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Surface morphology of the inner sulcus and related epithelial cells of the cochlea following acoustic trauma

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When the organ of Corti is severely traumatized by intense (160 dB) blast waves, such that as much as 7 mm of Corti's organ is torn loose from the basilar membrane, the cells of the inner sulcus respond to the altered biochemical milieu of the endolymph by a prolific elaboration of surface membrane, zeiosis and the development of numerous pseudopodia and microvilli. On the basis of our longitudinal study, this surface reaction appears to peak at approximately 10 days after trauma and may indicate that the inner sulcus cells are extremely active in the endocytosis of cellular debris. Signs of active changes on the surface of the inner sulcus cells occur immediately following trauma, and activity continues for as long as 30 days after exposure. The cells of Claudius, as well as other epithelial cells on the basilar membrane, are also capable of extreme membrane proliferation and mobility. Possible mechanisms for the unusual behavior and the role of the inner sulcus cells in the normal functioning cochlea are discussed.

endocytosis, inner sulcus cell, Claudius cell, endolymphatic sac

Introduction

Following a severe acoustic trauma, several millimeters of Corti's organ can be severely damaged or completely torn loose from its attachments on the basilar membrane [1,13]. The absorption or clearance of this cellular debris and the subsequent course of re-epithelialization of the basilar membrane have been the subject of a number of papers (e.g. [14,19,35]). Much of the pre-1970 literature on these two topics has been reviewed by Bohne [3]. While there is still no general consensus as to the primary route for clearance of debris, several possibilities exist, e.g.: (i) autolysosomal digestion – ample evidence for this mechanism exists [1], but the fate of the macromolecules thus produced has not been considered; (ii) phagocytosis by elements of the reticulo-endothelial system – however, these cells do not appear in sufficient quantity to handle the mass of debris which is often produced; (iii) endocytosis by the epithelial cells of the cochlear duct and of the endolymphatic sac – but once again sufficient endocytotic activity by these cells, to clear the mass of debris produced by the trauma, has not been unequivocally demonstrated.

The observation by Bohne [3] and others that large particles (clumps) of free-floating debris can persist for over a year in the endolymph, provided that the debris does not contact any of the duct epithelium, seems to question just how aggressively the cochlea 'tries' to eliminate such particulate matter. In a recent paper [13] we showed that extensive (5–7 mm) areas of organ of Corti that were torn loose from the basilar membrane following noise trauma and left free-floating in the scala media almost completely disappeared within 10 days of the exposure, although some small scattered particles of debris could be seen as long as 30 days after exposure. This finding would seem to indicate that, under certain conditions, a relatively rapid clearance or sequestering of debris is possible within the cochlear duct. With this in mind, we surveyed essentially all the epithelial

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cells of the cochlear duct and the endolymphatic sac in order to locate those cells most active in the endocytosis of cellular debris. This survey uncovered a very unexpected and rather dynamic behavior of the surface structures on the cells of the inner sulcus.

The primary purpose of this paper is to describe the surface morphology of the cells of the inner sulcus following an acoustic trauma, and to suggest that these cells with their clear and unexciting cytoplasm may, in fact, be capable of mobilizing their internal machinery in order to endocytose large quantities of endolymphatic material.

It is generally accepted that most epithelial cells will exhibit endocytotic behavior in varying degrees [24,33]. The cochlea is no exception and over the years a considerable body of literature has evolved on the topic of fluid and particulate transport across the epithelial boundaries of the membranous labyrinth. Duvall and Sutherland [7] and others [5,6,14] have described the morphology of most of the epithelial cells lining scala media, and through various tracer studies have tried to discover the functional role of many of these cell types. The inner sulcus cells have been conspicuously neglected in virtually all the detailed descriptions of the non-sensory epithelial cells of the cochlea. The work of Pedersen et al. [26] is an exception as they implicate the inner sulcus cells in the process of phagocytosis during the development of certain forms of hereditary deafness in the cat. However, their evidence is not compelling and their comments are only meant to be suggestive. The results of Duvall et al. [5] and others have shown that the epithelial layer of Reissner's membrane, the Claudius cells and the cells of the outer sulcus are important in the transport of particulate matter out of scala media. Their results are consistent with the description by Saxen [29] of the outer sulcus as the "sewer of the inner ear".

More recently, Hunter-Duvar [14] has shown micrographs that would indicate that Reissner's membrane is active in the endocytosis of comparatively large particles of cellular debris following an acoustic trauma.

The literature focusing on the role of the endolymphatic sac in the absorption/clearance of endolymph or the phagocytosis of cellular debris is plentiful (e.g. [12,23,30,31]). However, the specific role of the different regions of the sac and the different cell types is not at all clear. In general, when pathologies affecting the vestibular system are present, evidence for debris clearance in the sac is unequivocal. However, when the pathology is localized to the cochlea alone, as in acoustic trauma, the role of the endolymphatic sac in the clearance of debris has yet to be clearly established.

Many of the initial stages of endocytosis are surface-related phenomena, and thus there is considerable scanning electron microscopy literature on the surface morphology of the endocytotically active cell. It is from this literature that we will draw inferences and analogies about the possible role and very labile nature of the inner sulcus cell (ISC) surface.

Methods

The methodology employed in these studies has been detailed in an earlier publication [13]. The chinchilla was used as the experimental animal. The data presented in this paper were acquired from a survey of 32 binaural animals: 6 control and 26 experimental. The experimental animals were exposed to 100 blast waves having 160 dB peak equivalent SPLs. The impulses were presented at the rate of 2/min. The impulse consisted of a typical Freidlander wave having a duration of approximately 3.0 ms. Except for the noise exposure, the control animals followed the same protocol. The animals were killed by decapitation at 0, 1, 5, 10 and 30 days after exposure; the bullae were quickly removed and the cochleas perfused with cold 5% glutaraldehyde in veronal acetate buffer at pH 7.3 (630 mOs). Following 24 h of primary fixation, the cochleas were perfused with a glutaraldehyde/osmium mixture in a ratio of 5 : 2. The osmium was a 2% aqueous solution and secondary fixation lasted for 15 min. Following a partial dissection, the cochleas were dehydrated to 100% ethanol and critical point dried with liquid CO_2. Gold–paladium was sputtered onto each specimen and they were then viewed using a JEOL JSM-35 scanning electron microscope operating at 10–20 keV.

