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DISSECTION TECHNIQUE FOR COCHLEAS PREPARED FOR SCANNING ELECTRON MICROSCOPY

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Abstract

Scanning electron microscopy (SEM) permits a three-dimensional study of the surface morphology of the organ of Corti that is very useful in evaluating the condition of the apical end of the hair cells and the stereocilia. However, some laboratories have experienced problems with curling of the basilar membrane during critical point drying of cochlear specimens prepared for SEM evaluation using the Murakami or osmium thiocarbohydrazide-procedures. This curling of the basilar membrane can obstruct the view of the reticular lamina and the ciliary ends of the hair cells.

We have used a dissection method, referred to as the anchor technique, to overcome basilar membrane curling. This technique removes all the structures above the reticular lamina but leaves the basilar membrane attached to the spiral ligament and the lateral bone to which the spiral ligament is anchored. Individual cochlear turns are dissected in this manner and mounted on the same examination stub for SEM evaluation.

Maintenance of the lateral attachment of the basilar membrane requires additional dissection time but eliminates the problem of curling during critical point drying. An additional benefit is that mounting the individual turns on the same examination stub facilitates evaluation and photomicroscopy of the surface morphology.

The anchor technique has been used successfully on the guinea pig and should be appropriate for most mammalian cochleas.

KEY WORDS: Critical Point Drying, Reticular Lamina, Organ of Corti, OTO Technique, Scanning Electron Microscopy, Tannin-Osmium, Cochlea, Spiral Ligament, Glutaraldehyde, Hair Cell Status.

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Introduction

The revised Tannin-Osmium[6] (Murakami) and the modified thiocarbohydrazide[2] (OTO) techniques are frequently used to prepare cochlear tissues for evaluation by scanning electron microscopy (SEM). The Murakami method takes less time, but the OTO method facilitates subsequent evaluation with the transmission electron microscope (TEM).

With either method the basilar membrane may curl during critical point drying (personal communication, I. Hunter-Duvar and D.J. Lim). If the basilar membrane curls down toward the scala tympani, the hair cells usually can be evaluated. However, if the curling occurs in the opposite direction, towards scala vestibuli, the lateral margin of the basilar membrane hides the hair cells from view making it impossible to evaluate them.

There are reports of successful attempts to uncurl small areas of specimens processed with methods similar to that of Murakami (personal communication, D.J. Lim and B. Engström), but the brittle nature of OTO-processed tissue prohibits this. Due to curling difficulties, laboratories have developed other procedures.

Soudijn developed a technique for SEM evaluation of the cochlea which involves drilling and removing the otic capsule after critical point drying[8]. Although the goal of Soudijn's technique was to avoid removing the individual cochlear turns, his procedure should also reduce curling since the basilar membrane is securely fixed at the medial and lateral margins during critical point drying. However, the upper turns may interfere with the assessment of the lower turn morphology, and manipulating cochlear tissue after critical point drying increases the probability of dissection artefacts.

The SEM photographs of OTO-processed cochleas published by Hunter-Duvar[3,4] are quite impressive and show no curling problems. However, because he examined only the basal half of the cochlea, he was able to leave the bony labyrinth intact where the spiral ligament and basilar membrane were attached. With the basilar membrane anchored to the bone via the spiral ligament, it did not curl during critical point drying. The absence of the apical parts of the cochlea facilitated orientation of the specimen for photography and evaluation. Although Hunter-Duvar stated that the apical

turns were dissected in the same manner as the basal turns, no detailed description of the method or the results was given, so we do not know whether curling was a problem in the apical turns.

Hunter-Duvar's success with the basal turn prompted attempts to develop a similar approach for the upper cochlear turns. The following procedure, or anchor technique, has eliminated curling with the Murakami method and minimized curling with the OTO procedure to the point where it is not a problem in photomicroscopy or evaluation of morphology.

Materials and Methods

To perform the anchor technique dissection, the following instruments are necessary (Fig. 1): 1) Curved Derlacki knife (or a 0.5 mm blade), 2) Fine forceps, 3) Small sturdy forceps, 4) Iris scissors, 5) 2 mm diamond burr, 6) 0.5 mm diamond burr and 7) Reversible, variable-speed drill (not shown in Fig.1)

All specimens were fixed with 2% buffered glutaraldehyde and postfixed with 2% osmic acid. All cochleas were dissected during dehydration in 70% alcohol. With the Murakami method, dissection can be completed at any stage before processing with tannic acid. With the OTO method dissection must be completed before the thiocarbohydrazide stage.

Anchor Technique

It is critical that the bony labyrinth be thinned before any attempt is made to remove any of the cochlear turns. If the bone is too thick, it is difficult to control the direction in which the bone breaks as unnecessary sections of the otic capsule are removed. Figure 2 shows the cochlea of the guinea pig before (broken line) and after (solid line) the bony capsule is thinned. This thinning is done with the 2 mm diamond burr. Care should be taken, however, not to remove too much of the bone lateral to the junction of the scala tympani with the scala vestibuli of the turn below (arrow + bracketed areas in Fig. 2). This region of bone will form part of the anchoring for the basilar membrane.

