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REVIEW: MORPHOLOGICAL CHANGES ASSOCIATED WITH ENDOLYMPHATIC HYDROPS

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Abstract

Endolymphatic hydrops of the inner ear is identified as a swelling of the endolymphatic spaces. This morphopathology in man can only be confirmed at post-mortem examination although it is believed to underlie the auditory dysfunction and vestibular disturbances associated with Menière's disease. This is an illusive inner ear disorder characterized typically by the fluctuant hearing loss, tinnitus and episodes of vertigo. Menière's disease remains a major problem in otorhinolaryngology since the cause of the disease is not known and various treatments are recommended, often with unsatisfactory results. Experimentally induced endolymphatic hydrops in the animal model has been developed in order to understand better the consequences of this morphopathology on inner ear structure and function. Further investigations on the model might, in the future, lead to a more efficient management of the disorder.

Key Words: Endolymphatic hydrops, inner ear, cochlea, vestibule, Menière's disease, hearing, balance.

Introduction

The literature concerning endolymphatic hydrops is considerable. The present paper presents major findings in this field and in addition mentions those authors who later confirmed these data. An attempt is made to summarize what we believe to be the morphopathological repercussions of interruption of endolymph absorption, on the inner ear. Finally, we ask how can the animal model contribute to our understanding of the human inner ear pathology and where do we go from here with the animal model?

The inner ear is considered here in its entirety including the cochlea, the vestibule and the endolymphatic sac. The first two parts make up the sensory organs involved in hearing and equilibrium function, respectively, while the endolymphatic duct and sac are believed to participate in the absorption and/or secretion of endolymph-like fluid as well as have phagocytic and immunological functions. All three parts have their specific function but because they are interconnected by ducts, they appear to be interdependent for the regulation of fluid volume, although the exact mechanisms are not known (Fig. 1).

Clinical Context

Endolymphatic hydrops is the morphological swelling of the endolymphatic spaces of the cochlea and the vestibule. While all three parts of the labyrinth, as defined above, are interconnected, swelling of the endolymphatic sac has not been reported (Fig. 1). There is a reduction of the loose connective tissue around the endolymphatic sac as well as reduction in bone pneumatization (Hallpike and Cairns, 1938). The sac and duct are reported to be narrowed and filled with a thick colloidal mass (Yamakawa, 1938).

The association for the first time, of this inner ear pathology to temporal bones of Menière's patients (Menière, 1861) has been attributed to Hallpike and Cairns (1938) as well as to Yamakawa (1938). However, recognition and credit should be paid to Witmaack (1926), from Germany, who was probably the first to identify endolymphatic hydrops in human temporal bones. Indeed, according to their own report, Hallpike

and Cairns (1938) derived the term "endolymphatic hydrops" from Wittmaack's (1926) original description of a similar type of labyrinthine reaction "hydrops labyrinthi" to toxins. Yamakawa, no doubt, also heard of and saw this pathology during a study period with Professor Wittmaack which, according to Stahle (1989), took place between 1927 and 1929. It is also extremely interesting, as will be discussed below, that around this same period, Portmann published his basic research data on experimental induction of hypertension in the liquid of the inner ear in fish in 1921 and first reported on endolymphatic sac decompression in man in 1926 (Portmann, 1921, 1926).

Today, endolymphatic hydrops is taken to be the primary and characteristic morphopathological feature of Menière's disease which, by convention (Committee on Hearing and Equilibrium of the American Academy of Otolaryngology - Head and Neck Surgery AAO-HNO 1985), is associated with the occurrence of three symptoms: low frequency fluctuant hearing loss, tinnitus and vertigo. Menière's disease is idiopathic and while endolymphatic hydrops is likely to play a major role in contributing to the symptoms of the disease, it is still unknown whether the hydrops is primary or secondary in origin. On the other hand, endolymphatic hydrops is often associated with other pathologies such as labyrinthitis, otitis media, otosclerosis, head trauma, mumps, high blood pressure, diabetes, syphilis, meningitis (Kimura, 1976; Jongkees, 1979; Paparella, 1984, 1991) where it is likely to represent a secondary phenomenon.

Given the severe incapacitating nature of this inner ear disorder and the lack of information regarding its cause as well as absence of a satisfactory treatment, basic research including animal experiments remains essential.

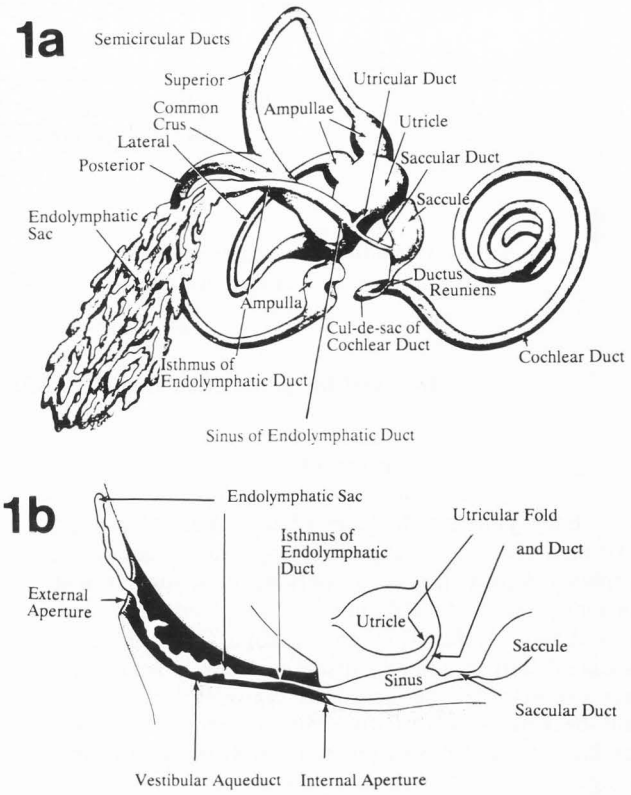

Figure. 1. (**a**) Sketch of the membranous labyrinth illustrating the cochlea, vestibule and the endolymphatic sac. (**b**) This sketch illustrates the interconnections between the cochlea, vestibule and the endolymphatic sac. (From Schuknecht and Guyla, 1986, Lea & Farber Company, Philadelphia).

Early Fundamental Research

One of the major limiting factors in the management of Menière's disease is that the cause of the disease is not known. In addition, it is not known whether endolymphatic hydrops is the result of an overproduction or an underabsorption of endolymph. In Menière's patients, the increased incidence of non-visualization of the sac in tomographical studies (Clemis and Valvassori, 1968; Stahle and Wilbrand, 1974) together with the observed hypoplasia of the vestibular aqueduct and endolymphatic sac (Egami *et al.*, 1978; Sando and Ikeda, 1984), perisaccular fibrosis of the sac (Yazawa and Kitahara, 1981; Schindler *et al.*, 1979) and poor bone pneumatization (Sando and Ikeda, 1984) suggest that there might be an underabsorption due to malfunction of the endolymphatic duct and sac. In recent years, fundamental research efforts in this domain have focused on experimental hydrops in the guinea pig induced by blocking the proximal/medial part of the sac or destroying the distal part of the sac (depending on the investigator) inducing, what has been considered to be, an underabsorption of endolymph.