Results

The blast wave exposure produced an extensive (5–7 mm) lesion of the organ of Corti which consisted of a strip of epithelium, containing the three rows of outer hair cells, Deiters' cells and Hensen cells, which was torn loose from the basilar membrane. The details of the resulting sensory cell pathologies thus generated have been described in a previous paper [13]. The series of micrographs presented in this report are intended to illustrate the dynamic changes taking place in the surface morphology of the inner sulcus cells and other non-sensory epithelial cells on the basilar membrane in a group of animals having very similar noise-induced lesions in the organ of Corti.

Fig. 1A illustrates approximately one-third of a turn of the cochlea from a control animal. The specimen was taken from a turn centered at approximately 40% of the distance from the apex of the cochlea. This is the area which was usually involved in the main blast wave lesion. The plate illustrates not only the typically uniform appearance of the inner and outer sensory cell region of the organ of Corti, but also the relatively uniform appearance of the inner sulcus epithelia. Drying artifacts usually manifest themselves by tissue cracks or, less frequently, by separations of epithelial cell junctions. Fig. 1B shows at a somewhat higher resolution view the same area of a cochlea, but from a noise-exposed animal whose tympanic membrane had ripped early in the exposure period. The animal suffered little hair cell loss and when killed at 30 days after exposure, the cochlea was very similar in appearance to the non-noise-exposed control cochleas. As in the controls, the inner sulcus had a relatively uniform appearance with only an occasional cell showing some surface disturbances (arrowhead). In this particular animal, some debris from degenerating outer hair cells was also visible (*). A higher resolution view of the surface of the inner sulcus cells (ISC) from control animals showed the typical surface distribution of the microvilli (Fig. 1C). The surface of the irregularly shaped inner sulcus cells is generally covered with a dense growth of microvilli with particularly dense accumulations along the cell junctions (J). The villi are not uniformly distributed on each cell. On some cells (Fig. 1C) the pattern of villi is irregular (patchy) with considerable surface area of the cell bare of villi as in cell (a); others show a relatively uniform surface distribution of villi, cell (b). 'Blebs' of comparatively small diameter (arrow) are generally seen on both types of cell surfaces.

Fig. 2A–C illustrates the characteristic appearance of the organ of Corti at post-exposure times of $t = 0$, 5 and 10 days, respectively. Fig. 2A illustrates how the primary lesion is formed immediately after exposure. A considerable mass of tissue containing the outer sensory and supporting structures of the organ of Corti is torn loose from its reticular lamina and basilar membrane attachments. The outer pillar (op) processes are clearly visible in this micrograph. In some preparations it is clear that even some of the Claudius cells are peeled off the basilar membrane. By 5 days after exposure (Fig. 2B), considerable debris (d) can be found on the basilar membrane; outer hair cells (OHC) are severely damaged (arrowhead) and degenerating inner hair cells (IHC) (arrow) are found interspersed with relatively normal appearing inner sensory cells. The cells of the inner sulcus begin to develop extensive growths of microvilli (m). Note the non-uniform distribution of microvilli on the inner pillar heads with the highest density in the region nearest the IHCs. Gentle convexities on some of the cells of the inner sulcus probably indicate the location of sub-surface nuclei. Fig. 2C, taken from a 10-day survival animal clearly illustrates 'scar tissue' (s) advancing from the area of the Claudius cells toward the modiolus. This scar tissue is in reality the spreading front of an extremely thin sheet of Claudius cell membrane (arrows) which covers large clumps of debris (d) and damaged or degenerating epithelial cells, causing comparatively large bulges (b) on the surface of the damaged organ of Corti. The advancing Claudius cell membrane essentially seals (or sequesters) debris as well as epithelial cells that are degenerating or undergoing autolysis between the connective tissue of the basilar membrane and the epithelial layer of the Claudius cell membrane. Note that a very thin cytoplasmic sheet of the Claudius cell membrane eventually covers normal appearing (except for a marked lack of microvilli) pillar cell heads (arrowheads). Debris is seen wedged between the artificially distorted tectorial

