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THE VESTIBULAR EPITHELIA IN EXPERIMENTAL HYDROPS

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Abstract

Morpho-pathological features, observed by scanning electron microscopy, in guinea pigs with endolymphatic hydrops of 4-14 months included shortening of the hair cell tufts, loss of tufts, retraction of sensory hair cells away from the surrounding tissue and hair cell loss. After 22 months of hydrops, there was complete loss of hair cells with indifferentiation of the epithelium. The loss of ciliary tufts involved loss of both stereocilia and kinocilia identified as short stubs and holes respectively. Control macular epithelia showed no hair tuft loss although ampullae in control ears could show some loss.

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Introduction

Experimental endolymphatic hydrops is of fundamental interest for the clinical understanding and management of inner ear disorders associated with such a pathology. In particular Menière's disease is most often cited in this context although other diseases may also be associated with endolymphatic hydrops (Schuknecht and Gulya, 1983; Paparella, 1984). Menière's disease is, by convention, characterized by the three classic symptoms of fluctuant low frequency hearing loss, episodes of vertigo, and tinnitus.

The surgical technique for experimental induction of endolymphatic hydrops, by blocking of the endolymphatic duct was first described by Naito (1950), extensively studied by Kimura and Schuknecht (1965), and widely employed by other researchers since that time. Indeed hydrops can be induced with 100% success in the guinea pig and many authors have described the morphological expression as a swelling of the endolymphatic spaces within the inner ear. It is now well established that the guinea pig model presents hearing dysfunctions similar to those observed in Menière's patients with early low frequency fluctuant losses (Horner and Cazals, 1987), then additional high frequency losses and in a later stage the mid frequencies are also affected (Horner et al., 1989b; Horner and Cazals, 1990; Horner, 1991).

Despite the remarkable similarity to the hearing deficit in Menière's disease, the model has often been criticized for lacking the appropriate vestibular dysfunction since episodes of vertigo are rarely witnessed in the guinea pig. Whilst asymmetry of the evoked rotatory nystagmus has been reported (Aran et al., 1984) and confirmed as being transient and associated with the first four months after induction of hydrops (Horner et al., 1989b), episodes of vertigo have only extremely rarely been observed by chance. On the other hand attacks of vertigo comprise one of the characteristic features of Menière's disease. Indeed there exists quite a body of literature describing pathological ultrastructural (transmission electron microscopy, TEM) features associated with the ampullae (Pietrantoni and Iurato, 1960; Ireland

and Farkashidy, 1963; Harada, 1973) and the utriculus (Friedmann *et al.*, 1963; Sanchez-Fernandez and Marco, 1975; Rosenhall *et al.*, 1977) from Menière's patients. Those studies have indeed confirmed the presence of morpho-pathological features. Those studies have described vacuolation of the cytoplasm of the sensory cells as well as partial or total loss of sensory hair cilia from the surface.

Although light microscope observations by Kimura and Schuknecht (1965) did not reveal any pathology associated with the vestibular sensory cells in hydropic ears, we considered it appropriate here to study the vestibular sensory epithelium at different lapses of time after the induction of hydrops using scanning electron microscopy (SEM). This study was particularly opportune since earlier SEM studies on the cochlea of hydropic inner ears has established that there is a morpho-pathology of outer hair cells which is apparently unique to hydropic inner ears. Those studies have shown that there is a selective atrophy of the short and middle stereocilia on the outer hair cells, whilst the tall ones remain upright and apparently unperturbed, throughout the three upper cochlear turns (Horner *et al.*, 1988, 1989a; Rydmarker and Horner, 1990, 1991).