Attention should be drawn to the early pioneering work of G. Portmann who was first to suggest the primordial absorptive function of the endolymphatic sac and went on to induce hydrops for the first time (in elasmobranch fish) in 1921 as well as to decompress the sac in a Menière's patient for the first time in 1926. Those data have steered the way to our present interest in the endolymphatic sac function in relation to Menière's disease. In the early 1900's, Portmann carried out numerous morphology studies on various animal species including elasmobranch fish, birds, and mammals (see summary in Portmann *et al.*, 1978). Those studies investigated the endolymphatic sac which Portmann concluded was universal to all and intuitively proposed that the sac might be involved in the regulation of inner ear fluid pressure (see Portmann, 1978). Portmann went on to carry out, for the first time, a surgical intervention to induce hydrops in fish by paraffin injection and cautery of the endolymphatic duct (Portmann, 1921). The fish were observed to have disturbed swimming equilibrium which indicated that there was a disturbance of the vestibule by the increased endolymph volume. Portmann confirmed histologically the blocking of the duct but did

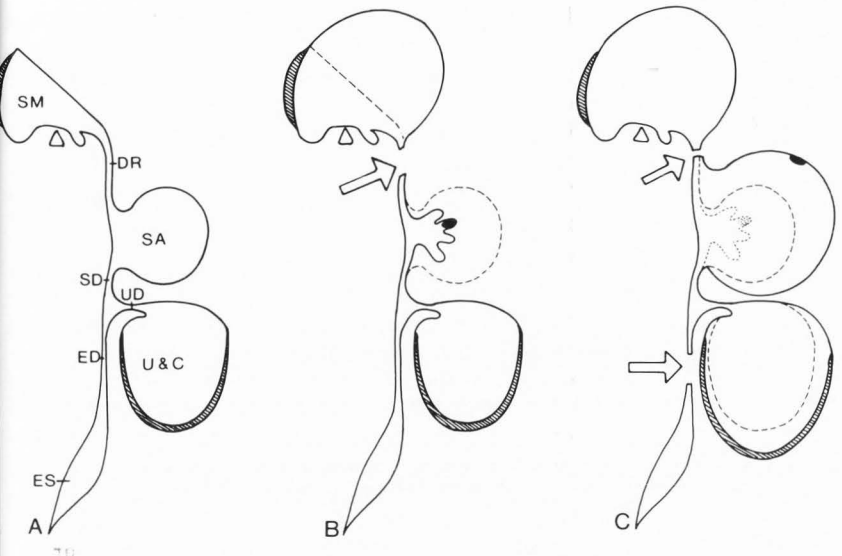

Figure. 2. (**a**) Normal positions of the labyrinthine membranes. (**b**) Changes after obliteration of the ductus reuniens show cochlear hydrops, saccular collapse with degeneration of the otolithic membrane (black dot) and normal utricle. (**c**) When the endolymphatic duct is blocked 2 months later, the saccular membrane distends beyond the original normal position and carries the remnant of the otolithic membrane with it. The utricle becomes hydropic. SM Scala media, DR ductus reuniens, SA saccule, SD saccular duct, UD utricular duct, U&C utricle and canals, ED endolymphatic duct, ES endolymphatic sac. The shaded areas represent the stria vascularis and dark cells. (After Kimura *et al.*, 1980, with permission).

not report on the morphological manifestation of endolymphatic hydrops of the inner ear. The endolymph flow experiments of Guild (1927) were in accord with these findings and he suggested that a failure of function of the pars intermedia of the endolymphatic sac would result in a blockage of the outflow of the endolymph and a distension of all parts of the membranous labyrinth. Indeed, years later, Arenberg and colleagues repeated those early Portmann experiments and confirmed that endolymphatic hydrops in elasmobranches does develop after cauterization of the endolymphatic duct (Arenberg *et al.*, 1972). Those fish with endolymphatic hydrops were shown to develop clumping of hair cell stereocilia in all sensory parts of the inner ear (Rauchbach and Arenberg, 1973).

Shortly after the first Portmann experiment on fish, McNally attempted to induce hydrops in rabbits by incising and cauterizing the sac. However, the very short post-operative observation period of hours or days was too short to comment on the development of hydrops (McNally, 1926). From that time, experimental induction of hydrops in animals appears to have been completely abandoned for more than 20 years. Lindsay then tried to induce hydrops in the monkey (Lindsay, 1947; Lindsay *et al.*, 1952). Schuknecht and Kimura (1953) attempted the same in the cat with no avail. The first attempts to induce hydrops in the guinea pig were carried out at this time also but with a low success rate (Naito, 1950, 1959). In those first experiments, the distal and medial portions of the sac were apparently destroyed but no mechanical blocking of the duct was performed. On the other hand, Harada (1959) reported the successful induction of hydrops, as determined by the bulging of Reissner's membrane, in the guinea pig following damage to the sac. In particular, Harada noted that total destruction of the sac did not produce hydrops if the flow of endolymph within the duct was not interrupted. Indeed, while Schuknecht and Seifi (1963) later reported the failure to induce hydrops in the cat when the sac was destroyed but the duct was not blocked, two years later, Kimura and Schuknecht (1965) reported systematic hydrops in the guinea pig when bone wax had been packed into the open duct.

Despite the disappointing early results, research on experimental endolymphatic hydrops reached a breakpoint between 1959 and 1965 when the experiments of Harada (1959) and Kimura and Schuknecht (1965) supported a longitudinal flow of endolymph towards the sac. This longitudinal flow as first postulated by Guild (1927) was elegantly demonstrated by Kimura *et al.* (1980) when they successfully obstructed the ductus reuniens and observed cochlear hydrops together with collapse of the saccule. Subsequent blocking of the endolymphatic duct resulted in hydrops in the vestibule (Fig. 2). Since 1960's, experimental endolymphatic hydrops has continued to be a hot topic. The guinea pig appears to be the favorite animal model.

Indeed, if the guinea pig is the favorite model, it is undoubtedly because of the high success rate for inducing hydrops. While failure to induce hydrops in the monkey was reported again by Kimura (1968) and Suh and Cody (1977), slight hydrops was reported by Swart and Schuknecht (1988). Only mild hydrops has since been observed in cats (Beal, 1968) and even after a survival time of 3 years by Schuknecht *et al.* (1968). Limited success in provoking hydrops has been reported for the rat (Manni *et al.*, 1986;1988) and the chinchilla (Kimura 1968; Lindsay 1947; Suh and Cody, 1974). On the other hand, it seems that endolymphatic hydrops can more successfully be induced in the rabbit (Beal, 1968; Suh and Cody, 1974; Martin *et al.*, 1983). The reason as to the variation in success rate between different animals is not clear.

Cochlea

Hydrops

The first description of inner ear morphology following experimental induction of hydrops in the guinea

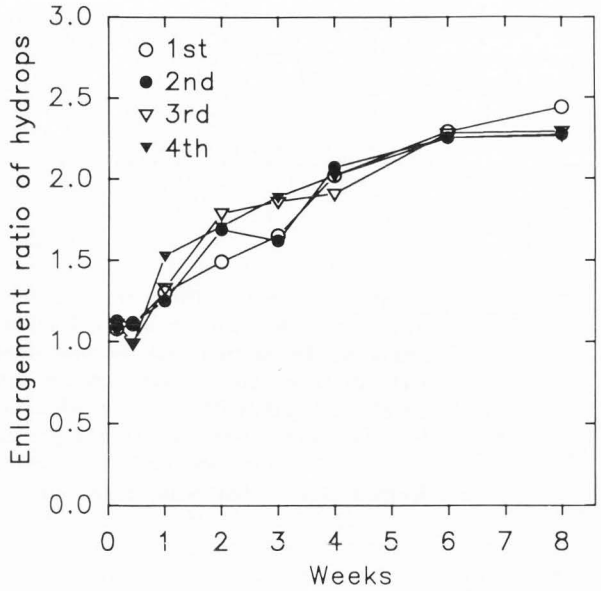

Figure 3. Enlargement ratio (S/s), bulging endolymphatic space (S) and original space of the scala media (s) as measured from serial sections of hydropic cochleae, for each of the four cochlear turns over an eight week period. (Drawn from the table of results published by Yazawa, 1990, with permission).