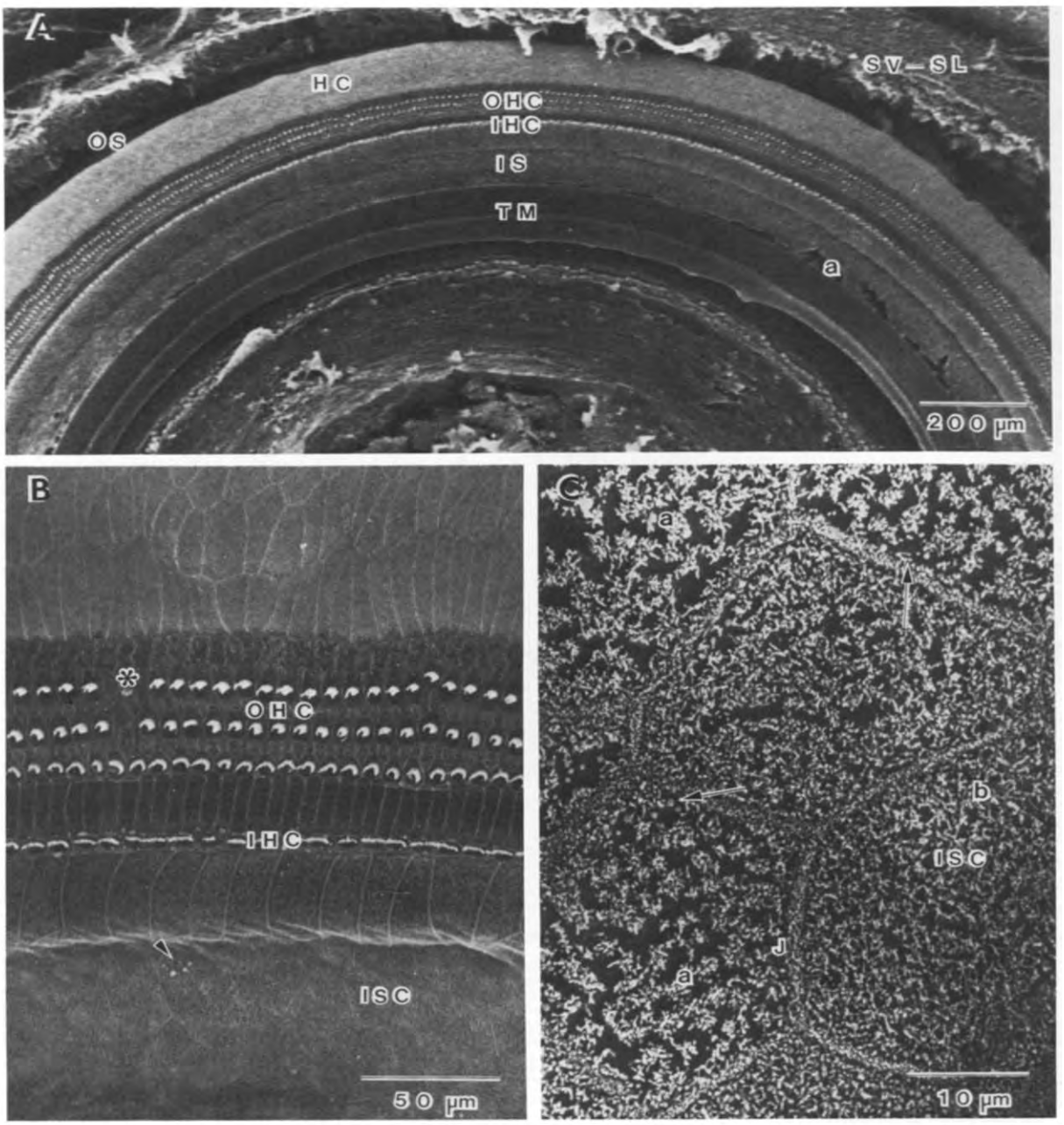


Fig. 1. (A) Approximately one-third of a turn of the normal (control) chinchilla organ of Corti from the region that lies approximately 40% down from the apex. a, shrinkage artifact; OHC, outer hair cells; IHC, inner hair cells; IS, inner sulcus, TM, tectorial membrane; OS, outer sulcus; HC, Hensen cells; SV–SL, stria vascularis/spiral ligament. (B) Higher resolution of a noise-exposed, but nearly 'normal' (see text) organ of Corti taken from the area approximately 40% down from the apex, 30 days post-exposure survival. ISC, inner sulcus cells; *, debris from degenerating hair cells; ▸, surface membrane growth on an inner sulcus cell. (C) Inner sulcus cells from the normal (control) chinchilla. a, cell with a patchy distribution of microvilli; b, cell with a uniform distribution of microvilli; →, blebs; J, dense microvilli associated with ISC cell junctions.

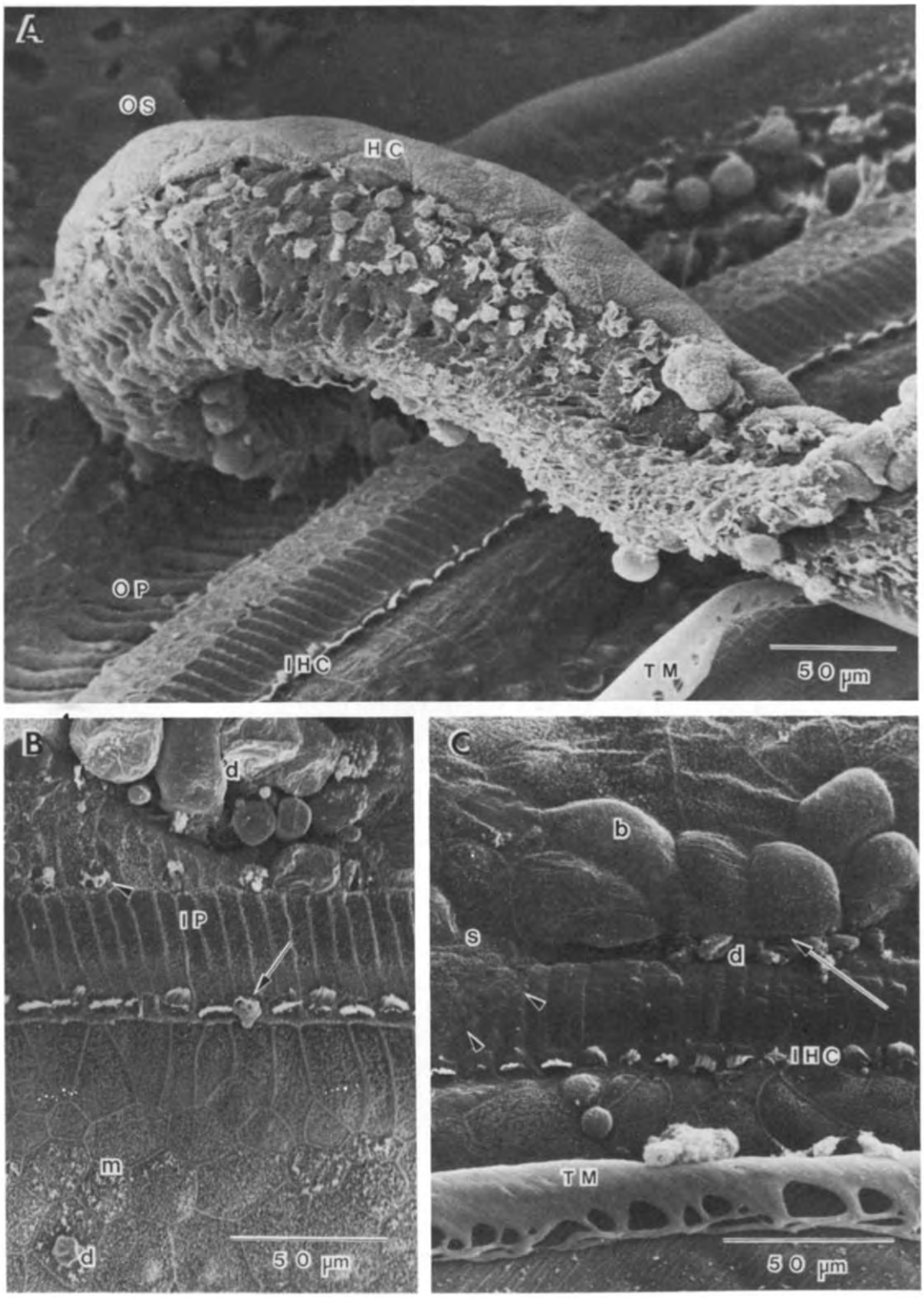
membrane (TM) and the peripheral edge of the inner sulcus.

The four plates in Fig. 3 illustrate with greater resolution the sheet of Claudius cell membrane shown in Fig. 2C as it advances over the debris and the pillar cells. The irregular bulges (b) seen in Fig. 2C and in Fig. 3A represent trapped debris and damaged epithelial cells beneath the advancing Claudius cell membrane. The leading edge of the Claudius cell advancing front is clearly marked by a line of contrasting density of microvilli (arrows and dashed line). In some areas (Fig. 3B–D) the rounded edge of the thin advancing membrane sheet can clearly be seen (*).