Materials and Methods

The guinea pigs were anaesthetized with ketamine (50 mg/ml) and xylazine (2%) (Ketalar/Rompun 1 mg/kg of the mixture at a volume ratio of 2:1). After opening the posterior fossa, an intradural approach to the endolymphatic sac and duct was made. Endolymphatic hydrops was surgically-induced by blocking the endolymphatic duct with bone wax. In all cases the left side was operated to induce hydrops and the right side served as a control. Animals were sacrificed after 4 months (n = 4), 8 months (n = 4), 14 months (n = 2) and 22 months (n = 1). In addition four non-operated animals served as controls.
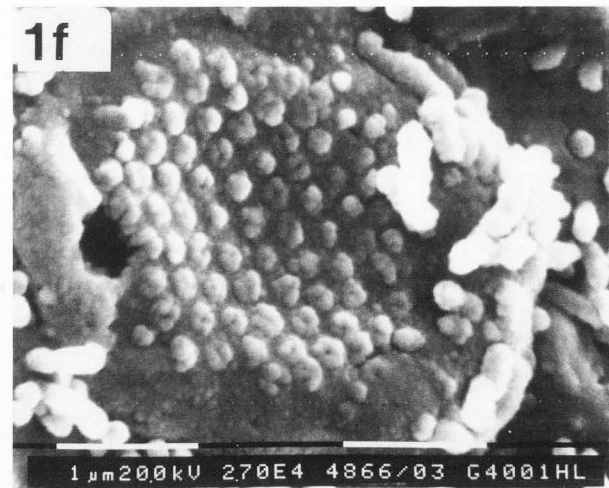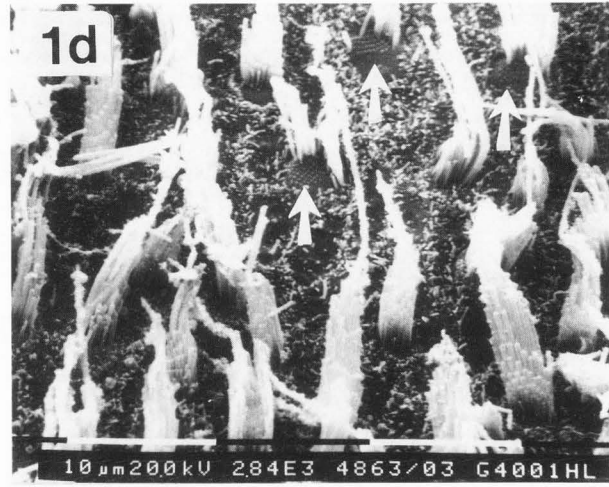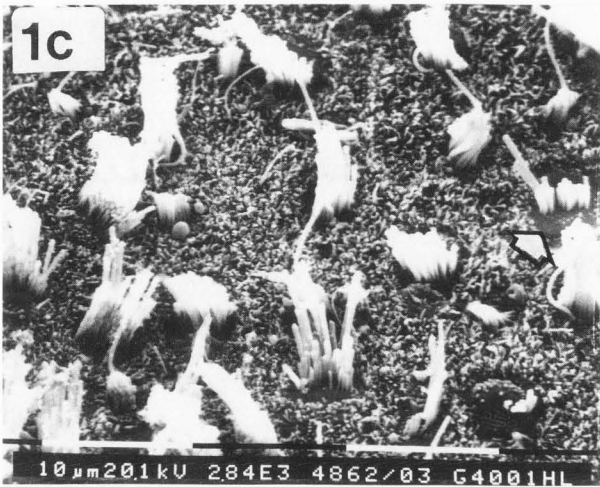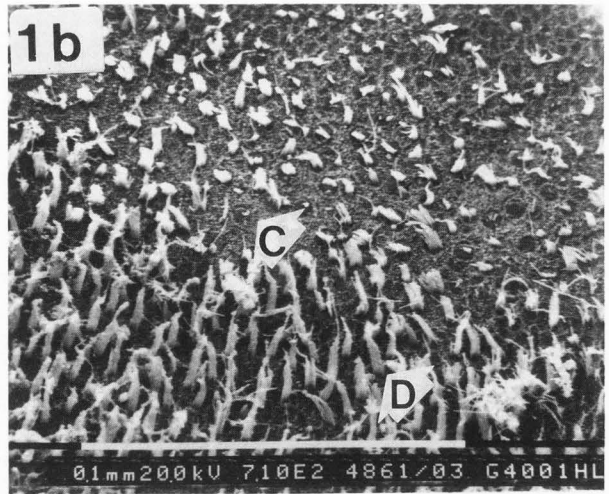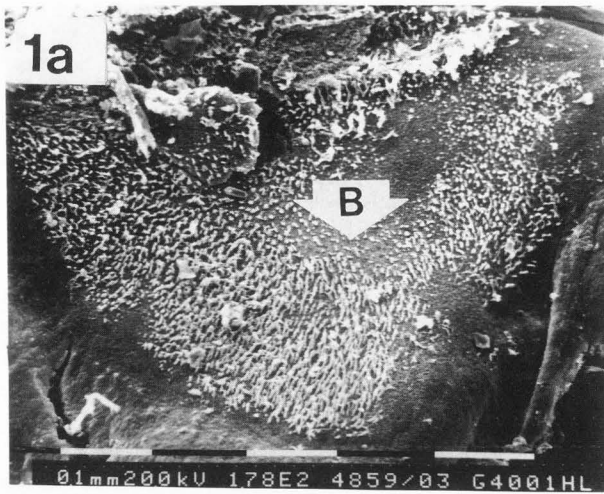
For sacrifice the animals were deeply anaesthetized with Ketalar/Rompun. Following decapitation the bullae were removed and perfused successively through the round and the oval windows with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and then left bathing in the fixative for 1 hour. The bullae were then rinsed by perfusion with the buffer and brought to 70% alcohol. The next day the vestibules were dissected and the presence of hydrops confirmed by the removal of a small portion of the cochlear wall at the base. The vestibules were brought to 100% alcohol for critical point drying with CO_2 (Balzers Union CPD 010). The specimens were then mounted on aluminum stubs and coated with gold/palladium (15 nm, Balzers Union SCD 030). Observations were carried out on a Philips 505 scanning electron microscope.