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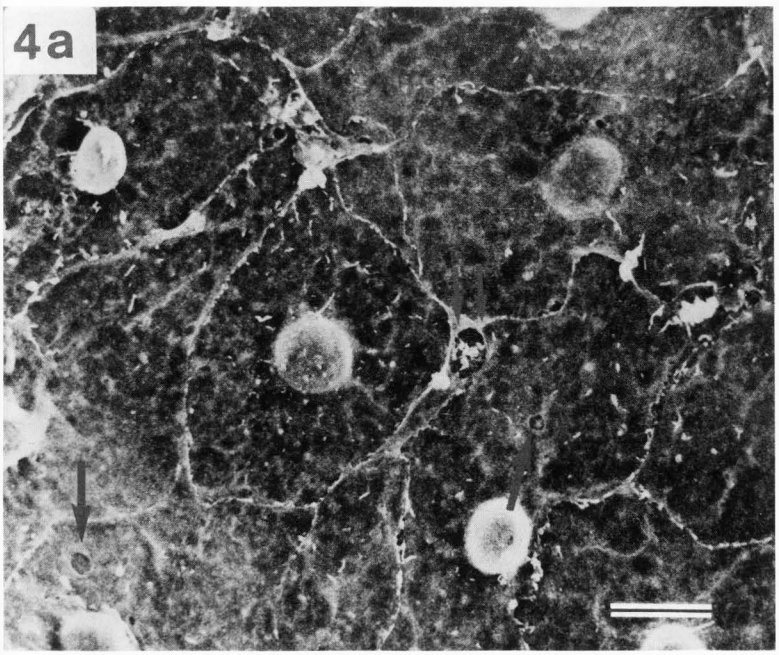
pig, was published by Kimura and Schuknecht (1965). Hydrops was found within the cochlea and the vestibule: Reissner's membrane and the saccular membranes being dilated as early as 24 hours post-surgery. After two weeks the saccular membrane was in contact with the stapes footplate. The observation of hydrops after only 24 hours was also reported by Suh and Cody (1974), Nakamura (1967) and Yazawa *et al.* (1990). In the study of Kimura and Schuknecht (1965), after three days, hydrops was limited to the basal and second cochlear turns and only later was observed in the apical turns. The evolution of increased endolymph volume was detailed recently by Yazawa (1990) who, after hydrops induction by cauterization of the sac with silver nitrate (Yazawa *et al.*, 1985) made serial sections of the temporal bones in order to assess the extent and evolution of hydrops (Fig. 3). Yazawa (1990) reported after one day a slight dilation in all turns, at 3 days the 3rd and 4th turns showed slight shrinkage and the basal turns continued to dilate. Yazawa interpreted these observations as indicating that the primary reaction was likely to be the result of the surgical intervention. The cauterization would progressively destroy the sac over a few days such that the day 3 modifications observed by him might correspond to the first day observations of Kimura and Schuknecht (1965). During the next 3 weeks the swelling in the higher turns exceeded that of the lower turns. These data were consistent with the findings of Kimura and Schuknecht (1965) and Yanagi (1973) but not with the reports of either Suh and Cody (1974), who found that hydrops developed near the apex and extended to the basal turn in about 2 weeks, nor of Ruding *et al.* (1987) who found that the hydrops started in the basal turn and proceeded rapidly towards the apex during the first month. Yazawa (1990) found that at 6 weeks, the hydrops was again equal in all turns. Finally at 8 weeks the Reissner's membrane had expanded to its maximum in the three upper turns and hence further dilation was only seen in the basal turn which had not yet reached the bony wall.

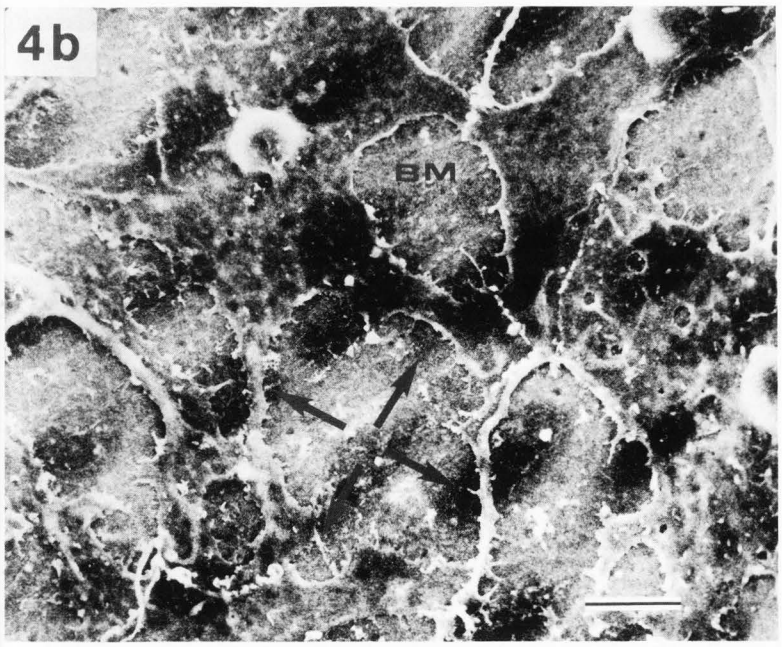
Reissner's membrane

The most striking morphopathology of the hydropic cochlea is the distended Reissner's membrane; this membrane is a bilayered structure consisting of an epithelium facing the scala medium and a mesothelium facing the scala vestibuli. Shinozaki and Kimura (1980) reported that distension of the Reissner's membrane occurred by irregular enlargement of epithelial cells by two to four fold. These observations on the model are in striking contrast with the little data which exist for human Reissner's membrane in endolymphatic hydrops. In particular, two recent publications describe significant proliferation in the number of epithelial cells (rather than stretching) of the Reissner's membrane in Menière's ears. In a group of 6 Menière's ears compared to 16 control ears (Quijano *et al.*, 1990) and in a group of 14 Menière's ears compared to 30 control ears (Yoon *et al.*, 1991) an increased cell density of Reissner's membrane was reported. These data support an earlier report from Johnsson (1971) who observed many small epithelial cells in the Reissner's membrane from a patient with severe hydrops and suggested that there might be a multiplication process in the distended membrane. On the other hand, an increase in epithelial cell size has been associated with the normal aging process in human specimens (Johnsson, 1971; Watanuki *et al.*, 1981; Quinjano *et al.*, 1990; Yoon *et al.*, 1991).

Kimura (1967) noted that there was frequently an increase in the intercellular gaps between mesothelial cells. Often in the hydropic ear, the Reissner's membrane lacked mesothelial cells on the perilymphatic side in the three upper turns while the endothelial cells remained intact and in contact with the basement membrane. Shinozaki and Kimura (1980), later confirmed this first report (Fig. 4) and also described outpouching and infolding of the membrane as well as the presence of small pits (also seen in control specimens) which increased in density on the distended Reissner's membrane. The observations have been confirmed by Albers *et al.* (1987a) in cochleae with hydrops of 1-3 months and extended to animals with hydrops of 8 months by Ruding *et al.* (1987). Ruding *et al.* (1987) reported on the presence of fistulae in Reissner's membrane which were mainly localized in the apical turn in cases of extensive hydrops. Fistulae in Reissner's membrane were also observed by Manni *et al.* (1988) in hydropic ears of rat.

Transmission electron microscopy (TEM) has revealed that the zonulae occludentes between epithelial cells in the Reissner's membrane were similar to those in controls (Shinozaki and Kimura, 1980; Albers *et al.*,





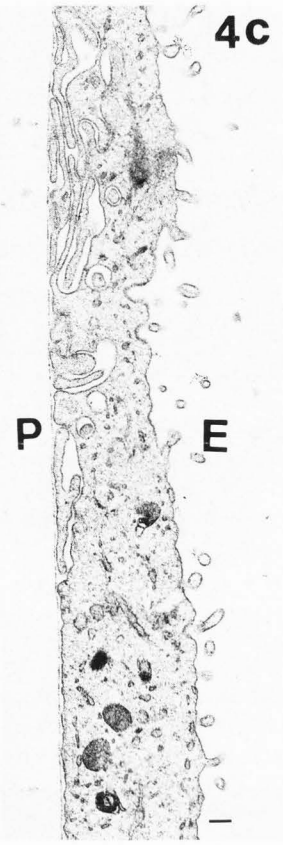


Figure 4. (**a**) Normal control Reissner's membrane. Mesothelial cells show small holes (arrows) in the cytoplasm and a gap (double arrows) between adjacent cells. (**b**) Reissner's membrane from the third cochlear turn of an animal with 1 month hydrops. Mesothelial cells are stretched. Gaps between cell junctions have become wider (arrows) and the basement membrane is exposed. (**c**) TEM of the Reissner's membrane from the apical turn of an animal with 4 months hydrops. Note the absence of mesothelial cells on the perilymphatic side (P); E: endolymphatic side. Bars = 10 μm (a, b); 1 μm (c). (After Shinozaki and Kimura, 1980, with permission).

