 mM Mg^{2+}, pH 7.4. Final osmolarity was 560 mOsM. We (Cohen and Fermin, 1978; Park and Cohen, 1982) explained the importance of fixative composition elsewhere. The method involved 5 steps: 1) cutting head in half along the dorsal midline; 2) removal of the brain; 3) injection of the fixative (perfusion); 4) trimming the skull; and 5) immersion and aeration of trimmed tissue. These 5 steps are illustrated with schematic diagrams and explained in more detail below.

First, immediately following decapitation, the head was cut in half along the dorsal midline. Prior to the cut, the razor was centered in the shallow recess on the cranial roof (midline sutures between the frontal bones) and aligned with the comb and beak. Then the razor blade was pressed down

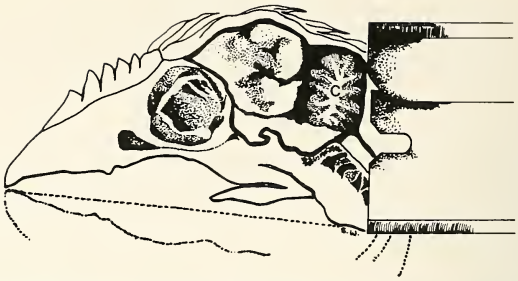

FIG. 1. The divided head. The head was sectioned with a razor blade. The right half is shown from the medial (inner) surface. The cerebellum (C) serves as an important landmark because of its proximity to the inner ear.

firmly in a smooth, continuous movement (Fig. 1). The head was split into equal halves and laid open.

Second, the brain was scooped out with a small spatula to expose the temporal bone (= periotic capsule) that encased the inner ear. As one half of the head was being prepared, the other half was temporarily placed in a beaker of cold (4 C) fixative.

Third, the locations of the semicircular ducts, though not visible through the temporal bone, were accurately estimated by following the outline of the bony canals. The latter were identifiable from the medial side as raised ridges lying directly lateral to the cerebellum. The needle tip (26 gauge) was carefully inserted through the wall of the bony canal and into the perilymphatic region (Fig. 2). The tip was positioned inside the canal but outside the membranous duct, a spatial relationship analogous to inserting a needle between a tire and a partially inflated inner tube. Once the needle tip was positioned within the canal, the cold (4 C) fixative (about 0.25 ml) was slowly injected from the syringe. The immediate outward flow of fixative from below the lagenar region (Figs. 2 and 3) indicated that the syringe was properly positioned within the perilymphatic duct. By comparison, the fixative did not readily flow from the base of the skull when the syringe was improperly inserted (i.e., into a blind sac) despite being forcefully injected from the syringe). The second set of injections delivered fixative (about 0.25 ml) directly above the saccule and utricle. This region is encased by the bony mound. Finally, the fixative (about 0.5 ml) was injected into the bony labryrinthic canal surrounding the lagena (Fig. 3). The lagenar macula (otolithic organ), a vestibular organ located at the tip (distal end) of the lagena, was usually visible through the bone in young chicks. It served as a marker to locate the lagena and guide the accurate insertion of the needles. To avoid puncturing the lagena during this injection, the needle was vertically positioned on either side of the inferred position of the lagena and

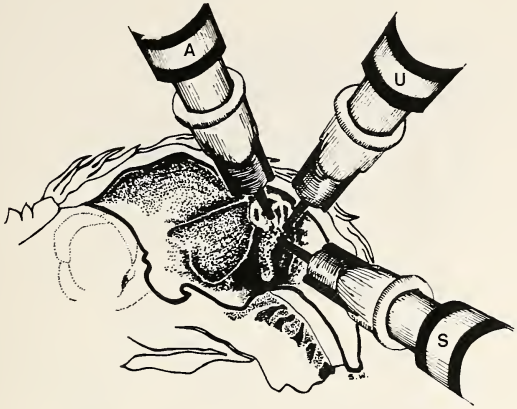

FIG. 2. Injection of fixative in and around the vestibular apparatus. The needle is inserted into 3 sites in temporal bone near the vestibular apparatus: 1) anterior vertical canal (A); 2) utricle (U); and 3) saccule (S). The lagena, which extends from the saccule, points downward. (Each syringe barrel is identified by a letter.)

