Cochlear and Vestibular Epithelia from a Patient with Meniere's Disease: A Case Study

K. C. Horner
Universite Bordeaux II

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K. C. Horner

Laboratoire d'Audiologie Expérimentale, Inserm U229,
Université Bordeaux II, Hôpital Pellegrin, Place Amélie Raba Léon,
33076 Bordeaux, France

Phone number: (33) 56 24 20 47 / FAX number: (33) 56 96 29 84

Abstract

Scanning electron microscopy observations were carried out on the cochlear and vestibular epithelia of the left temporal bone of a Menière's patient. There was almost complete absence of hair cells in the basal turn of the cochlea. The outer hair cells of the second turn presented an abnormal shortening of the shorter stereocilia within a tuft, reminiscent of the specific atrophy of the short and middle stereocilia in the ciliary tufts of outer hair cells in the guinea pig with experimental hydrops. The cilia of the inner hair cells showed fusion and giant cilia formation. Hair cells were observed in the apical turn which showed no pathological features in particular.

In the saccular epithelium there were a number of striking features including, loss of the kinocilium, loss of ciliary tufts, swelling of the sensory cells, holes in the epithelium, and sensory cells pushed out and lying on the surface. The utricular epithelium was less perturbed and showed only relatively small protrusions from the epithelial surface. Similar observations have earlier been made on the vestibular epithelium in experimental hydrops.

After taking into consideration the relatively long delay to fixation (12 hours) it appeared that the sacculus was more fragile and prone to autolysis than the other organs suggesting that the in-vivo pathology was manifested in particular in that organ as would be predicted from Menière's symptoms.

Introduction

Menière's disease is a relatively common inner ear disorder involving a classical triad of symptoms including a predominant low frequency (fluctuant in early stages) hearing loss, episodes of vertigo and tinnitus (Hood, 1983). The incidence has been estimated as being about 1 per 1000 of the adult population in Sweden (Stahle et al., 1978) and even as high as 1 per 100 in Britain (Hinchcliffe, 1961). Whilst the cause(s) of the disease remains obscure and there is no causal cure, observations of human temporal bones has demonstrated the swelling of the endolymphatic spaces (hydrops) of the inner ear (Hallpike and Cairns, 1938; Yamakawa, 1938), which now seems to be a systematic feature associated with Menière's disease (Paparella, 1991). However it appears quite anomalous that light microscope observations reveal only minor losses of hair cells associated with the apical turn despite extensive hearing deficit (Lindsay, 1968; Schuknecht, 1963).

In order to shed more light on the morphological repercussion of endolymphatic hydrops, it is appropriate to observe the ultrastructure of the inner ear tissue. However the availability of suitable material, fixed within the minimum delay, remains a severe impediment, in particular, in light of the recent reports on the importance of early fixation (Osborne et al., 1989; Comis et al., 1990). Very often ultrastructure studies on rare specimens, and in particular on temporal bones of Menière's patients, are based on case studies (as is the present) and hence the available published information is rather limited.

In contrast to the data from the light microscope studies mentioned above, Kimura et al. (1976) as well as Nadol and Thornton (1987) have observed by transmission electron microscopy (TEM) outer hair cell loss limited to the base of the cochlea and almost full recuperation in the upper turns of the cochlea. A few of those hair cells which remained demonstrated loss or fusion of stereocilia. Kimura et al. (1976) have pointed out that the extent of these modifications is too minimal to account for the severe hearing loss observed. On the other hand Kimura et al. (1976) have described many outer hair cells in the basal cochlear turn which were retracted

away from the reticular lamina and displaced towards the basilar membrane. Nadol and Thornton (1987) on serial sectioning across 15 outer hair cells in the middle cochlear turn, similarly observed 3 cells which did not make contact with the reticular lamina. It is perhaps for this reason that observations by light microscopy had in the past demonstrated a reduced number of hair cells at the apex of hydropic cochleas (Lindsay, 1968; Schuknecht, 1963).

Several TEM studies on the vestibule of Menière's patients including the ampullae (Pietrantoni and Iurato, 1960; Litton and Lawrence, 1961; Ireland and Farkashidy, 1963; Harada, 1973) and the utriculus (Friedmann et al., 1963; Hilding and House, 1964; Sanchez-Fernandez and Marco, 1975; Rosenhall et al., 1977) have described degenerative changes within sensory hair cells as well as partial or complete loss on the ampullae and the utriculus. Fitzgerald O' Connor et al. (1985) have described an abnormal thickening of the basal lamina lining the epithelial cells of the utricle. In addition brief scanning electron microscopy (SEM) observations of the vestibular epithelium from a Menière's patient by Johnsson (1980) showed loss of ciliary tufts as well as giant cilia.

Having acquired a single temporal bone from a Menière's patient it was considered here that SEM might be the most fruitful technique for the study of this rather rare specimen, in that light microscopy is very limited in resolution and with TEM, it is not possible to observe all the tissue and form an overall view. On the otherhand SEM can provide a rapid means of having an overall picture of all the sensory tissue which eventually can be subsequently processed for TEM observations (Barber and Boyde, 1968).

Experimentally-induced hydrops in the guinea pig (Harada, 1959; Naito, 1950, 1959; Kimura and Schuknecht, 1965) on the other hand, has provided an excellent model and both the cochlea (Horner et al., 1988, 1989; Rydmarker and Horner 1990, 1991) and the vestibule (Horner and Rydmarker, 1991) have been observed. The animal model has provided material rapidly fixed post-mortem such that the effects of endolymphatic hydrops alone could be discerned. Observations made on the human temporal bone with endolymphatic hydrops are presented here and compared with those made on the animal model with experimentally-induced hydrops.

Materials and Methods

Case history

The patient was seen first in 1971 at the age of 48 because of attacks of vertigo with hearing loss, nausea and vomiting, pressure and distortion of hearing and tinnitus for the preceding ten years. These attacks occurred about four to five times a year but were occurring less frequently when the patient was first seen in the office. However, at that time she was having rather constant unsteadiness. The attacks seemed to affect mainly the left ear, however, there was some question of some

activity in the right ear also. There was a purely sensorineural hearing loss averaging 68 dB in the left ear, slightly upsloping. An electronystagmogram revealed a 45% reduced vestibular response on the left side. She was found to be slightly hypothyroid and was placed on thyroid therapy supervised by her internist.

She showed some temporary improvement, but in 1972 began to have more attacks, and it was decided to perform a left subarachnoid endolymphatic shunt. Following the shunt her hearing improved to a level of 55 dB and her dizziness subsided. However, within one year the patient complained of her vertigo problem and subsequently underwent an allergic evaluation where it was found that she was sensitive to some inhalants and accordingly was started on allergy therapy.

In 1973, the vertigo become more severe and so it was elected to do a middle fossa vestibular nerve section. In this surgery, the superior and inferior vestibular nerves were sectioned. When last examined in 1976 she was no longer having vertigo and her hearing had been stabilized to around the 68 dB level (similar to that of 1971).

She died in April 1986 of an unknown cause. The temporal bones were removed 12 hours after death and immersed in formalin with a phosphate buffer.

In April 1991, the left temporal bone was sent to Bordeaux for SEM observations. The temporal bone was trimmed down by sawing and then drilled, under 70% alcohol, to remove the vestibule and the cochlea. The specimens were then dehydrated in alcohol and critical point dried with CO_2 (Balzers Union CPD 010). The specimens were mounted on aluminium stubs and coated with gold/palladium (15 nm, Balzers Union SCD 030). Observations were carried out on a Philips 505 scanning electron microscope.

Results

Cochlea

Previous observations on the cochlea in experimental hydrops revealed a hitherto undescribed stereocilia pathology - a selective atrophy of the short and middle stereocilia in the ciliary tufts of the outer hair cells in the upper three cochlear turns of the guinea pig cochlea (Horner et al., 1988; 1989; Rydmarker and Horner, 1990). This peculiar pathology is demonstrated in Fig. 1. The atrophy begins as a detachment and shortening of the short stereocilia (Fig. 1a) followed by detachment and shortening of the middle stereocilia (Fig. 1b) whilst the tall stereocilia remain upright and apparently do not shorten (Rydmarker and Horner, 1991). The present study on the human cochlea sought to compare the clinical pathology with the experimental.