Fig. 4A illustrates the region of the inner sulcus from an animal killed 10 days after exposure. In contrast to Fig. 1C, the surface of the inner sulcus cells now shows a variety of villi configurations and densities. Often, those closest to the IHC array (Fig. 4A) appear similar to control animals, while 1 or 2 cell diameters toward the modiolus, there is a profuse growth of villi on some cells (a), and large bleb formations (b) on others, while on still other cells the density of villi on the cell surface has decreased and large irregular areas of cell surface membrane without villi are evident (*). Many cells can be seen with a convex, bulging surface (c). In some areas of the primary lesion the growth of villi extends up to and includes an occasional inner border cell (Fig. 4B and C, arrows). As evidenced in Fig. 4C, the villi are capable of extensive growth and in this plate the long villi of the border cell interfere with the comparatively normal appearing stereocilia of the IHC. At higher resolution, Fig. 4D, the clumps of villi or pseudopodia appear to entrap particles of debris (arrow) and extensive membrane fusion and bleb formation appear to be taking place.

Similar surface dynamics and membrane proliferation are seen in the series of micrographs shown in Fig. 5 taken from a different area of an animal killed 10 days after exposure. Fig. 5A illustrates nearly complete 'scar formation' (s) in the region of the OHC. Numerous large and fine particles of debris (d) are present and many of the ISC are seen to contain an abundant crop of microvilli (m). Some of the ISC surfaces appear to be highly convex with a particularly dense growth of villi (arrow). At higher magnification (Fig. 5B–D) some of the details of the cell surfaces show an early stage in the development of a cell that will eventually display an extremely convex cell surface. In this series of plates, the surface has the typically dense population of microvilli and numerous distended membranous sacs (arrowhead). Opposing cell membranes of neighboring cells are easily delineated, as in normal animals, by a dense band of short microvilli (arrow). Some cells in the vicinity of surface-active cells appear quite normal (n) while others show a variety of villous formations interspersed by less dense areas of microvilli or completely bare surface membrane (*). Other areas of cell surfaces distinguished by having relatively few blebs (Fig. 5D) have extremely long, almost 'bird nest-like' entanglements (e) of villi. These unusually dense entanglements of villi were seen frequently in the 10-day animals.

The convex distension of the cell surface on some of the ISC in 10-day animals is best seen in Fig. 6A which illustrates the distension in differing degrees. The three cells labeled (a) show well-developed convex distortions while the two cells labeled (b) have such a severe bulge as to appear to be (almost) separate cells on the surface of the inner sulcus. However, as nearly as can be determined from our SEM analysis, this is not the case. The cell labeled (c) could not be identified as being part of the inner sulcus cell surface, and quite possibly could be a wandering cell of the reticulo-endothelial system. However, the similarity of the cell surfaces of each of these protrusions is striking. Furthermore, it is interesting to notice what appears to be an interaction (arrowheads) between closely opposing villi of the individual cell masses (a) and (b), to the extent that there is a noticeable distortion of the cell surface, while the two cells labeled (b) appear to be almost fused. ISC groups exhibiting this sort of convex distension of the cell surface with the dense villi population seen in Fig. 6A were more typical of 30-day survival animals, while in the 10-day animals, the distended cell surfaces more often took on a very non-uniform and very labyrinthine appearance, as seen in Fig. 6B–C. In Fig. 6C arrows identify areas of the cell surface that appear to have fused into sheet-like masses forming an extra-surface membrane system. Such extra-surface membranes are abundant on the two cells (*) illustrated in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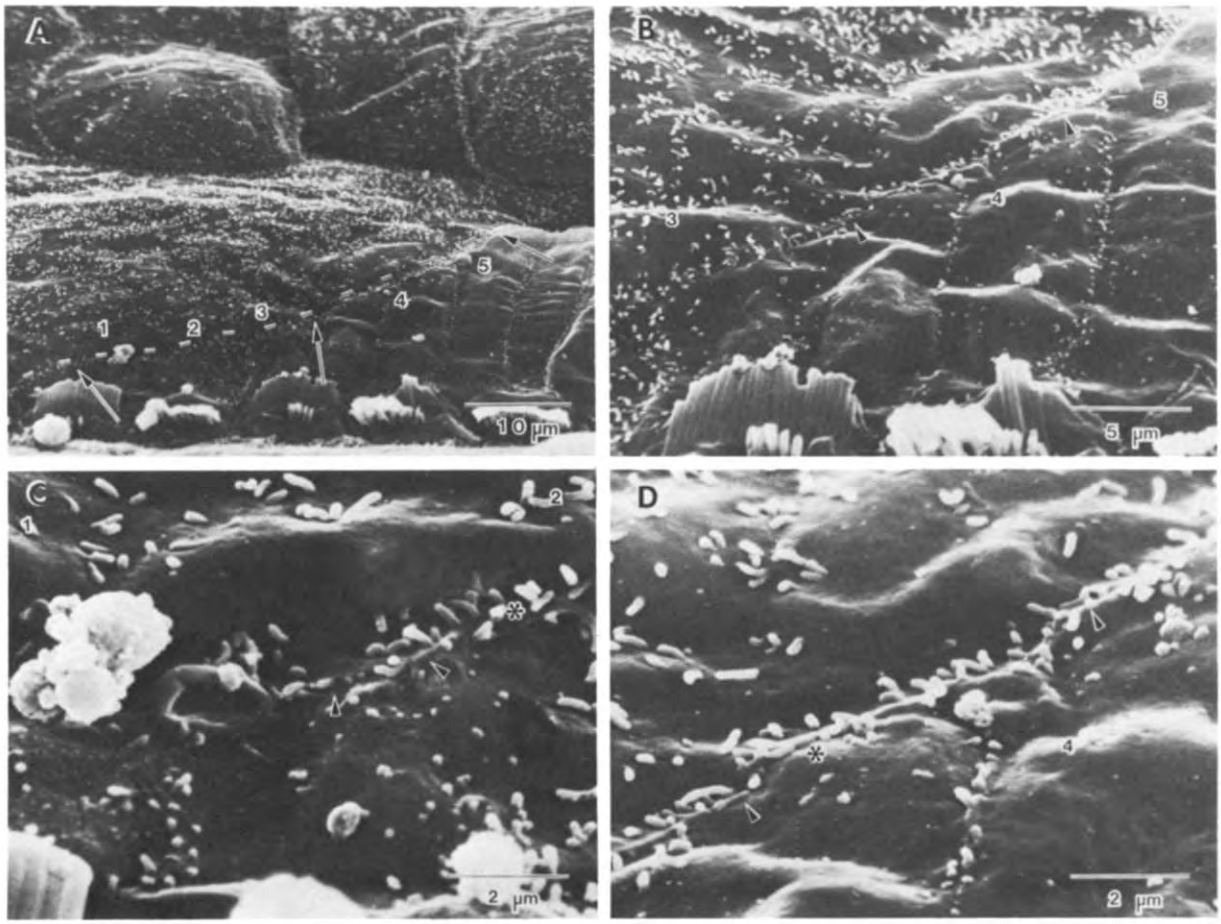
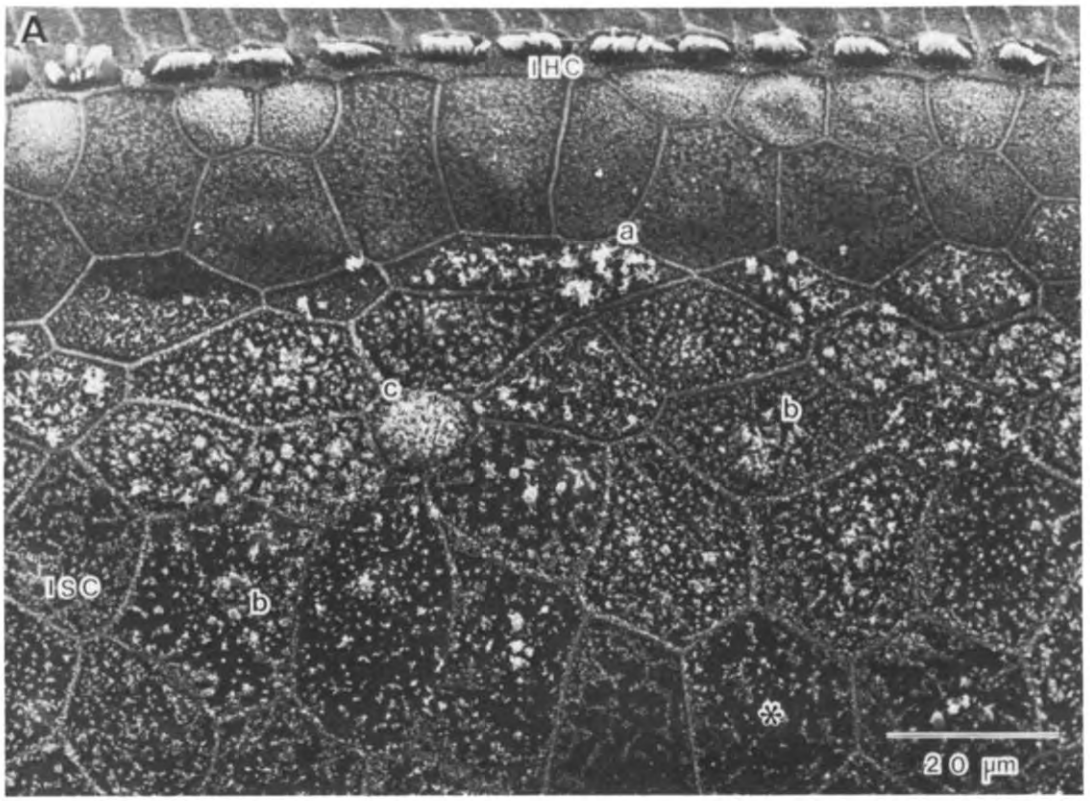