Figure 1. (**a**) The utricle from a guinea pig (I) with 4 months hydrops. Note the extensive area with reduced density of hair cell tufts. The transition zone from long cilia tufts to short cilia tufts is indicated with an arrow and shown at higher magnification in Fig. 1b. (**b**) There is an abrupt transition from an area with long cilia tufts to short tufts. Higher magnification of an area (arrow C) with short stereocilia tufts is presented in Fig. 1c and an adjacent area (arrow D) with long stereocilia is presented in Fig. 1d. (**c**) View of an area of the macula with short cilia tufts. Note the hair cell bundle (arrow) showing both shortened cilia and loss of cilia. (**d**) View of an area of the macula with long (normal) cilia tufts. The stairway arrangement of stereocilia is still respected. Within this area cells can be seen (arrows) which lack most of their cilia. (**e**) View of an area where most cells lack all cilia. Absence of one hair cell is indicated by a hole (around 3000 nm) on the sensory epithelium. (**f**) High magnification of the cuticular surface of a cell lacking all stereocilia (stubs with central depression which is presumably the location of the rootlet) as well as the kinocilium (hole of around 400 nm).
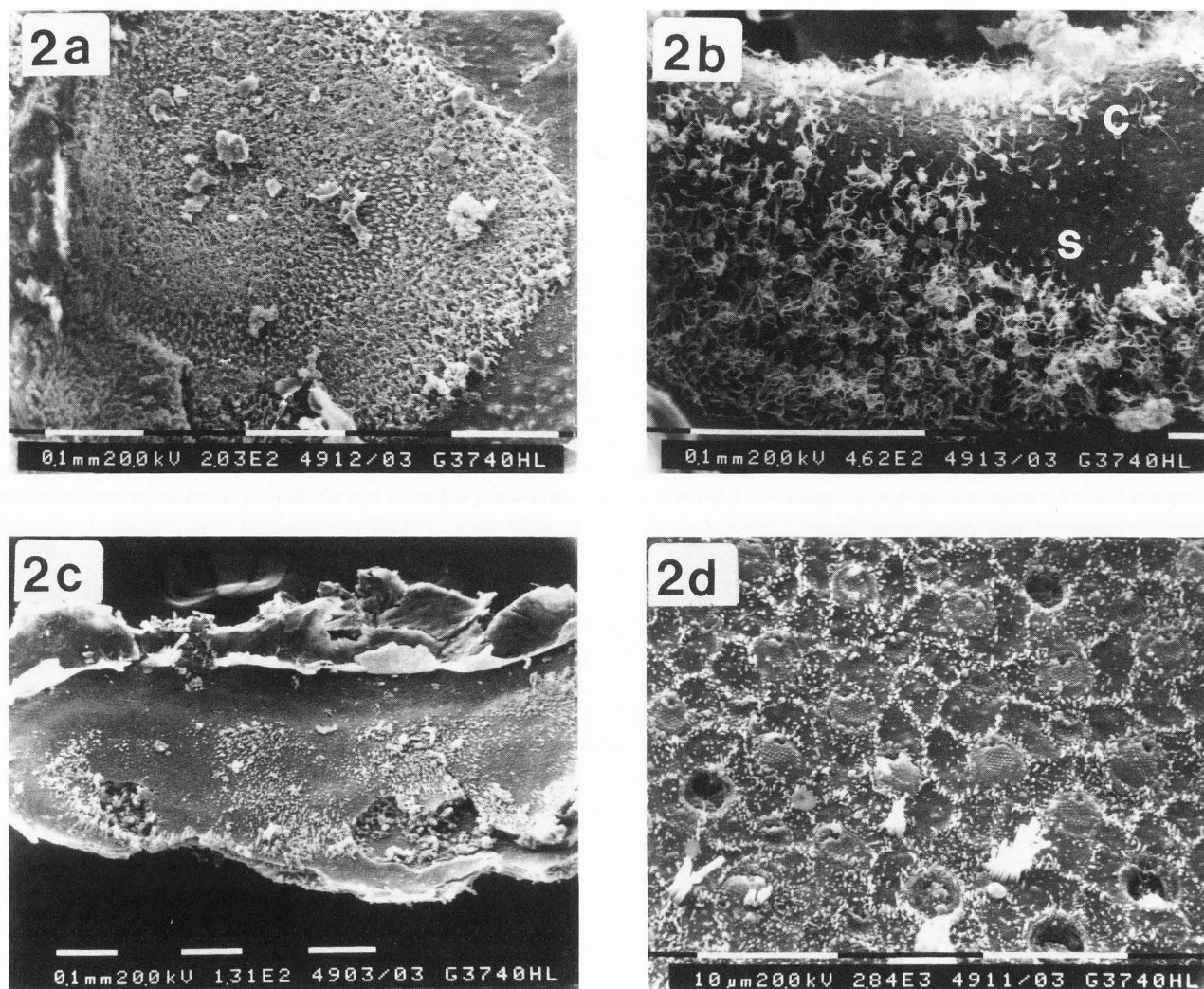