A small hole is drilled at the apical end of the cochlea with the 0.5 mm diamond burr. With the hole as a starting point, a small but sturdy pair of forceps is used to break away the bone covering, the scala vestibuli of the apical turn and most of the lateral wall of the scala media (Fig. 3a). This is done until the whole apical turn is

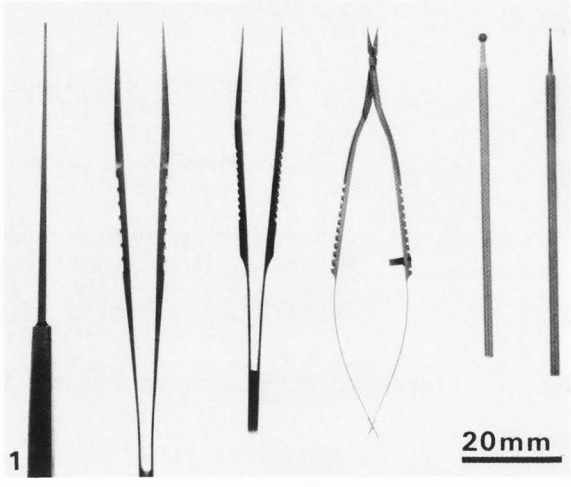
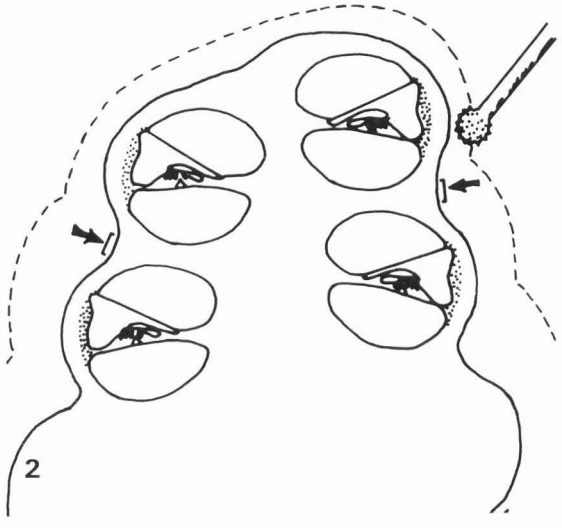


Fig. 1. Basic instruments used for anchor dissection. From left to right: curved Derlacki knife, fine forceps, small forceps, iris scissors, 2 mm diamond burr, 0.5 mm diamond burr.



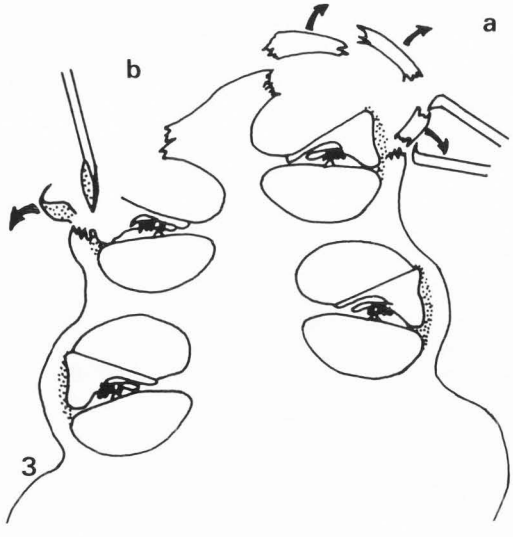


Fig. 2. Mid-modiolar section through the cochlea showing thickness of otic capsule before drilling (broken line) and after thinning with a diamond drill bit. Shaded areas next to the brackets demarcate the thicker bone that provides support for the finished specimen.

Fig. 3a. Sturdy forceps used to remove the bony capsule covering the scala vestibuli and upper-lateral margin of the spiral ligament.

Fig. 3b. A curved Derlacki knife used to cut away the upper spiral ligament and stria vascularis by "scissoring" the tissue between the knife and the bone lateral to the scala media.

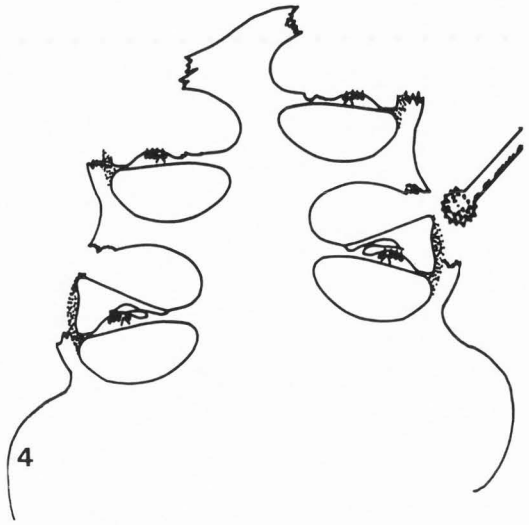


Fig. 4. A 0.5 mm diamond burr used to drill away the bone covering the scala vestibuli and upper scala media of the lower turn.

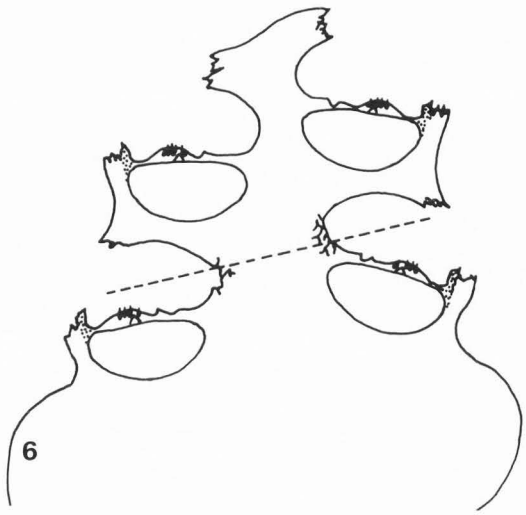


Fig. 6. Scissors (dotted line) aligned in order to cut the apical turn away from the cochlea.

opened. Repeating this procedure in each turn will result in approximately 360° turns and will eventually yield a basal turn free of overhanging remains from the second turn.

The curved Derlacki knife is used to remove the stria vascularis and the superior part of the spiral ligament to which it is attached by cutting the tissue between the knife and the superior edge of the remaining bone (Fig. 3b). Care should be taken not to remove all the spiral ligament above the level of its attachment to the basilar membrane. Removing too much will weaken the junction between the spiral ligament and the osseous capsule, and the objective of the anchor technique (i.e., maintaining an attachment to the lateral bony labyrinth) will be undermined. Fine forceps are used to remove Reissner's membrane.