1987a). Similarly, a freeze fracture study of the epithelial cells of Reissner's membrane as well as the vestibule showed no significant difference in zonulae occludentes between hydropic inner ears and control ears (Jahnke *et al.*, 1985) (Fig. 5). Indeed, these data receive support from a physiological study by Sakagami *et al.* (1991) who carried out intracochlear perfusion of horseradish peroxidase and studied the transport through Reissner's membrane in hydropic and control cochleae; they noted no difference between the groups, thus indicating that endolymphatic hydrops does not affect epithelial tight junctions despite the distension of Reissner's membrane.

Stria vascularis

Degeneration of the stria vascularis appears first in the apical and third cochlear turns. The marginal cells were observed by scanning electron microscopy (SEM) to have increased in surface area and the endolymphatic surfaces appeared to have flattened (Shinozaki and Kimura, 1980). Vacuolization of the marginal cells as well as a decrease in the number of ribosomes and mitochondria were reported (Kimura, 1967). Albers *et al.* (1987a) reported mild intercellular edema of both the marginal cells and also the intermediate cells in the apical turn of the cochlea one month after inducing

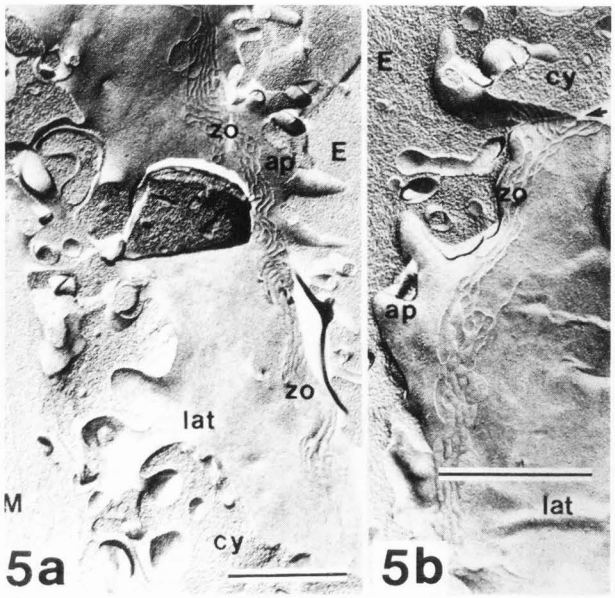


Figure 5 (above). (**a**) Freeze-fractured Reissner's membrane from an hydropic ear and (**b**) from a control ear. There are no significant differences in the number of strands and in depths of the zonae occludentes (zo). E cochlear duct, ap apical cell membrane with some microvilli, lat lateral cell membrane, cy cytoplasm of epithelial cell, M mesothelial cell. (After Jahnke *et al.*, 1985, with permission). Bars = 1 μm.

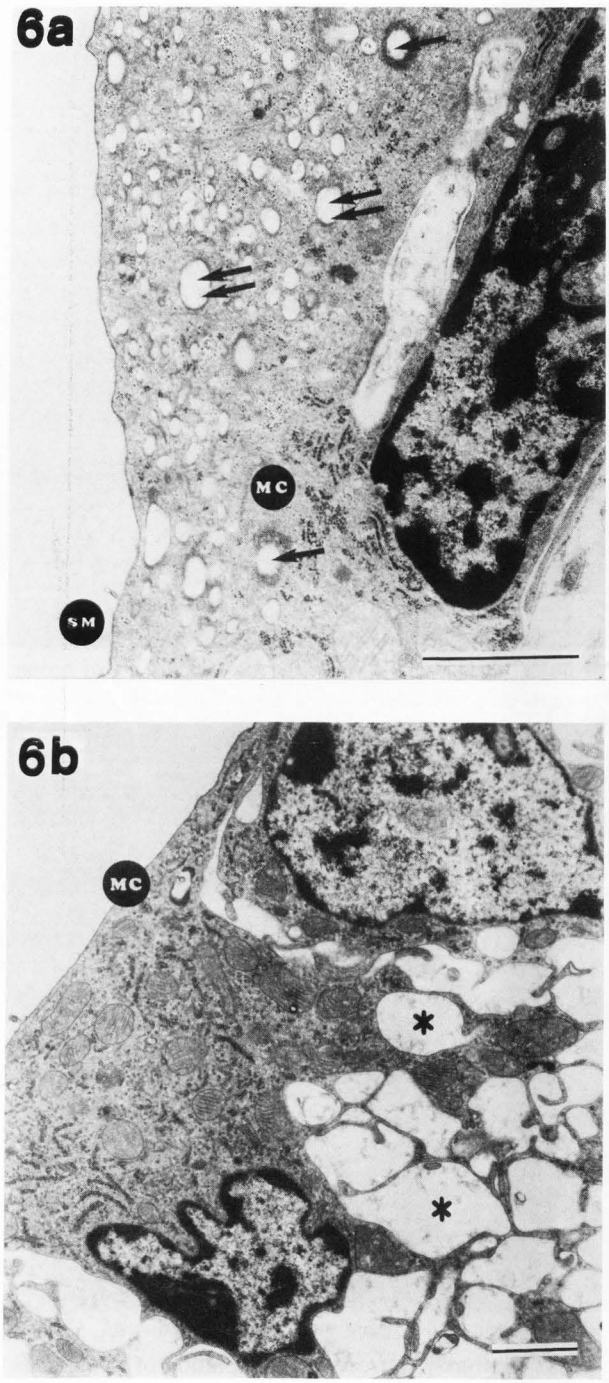


Figure 6 (at right). (**a**) Increase of coated (single arrows) and non-coated (double arrow) vesicles in endolymphatic zone of marginal cells (MC) in stria vascularis; SM represents the endolymphatic space. (**b**) Vacuolization (*) at basolateral side of marginal cells (MC). (After Albers *et al.*, 1987a, with permission). Bars = 1 μm.

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hydrops. In addition, they described an increase in coated and non-coated vesicles within the apical side (endolymphatic) of the marginal cells in the middle and lower turns of the cochlea (Fig. 6a). By two months, the vacuolization had progressed by a fusion of non-coated vesicles at the basolateral side of the marginal cells in the apex of the cochlea (Fig. 6b). Severe edema and decreased cytoplasmic processes of the marginal and intermediate cells were observed. After three months of hydrops, the atrophy of the intermediate cells, in the upper cochlear turns, was more extensive than that of the marginal cells so that direct contact between marginal and basal cells could be observed.

Several attempts have been made to determine whether inner ear blood circulation is modified in hydropic ears. Regional and total blood flow were studied by intracardiac injection of microspheres followed by their subsequent localization (Larsen *et al.*, 1988). No difference was detected between the hydropic ears (2, 4, 8 months) and the control ears. However, given the limitations of that technique, Larsen *et al.* (1988) stated that the data were inconclusive.

Observation of the blood vessels of the hydropic ear has been made by corrosion casting technique together with SEM, in guinea pigs with hydrops of 2 months (Nakai *et al.*, 1990). These authors reported vascular abnormalities which included either dilatation of the capillaries of the stria vascularis and narrowing of the arterio-venous anastomosis of the spiral ligament or conversely constriction of the capillaries of the stria vascularis and dilatation of the vessels at the arterio-venous anastomosis of the spiral ligament. Nakai *et al.* (1990)

noted also that the caliber of the vessels of the capillary plexus of the saccular macula were reduced. These data were interpreted as suggesting that endolymphatic hydrops gives rise to circulatory disturbances of the lateral walls of the cochlea and the saccular macula.

Yamamoto *et al.* (1991) used laser Doppler, hydrogen clearance, and oxygen tension measurements and found no difference in cochlear blood flow between hydropic ears and control ears. In addition, they attempted to modify systemic blood pressure by "bleeding" the animal (although the normal physiological condition of the animals was no longer assured). These authors reported that at severely low blood pressure, cochlear blood flow in hydropic ears was severely affected and did not recover to the same extent as the control contralateral ear. Yamamoto *et al.* (1991) interpreted these observations as suggesting that the auto-regulatory function in the hydropic ears is impaired.