The dissected human cochlea is shown in Fig. 2. Two preparation artifacts occurred at the basal turn (over about 6 mm), where the basalmost part and the hook region were not preserved, and the second turn (over about 3 mm). Observations could be made over the remaining organ with some difficulty at the apex of
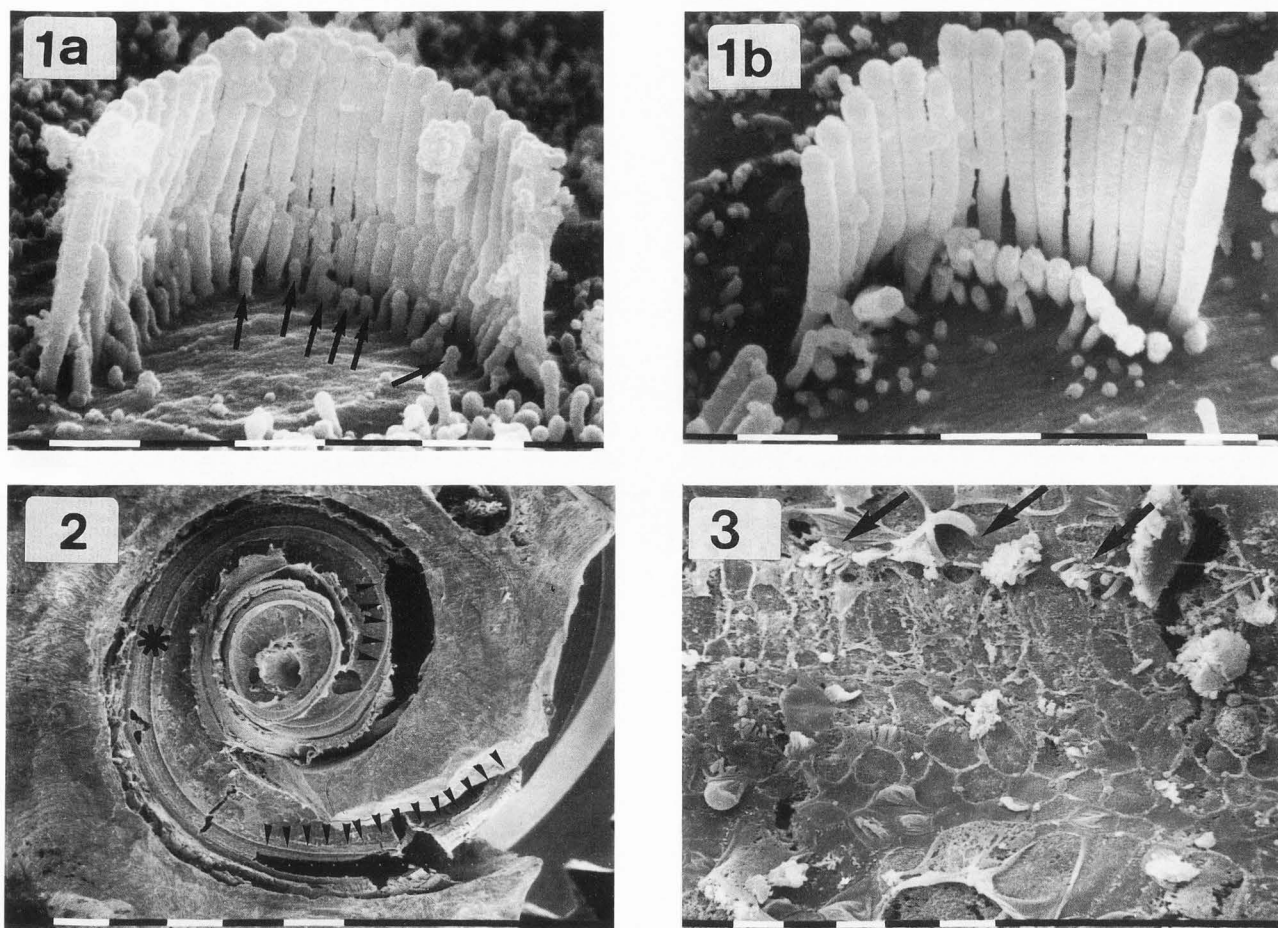
Figure 1. Guinea pig. **a**. Early stage atrophy of the short stereocilia on an outer hair cell. **b**. Advanced stage atrophy of the short and middle stereocilia on an outer hair cell. The arrows indicate several examples of atrophied short stereocilia. White bar = 1 μm.

Figure 2. Human cochlea. The arrow-heads indicate the extent of the two preparation artefacts. The area indicated by "*" is presented in Fig 3 at higher magnification. White bar = 1 mm.

Figure 3. Almost complete absence of sensory hair cells at the base of the cochlea. Several inner hair cells are present and indicated by the arrows. White bar = 10 μm.

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the cochlea because of curling of the tissue. The data are presented here starting from the base of the cochlea.

In the first cochlear turn there was almost complete absence of sensory hair cells. A low-magnification view is presented in Figure 3. A few dispersed inner hair cell stereociliary tufts could still be identified whilst the outer hair cell tufts were, for the most part, absent. Starting immediately after the artefact of the second cochlear turn hair cell ciliary tufts were plentiful but showed pathological features. Figure 4a presents a low-magnification view taken from the second cochlear turn. As described above, the best conservation of stereociliary tufts appeared to be those of the first row (OHC1) whilst those of the outer rows, in this particular view,

could not be assessed due to the forward folding of the tall stereocilia on the shorter ones. The white arrow in Figure 4a indicates a OHC1 presented in Figure 4b at higher magnification where no particular pathological feature could be seen in association with the buttress of short stereocilia. Several intra-row stereocilia links could be seen and the ciliary tuft appeared to be little perturbed. Figure 4c presents another low magnification view of the second cochlear turn where many hair cells appeared to be missing from the outer rows. Many hair cell ciliary tufts which could be observed, appeared to have some degree of shortening of the wedge of shorter stereocilia known as the "buttress". This pathological feature was similar, but not atrophied to the same extent,
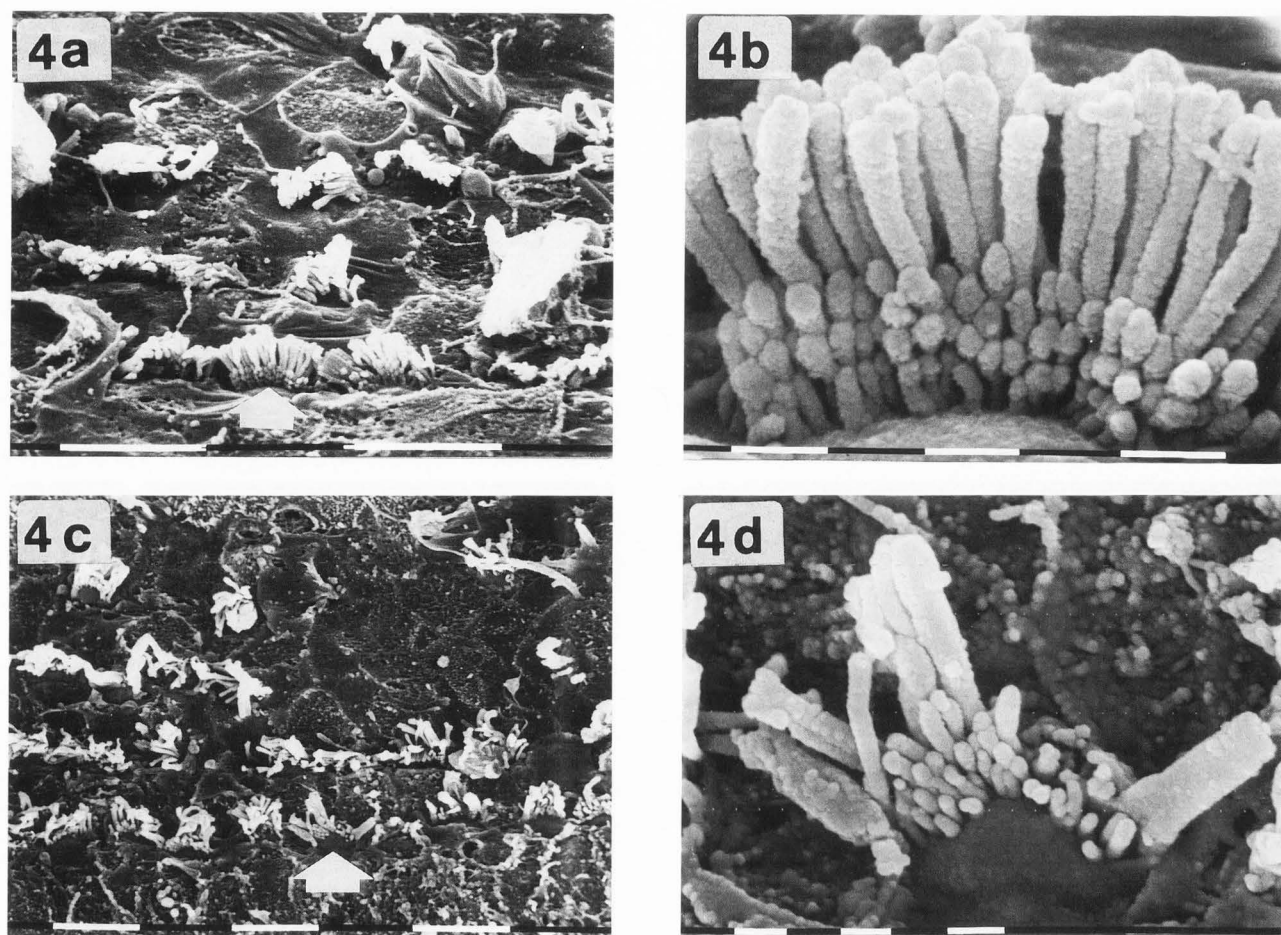
Figure 4. The second cochlear turn and above (starting immediately after the artefact) has variable degrees of hair cell loss and cilia perturbations with first row cells best preserved. (**a**) The white arrow indicates a OHC1 presented in Fig. 4b. (**b**) OHC1 presenting quite good preservation of the stereocilia tuft with intra-row links visible, the stereocilia surface does appear granular. (**c**) Another view of the second cochlear turn. Splaying of hair cell tufts can be seen but the buttress of shorter stereocilia is apparent for several hair cells. The white arrow indicates a cell presented in Fig. 4d. (**d**) OHC1 presenting shortening of the buttress of shorter stereocilia. Some of the taller stereocilia are missing and fusion is apparent between several of those remaining. White bars = 10 μm (a,c) and 1 μm (b,d).