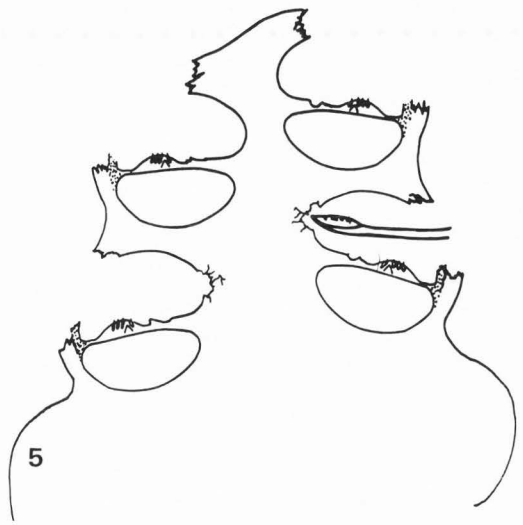


Fig. 5. The apical turn composed of the modiolus and a lateral ring of bone with the basilar membrane and organ of Corti stretched (or anchored) between them. A curved Derlacki knife is used to perforate the modiolus in order to facilitate a controlled break when the turn is separated from the remainder of the cochlea.

The surface of the organ of Corti is now clearly visible. The tectorial membrane is lifted at its most apical end with fine forceps and stripped away from the apical turn, but it is left attached at the upper part of the next turn since it is easier to make subsequent removals of the tectorial membrane if its free-floating end is available. Working with the free-floating end also reduces the likelihood that the hair cells will be damaged by the forceps.

A 0.5 mm diamond burr is used to open the bone lateral to the scala vestibuli of the next turn (Fig. 4). The small sturdy forceps are inserted into the drilled channel to remove that part of the osseous lateral wall covering the upper part of the scala media. The Derlacki knife is used to remove the stria vascularis and the upper portion of the spiral ligament in the same manner as it was removed from the apical turn. Reissner's membrane is removed and the tectorial membrane lifted from the turn.

The apical turn is now composed of the modiolus centrally, a ring of bone laterally, and the basilar membrane with the organ of Corti stretched between them. The apical turn must now be separated from the rest of the cochlea. It is advantageous to divide the cochlear turns along a line from helicotrema to the junction of the oval and round windows.

The 0.5 mm diamond burr is used to cut a notch in the lateral ring of bone at the point where the apical turn is to be separated from the remainder of the cochlea. Small iris scissors are used to make a radial cut in the Organ of Corti at this point. The turn is prepared for removal by using the Derlacki knife (or a needle) to make a perforated ring in the modiolus above the next (i.e., lower) turn (Fig. 5). The perforations

control the manner in which the modiolus breaks. Failure to make them often results in part of the lower turn coming off with the turn being removed. The scissors are positioned in such a manner as to align with the perforations in the modiolus (dotted line in Fig. 6) and to avoid damage to the lower turn. Once the turn is severed, it can be picked up by the bony ring and placed in the next processing solution.

Subsequent turns are dissected in the same manner. In the basal turn one needs to remove the material above the point where the plane of the basilar membrane intersects the bony lateral wall. Figure 7 shows the middle and apical cochlear turns when dissection is complete.

Results

Figures 8a, 8b, and 8c show the apical, one middle, and the basal turns of a guinea pig cochlea dissected with the anchor technique. Note the bony ring (B) to which the spiral ligament (S) and the organ of Corti (OC) were anchored before critical point drying. Since there are no overhanging turns, the surface morphology can be examined from either a modiolar (Figs. 9a,9b) or a lateral perspective (Figs. 10a,10b).

In 20% of our cochleas artefacts were noted but did not interfere with evaluations of morphology. Despite frequent solution changes during dissection, debris was occasionally noted (Fig. 11). Critical point drying caused cracks of both major (Fig. 12) and minor (Fig. 13) size between cells. Occasionally dissection resulted in dislocation of part of the organ of Corti (Fig. 14).

Problematic artefact was found in about 5% of our specimens. One was that the view of the organ of Corti could be obstructed if the spiral ligament was not trimmed close enough to its junction with the basilar membrane, or pulled away and curled up over the organ of Corti (* in Fig. 8a) or if the tectorial membrane was not completely remo-

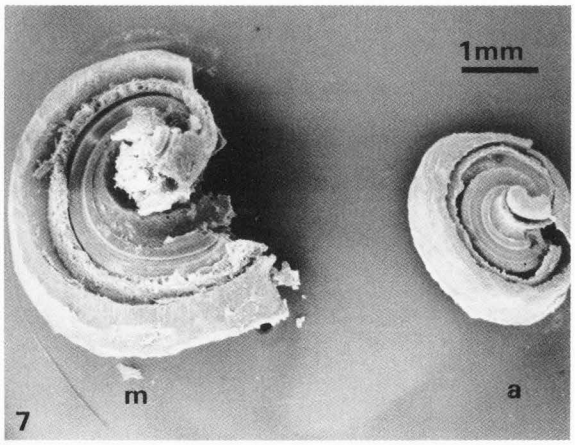


Fig. 7. Apical (a) and middle (m) turns of a left guinea pig cochlea that have been dissected using the anchor technique. The turns are glued to an aluminium stub. They lie in roughly the same plane, which facilitates photo microscopy and permits optimal working distance with the microscope.