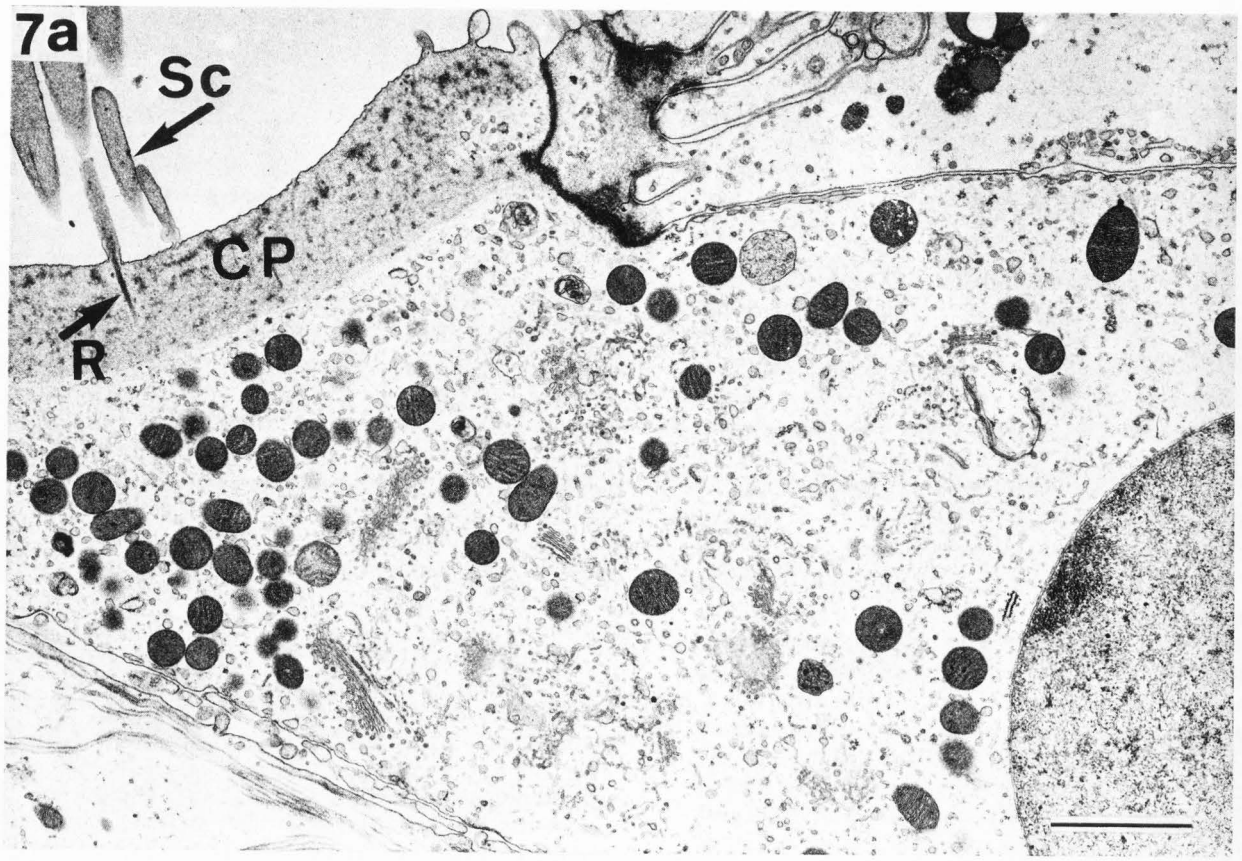
Sensory hair cells

Early ultrastructural changes have been observed starting near the apex of the cochlea. In the original study of Kimura and Schuknecht (1965) the animals were sacrificed after 1 day or up to 1 month. Despite the presence of hydrops, the sensory cells, the stria vascularis and the spiral ganglion, as seen by light microscopy, did not appear to be modified. Kimura (1967) later investigated the effects of hydrops up to 14 months post-surgery by TEM. In that study, he observed degeneration of sensory cells and spiral ganglia to be limited to the apical turn at two months. The degeneration progressed into the third cochlear turn during the next 4 months and between 7 and 14 months some degeneration could be observed in all cochlear turns (Kimura, 1967). Kimura pointed out that the distribution of hydrops, which could be observed throughout the cochlea, did not reflect the extent and distribution of sensory cell degeneration. The cytocochleograms presented by Aran *et al.* (1984) were quite similar to the data of Kimura (1967). In 2 and 3 months hydropic ears, outer hair cell (OHC) loss was restricted to the apical turn or the apical and third turns, respectively. Albers *et al.* (1987c) on the other hand suggested that the loss of hair cells was more rapid. They showed OHC loss restricted to the apical turn during the first month of hydrops, which progressed into the third turn after 2 months, and after 4 months the beginning of hair cell loss was observed in the second cochlear turn. These authors also reported the onset of inner hair cell loss in the apical turn after 4 months of hydrops. The cytocochleograms presented by Martin *et al.* (1983) for the rabbit suggest quite a different pathology; they noted that the degenerative changes were found in the tonotopic regions of the cochlea where electrophysiological thresholds were most elevated. Martin *et al.* (1983) reported functional losses for the very high and the low frequencies. Histological control after 3-6 weeks of hydrops showed hair cell loss at the base and the apex of the cochlea. In these pathological areas, degeneration of hair cells, supporting cells, stria vascularis and nerve fibers were observed. In particular, those authors reported that there was a more substantial loss of inner hair cells than outer hair cells.

Kimura (1967) observed, for the guinea pig, that OHCs were affected before the inner hair cells (IHCs). In the OHCs there was proliferation of the smooth endoplasmic reticulum (SER) and in more advanced cases, there was also chromatolysis of the nucleus, vacuolization of the SER, mitochondrial swelling, and lysosomes containing multilaminated membrane-bound structures as well as lipid droplets. Kimura (1967) pointed out that these early signs of degeneration were seen within the cell before the nerve endings were affected. Early signs of nerve fiber degeneration included swelling of the mitochondria. On the other hand, the IHCs in the upper cochlear turns showed a decrease in the quantity of tubular structures, Golgi apparatus, and mitochondria in the subcuticular zone, while the nucleus appeared to remain normal (Fig. 7). In long-standing hydrops Kimura (1967) observed vacuolization leading to degeneration of the nerve endings below IHCs together with marked reduction of the number of fibers in the osseous spiral lamina.

There is general agreement that even in long-standing hydrops in the guinea pig, substantial hair cell loss is limited to the upper 2 cochlear turns which corresponds tonotopically to frequencies below 2 kHz. However, long-term recording from chronically-implanted guinea pigs has shown that there is a strict sequence of sensitivity losses starting with the low frequencies during the first two months (all frequencies below 8 kHz). This is followed by a sensitivity loss at the very high frequencies after three months of hydrops (above 8 kHz). The mid frequencies (around 8 kHz) are affected last after four months of hydrops after which the audiogram becomes rather flat (Horner *et al.*, 1989b; Horner and Cazals, 1991). This evolution of hearing loss appears to echo that observed from Menière's patients for whom a "peak" audiogram has been described with the best frequency in the 1-2 kHz range (Paparella *et al.*, 1982). These observations would suggest that various factors might be involved in the development of hydrops and are likely to affect the apex and the base of the cochlea differentially (see Horner, 1991). In any case, it is evident that hair cell loss cannot account for the functional losses in the guinea pig model.

