Scanning Electron Microscopy of the Human Cochlea — Postmortem Autolysis Artefacts*

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Summary. Changes in the ultrastructure of the cochlea due to postmortem autolysis make the assessment of the normal or damaged anatomy difficult. Three methods of preserving the human cochlea were compared on the basis of the state of preservation of the sensory cell hairs of the organ of Corti as seen in the scanning electron microscope. Perfusion of the perilymphatic space with a glutaraldehyde-formaldehyde fixative within 40 min of death gave preservation as good as that seen in animal studies. Injecting formalin into the middle ear within 40 min of death allowed artefacts to develop when compared with the control ear which had been perfused with fixative. Refrigeration and early removal of the temporal bone gave poor preservation of surface structures.

Key words: Scanning electron microscopy — Organ of Corti — Hair cells — Postmortem autolysis

Scanning electron micrographs of the human cochlea are being increasingly found in the literature (e.g., Nomura 1978, 1979; Hoshino 1977). The difficulties involved in the production of temporal bone specimens suitable for any form of microscopy are many but the initial problem of limiting postmortem autolysis is one of the greatest. Most workers have devised methods to limit this autolysis although no one method has been universally successful (Rutledge 1969).

Bredberg (1968) thought that after death 10 h could elapse before fixation, yet still allow good preservation of the reticular membrane, with a distinct cell pattern visible under the light microscope. He did state, however, that this surface seemed to be more resistant to postmortem change than other elements of Corti's organ. Rutledge (1969) considered that temporal bones removed 8 h or more after death were often worthless for microscopic evaluation. Kimura (1964) using the transmission electron microscope and the squirrel monkey cochlea reported that little useful information could be

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obtained from the sensory cell area of specimens fixed 5 h after death. However, if the cochlea was fixed by instilling Osmium tetroxide into the oval window 1 h after death and the temporal bone was removed from the body 5 h after death, then preservation was remarkably good.

Fernandez (1958a, b) showed that refrigeration of the body very shortly after death reduced the degree of autolysis. He also demonstrated that formalin injected into the human middle ear immediately after death again lowered the degree of autolysis seen by light microscopy. This finding was confirmed by Schuknecht (1979).

Methods used to prepare specimens for studying the normal or altered anatomy of the human cochlea under the scanning electron microscope must reduce autolysis to the minimum, as autolytic changes (Schuknecht 1974) might be difficult to distinguish from the changes caused by ototoxic drugs and acoustic trauma. Three methods of postmortem fixation have been compared on the basis of the state of preservation of the sensory cell hairs of the organ of Corti. The sensory hairs have been chosen for comparison as they appear to be the most sensitive of the surface structures easily accessible in the human cochlea, and because of the wealth of animal data that exists for comparison.

**Methods**

Prior to removal of the temporal bone from the body, three methods were used to attempt to reduce autolysis.

*Perilymph Perfusion*

Within 40 min of death a tympano-meatal flap was lifted, the stapedius tendon cut, and the round window membrane perforated with a curved needle. The incudo-stapedial joint was dislocated and the stapes gently extracted. A fine curved cannula was fitted into the round window niche and the cochlea gently perfused with 5 ml of fixative, the excess escaping via the oval window. The fixative used was 2.5% glutaraldehyde with 2% formaldehyde in 0.1 m cacodylate buffer, pH 7.25 (Karnovsky 1965). The middle ear was then filled with a self-curing silicon elastomer (Xantopen: Bayer) to seal the inner ear and prevent leakage.

*Fixative Introduced into the Middle Ear*

Within 40 min of death a hypodermic needle was used to pierce the tympanic membrane and 10% aqueous formalin was injected into the middle ear. The external auditory meatus was then sealed with a pledget of cotton wool soaked in the formalin.

*Refrigeration Only*

No attempt was made to fix the fine structures of the cochlea prior to removal of the temporal bones, apart from ensuring that the body was refrigerated at 4°C as soon as possible after death.

Once permission for postmortem had been granted, the skull was opened and a small part of the temporal bone containing the cochlea was removed. The time delay was never more than 15 h. The silastic or cotton wool, if present, was removed. The cochlea was then perfused with the buffered glutaraldehyde-