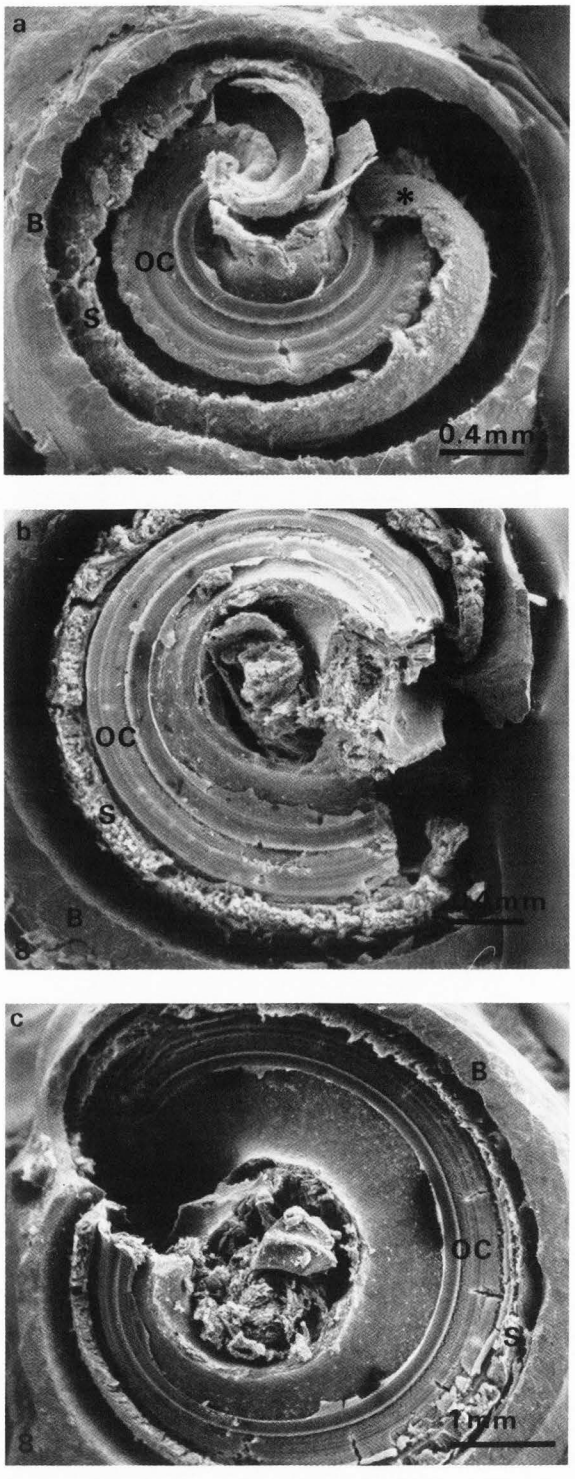


Fig. 8. The apical turn (a), one middle turn (b) and the basal turn (c) of a guinea pig. The organ of Corti (OC) and the spiral ligament (S) are anchored to the bony ring (B) before the critical point drying. Note in the apical turn that the spiral ligament is not trimmed enough at the basal end. (*)

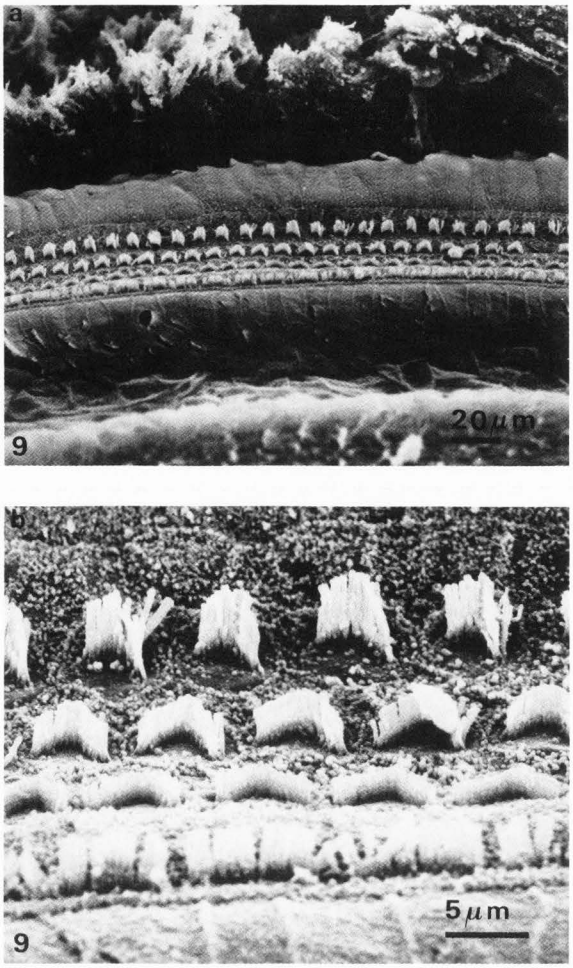


Fig. 9. A middle turn from modiolar perspective in (a) low magnification, and (b) high magnification.

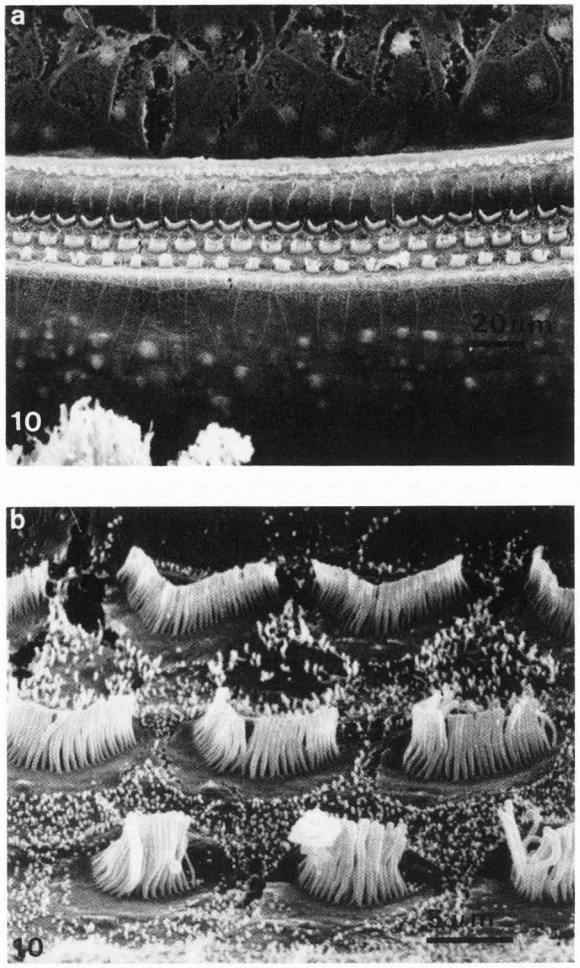


Fig. 10. A middle turn from lateral view in (a) low magnification, and (b) high magnification.