The loss of sensory hair cells from the cochlea of Menière's patients has also been observed by light microscopy, to be minimal and limited to the apex of the cochlea while the hearing loss can be extensive and cover most of the audible range of frequencies (Lindsay, 1968; Schuknecht, 1963, 1968, 1975; Schuknecht *et al.*, 1962, 1968). In contrast to these data from light microscopy, Kimura *et al.* (1976), and Nadol and Thornton (1987) observed temporal bones from Menière's patients and described OHC loss at the base of the cochlea with minimal loss at apex by TEM. Some fusion of the stereocilia was noted, but Kimura *et al.* (1976) pointed out that this pathology was too minimal to account for the hearing loss. Both Kimura *et al.* (1976), and Nadol and



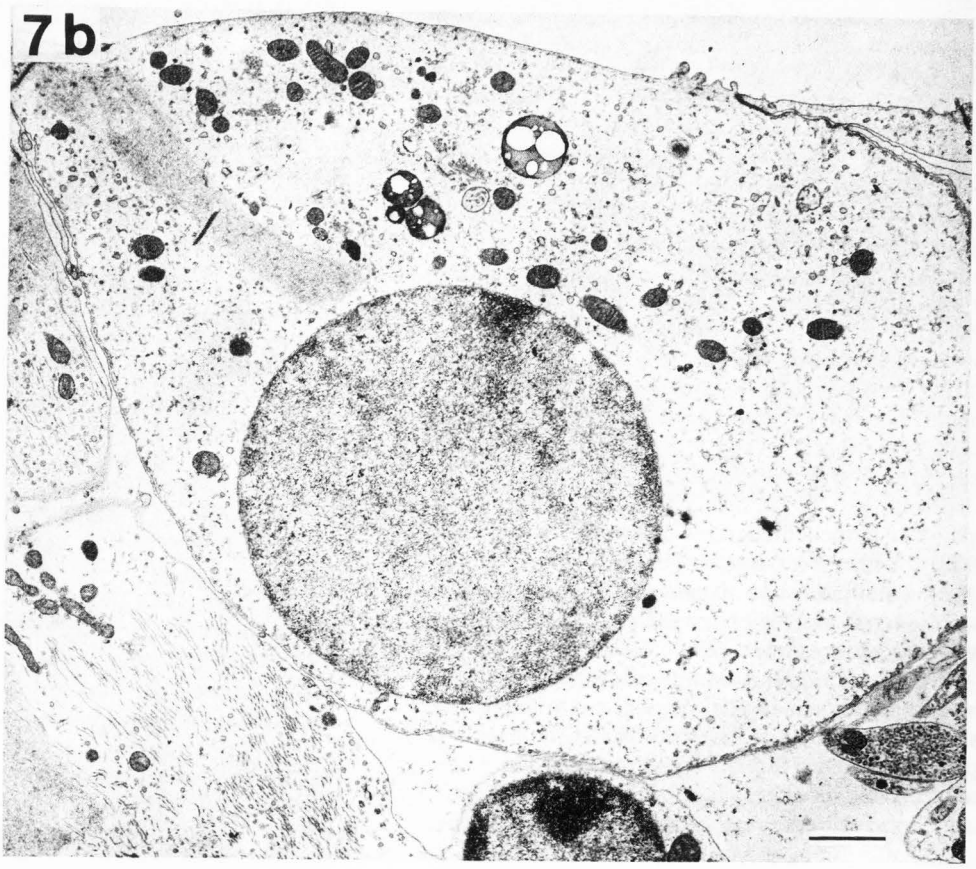


Figure 7. (**a**) Normal inner hair cell; Sc: stereocilia; CP: cuticular plate; R: rootlets. Bar = 1 μm. (**b**) Atrophic inner hair cell with unusually wide apical free surface and displaced cuticular plate with hair rootlet. Bar = 1 μm. (After Kimura, 1967, with permission).

Thornton (1987) described, in addition, the retraction of OHCs away from the reticular lamina to such an extent that certain hair cells no longer reached the surface of the epithelium. No similar pathology has been reported in the animal following the experimental induction of hydrops.

Fundamentally, it is extremely encouraging that the model can imitate the human cochlear pathology to such an extent that the lack of correlation between hair cell loss and the sensitivity loss becomes a characteristic feature of the two. However, we are still left wondering as to the underlying cause of the hearing loss associated with endolymphatic hydrops and Menière's disease in particular.

Stereocilia

For the guinea pig, the discrepancy between the extent of the functional deficit and the morphopathology persisted until the report of a new outer hair cell stereocilia pathology, which could be identified throughout the three upper turns of the cochlea (Horner *et al.*, 1988) and which correlated precisely in time with the tonotopic functional losses (Horner *et al.*, 1988; Rydmarker and Horner, 1991) (Fig. 8). Those authors described, in hydropic cochleae, selective atrophy of the short and middle stereocilia on OHCs, while the tall stereocilia remained apparently unperturbed. The IHCs did not demonstrate this type of stereocilia atrophy. The resemblance to a retrograde step in the development of stereocilia was discussed (Horner *et al.*, 1989a). Further investigations, by the same group (Horner *et al.*, 1989a; Rydmarker and Horner, 1990), confirmed the initial findings for long-standing hydrops and extended the study to early hydrops of 1 to 4 months (Rydmarker and Horner, 1991). The observed elevation of the tectorial membrane, in hydropic cochleae, was proposed to be implicated in the process leading to the atrophy of stereocilia (Rydmarker and Horner, 1990).

As pointed out above, the lack of correlation between hair cell loss and the sensitivity loss has been a common feature of endolymphatic hydrops in man and in the experimental model. A specific stereociliary pathology, although theoretically considered (Tonndorf, 1980), had not been investigated earlier. Further studies of the atrophy process in OHCs, if confirmed to be specific to hydropic cochleas, might lead to a better understanding of the pathology. In the first phase of hydrops, the sensitivity loss is restricted to the low frequencies which extends over the three upper cochlear turns. In particular the loss is fluctuant (Horner and Cazals, 1987a). Horner *et al.* (1988) have proposed that the fluctuations might be due to stretching of the inter-row bridges during the atrophic process, which might intermittently disturb transduction processes. Once the short and middle stereocilia have atrophied to an extent that the inter-row bridges are no longer attached, the sensitivity loss would be non-fluctuant.

Horner *et al.* (1988) pointed out that this peculiar morphopathology had never before been reported since few authors have carried out a detailed SEM study of hydropic cochleas. In addition, it is essential to observe the stereocilia tufts from the short stereocilia towards the tall rather than observing the backs of the tall stereocilia as other authors appear to have done (Shinozaki and Kimura, 1980; Ruding, 1988). Unfortunately, however, this selective atrophy of short and middle stereocilia was not reported in a recent publication of Ruding *et al.* (1991). Those authors observed hydropic cochleas at 1, 2, 4, and 8 months after endolymphatic sac obliteration, but only presented micrographs from hydropic cochleas at 1 month, a stage at which the atrophy process only first becomes evident.

It is of interest to note that despite the atrophy of stereocilia, fusion of stereocilia is not a feature in hydropic cochleae according to most SEM studies (Horner *et al.*, 1988, 1989a; Rydmarker and Horner, 1990, 1991) although fusion and formation of giant cilia have been reported (Ruding *et al.*, 1991). The fusion of stereocilia after gentamicin treatment is thought to be due to the loss of the glycocalyx (Takumida *et al.*, 1989). On the other hand, it seems that the glycocalyx is not lost, although it is less contrast stained, in hydropic ears after 3 months of hydrops (Albers *et al.*, 1987b).

In the context of Menière's disease, these results are significant only if a similar pathology exists in human temporal bones. Horner (1992) has recently observed, for the first time by SEM, a single human temporal bone with a long-standing hydrops. That specimen had suffered, however, from a post-mortem fixation delay of 12 hours. Despite fixation artifacts, it seems that shortening of stereocilia might well be a feature associated within the second cochlear turn although it is too early to draw definite conclusions from the preliminary data (Horner, 1992).

Vestibule

While vertigo is one of the characteristic features associated with Menière's disease, episodes of imbalance are very rarely observed in the guinea pig model of endolymphatic hydrops. It is perhaps for this reason, that few studies have dealt with the morphology of the vestibule in hydropic inner ear. Aran *et al.* (1984) reported that the nystagmogram response to horizontal rotation could be asymmetrical in the guinea pig with hydrops. Horner *et al.* (1989b) confirmed that the nystygmographic response was asymmetric during a certain period in most guinea pigs with unilateral hydrops. The asymmetry reached a maximum after about 2 months of hydrops, but after 4 months the response was again symmetrical. Manni *et al.* (1988) reported static labyrinthine function to appear normal. There were abnormal canal responses in only 6 out of 19 rats with endolymphatic hydrops despite fistulae and dislocation of the saccular otolithic membrane. In contrast to the guinea pig and the rat, behavioral vestibular dysfunction appears to be common in the rabbit (Martin *et al.*, 1983). Those authors reported that in some animals there was post-operative spontaneous nystagmus towards the operated side and postural

imbalance with torsion of the neck which worsened over the survival time. In the late stage, periodic attacks of vertigo once every 2-3 hours were observed. A caloric test revealed, in some cases, abnormal vestibular function. Those authors concluded that destruction of the sac and duct produced extremely variable vestibular effects.