Fig. 3. (A) Higher resolution views of the re-epithelialization illustrated in Fig. 2C. Numbers refer to enlarged areas illustrated in the succeeding plates B–D. Dashed line and arrows highlight the advancing epithelial sheet. (B–D) Enlargement from Fig. 3A identified by number. Note the paucity of microvilli on the surface of the inner pillar cell complex. ▶ and * point out the advancing epithelial front and the rounded leading edge of the front, respectively.

Fig. 6C. At higher resolution, such cells have the appearance seen in Fig. 6B. On its surface, this distended cell has formed whorl-like folds (arrow) and labyrinthine formations of surface membrane with microvilli. This membrane elaboration, forming a labyrinthine-like canal at the cell surface represents an extreme example of the extent of the surface membrane changes that can be seen in the 10-day survival animals. In general, the surfaces of such cells display prominent pleomorphic folds

Fig. 2. (A) Immediately following ($t = 0$) blast wave exposure, the strip of epithelium containing the outer hair cells and their supporting elements are torn loose from the basilar membrane. OS, outer sulcus; HC, Hensen cells; OP, outer pillar cell processes; IHC, inner hair cells; TM, tectorial membrane. (B) Segment of the noise-damaged organ of Corti and the inner sulcus; 5 days post-exposure survival. d, cellular debris; IP, inner pillar cell heads; m, growths of microvilli on the ISC; ▶, degenerating OHC; →, degenerating IHC. (C) Segment of the noise-damaged organ of Corti illustrating one mechanism of scar formation and debris entrainment associated with spreading of the Claudius cell membrane. S, 'scar formation' or re-epithelialization by a thin sheet of advancing Claudius cell membrane; ▶ leading edge of the advancing epithelial sheet; d, debris; →, the advancing epithelial sheet as it entrains debris; b, surface bulges on the basilar membrane caused by the debris trapped below the sheet of Claudius cell membrane. IHC, inner hair cells; TM, tectorial membrane.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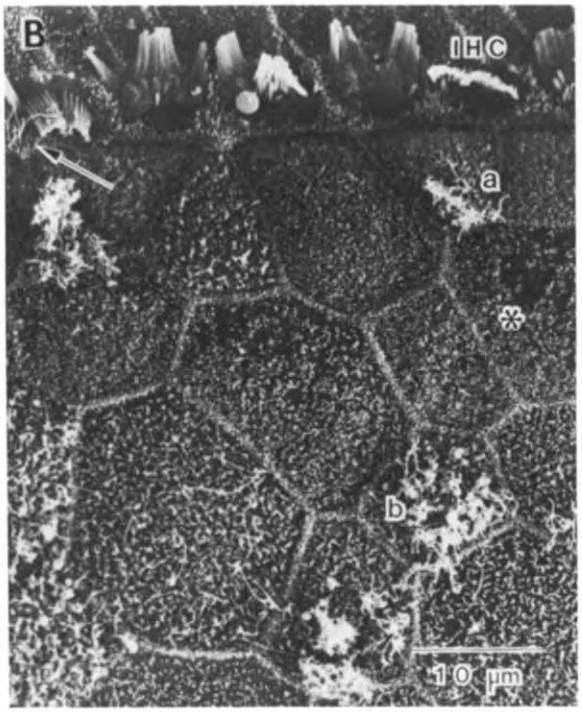
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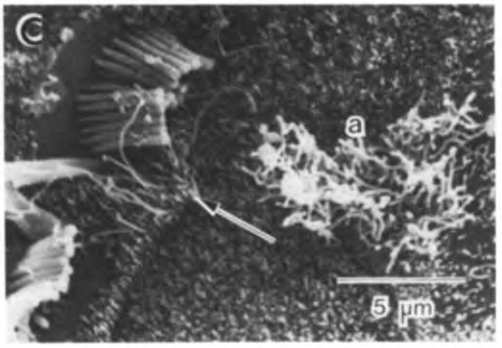
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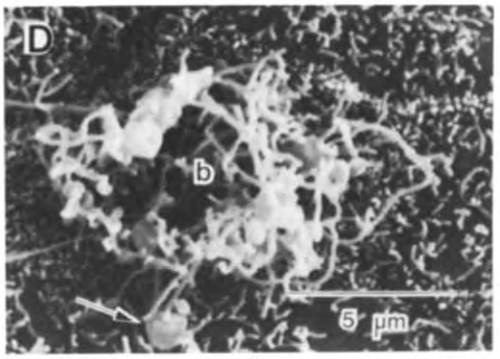
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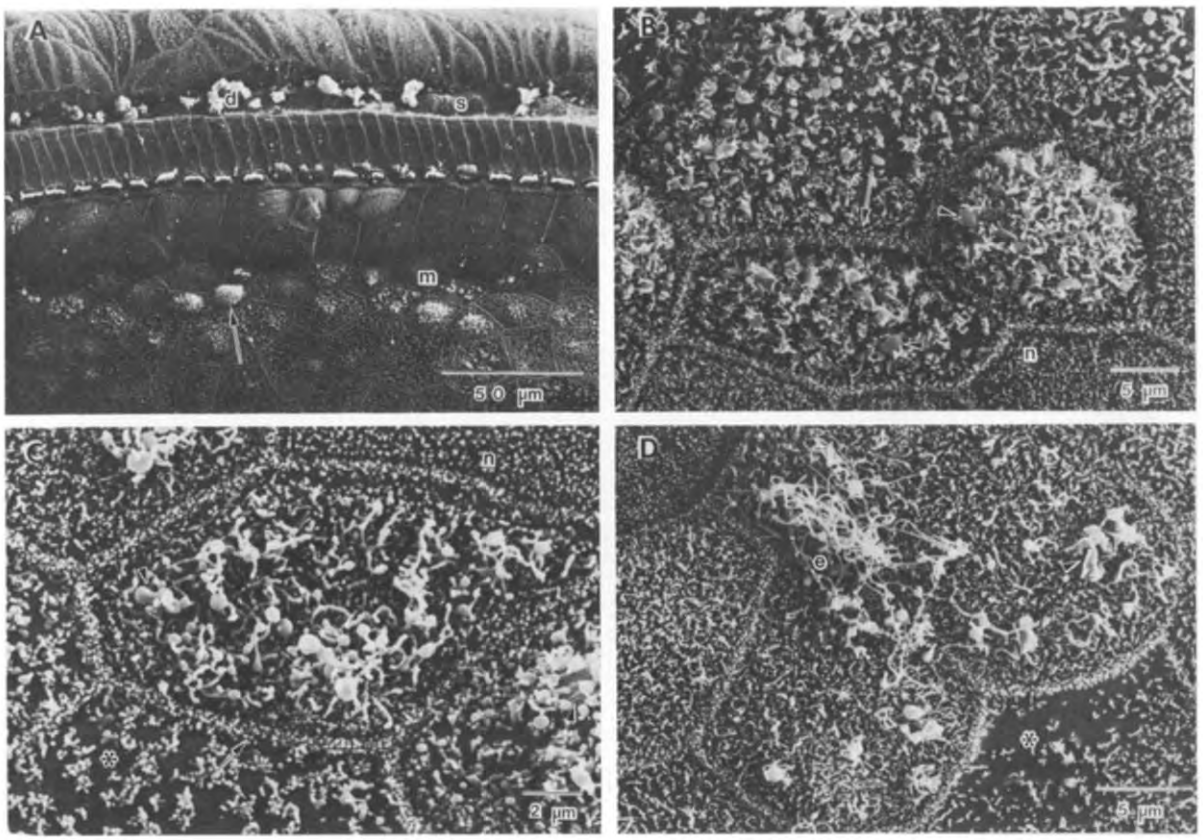

Fig. 5. (A) Scar formation(s) in the region of the outer hair cells is nearly complete and a variety of surface membrane proliferation similar to those shown in Fig. 4 can be seen. →, highly convex ISC surface; m, microvilli growth; d, debris. (B–D) Higher resolution illustrations of ISC that show a variety of surface membrane dynamics. →, indicates the typical, unaltered microvilli morphology of the ISC junctions; ▶, indicates a variety of membranous pleomorphic forms on the ISC surface, indicative of endocytotic events; e, commonly observed 'bird nest-like' entanglements of unusually long microvilli; *, indicates cells with a distinct paucity of microvilli.

and ridges which vary in local density and distribution. Fig. 6D illustrates that the variety of cell surfaces that have been previously discussed can all exist in the proximate vicinity of each other.

Fig. 7 illustrates, with greater resolution, the relationship between individual villi and what are most likely particles of debris which develop into bleb-like irregularities. These large blebs appear to be formed by a process that is suggestive of a very viscous fluid-like spreading or flowing of villi membrane during the incorporation or entrainment of debris.

Fig. 7A and the upper insert show a higher magnification of the surface of the cell identified

Fig. 4. (A) A view of the inner sulcus from a 10-day survival animal. Note the profuse growth of microvilli with a variety of surface morphology on different cells of the inner sulcus. IHC, inner hair cells; ISC, inner sulcus cell; a, profuse growth of microvilli; b, inner sulcus cell with an extensive membranous sac formation; c, cell with a particularly dense growth of microvilli and a rather distended convex surface; *, cell with an unusually low density of microvilli. (B–C) This pair of micrographs illustrates a similar spectrum of surface morphology as the previous plate; however, note the involvement of an inner border cell (arrow) in the extreme proliferation of microvilli. (D) Higher resolution of an ISC whose villi entanglements with debris (arrow) has resulted in the formation of a number of membranous sacs or membrane fusion.