Results

In inner ears of guinea pig having hydrops during 4-22 months several morpho-pathological features of the vestibular epithelia were identified. Representative characteristic features are presented in Figure 1 for the utricle of a guinea pig (GP4001) with four months hydrops. There was total or partial loss of hair cell ciliary tufts over a substantial area of the utricular epithelium (Fig. 1a). The loss of cilia appeared not to be associated, in particular, with the striola but was rather dispersed throughout the sensory epithelium. In those areas affected, the length of the ciliary tufts appeared to be shorter (Figs. 1b, 1c) than normal (Fig. 1d) or completely absent (Fig. 1e). In addition intact ciliary tufts with long cilia could be observed in close proximity to tufts which lacked some cilia (Fig. 1d). Those hair cell bundles which were intact or even lacked some cilia did not show any pathology such as fusion of stereocilia or splaying. Loss of stereocilia was indicated by 10/12 parallel rows of 6-9 stubs on the cuticular plate (Fig. 1f). Each stereocilia stub appeared as a promontory (around 100 nm in diameter) with a minute depression in the center which was presumably the location of the rootlet. The position of the kinocilium appeared as a hole (around 400 nm in diameter). Complete hair cell loss was identified as a hole (around 3000 nm in diameter) on the surface of the epithelium (Fig. 1e). At this stage (4 months hydrops) the utricle appeared to be affected to a greater extent than the saccule. Loss of ciliary tufts on the ampullae was also identified and
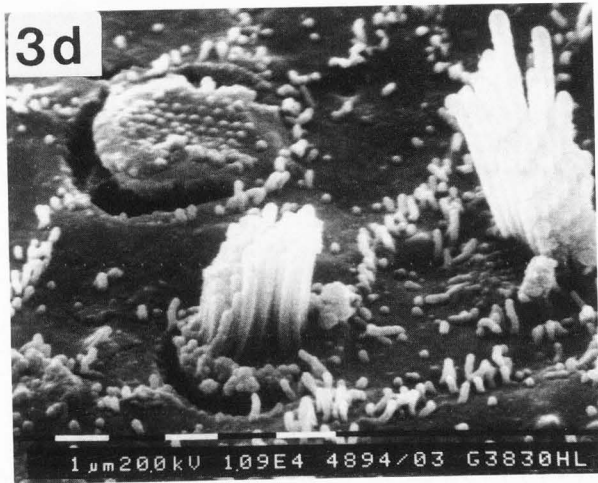
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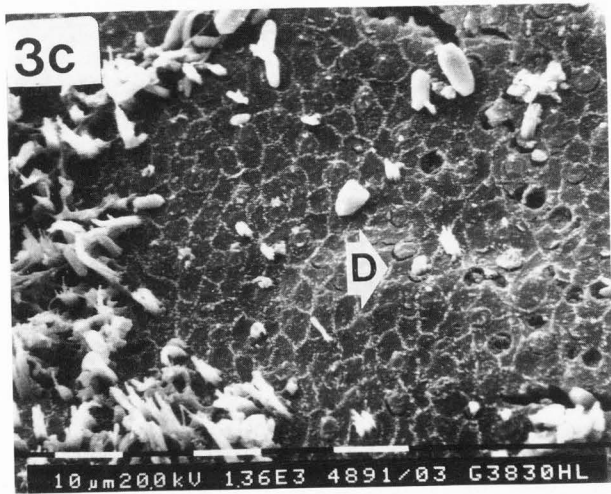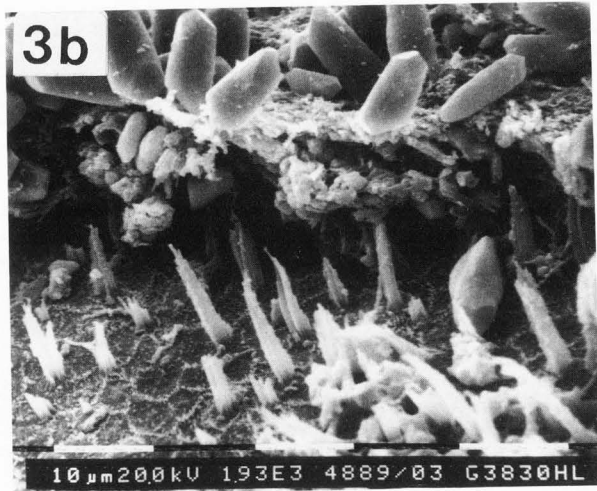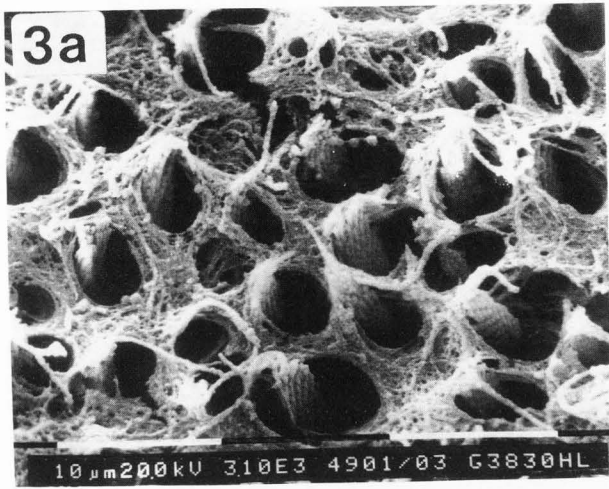See facing page for legend of Figure 1.

Figure 2. (a) The utricle from a guinea pig (GP3740) with 8 months hydrops covered for the most part by an otolithic membrane net but which apparently does not lack cilia. (b) An ampulla showing loss of cilia from the side (S) as well as the crest (C). (c) The saccule with substantially reduced density of ciliary tufts. The loss of cilia does not appear to be associated in particular with the striola. (d) The same saccule as in Fig. 2c at higher magnification to demonstrate the almost systematic loss of all ciliary tufts from the sensory cells. In each case the stubs of the stereocilia and the hole representing the location of the kinocilium can be identified.

Figure 3 (on the facing page). (a) The utricle from a guinea pig (GP3830) with 8 months hydrops, which was almost completely covered by the otolithic net. Apparently normal ciliary bundles can be seen projecting from the holes. (b) The saccule showing the sensory hair cells partially covered with the otolithic membrane and otoliths. Note the proximity of short stereocilia tufts to long tufts. The otoliths in general do not appear pathological. (c) A different view of the same saccule as in Fig. 3b showing a substantial area of complete loss of ciliary tufts. Higher magnification of three cells (arrow D) is presented in Fig. 3d. (d) Three stages in the apparent degeneration process are shown. One cell shows the loss of 20-30 stereocilia whilst the remaining stereocilia appear quite normal. Another cell shows shortening of the ciliary tuft. The third cell totally lacks all stereocilia. In addition note the cracks which surround the cuticle. (e) An ampulla from the same guinea pig showing extensive hair cell ciliary tuft losses particularly on the side (S) as well as the crest (C). (f) High magnification view of the same ampulla as in Fig. 3e showing not only ciliary tuft loss but also holes on the surface of the sensory epithelium indicating the loss of hair cells.