Hydrops

Kimura and Schuknecht (1965) observed hydrops of the saccule 24 hours after blocking the endolymphatic duct and the saccular membrane was observed to touch the stapes footplate after two weeks. A TEM study of the saccular membrane revealed gaps between mesothelial cells which exposed the basement membrane to the perilymph, although there appeared to be less degeneration of the mesothelial cells in the saccular membrane than Reissner's membrane. The epithelial cells increased in size and reached up to four times that of the normal cells (Shinozaki and Kimura, 1980).

Secretory epithelium

A new aspect concerning experimental endolymphatic hydrops was revealed by Meyer zum Gottesberge-Orsulakova and Kaufmann (1986). Those authors described modification of the intracellular ionic composition and especially an increase of Ca^{++} in the light cells and the melanocytes of the vestibular organs (laser-induced microprobe mass analyzer determination). The extracellular cochlear calcium concentration has also been shown to increase (Ninoyu and Meyer zum Gottesberge, 1986; Salt and DeMott, 1992). The calcium concentration was shown to be inversely related to the endocochlear potential (Ninoyu and Meyer zum Gottesberge, 1986; Meyer zum Gottesberge and Ninoyu, 1987). These ionic changes are accompanied by morphological modifications of the light cells, the melanocytes, and the dark cells.

The light cells were reported to increase in size one hour following the surgical blocking of the duct. The increase in size continued during 4-7 days where maximal cellular alterations were reported while no changes were observed for the dark cells at this early stage (Meyer zum Gottesberge, 1988a). In long-standing hydrops the light cells showed an increase in cell volume and the dark cells showed flattening of the basal infoldings in coincidence with pronounced interstitial edema and an increase in intracellular vesicles and vacuoles (Meyer zum Gottesberge-Orsulakova and Kaufmann, 1986; Meyer zum Gottesberge and Ninoyu, 1987). In addition oval shaped otoconia-like bodies were observed lying on the top of dark cells which were thought to represent degenerative changes in otoliths (Meyer zum Gottesberge and Ninoyu, 1987).

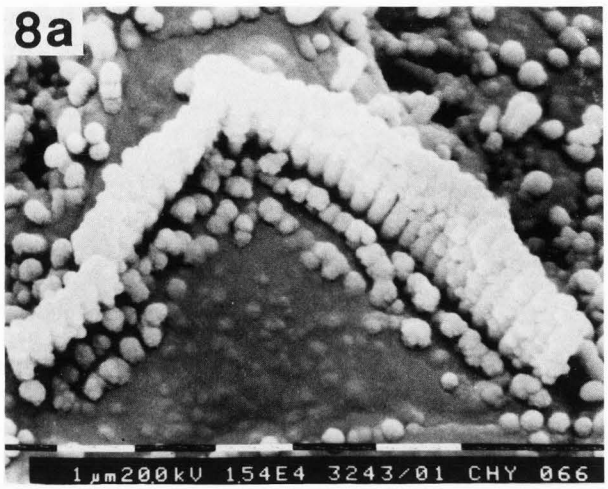
The melanocytes were observed to change in size with hyperpigmentation. The dendritic processes of the melanocytes were observed to migrate progressively from the normal subepithelial position up towards the basement membrane and finally to penetrate the basement membrane and to lodge between the infoldings of the epithelial dark cells (Meyer zum Gottesberge and Ninoyu, 1987). Shape changes were described as "motile" effects (Meyer zum Gottesberge, 1988b). Direct adherence of melanocytes to the surrounding blood vessels in hydropic vestibules was observed in the hydropic ears while in control ears there is always a membrane interposed between the melanocytes and the capillary endothelium (Meyer zum Gottesberge, 1988a).

The endolymphatic sac and duct have earlier been implicated in the degradation of otoconia in fetal guinea pig (Imoto *et al.*, 1983) and in human temporal bones (Friberg *et al.*, 1984). When the duct is blocked it is suggested that the extracellular calcium concentration increases which is partially counteracted by the active uptake of calcium ions by the light cells and the melanocytes (Meyer zum Gottesberge-Orsulakova and Kaufmann, 1986).

These data are particularly interesting since the observed increase in calcium is almost certainly related directly or indirectly to the inner ear functional disorders. Meyer zum Gottesberge and Ninoyu (1987) have pointed out that calcium is now recognized as the most fundamental regulating factor for various cellular and enzyme functions. The disturbance in calcium homeostasis may lead to an increase in osmotic pressure and endolymphatic hydrops. They have further suggested that the disturbed calcium homeostasis may affect the release of neurotransmitter and consequently, modulate inner ear function.

Sensory hair cells

The population of sensory cells of the vestibule was reported to be normal as observed by light microscopy for the guinea pig (Kimura and Schuknecht, 1965; Kimura, 1968) and the rat (Manni *et al.*, 1988). In the SEM study of Shinozaki and Kimura (1980) of the saccule "very little change" of the surface structures of the saccular hair cells was reported, although no micrographs were presented. On the other hand, Meyer zum Gottesberge and Ninoyu (1987) reported pronounced ultrastructural changes in the utriculus and the cristae ampullaris as seen by TEM, in guinea pigs after 18 months hydrops. They described vacuolization of the sensory cells as well as the supporting cells. In addition, they noted the cystic separation between hair cell type I and the nerve chalices. In guinea pigs with 4-22 months hydrops Horner and Rydmarker (1991) recently reported substantial loss of hair cells and various hair cell cilia morphopathologies from both the utricle and the saccule which did not appear to be associated in particular with the striola. Those authors described retraction of some sensory hairs away from the surrounding tissue. In addition, hair cell loss apparently left wide holes in the surface of the epithelia. A series of different cilia pathologies was described including loss of kinocilia and within a stereociliary tuft there could be either loss of all stereocilia, loss of part of the tuft, or shortening of the tuft (Fig. 9). A recent study by Oda *et al.* (1992) of the vestibular hair cells in hydropic inner ears also mentioned that some hair cell bundles can show partial loss



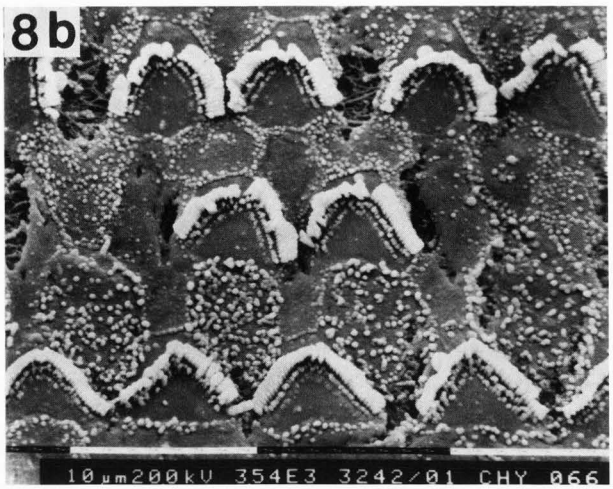


Figure 8. (**a**) An outer hair cell presenting atrophy of the short and middle stereocilia. This scanning electron micrograph was taken from a cochlea with hydrops of one year duration (bar = 1 μm). (**b**) The atrophy of stereocilia is observed on all three rows of outer hair cells (bar = 10 μm). (After Horner *et al.*, 1988, with permission).

Figure 9. (**a**) The utricle from a guinea pig with 4 months hydrops. Large areas, not limited to the striola, present abnormal stereociliary tufts. Note hair bundles with short cilia adjacent to bundles with long cilia (arrow). Partial loss of cilia within certain bundles is also seen (double arrow) (bar = 10 μm). (**b**) View of an area where most cells lack all cilia. Absence of one hair cell is indicated by a hole of around 3 μm (asterisk) in the sensory epithelium. Note also the absence of the kinocilia (arrows) and of the stereocilia (geometrically arranged stubs with central depression which is presumably the location of the rootlet) (bar = 10 μm). (After Horner and Rydmarker, 1991, with permission).