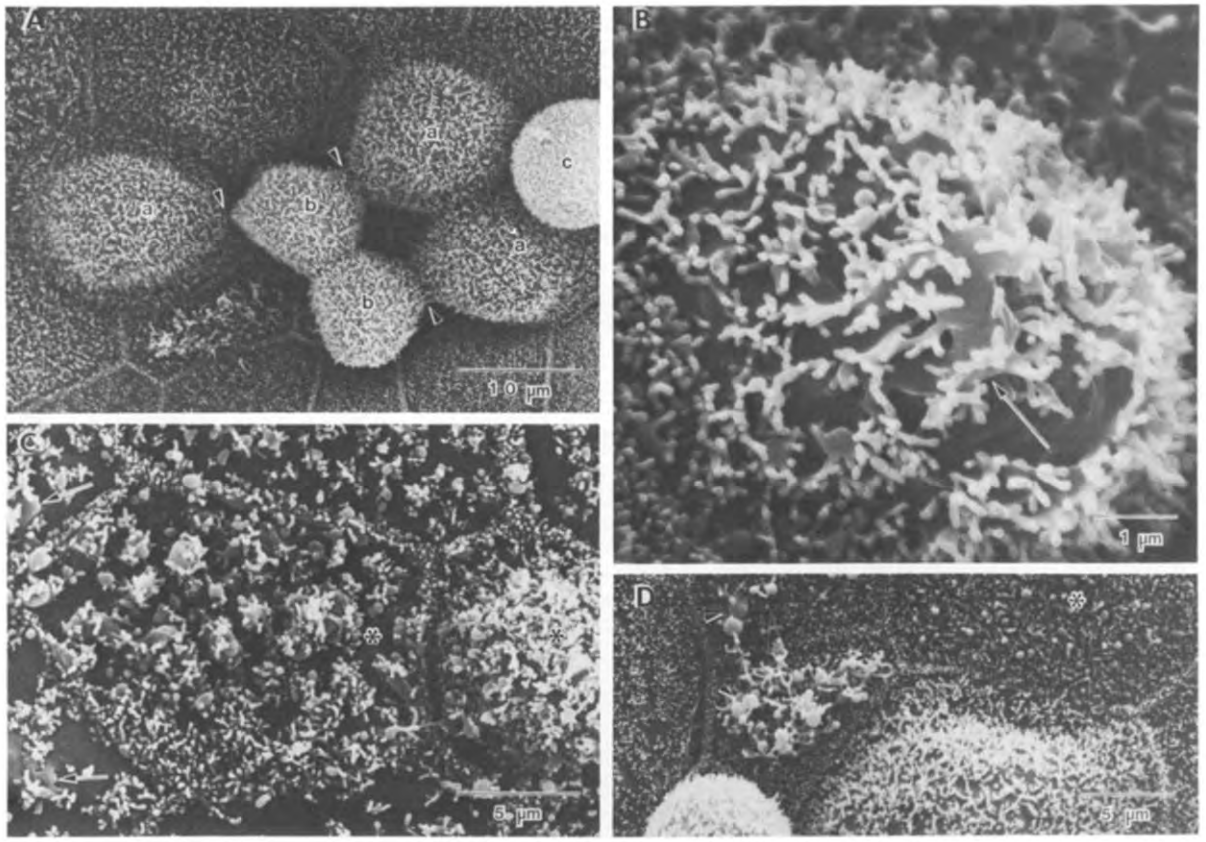

Fig. 6. (A) Extreme convex distensions of the ISC in a 10-day survival animal. a, ISC with a moderate surface convexity; b, ISC with extreme surface convexity; c, cell of unknown origin, possibly a wandering cell of the reticulo-endothelial system. ▶, indicates sites of apparent cell interactions. (B) High resolution micrographs of an ISC with a highly convex surface and a highly developed surface membrane system. →, indicates labyrinthine-like membranous folds. (C) Illustrations of more subtle 'extra surface' membrane systems (arrows). Note the particulate matter under the surface of the structure indicated by the lower left arrow. *, indicates a pair of ISCs whose surface has an unusually rich accumulation of 'extra surface' membrane. (D) Illustration of the variety of different surface morphologies that can exist on adjacent cells. From relatively normal appearing to the zeiotic types identified by *, which are probably indicative of later stages or cycle of an endocytotically active cell.

with arrowheads in Fig. 6D. The arrows in Fig. 7A indicate extra-cellular masses which appear to have taken up a coat of cell membrane associated with the local microvilli. The upper insert illustrates more clearly what appears like a 'viscous flow of membrane' over, or an encapsulation of, an extraneous particle by microvilli (arrowheads). In Fig. 7B and C the villi entanglements associated with what appears to be the entrapment of extra-cellular particles of material can be seen clearly, while the lower insert illustrates a single villus wrapping itself around a particle. A conceptual sketch which reflects the events described in Fig. 7 is illustrated in Fig. 8. The series of micrographs in Figs. 4–7 illustrate the extreme surface reactivity of the inner sulcus cells. However, as shown earlier other cells of the cochlear duct appear to be capable of such surface activity, but to a much less marked degree; for example, the inner border cells shown in Fig. 4C. Other cell types shown in Fig. 9 that have an elaborate population of microvilli include the Deiters cells (D) (Fig. 9A) and the Claudius cells (C) near the outer sulcus (Fig. 9B and C). In Fig. 9B, this particular Claudius cell has a rather vigorous growth of very long villi, while in another area the Claudius cell surfaces such as cell (a) and