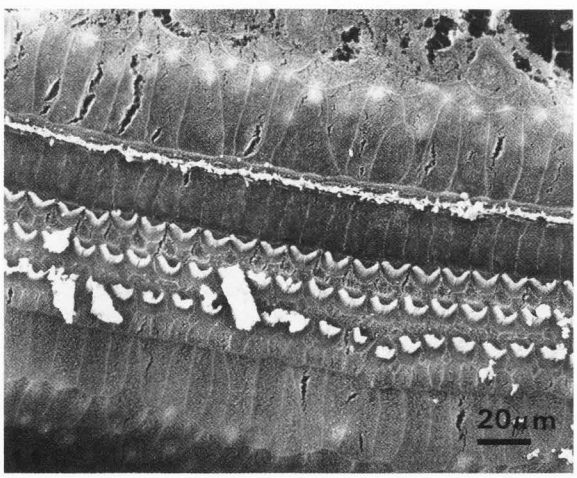


Fig. 11. Occasionally debris is found on the organ of Corti.

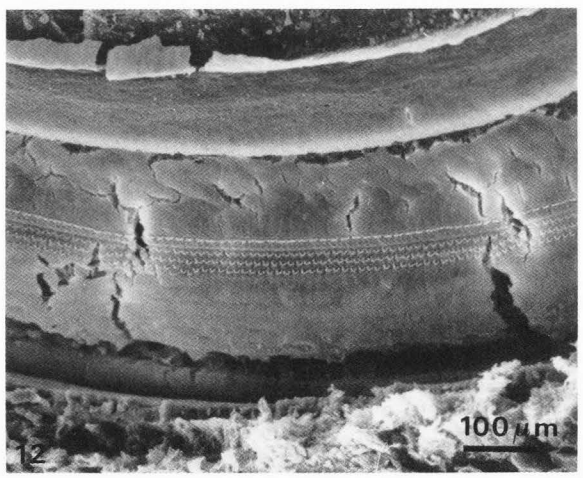
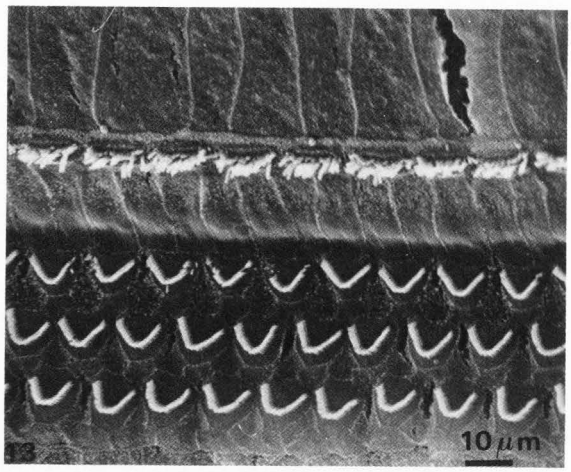
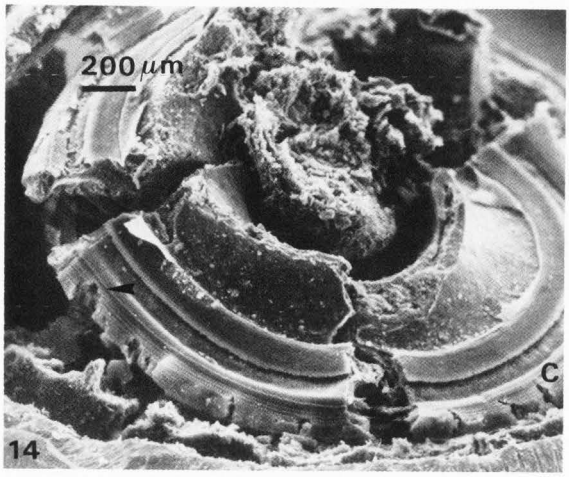


Fig. 12. Major radial cracks in the organ of Corti after critical point drying.





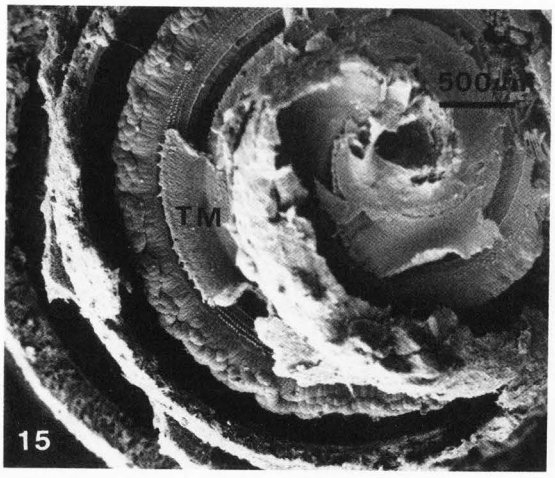


Fig. 13. Small cracks between the hair cells.

Fig. 14. Major cracks (C) and dislocation of a part of the organ of Corti. Some dissector errors in this middle turn (arrow).