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to that seen in the experimental model (Fig. 1). The white arrow in Figure 4c indicates a cell clearly showing this shortening of the buttress of stereocilia and presented at higher magnification in Figure 4d. For this cell there was, in addition, some loss of the tall stereocilia as well as some fusion of those remaining.

The stereocilia tufts appeared to range from being almost normal to being clearly pathological. Figure 5a presents a OHC3 which appeared to be quite normal, with multiple rows of tall stereocilia and a buttress of progressively shorter cilia. Figure 5b shows a OHC1 where the buttress of shorter stereocilia was not regular such that the cilia appeared to have variable heights and thinning of their girths towards the base of each stereocilia. Several swollen blebs on the surface of the stereo-

cilia (arrows) were considered to represent the swelling of inter-row stereociliary links. Figure 5c presents an OHC1 and Figure 5d an OHC2, both of which demonstrated an apparent shortening of the stereocilia buttress. In both cases the tall stereocilia were also somewhat splayed but they appeared to consist of a single row rather than of multiple rows. The OHC1 presented in Figure 5e showed shortening of the buttress together with collapse of some tall stereocilia. In some cases, as illustrated in Figure 5f, the tall stereocilia could be seen to be collapsed and to be fused into the surface of the reticular lamina.

The inner hair cell stereocilia tuft appeared not to be normal yet presented a pathology different to that of the outer hair cells. Figure 6 presents a representative
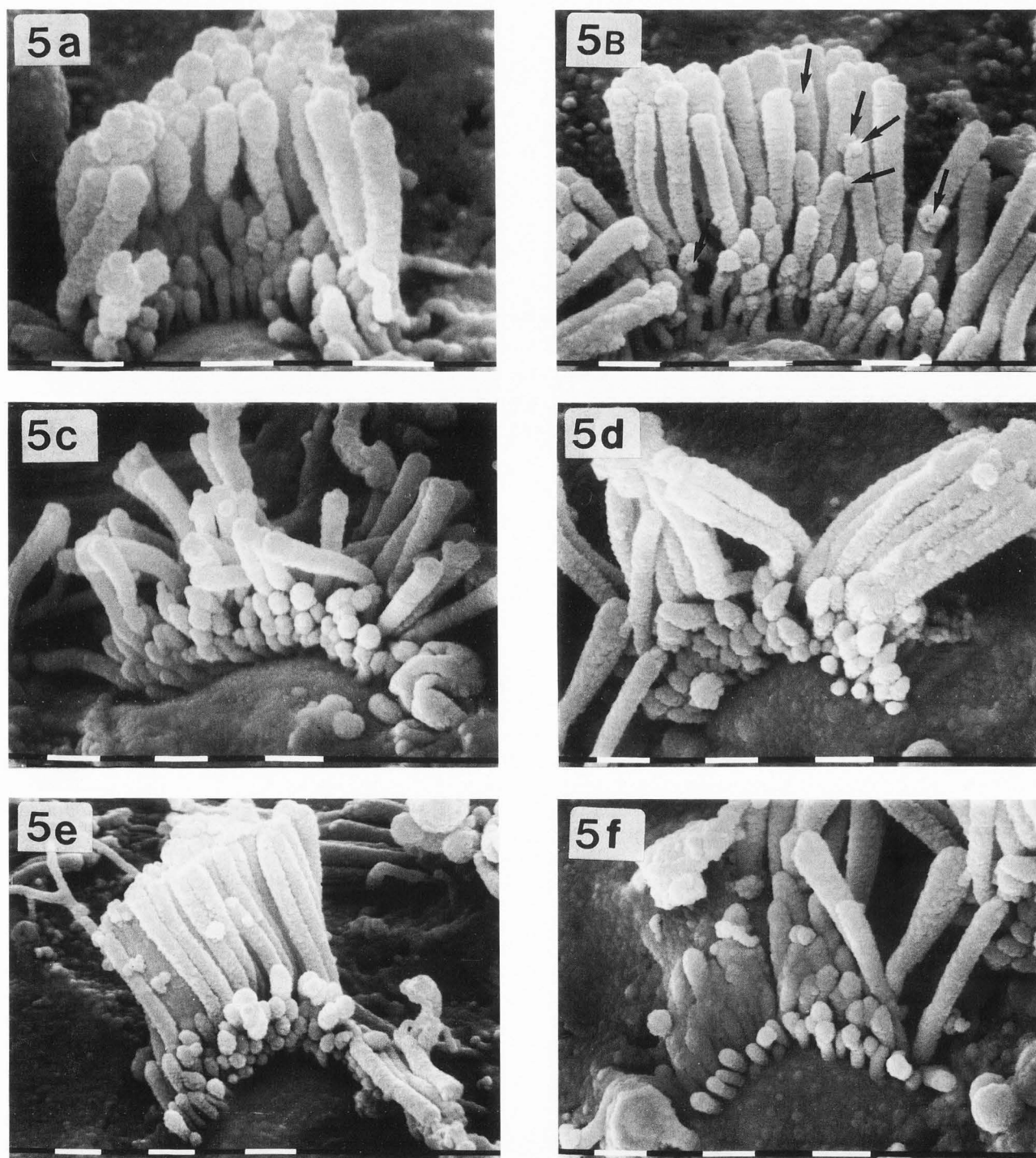
Figure 5. (a) OHC3 presenting no particular pathological features. (b) OHC1 showing irregular heights and thinning of the girth of the shorter stereocilia. The small blebs on the surface (arrows) almost certainly represents the swelling of inter-ciliary links. (c) OHC1 presenting shortening of the buttress of shorter stereocilia and disorganization of the taller stereocilia. (d) OHC2 presenting shortening of the buttress of the shorter stereocilia and splaying of the tall stereocilia. (e) OHC1 presenting shortening of the buttress and some tall stereocilia have flopped forward. (f) OHC2 presenting pronounced shortening of the short ciliary buttress. The majority of taller stereocilia have flopped backwards and fused with the reticular lamina which might be due to the post-mortem fixation delay. White bars = 1 μm.
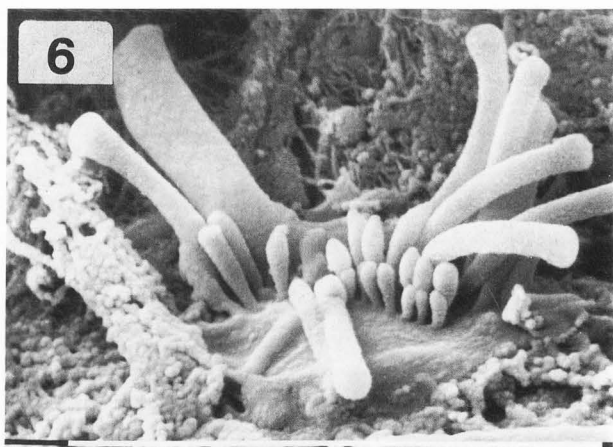
Figure 6. Inner hair cell stereocilia showed variable degrees of perturbation but often with some fusion of the stereocilia as represented here. This stereocilia appear less granular than that of the outer hair cells. White bar = 10 μm.
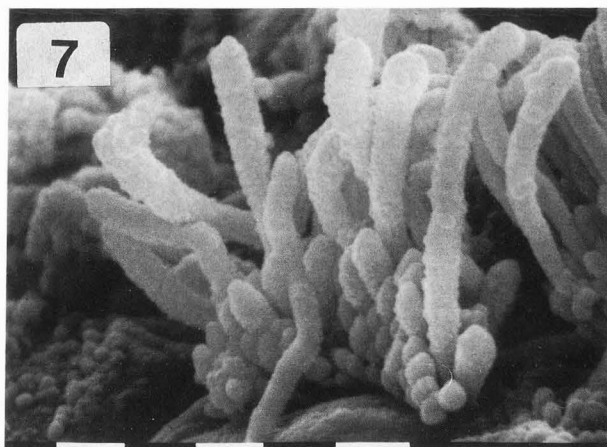
Figure 7. The stereocilia of OHCs in the apical turn were quite well preserved and did not present the shortening of the short cilia buttress as seen in the second cochlear turn. White bar = 1 μm.

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inner hair cell tufts where some fusion and collapse of stereocilia was observed. The membrane surface appeared less granular than that of the outer hair cells.

Despite some curling of the tissue the apical turn could be observed along some of its length. In general, there was neither fusion nor shortening of the short stereocilia buttress were apparent as indicated from the representative OHC presented in Figure 7.

Vestibule

Two ampullae as well as the sacculus and the utriculus were observed. No micrographs, however, of the ampullae are presented here since one was covered with debris and the other was completely denuded of sensory epithelium which is likely to be due to the preparation *in vitro* rather than representative of the *in vivo* situation.

Sacculus A low magnification view of the sacculus (Fig. 8a) shows three different aspects to be presented: surface features of the epithelium, the type of cells identified within cracks on the epithelium and pathological swelling of cells around the periphery of the epithelium.