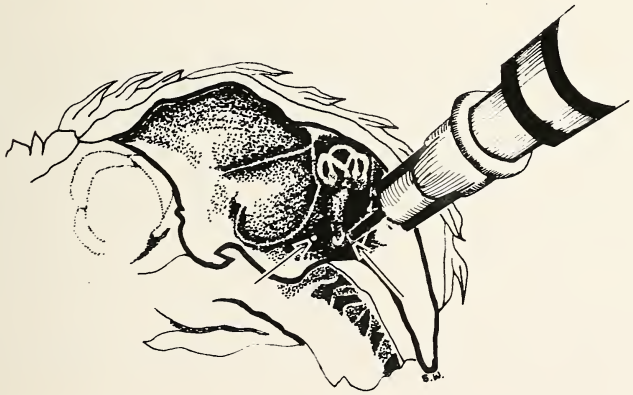

FIG. 3. Injections of fixative around the lagena. The fixative (about 0.25 ml) was injected on each side of the lagena (arrows).

midway between the bony mound and the distal tip. When the lagenar macula (otolithic organ) was not visible, injections were made approximately 2 mm below the lateral edges of the bony mound encasing the utricle and saccule.

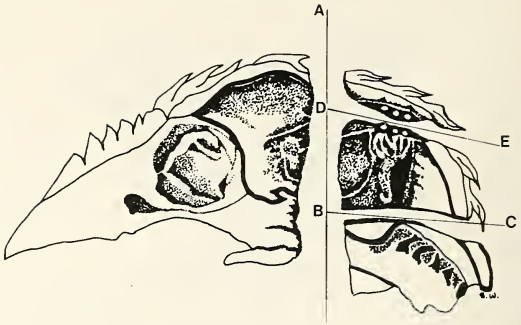

Fig. 4. Trimming procedures. The skull is first cut vertically along the ADB plane and is then cut along horizontal planes BC and DE. Note that cut DE either exposes or lops off the tops of the canals, depending upon the cutting angle.

Fourth, immediately after injection of fixative into both halves of the skull, each half was trimmed to remove extraneous bone. The trimming required 3 major cuts. The first, an oblique cut along line ADB (Fig. 4), removed the eye and the rostral portion of the skull (Fig. 4). The razor blade was angled slightly away from the inner ear to avoid damaging it. The second cut, also oblique along line BC (Fig. 4), removed the basioccipital bone, the ventral portion of the temporal bone, and the neck stump. It was very important when making this second cut to avoid trimming off the distal end of the lagena. To avoid excessive trimming, the ridge of the adjacent cerebellar cavity was located and followed dorsoventrally until it curved caudad. The cut was then made just below this portion of the ridge. The third cut (along line DE) across the top of the canals and through the semicircular ducts allows the fixative to penetrate directly into the endolymphatic regions. In addition, the lagena was also split open by a small transverse cut extending halfway through the trimmed specimen the trimmed specimen made with the razor positioned perpendicular to the long axis of the lagena. Then tissues on the external surface (i.e., muscle, skin, and feathers) were trimmed away. The completely trimmed specimen (Fig. 5) contains the intact membranous labyrinth.

Fifth, the specimens were then immersed in cold (4 C), aerated fixative. Following 10 min of aeration by continuously bubbling atmospheric O_2 through the fixative, the immersed specimens were refrigerated at 4 C for about 24 hr before beginning the final isolation of the inner ear from the temporal bone. Because the original injection holes in the temporal bone remained visible indefinitely, they served as landmarks for locating various inner ear regions during isolation.

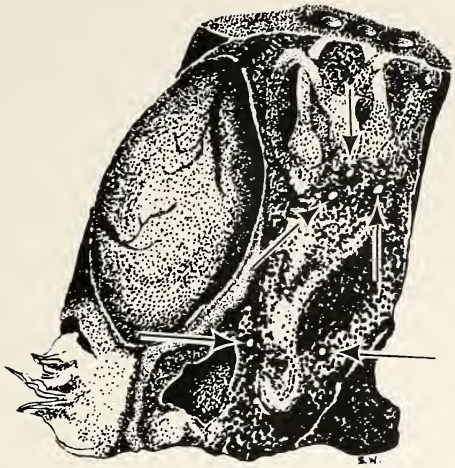

FIG. 5. A trimmed specimen. Note the injection holes (arrows) in and around the inner ear. In this diagram, the tops of 2 canals have been cut off.