Fig. 15. In this apical turn of a guinea pig cochlea the tectorial membrane (TM) is not completely removed.

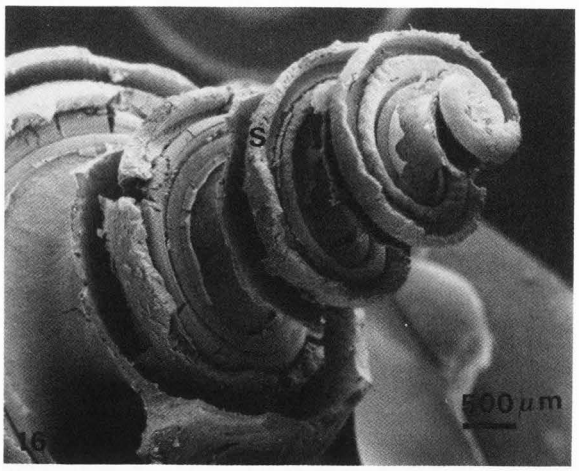


Fig. 16. A guinea pig cochlea with all turns in place and with only parts of the spiral ligament (S) removed. The apical turn restricts the view of the curled middle turn below. Also, part of the apical turn is difficult to examine because of curling of the spiral ligament and basilar membrane.

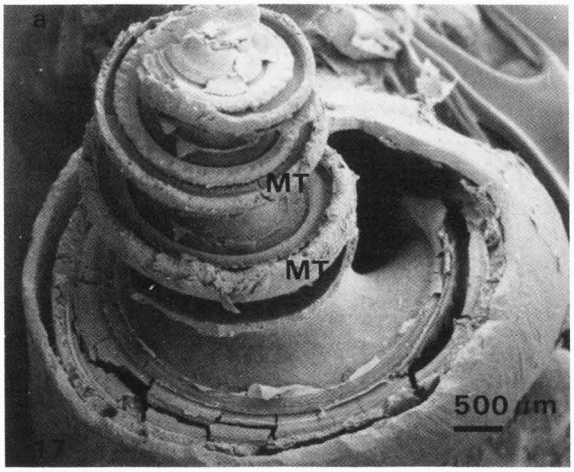
ved (Fig. 15). The other source of the artefact that limited morphologic evaluation was dissector error (Fig 14, arrow). Damage to hair cells when the organ of Corti was radially cut did not exceed three cells per row.

Before adopting the anchor technique, we tried other approaches to minimize curling of the basilar membrane. In one of these procedures we used Murakami's method in which the dissection procedure leaves all the cochlea turns in place. When the spiral ligament was left in place and trimmed down to the level of the basilar membrane, curling inward of the spiral ligament and basilar membrane obstructed the view of the organ of Corti in the apical and middle turns (Fig. 16). With the same dissection technique but using OTO, the spiral ligament still, curled inward (Figs. 17a, 17b). When the spiral ligament was removed in cochleas prepared by Murakami's method (Fig. 18a), the upper turns restricted the view of the reticular lamina of the lower turns and the basilar membrane curled inward (Fig. 18b), which seriously limited the view of the organ of Corti.

Another approach to reduce curling of the basilar membrane was made by drilling away the bone of the scala vestibuli and scala media without removing the individual turns (Fig. 19). The result with the guinea pig was that the basilar membrane remained flat, but the bony ring from the turn above often made it difficult to evaluate the cells because it seriously restricted the angle for viewing and photographing them.

Discussion

Using the anchor technique, we detected dissection artefacts in approximately 20% of our cochleas, but morphologic evaluation was still possible in 95% of the cochleas. Less than 5% of specimens exhibited areas of artefact that precluded accurate morphologic evaluation. With the Murakami method and when we analyzed the whole



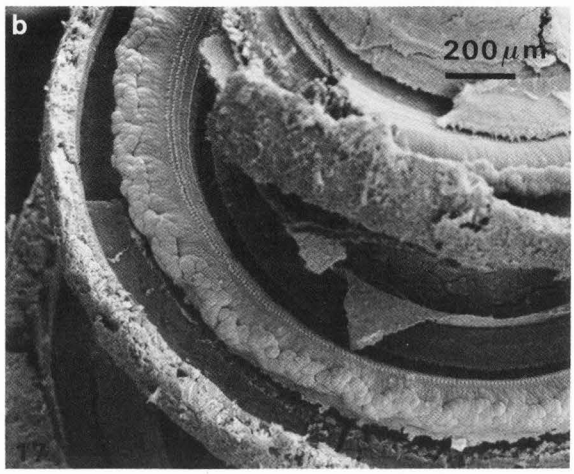
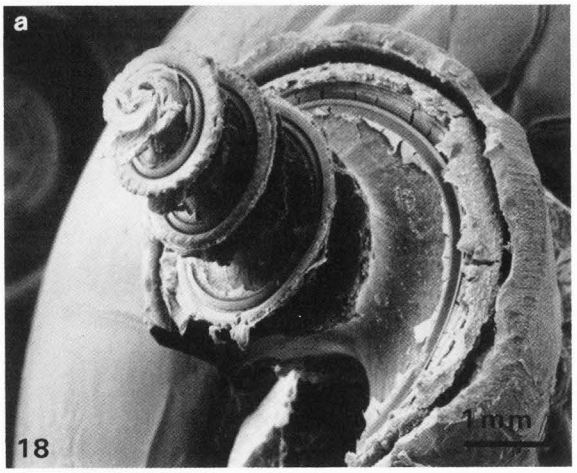


Fig. 17. A guinea pig cochlea processed with OTO (a). The two middle turns (MT) are both curled too much for satisfactory examination. In (b) one middle turn. Examination of all of the hair cells is not possible as the turn above restricts the view.