See facing page for legend of Figure 3.

Figure 4 (in right column). (a) The utricle from a guinea pig (GP3510) with 22 months hydrops. There is almost total absence of ciliary tufts except for a small area (star) presented in Fig. 4c. (b) High magnification of the utricular epithelium showing the almost total lack of sensory cells (except for the small surface shown in Fig. 4c with no apparent differentiation of the tissue. (c) A small area of the sensory epithelium with a few remaining hair cells with ciliary tufts. In addition protrusions from the sensory epithelium can be seen which are likely to represent the protrusion of the remains of the degenerated sensory cells.

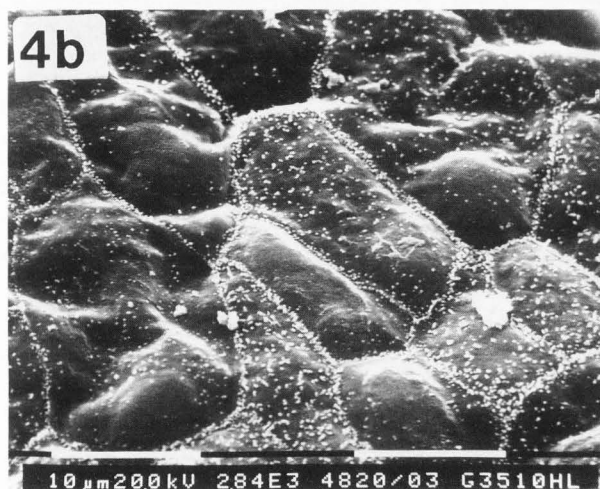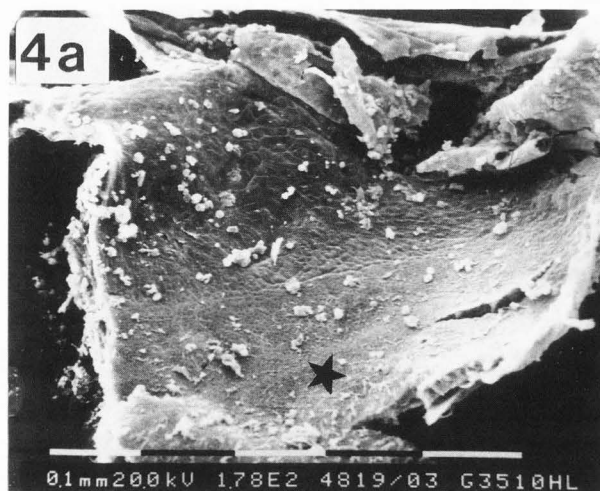--

appeared not to be associated with the crest in particular (see below for illustration).
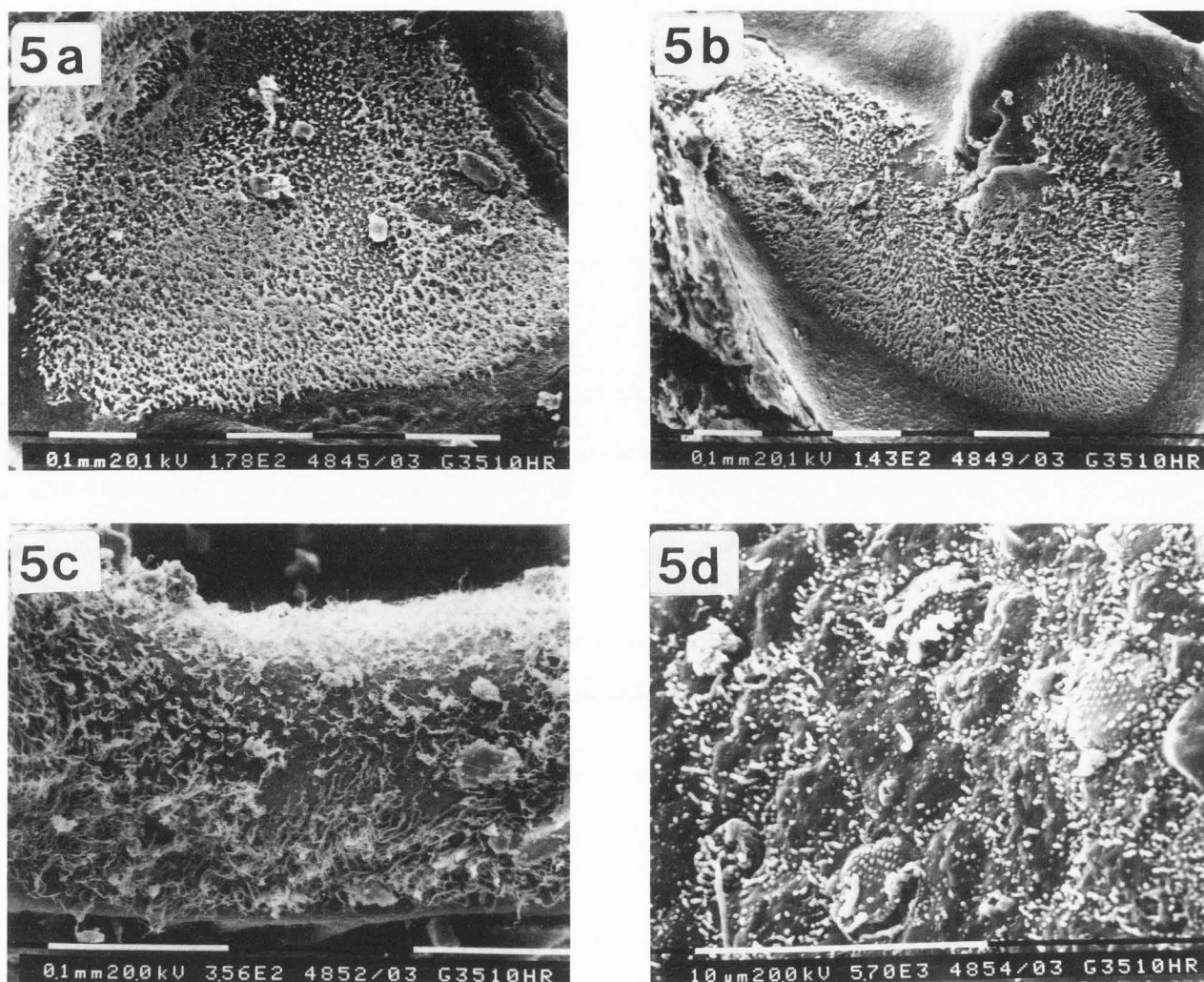
After 8 months of hydrops the hair cell ciliary tuft loss on the saccule (Fig. 2c) appeared to have evolved more rapidly than on the utricle (Fig. 2a) such that almost the whole surface of the saccule was affected as shown here for one guinea pig (GP3740) but confirmed for all four animals in this group. The density of ciliary tufts appeared variable over the surface of the epithelium and as was noted for the utricle, the loss appeared not to be associated in particular with the striola. In addition the ciliary tufts of both cell type I and II appeared to be affected similarly (2d). The ciliary tuft loss in the ampullae was for the most part associated with the sides rather than with the crest of the crista (Fig. 2b).