DISCUSSION—The mammalian inner ear is embedded in the petrous (stony) temporal bone. The bone is difficult to puncture quickly by routine procedures. By comparison, the avian temporal bone is porous (pneumatized) (Jollie, 1958). Thus, we could puncture the chick's soft temporal bones with the syringe needle to perfuse the fixative directly through the perilymphatic spaces. With practice, we routinely perfused and trimmed the specimen in 45-60 sec, which ensured the rapid delivery of the fixative before the onset of degradative changes. After the tissue was fixed, we isolated the membranous labyrinth from the temporal bone. With slight modifications, we employed this method for preserving the inner ears of mice.

Our method is considerably faster than conventional techniques of perfusion through the oval and/or round windows, or intralabyrinthine perefusion (Takasaka and Smith, 1971). Although these methods preserve well (Anniko and Lundquist, 1977), they are slower and more difficult to perform.

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LITERATURE CITED

ANNIKO, M., AND P. G. LUNDQUIST. 1977. The influence of different fixatives and osmolality on the ultrastructure of the cochlear neuroepithelium. Arch. Oto-Rhino-Laryngol. 218:67-78.

Cohen, G. M., and C. D. Fermin. 1978. Development of the embryonic chick's basilar papilla. Acta Otolaryngol. (Stockh.) 78:342-358.
Fermin, C. D., and G. M. Cohen. 1983. Prenatal ototoxicity of kanamycin in the chick: Damage to the basilar papilla. Acta Otolaryngol. (Stockh.), in press.
_____., J. C. Park, and G. M. Cohen. 1980. Pre-and post-natal ototoxicites of kanamycin and streptomycin in the chick. Third Midwinter Res. Meet. Assoc. Res. Otolaryngol. Abstracts. St. Petersburg Beach, Florida.
Hirokawa, N. 1978. The ultrastructure of the basilar papilla of the chick. J. Comp. Neurol. 181:361-374.
Jollie, M. T. 1957. The head skeleton of the chicken and remarks on the anatomy of this region in other birds. J. Morphol. 100:389-436.
Park, J. C., and G. M. Cohen. 1982. Vestibular ototoxicity in the chick. I. Effects of streptomycin on equilibrium and on ampullary dark cells. Amer. J. Otolargnol. 3:117-127.
_____., B. Culliney, and G. M. Cohen. 1982. Comparative actions of gentamicin and streptomycin in the chick. Fifth Midwinter Res. Meet. Assoc. Res. Otolaryngol. Abstracts. St. Petersburg Beach, Florida.
Takasaka, T., and C. A. Smith. 1971. The structure of the pigeon's basilar papilla. J. Ultrastruct. Res. 35:20-65.
Tanaka, K., and C. A. Smith. 1978. Structure of the chicken's inner ear: SEM and TEM study. Amer. J. Anat. 153:251-261.

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Biological Sciences

STATUS OF THE MOUNTAIN MULLET IN SOUTHERN FLORIDA

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Abstract: *The mountain mullet* (Agonostomus monticola) *is a rare component of the Florida ichthyofauna. Until recently, records for the southern half of the peninsula were nearly nonexistent. We document and summarize the range, size distribution, and habitat occurrence of the mountain mullet in southern Florida. Florida populations are characterized by low numbers of small individuals that are probably recruited from the West Indies via surface ocean currents. Its rarity in southern Florida seems due to the absence of suitable freshwater stream habitats. Mountain mullet persistently occur in southern Florida in an artifically maintained stream with access to salt water. We collected 5 mountain mullet from this population, including the largest documented United States specimen. We also observed additional adult-sized mullet there. Based on current understanding of the life history of* A. monticola, *we discuss the possibility that Parrot Jungle may hold the first breeding population for the United States.*

The mountain mullet *(Agonostomus monticola)* commonly occurs in high-gradient freshwater streams in the West Indies, Central America, and northern South America (Hildebrand, 1938; Gilbert, 1978). The larvae undergo a period of development in salt water before entering fresh water