There was considerable loss and perturbation of stereocilia from the whole saccular epithelial surface. Several particular surface features were observed as illustrated in Figure 8b. Holes were seen, out of which material (asterisk), which could sometimes be identified as a cell (white arrow), appeared to be ejected. In some cases the hole exposed the lateral wall of adjacent sensory cells (black arrow) as presented in Figure 8c. This figure illustrates the apparent loss of a cell, the lateral wall of a sensory cell and the apical surface of a different sensory cell. This latter cell presents a tuft of perturbed stereocilia from which the kinocilium was missing. This feature was identified on several cells and illustrated for another in Figure 8d.

A crack in the epithelium (Fig. 8a) exposed the cell bodies of a band of cells as shown in Figure 9a. Two types of cells could be identified: short (10 μm) flask shaped cells likely to be type I and longer (30 μm) columnar cells likely to be type II. The calyx of type I cells could sometimes be recognized as shown in Figure 9b. However an intermediate type of cell was, in addition, identified as having a bulbous base, presumably containing the nucleus, together with a long thin stalk (neck) on the end of which was the cuticular plate with the ciliary tuft (Fig. 9c).

The periphery of the saccular epithelium presented a particularly pathological picture. The pathology appeared as material, with a relatively flat surface, being pushed up above the surface of the epithelium (Fig. 8a). Further towards the periphery there were so many protrusions that the surface looked like flat interlocking paving-stones and only very few "normal-looking" sensory cells could be identified adjacent to or lying on the surface of the epithelium (Fig. 9d). The swollen protrusions were identified as being the swollen cuticular plate of the sensory cells completely denuded of ciliary tufts although the whole cell body of the sensory cells appeared swollen in some cases.

Utriculus A low magnification view of the utriculus (Fig. 10a) indicated three different aspects presented here: surface features of the epithelium, the curled up peripheral edge of the epithelium exposing the base of the cells and the type of cells identified within the cracks on the epithelium.

Figure 10b presents a higher magnification of the surface of the epithelium illustrating the distribution of ciliary tufts which appeared more dense than within the saccular epithelium seen above (Fig. 8a). Indeed the surface of the epithelium appeared less perturbed than that of the sacculus. Whilst rounded elevations on the
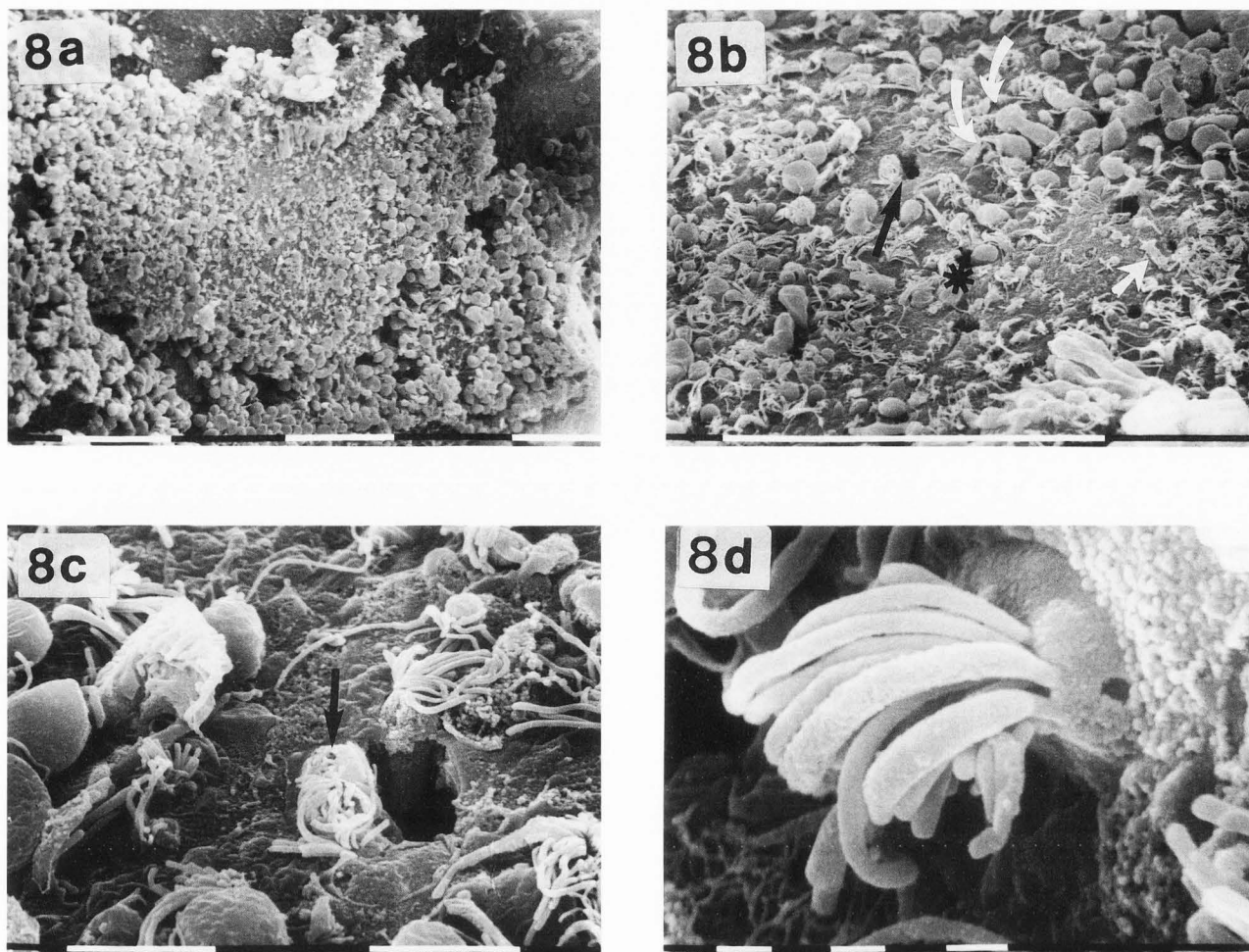
Figure 8. Sacculus. **(a)** Low magnification view showing extensive stereocilia loss and perturbation throughout the epithelium including swollen cells at the periphery. **(b)** Higher magnification showing loss of stereocilia, holes on the surface out of which tissue was projecting (*) and sensory cells were lying free on the surface (white arrow). Holes on the surface (around 10 μm in diameter) exposed the lateral wall of other sensory cells (arrow in b; presented at arrow in Fig. 8c at higher magnification). **(c)** Holes on the epithelial surface exposed the cell body of adjacent cells. Some hair cells had no kinocilium whilst some stereocilia remained. **(d)** Another sensory cells presenting loss of the kinocilium whilst the stereocilia bundle persisted. White bars = 0.1 mm (a,b), 10 μm (c), and 1 μm (d).

epithelium surface could be observed throughout and some sensory hair cells could be seen lying on the surface (arrow) neither swelling of cell cuticles nor holes on the surface were observed in contrast to the sacculus (Fig. 10b). The periphery of the epithelium presented a curled-up edge and gave the possibility to look at the underside of the sensory cells (Fig. 10c) which appeared for the most part with flat bases although some cells appeared to be coated with a net-work of fibres.

The utricular epithelium had a wide preparation artefact extending from the center (striolar region) through to the edge (Fig. 10a) and presented the fortuitous possibility to observe the types of hair cell bodies. Different hair cell types could be identified as presented in Figure 10d. Two types of cells could be distinguished: short (10 μm) flask-shaped cell type I and long (30 μm) columnar type II similar to those presented above as observed in the sacculus. Figure 10d illustrates these two types and in addition presents an intermediate type of cell which had a bulbous region towards the base and a long stem reaching up to the cuticular plate similar to that observed in the sacculus (Fig. 9c). Fine (nerve) fibres could be seen to terminate towards the base of several of these intermediate type cells (Fig. 10d).