Another example of an 8 month hydrops vestibule is presented in Figure 3. In this case (GP3830) a fine otolithic membrane net covered most of the surface of the utricle but the intact hair cell tufts could be seen projecting out of the net (Fig. 3a). Extensive ciliary tuft loss was on the other hand evident on the saccular surface. Figure 3b illustrates the proximity of short ciliary tufts to adjacent long tufts. This figure also shows some otoconia which for the most part did not appear different from normal control specimens. In those areas of the macular epithelium where there were holes on the surface, cracks surrounding several hair cell cuticles could also be identified (Fig. 3c). Higher magnification (Fig. 3d) suggested that the hair cell body or at least the cuticle was retracted away from the surrounding supporting tissue. Figure 3e illustrates the extensive hair cell ciliary loss on the wall of an ampulla. Indeed holes could be identified on the ampulla wall as shown in Figure 3f.

After fourteen months of hydrops the loss of stereociliary tufts was more extensive but similar to that observed at 8 months. However, even at this relatively advanced stage of hydrops, the stereocilia remaining within a tuft which showed some loss, did not exhibit any obvious fusion or splaying.

In the most advanced stage of hydrops observed here (22 months) the utricular surface showed not only

Figure 5. (a) The right control ear of guinea pig (GP3510) whose left hydropic epithelia are presented in Figure 4. The utricle shows no hair cell ciliary tuft loss apart from the small preparation artefact. (b) The saccule where no hair cell ciliary tuft loss is evident. (c) An ampulla showing some hair cell ciliary tuft loss. (d) High magnification of the limited area on the surface of the ampulla where hair cell ciliary tufts were absent. Loss of the ciliary tufts is indicated by the stubs on the surface of several cuticles. On the other hand other cells lacking cilia show smooth cuticles with, in some cases, a single cilium remaining which can be distinguished from the kinocilium which is absent and whose former location can be identified as a hole on the surface of the cuticle.

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total loss of ciliary tufts but also loss of the hair cells (Fig. 4a).

The epithelium tissue appeared to be undifferentiated over the whole surface of the epithelium (Fig. 4b) with the exception of a small region on the periphery where a few hair cell ciliary tufts could be identified (Fig. 4c). In this region there were also dense microvilli and rounded protrusions from the epithelium surface (Fig. 4c) suggesting that the remains of the hair cells might be expulsed from the epithelium. The morpho-pathologies

described here were thought not to be due to an aging process since the right control ear of this same animal (22 months hydrops - operated at 3 months old) showed completely intact macular epithelia (Figs. 5a, 5b). On the other hand some ciliary tuft loss could be identified to some extent on the ampullae on the control side although it was usually associated with the crest (Fig. 5c). On close observation the ciliary tuft loss appeared to be similar to that observed in the hydropic macular epithelia (Fig. 5d).