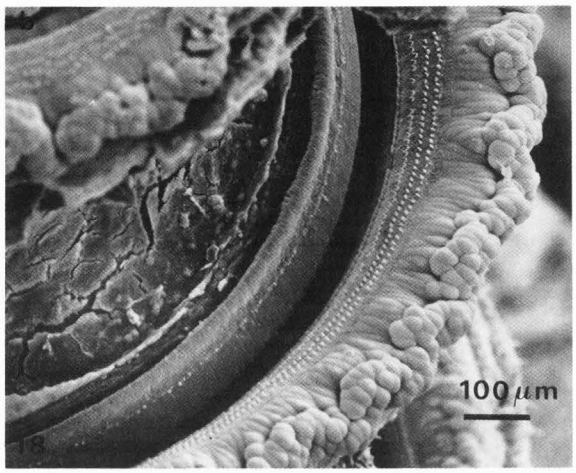
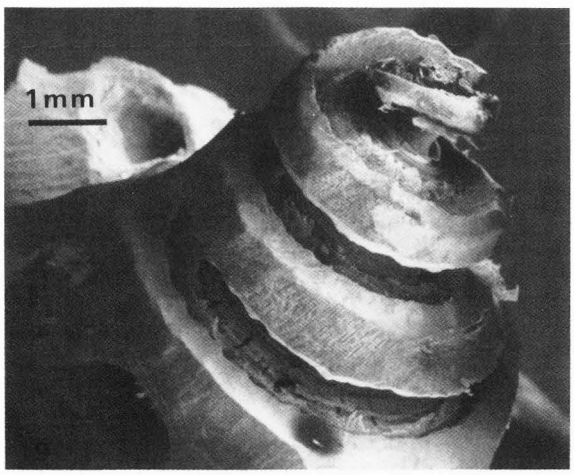


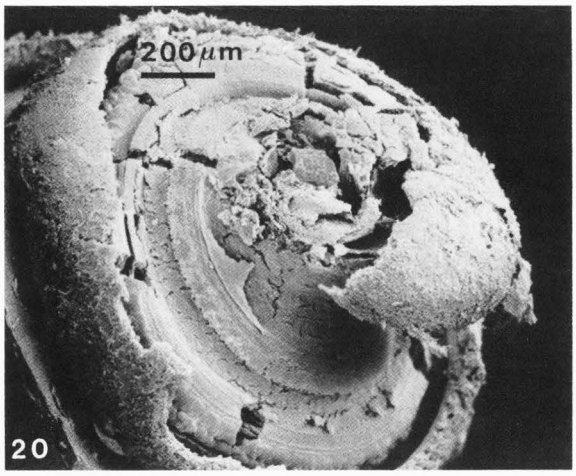
Fig. 18. All of the spiral ligament is removed, but all turns are in place (a). The upper turns restrict the view of the hair cells of the lower turns (b). The middle turns are curled inwards (b).

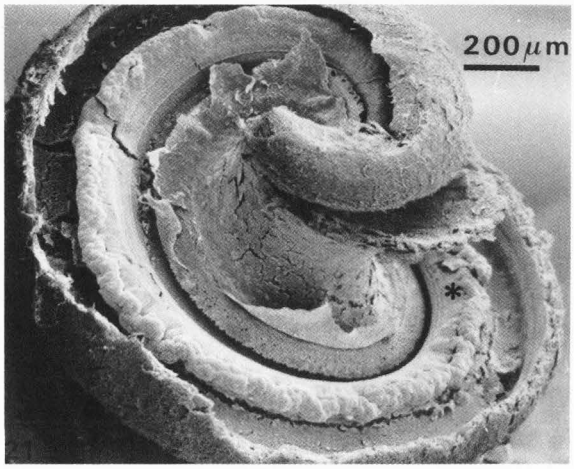


cochlea with all turns in place the view was so obstructed that only the basal (Fig. 18) and sometimes the apical turns (Fig. 20) could be investigated. In several cochleas curling of the most basal of the middle turns was within tolerable limits if the spiral ligament was not removed (Fig. 16). With the OTO method and with the same dissection technique with all turns in place, the view of the reticular lamina of the two middle turns (Fig. 17a) and often the apical turn (Fig. 21) was obstructed by upward curling of the spiral ligament and the basilar membrane. Thus, both the middle turns and the apical turn need to be dissected with the anchor technique to prevent curling.

Both large and small cracks caused by critical point drying seemed to occur more often with

Fig. 19. Guinea pig cochlea drilled in the manner of the anchor technique without removing the individual turns.





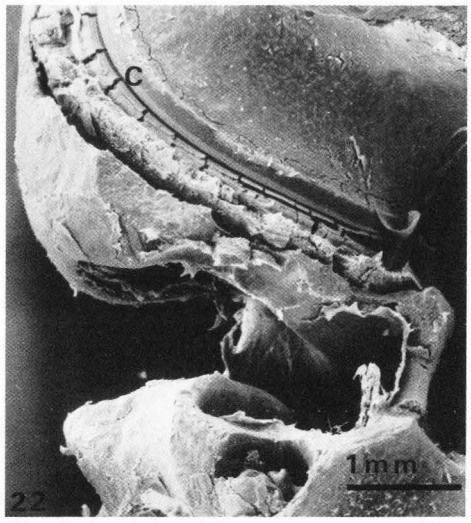


Fig. 22. The hook area in a critical point-dried guinea pig cochlea. Several major radial cracks in the organ of Corti (C).

Fig. 20. The upper turn dissected without using the anchor technique and using the Murakami method.