Discussion

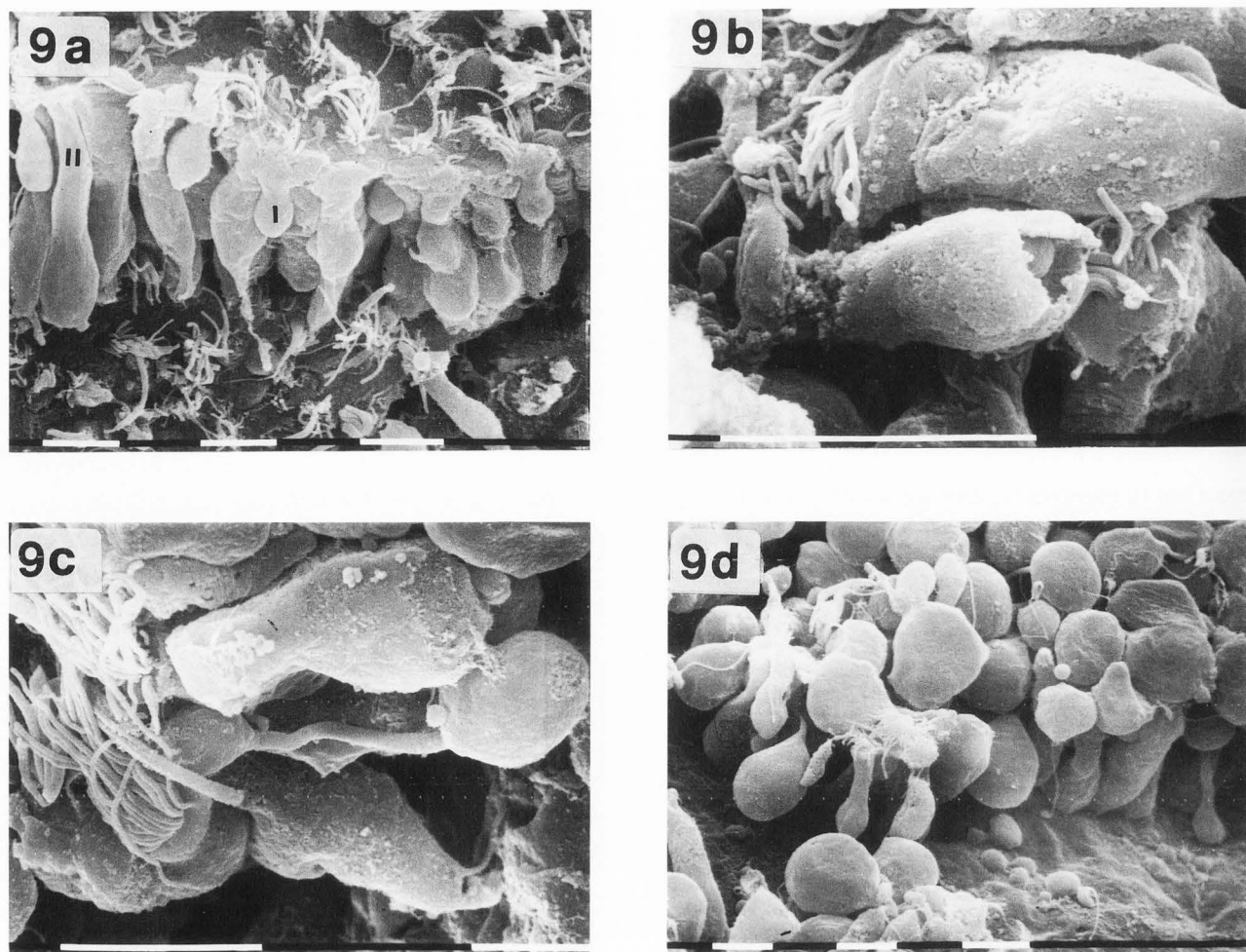Scanning electron microscope observations from

Figure 9. Sacculus. **a**. An open crack on the epithelial surface allowed to identify two types of sensory hair cells. The short flask shaped cells with a neck are likely to be type I whilst the long columnar cells are likely to be type II. **(b)** High magnification of two sensory cells which are almost certainly type I. The calyx surrounding one cell is open exposing the base of the cell itself. **(c)** An intermediate type of cell is shown here as having a bulbous base a long stem and a regular cuticular plate with stereocilia (probably type II, see discussion). **(d)** The periphery of the saccular epithelium showed extensive swelling of cells with complete loss of ciliary bundles. White bars = 10 μm.

the cochlea and the vestibular epithelia from the temporal bone of a Menière's patient have been presented here.

The patient had undergone two surgical interventions (subarachnoid shunt and vestibular nerve section) but the audiogram was essentially the same, when last tested in 1976, as that made in 1971 before any treatment. It seems unlikely, therefore, that these operations contributed substantially to the pathologies described.

Critical review of the data is likely to point out that the time delay to fixation was excessively long (Rutledge, 1969; Bredberg *et al.*, 1972; Hoshino, 1977; Wright, 1980; Gleeson 1985). Indeed, by high resolution SEM it has been shown that autolysis can be identified in human inner ear material as early as 15 minutes

post-mortem (Osborne *et al.*, 1989; Comis *et al.*, 1990). On the other hand it is not often feasible to attain such ideal conditions when working with human material and it is extremely difficult to obtain suitably fixed human temporal bones, and in particular those of Menière's disease. A comprehensive study by Bagger-Sjöbäck and Engström (1985) on cochleas from 16 patients investigated the effect of post-mortem delay to fixation of 75 minutes to 12 hours. These authors demonstrated that whilst a short delay to fixation is ideal, some human cochleas fixed as long as six to eight hours post-mortem could not be distinguished from those fixed with two hours of death. In addition, given the large inter-individual variations in preservation, these authors did not exclude the possibility that valuable information might
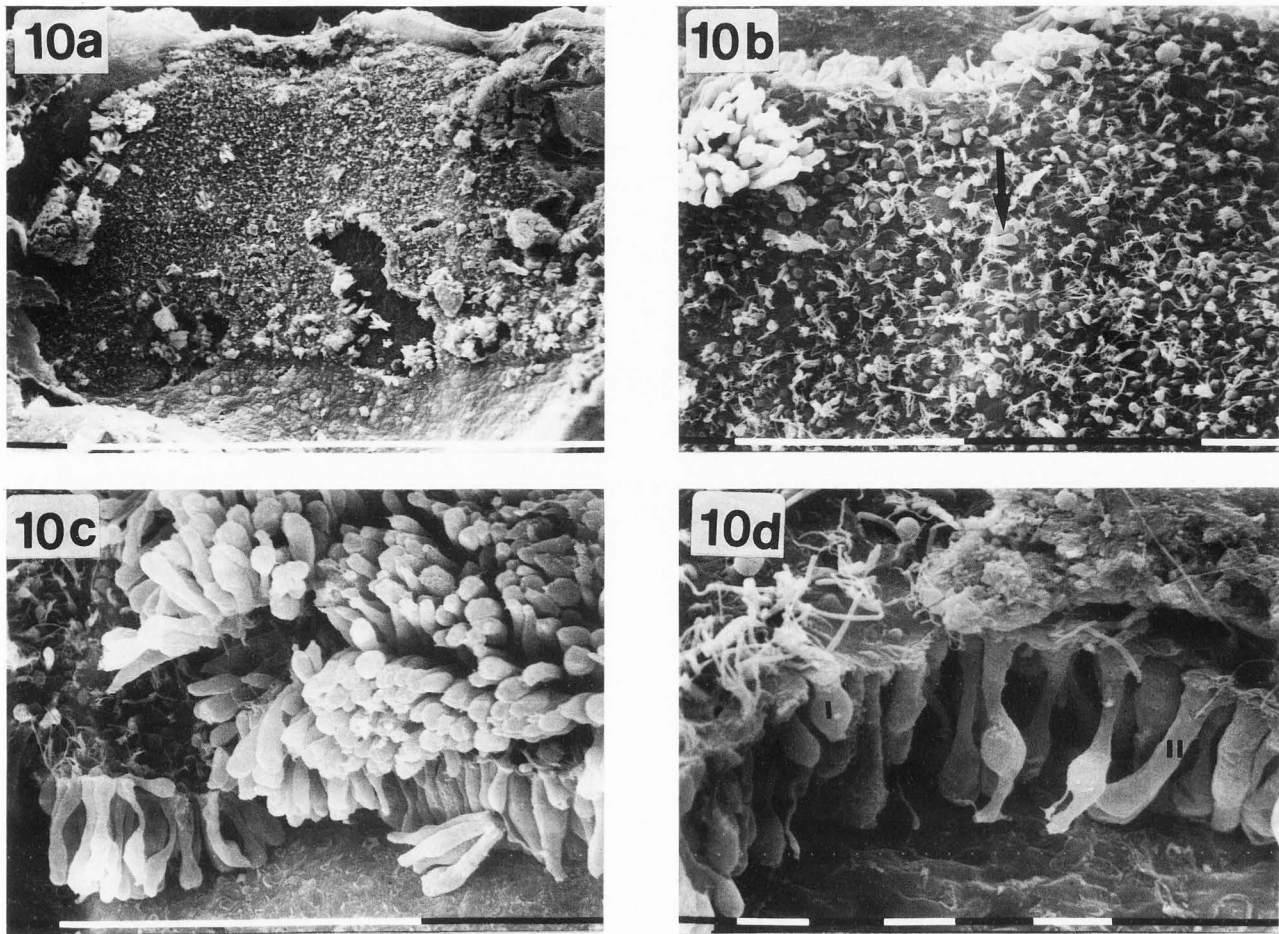
Figure 10. Utriculus. (a) Low magnification view of the epithelium showing a preparation artefact with loss of a small area of the epithelium. In addition the edge of the epithelium has curled up and presented in Fig. 10c. (b) The surface of the epithelium with stereocilia disarray, debris and some sensory cells (arrow) lying on the surface. (c) The edge of the epithelium curled up by 180 degrees exposing the base of the sensory cells and allowing the identification of different types of cells. (d) The preparation artefact on the epithelium allowed the identification of three different forms of cells: short flask shaped cells (type I), long columnar cells (type II) and an intermediate form with long body and a bulbous section (probably type II, see **Discussion**). White bars = 1 mm (a); 0.1 mm (b,c); and 10 μm (d).

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be derived from specimens with a much longer post-mortem fixation interval.