Discussion

We have described here scanning electron microscopy (SEM) observations of the vestibular sensory epithelia in hydropic inner ears of guinea pigs. Indeed this study, to the best of our knowledge, represents the first SEM study of the vestibular epithelium in experimental endolymphatic hydrops. We have described here hair cell cilia loss, including the kinocilium as well as the stereocilia, extending over a substantial area of the ampullae and the macular epithelia in hydropic inner ears. It appeared here that cilia loss is preceded by shortening of the cilia tuft. Sensory cells with short cilia could be observed throughout the maculae including the periphery, which in normal maculae is associated with long cilia, and always in close proximity to an area completely lacking cilia. The shortening of cilia described here for hydropic specimens is clearly different from that observed in the central part of the normal macula (Lindeman, 1969). This shortening might very well be due to the presence of hydrops. It is now well established that hydropic cochleas are associated with an atrophy of stereocilia which is unique to hydropic ears. In the cochleas in fact there is a progressive shortening of the short and middle rows of stereocilia on the cilia tufts on the three upper cochlear turns, whilst the tall ones are preserved (Horner et al., 1988; 1989a; Rydmarker and Horner, 1990; 1991). The mechanisms underlying the selective atrophy of short and middle stereocilia and the preservation of the tall stereocilia in the cochlea are not known but discussed by Horner et al. (1988). In those cochlear specimens with hydrops of less than four months duration, the short cilia apparently become detached from the middle stereocilia and the middle from the tall before atrophy takes place (Rydmarker and Horner, 1991). The present study on the vestibular epithelia has dealt only with animals with hydrops of more than four months which corresponds to a stage of well developed hydrops. It is likely that more complete information regarding the sequence of phenomena leading to cilia tuft loss, can be gained in future studies dealing with hydrops of shorter duration.

The present study has, in addition, described some hair cell loss. These data appear at first sight to be in conflict with the data of Kimura and Schuknecht (1965) who concluded that the sensory cells in the maculae utriculi and cristae ampullares in early hydrops were normal. However those authors' conclusion was based on light microscope observations where certain pathologies may not have been detected.

Loss of cilia on the vestibular epithelia has earlier been reported in animals following ototoxic drug treatment (Duvall and Wersall, 1963; Lindeman, 1969; Wersall et al., 1971; Aran et al., 1982). However it is not clear whether those losses are the same as those described here in hydropic vestibules. The present study has demonstrated that in the vestibular epithelia of hydropic ears absence of the stereocilia is represented by stubs, each with a central depression (presumably representing the location of the rootlet), whilst the kinocilium leaves a hole on the surface of the cuticle. However, a similar morpho-pathology could be seen in control specimens of ampullae as shown in Fig. 5d. In addition, Lim (1971) has presented a micrograph of a sensory cell from the utricle of what was apparently a normal control guinea pig, which presented a similar morpho-pathology. On the other hand Takumida et al. (1989b) has described the same in vestibular epithelia following gentamicin toxicity. After drug treatment the loss of cilia is preceded by splaying and/or fusion between cilia as well as giant cilia formation which is thought to be due to loss of the glycocalyx (Takumida et al., 1989a, b). Those types of cilia changes were not observed here in hydropic vestibules and indeed even when only a few cilia of a tuft remained on the cuticle the surface of those cilia appeared smooth and unperturbed. In addition the vestibular hair cell cilia pathology following the administration of different ototoxic drugs is reported to be essentially localized in the striolar region of the macula. This seems also to be the case in certain genetic disorders affecting the vestibule such as in the Waltzing guinea pig (Sobin and Wersall, 1983) and in the Dancer mouse mutant (Wenngren and Anniko, 1989). In contrast, the ciliary loss observed in hydropic macula epithelia were not associated, in particular, within the striola, but rather affected large areas of the epithelium and, in particular, the periphery. Although this particular morpho-pathology of the sensory cells may not be unique to hydropic vestibules the fact that the cilia are lost without splaying or fusion occurring indicates that the mechanisms leading to the cilia loss in hydrops and drug toxicity are likely to be different.

As pointed out above, the present study is apparently the first SEM study of the vestibular epithelium in experimental hydrops and hence the present findings cannot be compared with similar studies. However it is of particular interest that observations presented here show a striking similarity to those reported from vestibular epithelia in patients with Menière's disease. From transmission electron microscopy (TEM) observations several authors have reported evidence for degenerative changes within sensory hair cells as well as partial or complete cilia loss on the ampullae and the utriculus (Pietrantoni and Iurato, 1960; Ireland and Farkashidy, 1963; Friedmann et al., 1963; Harada, 1973; Sanchez-Fernandez and Marco, 1975; Rosenhall et al., 1977). The TEM observations of Rosenhall et al. (1977) have demonstrated a peculiar morpho-pathology associated

with Type I sensory hair cells. Those authors have described retraction of the hair cell body away from the chalice with the formation of a cystic cavity. Because two different techniques were employed it is not possible to draw a close correlation between those data (TEM) and the findings presented here for the animal model (SEM). However the present SEM study has demonstrated the apparent retraction of certain sensory hair cell cuticles away from the surrounding tissue (Fig. 3c) which might suggest that those cells are particularly sensitive and they might correspond to cell type I. Future TEM studies on the vestibular epithelia of hydropic ears are necessary in order to compare the intracellular ultrastructural changes in experimental hydrops with those reported in Menière's disease and possibly to identify the hair cell type associated with cuticular retraction in the animal model.

The data presented here suggest that endolymphatic hydrops might induce a specific morpho-pathology associated with the loss of the sensory hair cell cilia which might indeed be modelled in experimental hydrops.

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Discussion with Reviewers

M.D. Ross: Do you have any information concerning whether the kinocilium is lost prior to or following stereociliary degeneration?
Authors: It seems that the kinocilium is affected before the stereocilia. The additional micrographs presented below are taken from a utricle (Fig. 6a) of a guinea pig with 14 months hydrops, and indicate that the kinocilium can be partially degenerated or lost from a ciliary bundle while some stereocilia still remain (Fig. 6b).