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of cilia. That study described degeneration of the glycocalyx, ciliary interconnections and tip links.

TEM studies of the vestibular epithelia in Menière's patients have described degenerative changes in sensory cells as well as partial or complete cilia loss on ampullary hair cells (Pietrantoni and Iurato, 1960; Irelande and Farkashidy, 1963; Harada, 1973) and the utriculus (Friedmann *et al.*, 1963; Sanchez-Fernandez and Marco, 1975; Rosenhall *et al.*, 1977). Indeed, Rosenhall *et al.* (1977) described retraction of the type I hair cell body away from the chalice with the formation of a cystic cavity in an ampulla from a patient with Menière's disease, similar to the report by Meyer zum Gottesberge and Ninoyu (1987) and perhaps corresponding to the retraction of hair cells as observed in SEM by Horner and Rydmarker (1991). In addition, Fitzgerald O'Connor *et al.* (1985) have described a specific thickening of the basal lamina.

Preliminary SEM observations of a Menière's temporal bone has shown loss of kinocilia, loss of ciliary

tufts, holes in the epithelium, and sensory cells pushed out onto the surface (Horner, 1992). In that study, the saccule appeared to have more morphopathological features than the utricule which suggests either that the endolymphatic hydrops affected the saccule to a greater extent than the utricule which would be in keeping with this inner ear pathology, or that the saccule was more sensitive to postmortem autolysis.

Endolymphatic sac and duct

Little has been published regarding the morphological modifications of the endolymphatic sac following the blocking of the duct or the proximal/medial part of the sac. Kimura (1976) has mentioned that, in the guinea pig, the part of the sac distal to the point of interruption remains intact. The sac is, in general, collapsed although the lumen can still be recognized. In the rat the endolymphatic sac was reported to be collapsed shortly after obstruction. With time, the number of lysosomes and resorption vacuoles decreases and the lumen narrows (Kuijpers *et al.*, 1988). In addition, Manni *et al.* (1986) reported that the lumen of the intermediate and distal parts of the sac is filled with a coagulum which stains homogeneously positive with Periodic acid-Schiff suggesting the active involvement of the endolymphatic duct in the migration of endolymph towards the sac. These latter data appear to have been confirmed in the guinea pig, by Tsujikawa *et al.* (1992). Those authors observed histologically-stainable substances within the endolymphatic sac lumen beginning 1 hour after obstruction of the endolymphatic duct and confirmed 3-6 hours, 1 day, 3 days and 7 days later. From 1 day onwards, the DC potential recorded within the sac lumen was significantly reduced compared to control or sham-operated ears.

Interesting comparative data on modifications to sac morphology have been revealed following several experimental manipulations in the mouse, such as after labyrinthectomy (Friberg *et al.*, 1986; Erwall *et al.*, 1988a), in a mouse mutant with cochleo-saccular pathology (Rask-Andersen *et al.*, 1987), following treatment with the loop diuretics ethacrynic acid (Takumida *et al.*, 1988) as well as furosemide (Erwall *et al.*, 1988b), or glycerol, mannitol, and urea (Erwall 1988, Erwall *et al.*, 1988c; Rask-Andersen *et al.*, 1989). All these studies have shown that following treatment numerous epithelial cells present a transient increase in intracellular granules which is concomitant with the filling of the lumen with a stainable substance. The general opinion of those authors (from the same research group) is that the increase in granule containing cells provides evidence for a secretory role of the sac which would be triggered into functioning when necessary in order to counteract homeostatic insults to the inner ear.

What Has the Model Contributed and How Can it Serve in the Future to Expand our Knowledge of Menière's Disease?

Unfortunately, despite more than thirty years of research by different international teams, relatively little progress has been made in the understanding of the pathology and in the management of endolymphatic hydrops and Menière's disease. Despite this, we have seen in recent years that there is a striking resemblance between the cochlear sensitivity losses in the model (Horner *et al.*, 1989b; Horner and Cazals, 1991) and in Menière's disease (Paparella *et al.*, 1982). Both present a "peak" audiogram in the early phases of the pathology. Neither present a correlation between the sensitivity loss and the hair cell loss. The animal model does however, show a peculiar cochlear stereocilia atrophy limited to the two smaller rows and extending throughout that part of the cochlea where there is a sensitivity loss. This morphopathology has been likened to a retrograde step in the development of the ciliary bundles (Horner *et al.*, 1988). Future studies should aim, first at reproducing these data from the guinea pig model, and then proceed to investigate this type of pathology in human specimens.

While atrophy of stereocilia in the model and in human temporal bones awaits to be confirmed by other groups and in other specimens, the possibility of selective atrophy of stereocilia of the outer hair cell as a primary pathology associated with the hearing loss would certainly swing popular thinking away from membrane fissure (Lawrence and McCabe, 1959, Schuknecht, 1968) and endolymph/perilymph mixing (Dohlmann, 1965), and towards some other micromechanical or discrete biochemical modifications which might underlie this particular morphopathology (Horner, 1991).

As far as the vestibular function is concerned, various ciliary morphopathologies of the sensory epithelia have been described (Horner and Rydmarker, 1991; Oda *et al.*, 1992). However, the animal model fails to manifest overt problems of equilibrium while attacks of vertigo are typical of Menière's disease. Some theories on the cause of these attacks in man suggests that the sac may play a prominent role. The sac is believed to secrete proteinaceous substances including hyaluronic acid (Godlowski, 1972; Friberg *et al.*, 1989) in reaction to the hydrops which would tend to draw the excess endolymph towards the sac. The theory of Gibson and Arenberg (1990) holds that when the duct and sac can no longer cope with the flow, there would be, from time to time, a back-flow of endolymph into the vestibule via the utricular valve of Bast and resulting in an attack. This theory might, however, be further elaborated to take into account the proposed pressure regulatory role of the sac (Friberg *et al.*, 1985; Bagger-Sjöbäck and Rask-Andersen, 1986) as supported by the presence of elastic fibers in the subepithelium of the endolymphatic sac in man (Bagger-Sjöbäck *et al.*, 1986) as well as in gerbil (Barbara *et al.*, 1987). Hence from time to time the sac might contract and force endolymph into the vestibule via the valve of Bast resulting in an attack of vertigo.

However, as pointed out above, the animal model of endolymphatic hydrops is deficient in that the sac is isolated from the rest of the labyrinth and hence any

pressure control reaction mechanism which might occur, as a result of hydrops, cannot be assessed. Isolation of the sac from the rest of the inner ear, in the animal model of endolymphatic hydrops, might well underlie the apparently anomalous deterioration of auditory sensitivity following glycerol intake, in the guinea pig model (Horner and Cazals, 1987; Kusakari *et al.*, 1989) while there is often a hearing improvement in Menière's patients.

In order to fully appreciate the role of the endolymphatic sac in volume control of the inner ear, future research should develop alternative models where the sac is at least partially functioning. Indeed a very interesting animal model having a partially functioning endolymphatic sac has been developed recently and described in a series of intuitive experiments by the Swedish group (Takumida *et al.*, 1989a, 1989b, 1991). Glycerol administration resulted in the formation of secretory granules in the light epithelial cells of the endolymphatic sac, transitory decrease in lumen size followed by the filling of the lumen with a stainable substance (Erwall, 1988). Those changes are believed to reflect the secretory function of the sac. In another study, partial dysfunction of the endolymphatic sac was induced by the administration of colchicine, which is known to interfere with carbohydrate secretion. In that experimental condition, glycerol administration resulted in the formation of intracellular granules in the light cells but the collapse of the sac lumen was retarded at onset, increased in duration, and frequently no stainable substance was observed within the lumen. In addition, a temporary bulging of Reissner's membrane was observed (Takumida *et al.*, 1989a, 1989b), providing convincing evidence in favor of the theory that the endolymphatic sac contributes to fluid homeostasis of the inner ear.

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.