Despite the unfavorable conditions of fixations, the human inner ear sensory epithelium observed here appeared surprisingly well preserved. Indeed, cracks in the surface of both the sacculus and the utriculus allowed observation and identification of different types of sensory cells, short (10 μm) flask-shaped type I and long (30 μm) columnar type II. These observations appear very similar to the cell types observed and briefly presented by Johnsson *et al.* (1984). In addition the present study described an intermediate long (30 μm) cell type with a bulbous base and a long neck which was sometimes seen to be in contact with fine (nerve) fibres which

is likely to represent the bouton endings on type II cells associated with the extra-striolar region (Goldberg, 1991). The relatively good condition of the cochlear tissue is even more striking given the fact that the cochlea observed was from a Menière's patient where biochemical changes have certainly occurred *in vivo*. Indeed, electrochemical analysis of the endolymph from patients with Menière's disease, carried out during surgery, has demonstrated variable data but in general there was a decrease in osmolality whilst in two cases the total calcium had increased (Tran ba Huy *et al.*, 1989). These data from patients with Menière's disease are in agreement with recent data on the animal model which has also demonstrated a decrease in the K^+ concentration and a decrease in the calculated total osmolality (Sziklai *et al.*,

1989) whilst the calcium content of the cochlear endo-lymph has augmented by a factor of 10 (Ninoyu and Meyer zum Gottesberge, 1986). Current interpretation of such data suggests that the imbalance in the calcium homeostasis might be responsible for the swelling vol-ume of endolymph (Meyer zum Gottesberge and Kaufmann 1986, Meyer zum Gottesberge and Ninoyu, 1987) or for the disturbance in the osmolality (Thalmann *et al.*, 1989). In any case it would be expected that in Menière's patients with long-standing hydrops, as was the case examined here, deterioration of the sensory epi-thelium might occur already before death which would almost certainly be accentuated by post-mortem delay to fixation.

When comparing the cochlear tissue to that from the vestibule it was noted here that the saccule was the most perturbed with severe swelling of the sensory cells with complete loss of the ciliary tufts, in particular from the periphery of the epithelium. This feature was not seen in the utricular epithelium nor in the cochlea. Gleeson (1985) in his SEM study on the rat, of the effect of post-mortem delay of fixation has presented a micro-graph of the organ of Corti which is reminiscent of those presented here for the sacculus, with swollen paving-stone representation of sensory cells denuded of cilia raised above the reticular lamina. In that study the ves-tibule was not observed. The surgical interventions which the patient underwent are unlikely to have contrib-uted to this selective sensitivity of the sacculus over the other sensory epithelia. It seems likely, therefore, that the excessive swelling of peripheral cells within the sac-culus as described here are due to the effect of the inner ear pathology *in vivo* together with the long post-mortem delay to fixation.

The sacculus also showed additional features which were thought not to be due to the post-mortem de-lay to fixation. There was loss of ciliary tufts through-out the epithelium and of interest was the fact that the kinocilium appeared to be missing from some hair cell tufts, leaving a hole on the cuticular plate. In addition, holes having approximately the same diameter as the sensory cells, were observed scattered throughout the epithelium. In addition sensory cells were observed lying on the surface. Very similar features have been reported recently in the vestibular epithelia of the guinea pig with experimental hydrops where the delay to fixa-tion is in the order of minutes (Horner and Rydmarker, 1991) and, therefore, the observations described here are not likely to be due to the delay to fixation. On the other hand, TEM observations by Rosenhall *et al.* (1977) on temporal bones from Menière's patients have in-cluded the description of degenerating type I hair cell with the body retracting away from the chalice leaving a cystic cavity. Meyer zum Gottesberge and Ninoyu (1987) have also reported cystic separation between type I hair cell and the nerve chalices in long-standing hydrops in the guinea pig.

As far as the cochlea from the Menière's patient is concerned, there was almost complete loss of hair cells from the basal turn of the cochlea. It might be considered that the surgical interventions, which the pa-tient had undergone, might involve bone drilling and hence, hair cell damage in particular at the base of the cochlea. However, similar findings have earlier been reported by other authors using TEM on temporal bones from Menière's patients (Kimura *et al.*, 1976; Nadol and Thornton, 1987). On the other hand, it might be that long-standing hydrops results in this hair cell loss from the base of the cochlea as a result of a certain intracoch-lear pressure. In the guinea pig where endolymphatic pressure was augmented directly, via a cannula intro-duced into the endolymphatic duct, complete loss of cells from the basal turn of the cochlea was induced (Horner and Cazals, 1991). Simultaneous electrophysiological recording of the compound action potential at the round window demonstrated that this morpho-pathology was associated with a high frequency sensitivity loss (Horner and Cazals, 1990) and suggested that endolymphatic pressure was likely to be a feature of long-standing hy-drops and could result in a loss of cells from the basal turn of the cochlea (Horner and Cazals, 1991).

Concerning the more discrete morpho-pathology of the ciliary tufts, fusion and giant cilia were often observed in association with the inner hair cells (IHCs) and sometimes with the outer hair cells. Several blebs on the surface of the stereocilia were considered to rep-resent the swelling of inter-row stereociliary lines (Fig-ure 5b). In addition, the tall stereocilia of a ciliary bundle, in the outer rows of outer hair cells, were often observed bent forward over the shorter cilia. Similar pathological features have earlier been described as being due to post-mortem fixation delay (Osborne *et al.*, 1989). However, as far as the present material is con-cerned it is not certain that such changes are wholly due to the delay to fixation. As pointed out above the ionic composition of the cochlear liquids had almost certainly changed during the patients life time and hence these modifications might be also due to Menière's disease.

There was, on the other hand, some evidence for shortening of the buttress of shorter stereocilia within the tuft on OHCs of the second cochlear turn. Whilst the observations are limited here to one specimen with Menière's disease, the possibility of atrophy of the shorter stereocilia as a feature particular to hydropic inner ears is very intriguing, since it appears to be a feature characteristic of experimental hydrops in the guinea pig. Stereocilia atrophy with the simultaneous stretching of the intra-ciliary row tip links was consider-ed as a possible mechanism to account for the fluctuant hearing loss in early stages of hydrops (Horner *et al.*, 1988). Indeed, these authors have pointed out that ad-vanced atrophy of the buttress of stereocilia away from the tall stereocilia with the breaking of tip links would almost certainly lead to the non-fluctuant flat losses as-sociated with long-standing hydrops.

The shortening, however, of the stereocilia, was not as striking as that observed earlier in the experi-mental model of endolymphatic hydrops (Horner *et al.*,

1988, 1989; Rydmarker and Horner, 1990). In order to understand why this might be so it is necessary to recall the hypothesis put forward to explain the atrophy process in the animal model.

The specific atrophy of the shorter stereocilia in the OHC tufts of the guinea pig was observed starting above 6 mm from the base of the cochlea (Rydmarker and Horner, 1991). Abnormal elevation of the tectorial membrane in hydropic ears was observed (Rydmarker and Horner, 1990) and these authors suggested that since the tall stereocilia are imbedded within the tectorial membrane (Lim, 1980) that inter-row stereociliary bridges (Pickles et al., 1984) might be stretched and finally broken. Once broken, and the shorter stereocilia detached, the atrophy process might begin (Horner et al., 1988). In order to account for the lack of atrophy in the basal turn, it was suggested at that time that lateral attachment of the tectorial membrane being more substantial in the basal turn (Lim, 1980; Lawrence and Burgio, 1980) could be more resistant to the mechanical lifting force. In view of the present SEM observations on a Menière's patient this hypothesis can be further elaborated. Shortening of the shorter stereocilia within the ciliary tufts in the second cochlear turn was observed in the present investigation. However, there appeared not to be complete atrophy of the buttress as in the guinea pig. Comparative SEM morphological data from normal human and guinea pig cochleae might be the key to the explanation of not only the lack of complete atrophy in human, but also of the complete atrophy in the guinea pig. Indeed, Wright (1984) has shown in the guinea pig, starting above about 6 mm from the base of the cochlea, that the longest stereocilia of OHC3 are longer than those of IHCs and those of OHC2 are about the same length as the IHCs whilst those of OHC1 are shorter than the IHCs. That author has shown that the relative stereocilia lengths are very different in man where the length of the tallest stereocilia decreases from the IHCs to OHC3, to OHC2 and finally to OHC1 (Wright, 1984). In addition, within each tuft, there is a gradation in ciliary lengths and the gradation is greatest in the upper cochlear turns (Lim, 1980). Furthermore, recent evidence suggests that the graphical representation of length of the hair cells as a function of distance from the base of the cochlea, in the guinea pig, has two gradients with an inflection point for OHC3 starting at about 7.5 mm from the base of the cochlea (Pujol et al., 1992). Hence, if the endolymphatic hydrops introduces, to the organ of Corti, some micromechanical or biochemical modification which forces a separation between the hair cell tips and the tectorial membrane, as suggested in the hypothetical model of Tonndorf (1981), then the breaking of inter-row bridges might occur more easily where the cilia are relatively the longest. In the case of the guinea pig this would occur above about 6 mm from the base of the cochlea, and starting at OHC3, which does, in fact, seem to be the case (Rydmarker and Horner, 1991). If one assumes that the same forces are involved in the case of the human cochlea, then complete atrophy of shorter stereocilia might be opposed, not only by the relative length of the longest stereocilia, but also by the composition of each buttress. In the human organ of Corti, within the tuft of cilia on the outer hair cells there are normally several rows of tall stereocilia with a buttress of shorter stereocilia made up of 4 to 5 rows. In the guinea pig, on the other hand, there is only one row of tall stereocilia and the buttress of shorter stereocilia is made up of only 2 additional rows of cilia. Hence the micromechanical forces within a ciliary bundle of an OHC in man and GP are likely to be different and might account for difference in the extent of atrophy of stereocilia reported here.