Fig. 21. The apical turn dissected without using the anchor technique but using OTO. Because of curling of the organ of Corti, it is difficult to examine all hair cells (*).

the anchor technique than with techniques in which both the bony ring and the spiral ligament were removed. A shrinkage of about 20% may be an inevitable consequence of the critical point drying. The techniques used will then "determine" the type of shrinkage. Removing the bone and spiral ligament results in more curling and less cracking, whereas the anchor technique causes more cracks and less curling. Since the area of the basal turn is larger, this could cause a higher proportion of large cracks, whereas small cracks would be more abundant in the apical turns. This difference is particularly obvious in figure 19. This is in agreement with Hunter-Duvar[4] who described large cracks, which however, also may be caused by using the OTO-method. Bohne's[1] description of lesions in the reticular lamina does not resemble the minor cracks in our preparations. Since our purpose was primarily to evaluate the stereocilia we therefore found the anchor technique to better suit our intentions.

Because of the drilling, bone dust was more common with the anchor technique, but it did not cause any major evaluation problems (Fig. 11). Since the anchor technique, unlike other dissection techniques we tried, includes cutting the organ of Corti, some but very few hair cells are lost.

With Soudijn's technique, cutting the cochlea into separate sections is avoided,[8] however, with this procedure the upper turns may interfere with the evaluation of lower turn morphology. The difficulty of removing undesired tissue without damaging the organ of Corti after critical point drying is probably another reason this technique has limited use. The individually mounted turns produced with the anchor technique do not require manipulation of the tissue after critical point drying. Compared to the other techniques we have tried, including the Soudijn's technique, the anchor technique seems to cause less serious artefacts.

Another benefit of the anchor technique is that several turns can be mounted on the same specimen stub and will lie in approximately the same plane (Fig. 7). This permits optimal working distance with the scanning electron microscope and allows viewing of several turns without having to replace stubs and wait for the microscope to re-establish its vacuum. Photography is also simplified because there are no structures overhanging the area being photographed. If the spiral ligament had curled inward, this technique offers a possibility of tilting the specimen and "looking beneath" the spiral ligament.

The basal tip or "hook area" presents a special problem for SEM evaluation because of its almost perpendicular orientation relative to the other turns. We did not evaluate this area routinely because our auditory sensitivity measures

and the correlated hair cell morphology occurred apical of the hook region. Although we attempted to examine the hook area on a few cochleas, we had limited success because the membranous labyrinth was very fragile (Fig. 22).

The anchor technique has been successfully used on guinea pigs, chinchillas[2], and cats. It should be equally appropriate for other mammalian cochleas. It requires additional dissection time, but once mastered, it yields results that justify the effort since a complete evaluation of the structures of the reticular lamina is possible from the lateral or modiolar side. We recommend this technique when quantification of cochlea surface morphology is required.

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References

1. Bohne BA, Clark WW. (1982). Growth of hearing loss and cochlear lesion with increasing duration of noise exposure. In: New perspectives on noise-induced hearing loss. Eds. Hamernik RP, Henderson D, Salvi R. Raven Press, NY, 283-302.
2. Dunn DE, Ferraro JA, Lim DJ. (1979). Electrophysiological and morphological correlates of TTS in the chinchilla. Association for Research in Otolaryngology, ARO, St Petersburg Beach, Florida. Abstracts of the second mid-winter research meeting, p. 37.
3. Hunter-Duvar IM. (1977). A scanning study of acoustic lesions of the cochlea. Inner Ear Biol. 68:385-396.
4. Hunter-Duvar IM. (1977). Morphology of the normal and the acoustically damaged cochlea. Scanning Electron Microsc. 1977; II:421-428.
5. Malik LE, Wilson RB. (1975). Evaluation of modified technique for SEM examination of vertebrate specimens without evaporated metal layers. Scanning Electron Microsc. 1975: 259-266.
6. Murakami T. (1974). A revised tannin-osmium method for non-coated scanning electron microscope specimens. Arch. Histol. Jap. 36:189-193.
7. Nilsson P, Rydmarker S, Dunn D, Lindqvist C. (1985). Methods for morphological evaluation of the inner ear. University of Lund Report/-LUMEDW/(MERM-3007)/1-34/(1985).
8. Soudijn ER. (1976). Scanning electron microscopic study of the organ of Corti in normal and sound-damaged guinea pigs. Ann. Oto. Rhin. & Laryngol. Suppl. 29, vol 85, No. 4, part 2,1-58.

Discussion with Reviewers

Reviewer I: Have specimens prepared by the anchor technique ever been subsequently examined by TEM? If so what was the quality of the results?
Authors: None of our specimens have undergone TEM evaluation. Since our tissue was prepared using either the OTO or Murakami method, we would expect the quality of our tissue to be comparable to that of other labs using these methods. However, attempting to remove the bony ring and spiral ligament from the unembedded dried tissue will probably result in dissection artefact.

Reviewer I: How much artefact occurs at the cut edges of the OC? Are quantitative data collected on specimens prepared in this fashion?
Authors: Light microscopic evaluation of practice cochleas before and after scissor cuts revealed that fewer than three hair cells per row are lost (Nilsson et al 1985)[7]. Using this technique, we have made both quantitative and qualitative measures of noise-exposed cochleas (with the exception of the hook) and are preparing the results for publication.

Reviewer IV: The method includes increased drilling and dissection of the cochlea and also exposure of the opened coils for bone dust. How do you deal with that? How do you remove the bone dust?
Authors: Frequent changes of the specimen bath are made during drilling to limit the amount of bone dust. Most of the heavy drilling is done with the otic capsule closed (Fig. 2) so that the bone dust is not able to enter the scala media. The light drilling is done mostly with Reissner's membrane intact (Fig. 4). The layer of bone dust is flushed from Reissner's membrane before the membrane is removed. During the drilling to open the scala vestibuli of the lower turn, it is critical that solution changes be made regularly.

Reviewer IV: How do you get enough space to get the iris scissors between the coils?
Authors: There is ample space between the turns to make the radial cut with the iris scissors. The important step is to cut a notch in the lateral bony ring at the point where the organ of Corti is to be divided.