M.D. Ross: How do you explain an apparently greater involvement of the utricular macula in early stages of the hydrops in the experimental animals? Is this possibly due to experimental blocking of the endolymphatic duct? If so, please evaluate this type of experimentally induced hydrops as a model for Menière's disease.
Authors: From the specimens available it appears that the utricle is affected to a greater extent than the saccule in early hydrops which is no doubt due to the blocking of the endolymphatic duct. At this time we can only speculate as to the reason. For example when the endolymphatic duct is blocked the flow of endolymph from the cochlea as well as from the vestibule will be interrupted. The endolymph is produced from the stria vascularis in the cochlea and the dark cells of the ampullae and the utricle. On the other hand the saccule lacks dark cells (Kimura, 1969). Hence when the duct is blocked those end organs producing endolymph might suffer the consequences immediately whilst the saccule might not be affected until after a certain delay.

The experimental model presented here is certainly one excellent model to investigate the consequences of endolymphatic hydrops on the inner ear. However it is obvious that there is not one type of Menière's patient and the recent article of Schuknecht and Rüther (1991) exemplifies this. Those authors have shown that in temporal bones from Menière's patients not only can there be blocking of the endolymphatic duct (as modelled here) but also of the endolymphatic sinus, utricular duct, saccular duct and ductus reuniens all of which will have different consequences on inner ear function.
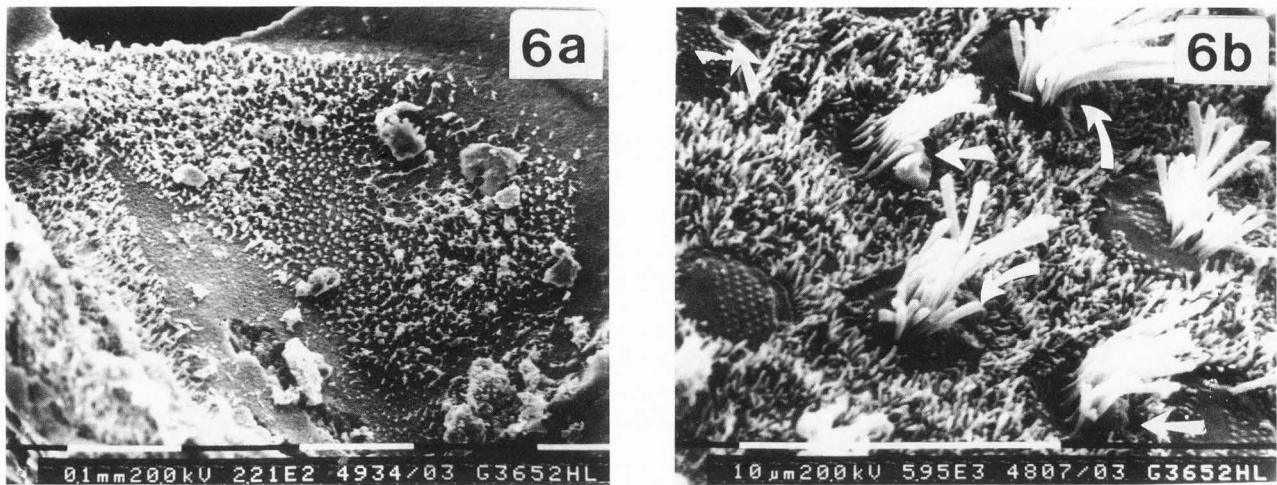
D. Bagger-Sjoback and J. Wersall: It would be interesting to hear your view or suggestion as to the etiology and pathophysiology behind the mentioned changes.
Authors: At this time it is difficult even to speculate. However we cannot ignore the fact that a unique form of stereocilia atrophy exists in the hydropic cochlea in the upper cochlear turns and so the presence of a particular ciliary pathology in the vestibule is not entirely surprising. The changes might, for example, be due to biochemical modifications of the endolymph or to micromechanical changes due to pressure within the inner ear or to both of these factors which themselves might be peculiar to hydrops.

G.K. Martin: In Kimura's work on this model considerably less sensory cell loss was observed. Do you have any explanation for this discrepancy?
A.N. Salt: Although SEM studies may not have been performed, a number of groups (including Kimura's) have examined the vestibular labyrinth of hydropic animals or Menière's patients by conventional methods. I think the consensus of this literature is that there are no major sensory cell abnormalities in hydropic animals. Please comment.
Authors: There is really no discrepancy. Kimura and Schuknecht (1965) used light microscopy and observed specimens of up to one month hydrops. In that case they observed no hair cell loss. Shinozaki and Kimura (1980) observed hydropic ears of up to 8 months hydrops and made one mention to saccular cells "Sensory cells of the sacculus showed normal surface structure in severely hydropic inner ears". However, that particular study dealt almost entirely with observations on the Reissner's and saccular membranes and neither details nor micrographs of the saccular epithelia were presented. It is possible that those authors overlooked the cilia loss as well as hair cell loss, since, as we point out hair cell loss at 8 months hydrops is not a dominant feature.

Figure 6. (a) The utricle from a guinea pig (GP3652) with 14 months hydrops. Note the particular loss of cilia on the periphery of the macula. (b) View of eight sensory cells, four of which lack all cilia, one cell lacks about 30 stereocilia and all cells appear to have partially or completely lost the kinocilium (arrows).

Additional References

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