In conclusion, it seems that the inner ear of Menière's patients might present cochlear and vestibular epithelial features particular to that pathology. In order to shed new light and advance our understanding of the disease, this preliminary study merits to be followed up with further SEM observations on temporal bones from other Menière's patients under improved conditions of fixation.

References

Bagger-Sjöbäck D, Engström B (1985) Preservation of the human cochlea. Anns. Otol. Rhinol. Laryngol. **94**, 284-292.

Barber VC, Boyde A (1968) Scanning electron microscopic studies of cilia. Z. Zellforsch. **84**, 269-284.

Bredberg G, Ades HW, Engström H (1972) Scanning electron microscopy of the normal and pathologically altered organ of Corti. Acta Otolaryngol. (Stockh.) Suppl. **301**, 1-48.

Comis SD, Osborne MP, O'Connell J, Johnson AP (1990) The importance of early fixation in preservation of human cochlear and vestibular sensory hair bundles. Acta Otolaryngol. (Stockh.) **109**, 361-368.

Fitzgerald O'Conor AF, Bagger-Sjöbäck D, Friberg U, Rask-Andersen H (1985) Basal lamina pathology of the utricle and endolymphatic duct in Menière's disease. ORL. **47**, 288-293.

Friedmann I, Cawthorne T, Mc Lay, Bird ES (1963) Electron microscopic observations on the human membranous labyrinth with particular reference to Menière's disease. J. Ultrastruct. Res. **9**, 123-138.

Gleeson MJ (1985) A scanning electron microscopy study of post-mortem autolytic changes in the human and rat cochlea. Acta Otolaryngol. (Stockh.) **100**, 419-428.

Goldberg JM (1991) The vestibular end organs: morphological and physiological diversity of afferents. Current Opinion in Neurobiology **1**, 229-235.

Hallpike C, Cairns H (1938) Observations on the pathology of Menière's syndrome. J. Laryngol. Otol. **53**, 625-655.

Harada Y (1959) Experimental studies on the disturbance of the circulation of the labyrinthine fluid (in Japanese) J. Otolaryngol. Jpn. **62**, 319-330.

Harada Y (1973) Comparison between the lateral semicircular canal lesion in Menière's disease and vestibular lesion with ototoxic agents. Equilibrium Res. **3**, 30-40.

Hilding DA, House WF (1964) An evaluation of the ultrastructural findings in the utricle in Menière's disease. Laryngoscope **74**, 1135-1148.

Hinchcliffe R (1961) Prevalence of the commoner ear, nose and throat conditions in the adult rural population of Great Britain. A study by direct examination of two random samples. Br. J. Prev. Soc. Med. **15**, 128-139.

Hood JD (1983) Audiology. In: Menière's disease: A comprehensive appraisal. Oosterveld WJ (ed.), John Wiley & Sons Ltd., Chichester, U.K., 35-53.

Horner KC, Cazals Y (1990) Alteration of CAP audiogram by increased endolympahtic pressure and its relation to hydrops. Hearing Res. **45**, 145-150.

Horner KC, Cazals Y (1991) Contribution of increased endolymphatic pressure to hearing loss in experimental hydrops. Anns. Otol. Rhinol. Laryngol. **100**, 496-502.

Horner KC, Cazals Y, Guilhaume A (1989) Round window cochlear microphonics and atrophy of short and middle stereocilia on outer hair cells in hydropic cochleae in guinea pigs. In: Mechanics of Hearing, Wilson JP, Kemp DT (eds.), Plenum Press, New York, 207-215.

Horner KC, Guilhaume A, Cazals Y (1988) Atrophy of middle and short stereocilia on outer hair cells of guinea pig cochleas with experimentally induced hydrops. Hearing Res. **32**, 41-48.

Horner KC, Rydmarker S (1991) The vestibular epithelia in experimental hydrops. Scanning Microscopy, **5**, 755-765.

Hoshino T (1977) Contact between the tectorial membrane and cochlear sensory hairs in the human and the monkey. Arch. Otorhinolaryngol. **217**, 53-60.

Ireland PE, Farkashidy J (1963) Electron microscopic studies of Menière's disease: twelve fresh specimens taken at operation. Trans Am. Acad. Ophthalmol. Otolaryngol. **67**, 28-36.

Johnsson LG (1980) Hydrops of the membranous labyrinth as seen by microdissection. In: Proc. Sixth Shambaugh Intern Workshop on Otomicrosurgery and Third Shea Fluctuant Hearing Loss Symposium, Shambaugh GE, Shea JJ (eds.), The Strode Publishers Inc, Alabama, USA, 219-227.

Johnsson LG, Hawkins JE, Rouse RC, Kingsley TC (1984) Four variations of the Mondini inner ear malformations as seen in microdissections. Am. J. Otolaryngol. **5**, 242-257.

Kimura RS, Ota CY, Schuknecht HF, Takahashi T (1976) Electron microscopic cochlear observations in bilateral Menière's disease. Anns. Otol. Rhinol. Laryngol. **85**, 1-11.

Kimura RS, Schuknecht Hf (1965) Membranous hydrops in the inner ear of the guinea pig after obliteration of the endolymphatic sac. Pract. Otorhinolaryngol. **27**, 343-354.

Lawrence M, Burgio PA (1980) Attachment of the tectorial membrane revealed by scanning electron microscopy. Ann. Otol. Rhinol. Laryngol. **89**, 325-330.

Lim DJ (1980) Cochlear anatomy related to cochlear micromechanics. J. Acoust. Soc. Am. **67**, 1686-1695.

Lindsay JR (1968) Histopathology of Menière's disease as observed by light microscopy. Otolaryngol. Clin. North Am. **1**, 319-329.

Litton WB, Lawrence M (1961) Electron microscopy in Menière's disease. Arch. Otolaryngol. **74**, 32-39.

Meyer zum Gottesberge AM, Kaufmann R (1986) Is an imbalanced calcium homeostasis responsible for the experimentally induced endolymphatic hydrops? Acta Otolaryngol. (Stockh.) **102**, 93-98.

Meyer zum Gottesberge AM, Ninoyu O (1987) A new aspect in pathogenesis of experimental hydrops: Role of Calcium. Aviation, Space and Envir. Med. Suppl. **58**, A240-A246.

Nadol JB, Thornton AR (1987) Ultrastructural findings in a case of Menière's disease. Anns. Otol. Rhinol. Laryngol. **96**, 449-454.

Naito T (1950) Experimental studies on Menière's disease. Jpn. J. Otol. **53**, 19-20.

Naito T (1959) Clinical and pathological studies on Menière's disease. Proc. 60th Annual Meeting of Oto-Rhino-Laryngol. Soc. Jap., Tokyo, 1-66.

Ninoyu O, Meyer zum Gottesberge AM (1986) Changes in Ca^{++} activity and DC potential in experimentally induced endolymphatic hydrops. Arch. Otorhinolaryngol. **243**, 106-107.

Osborne MP, Comis SD, Johnson AP, Jeffries DJR (1989) Post-mortem changes in hair bundles of the guinea pig and human cochlea studied by high-resolution scanning microscopy. Acta Otolaryngol. (Stockh.) **108**, 217-226.

Paparella MM (1991) Pathogenesis and pathophysiology of Menière's disease. Acta Otolaryngol. (Stockh.) Suppl. **485**, 26-35.

Pickles JO, Comis SD, Osborne MP (1984) Cross-links between stereocilia in the guinea pig organ of Corti and their possible relation to sensory transduction. Hearing Res. **15**, 103-112.

Pietrantoni L, Iurato S (1960) Some initial electron microscopic investigations of case of Menière's syndrome. Acta. Otolaryngol. (Stockh.) **52**, 15-26.

Pujol R, Lenoir M, Ladrech S, Tribillac F, Rebillard G (1992) Correlation between the length of the outer hair cells and the frequency coding of the cochlea. In: Advances in Biosciences, Vol 83, Auditory Physiology and Perception. Cazals Y, Demany L, Horner KC (eds.), Pergamon Press, Oxford, 45-51.

Rosenhall U, Engstrom B, Stahle J (1977) Macula utriculi in four cases with Menière's disease. Acta

Otolaryngol. (Stockh.) **84**, 307-316.

Rutledge LJ (1969) Histological study of the perfused human temporal bone. Laryngoscope **79**, 2104-2125.

Rydmarker S, Horner KC (1990) Morphological changes of hair cell stereocilia and tectorial membrane in guinea pigs with experimentally induced hydrops. Scanning Microsc. **4**, 705-714.

Rydmarker S, Horner KC (1991) Atrophy of outer hair cell stereocilia and hearing loss in hydropic cochleae. Hearing Res. **53**, 113-122.

Sanchez-Fernandez JM, Marco J (1975) Ultra-structural study of the human utricular macula and vestibular nerve in Menière's disease. Acta. Otolaryngol. (Stockh.) **79**, 180-188.

Schuknecht HF (1963) Menière's disease: A correlation of symptomology and pathology. Laryngoscope **73**, 651-665.

Stahle J, Stahle C, Arenberg IK (1978) Incidence of Menière's disease. Arch. Otolaryngol. **104**, 99-102.

Sziklai I, Horner KC, Ferrary E, Sterkers O, Amiel C (1989) Electrochemical composition of cochlear fluids in early experimental hydrops. Acta Otolaryngol. (Stockh.) **107**, 371-374.

Tran ba Huy P, Ferrary E, Roinel N (1989) Electrochemical and clinical observations in 11 cases of Menière's disease. In: Menière's Disease, Nadol JB (ed.), Kugler & Ghedini, Amsterdam, 241-246.

Tonndorf J (1981) Stereociliary dysfunction, a cause of sensory hearing loss, recruitment, poor speech discrimination and tinnitus. Acta Otolaryngol. (Stockh.) **91**, 469-479.

Thalmann R, Salt AN, de Mott J (1989) Endolymph volume regulation: Possible mechanisms. In: Second International Symposium on Menière's Disease. Nadol JB (ed.), Kugler & Ghedini, Amsterdam, 55-60.

Wright A (1980) Scanning electron microscopy of the human cochlea - postmortem autolytic artefacts. Arch Otorhinolaryngol. **228**, 1-6.

Wright A. (1984) Dimensions of the cochlear stereocilia in man and the guinea pig. Hearing Res. **13**, 89-98.

Yamakawa, K. (1938) The inner ear of a patient with Menière's syndrome. J. Otorhinolaryngol. Soc. Jpn. **44**, 2310-2312.

Discussion with Reviewers

D. Bagger-Sjöbäck: Although some case reports and inner ear material from patients like this have been presented earlier, this is the type of material and report so badly needed in order to further understand the events occurring in patients with Menière's disease. Therefore even a single case report may be of value.

As usual, in material like this, it is extremely difficult to distinguish between true pathological findings and postmortal changes. The latter may emanate from true autolysis due to the delay between death and fixation, it may be due to preparation artifacts and also the fact that the specimens were immersed in fixative for several years. It has been shown that changes, although discrete, may occur if the specimen is kept too long in the fixative. This fact deserves further penetration.

D.J. Lim: The primary weakness of the study is the postmortem changes that are expected and may cloud the interpretation of the pathologies. Having a parallel experimental postmortem study could have strengthened the interpretation of the present study. However, even taking into consideration of the expected postmortem changes, the present study has a merit, as striking pathologies involving the cochlea and saccule are too specific to be entirely due to postmortem.

Because this study deals with the postmortem material, one should also consider fixation and dissection artifacts in this type of preparation. The swelling or the "bleb" formation on stereocilia has been reported in both pathology as in the normal animal specimen that were poorly fixed. Therefore, one should be careful in interpreting the data and an experimental postmortem study could have helped.

M.P. Osborne and S.D. Comis: We have one serious reservation, which is that appropriate control material was not examined. If the author would be prepared to compare the material with a "normal" cochlea obtained from a cadaver which was age-matched etc. and had been prepared for scanning microscopy under identical conditions as the diseased ear, we would be more than happy to recommend the paper for publication. Until this is done, it is very difficult to distinguish pathological damage caused by Menière's disease from that due to post mortem artifacts, although we would concede that total absence of hair cells is more likely to be pathological than post mortem in origin.

Y. Harada: Unfortunately in this temporal bone, artifacts (post mortem changes) are quite severe; I think it is very difficult (or impossible) to compare the findings, especially in the fine structure, obtained in this study, with those in animal model.

A.N. Salt: The paper may be valuable in demonstrating stereociliary atrophy, similar to that seen in animals, in a human Menière's case. I agree the author is cautious and attribute many observations to possible fixation artifacts. However, the important points will be lost amidst the confusion regarding fixation artifacts. Further, there is no attempt to incorporate any type of control ear, fixed under similar conditions, to help judge exactly what is fixation artifact.

In my opinion, Menière's disease is not that rare a condition (stated as 1:100 in Britain in the **Introduction**) that it would not be possible to obtain better-fixed material. However, I accept that this may be misleading and these temporal bones are indeed extremely difficult to obtain.

Author: I appreciate the reviewers' concerns about fixation artifacts and suitable control material. As already pointed out in the **Discussion** "it is not often feasible to attain ideal conditions (fixation) when working with human material and it is extremely difficult to

obtain suitably fixed human temporal bones, and in particular those of Menière's patients".

In addition, I would like to take this opportunity to emphasize the difficulty encountered when trying to recuperate human temporal bones. Despite the reported existence of a European Bone Bank, the specimens are rare and those of Menière's patients have been reserved for a few "well-placed" individuals.

D. Bagger-Sjöbäck: A major problem is that the circumstances around the death of the patient is unknown. It has been showed by several authors that the way the patient dies is of great importance when observing the inner ear epithelia. Thus, a pyrrhectic patient dying of prolonged disease may present in a different way from patients having died abruptly without prior disease.

Author: The patient was last examined in 1976. She died in April 1986. There was no communication from her between her last visit and the time of her death. The cause of death is unknown.

D. Bagger-Sjöbäck: Last pure tone audiogram is not presented; it would be of great help to the reader to correlate the cochlear findings with the actual pure tone audiometrical findings.

Y. Harada: In the case history, if audiogram of this patient examined in 1976 is available, it will be better. Also, this patient died at age of 63. I think age should be taken into consideration particularly on the morphology of the cochlea. Do you have any data about the hearing level of right (unaffected) ear?

Author: The hearing level (dB) in 1976, in the left ear, was 75, 75, 65, 65, 65, and 75 at frequencies (in kHz) 0.25, 0.5, 1, 2, 4, and 8, respectively. The hearing level (dB) at that time, in the right ear, was 25, 30, 30, 45, 30, and 50 at the same frequencies. Hence there is no clear correlation between the hair cell loss and the pure tone audiogram.

D. Bagger-Sjöbäck: One interesting finding is shown in Figure 9a where the author describes the two types of sensory hair cells to be different in size. There should be a relationship of cell length between the two types with a ratio of 1 to 3. This has to my knowledge not been found earlier to be so large and it would be interesting to hear the author speculate or give some references supporting this finding. I do not deny, however, that the cell bodies shown in the Figures may well be type 1 and type 2 cells but this still is a new finding which ought to be discussed in greater detail.

Author: At least two types of cells were observed in this investigation: short flask shaped cells and longer columnar shaped cells which were tentatively identified as type I and II respectively.

Johnsson *et al*. (1984) presented one micrograph of a fractured area from each a saccular and a utricular human sensory epithelium; they stated that both types I and II hair cells were represented but did not identify those particular cells. From their micrographs, however, it can be seen that indeed, the tall cells were at least 2 times longer than the short cells.

In addition, a recent study by Rennie and Ashmore (1991) on isolated hair cells from the vestibule of the guinea pig has identified three types of sensory cells based on size as well as shape [Rennie KJ, Ashmore JF (1991) Ionic currents in isolated vestibular hair cells from the guinea pig cristae ampullaris. Hearing Res. **51**, 279-292]. These authors identified type I cells as having a neck of uniform diameter throughout the cell length until joining the spherical base of the cell or it would show some tapering as it approached the cuticular plate. Type II cells were identified by the lack of a constricted neck region and were either tall (15-33 μm) or short (4-15 μm).

D. Bagger-Sjöbäck: As regards the cell in Figure 9c it cannot be concluded that this is one cell as shown in this particular micrograph. The bulbous swelling cannot be proven, as shown from this angle, to be directly connected to the thin stalk. This type of cell body has not been shown by other authors and I would like to see more convincing proof before accepting this third cell type.

Y. Harada: The author observed intermediate type cell in the otolithic organ. Could you observe the sensory hairs on this type of cell? Is there any possibility that this cell is the supporting cell?

Author: The micrograph presented in Figure 9c was taken in order to demonstrate the presence of cilia on the cell with the long neck and bulbous swelling. However, I admit that this is not really proven here, although, Figure 10d illustrates at least three types of cells. As stated in the text, fine fibres appear to make contact with the bases of some cells, and in particular, the cell with the bulbous base which suggests that these cells might be type II.

It should be remembered that Wersall, already in 1956, described different types of type II cells in the vestibule of the guinea pig [Wersall J (1956) Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig. Acta. Otolaryngol. Suppl. 126, pp 85]. Wersall described type II cells as being fairly regular cylindrical shape with a rounded lower part and being longer than type I. Wersall stated: "Some hair cells of type II nevertheless show a more irregular structure, the lower part being narrower than the upper part and, in some cases, having only one-third of the latter's diameter."

However, at this time, one cannot reject the possibility that the third type of cells is supporting cells.

Y. Harada: The author did not state about the presence or degree of hydrops in the cochlea or the vestibule. Was there any hydropic findings such as distention of Reissner's membrane or saccular membrane?

Author: During the microdissection step, care was taken to confirm the convexity of the Reissner's membrane. In fact, however, the Reissner's membrane was not visualized and it was assumed that it had been in contact with the bony wall which was removed. The saccular membrane did appear somewhat distended.