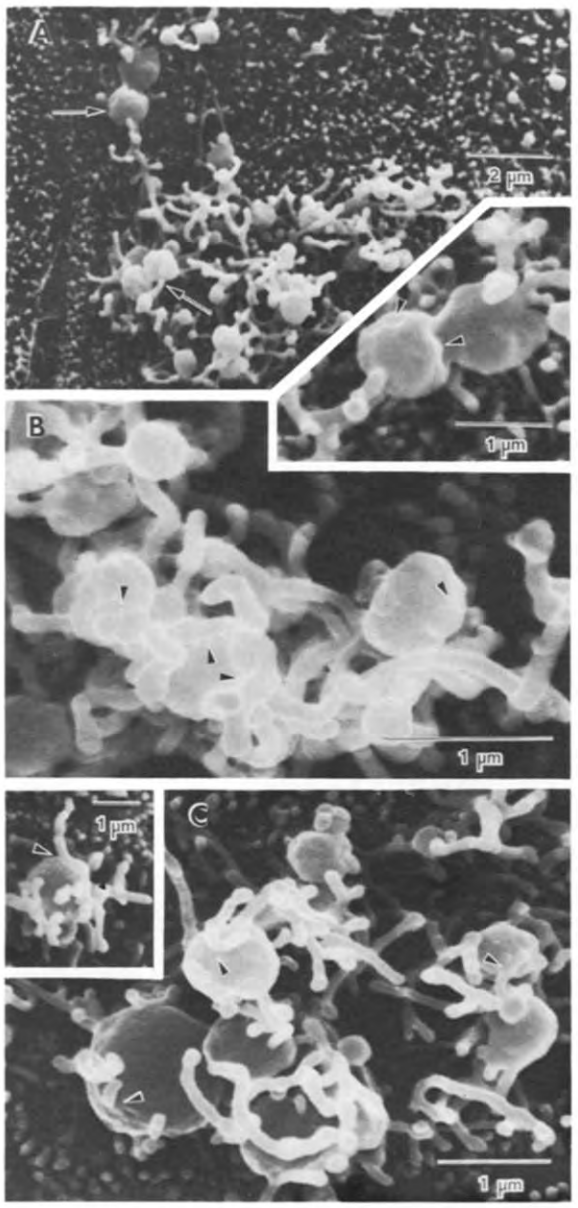

Fig. 7. (A–C) High resolution micrographs illustrating what might be referred to as the mid-cycle of the endocytotically active cell surface. Plate A is an enlargement of the cell identified by the arrowhead in Fig. 6D. Collectively, this figure illustrates the relationships among the particulate matter and the enveloping pseudopodia (→) resulting in what appears to be a membrane flow around the particle (e.g., upper and lower insert – arrowheads).

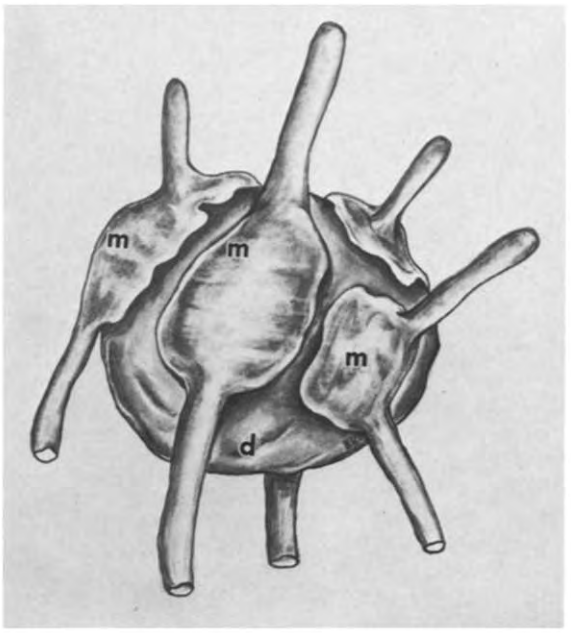

Fig. 8. Schematic of the 'flow' of microvilli membrane (m) during encapsulation of a sphered particle of debris (d).

(b) in Fig. 9C are similar in their surface morphology to normal inner sulcus cells, but cell (c) has noticeably longer villi. However, this surface behavior of cells located on the basilar membrane per se was infrequently observed and most definitely not as prolific as the surface reaction of the inner sulcus cells.

In the animals killed after 30 days (Fig. 10), small particles of debris (d) are still seen on the basilar membrane. The inner sulcus cells usually do not have the extreme villous growths and membrane systems seen in the previous micrographs taken from the 10-day animals. However, many cells of the inner sulcus did exhibit a pronounced surface distension (insert) with higher than normal microvilli density. Frequently, debris was attached (arrowhead). These types of distended cells were invariably localized to the specific regions of the organ of Corti that were lesioned (Fig. 10A). Distant from the lesion the inner sulcus generally appeared normal. This localization of the ISC reaction is quite dramatic. Furthermore, as mentioned previously, the surface protrusions frequently appear to interact among themselves (arrowheads, Fig. 10B), as well as with cells of unknown origin (*). Some of these anomalies at the cell surface give the appearance of a mitotic

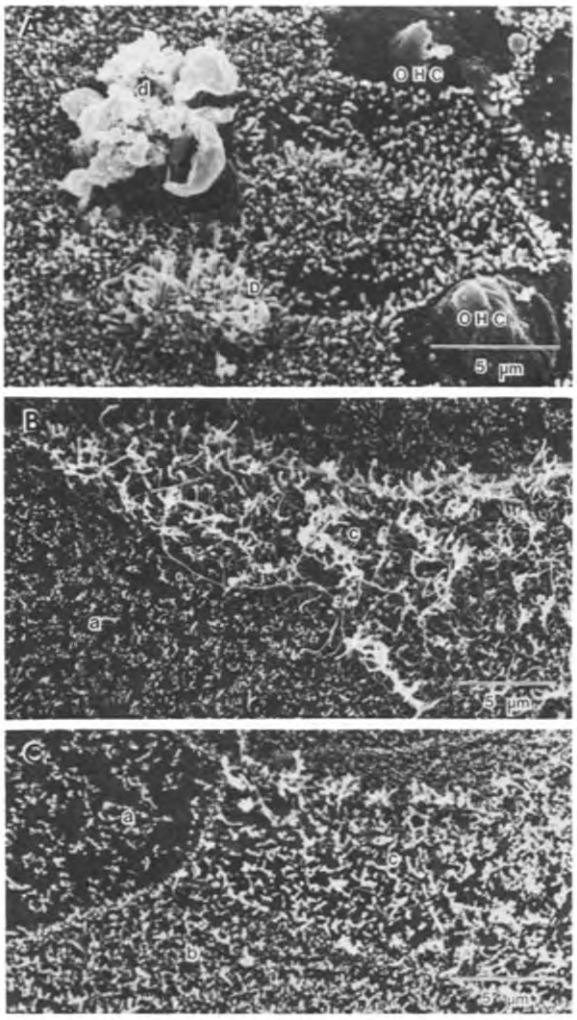

Fig. 9. (A) Region of the outer hair cells (OHC) that are adjacent to the primary lesion in a 10-day survival specimen; d, debris from a damaged OHC; D, Deiters cell head with a prolific growth of microvilli. (B) Claudius cells showing a variety of microvilli configurations. a, normal appearing microvilli with a 'patchy' distribution; c, Claudius cell surface with a particularly vigorous growth of microvilli. (C) Another area of Claudius cells whose microvilli sizes and densities are variable. a, normal but 'patchy'; b, relatively normal slight zeiotic; c, longer than normal with some fusion.

event; however, definite identification of these 'extra' sulcus cells must await a transmission electron microscopy analysis which is in progress. The sequence of plates of Fig. 10A–C illustrates different areas of the lesion: in Fig. 10A, the apical edge; in Fig. 10B a basal section where IHC are relatively normal in appearance and the pillar cells are all present; and Fig. 10C where all elements of the organ of Corti have been replaced by Claudius or

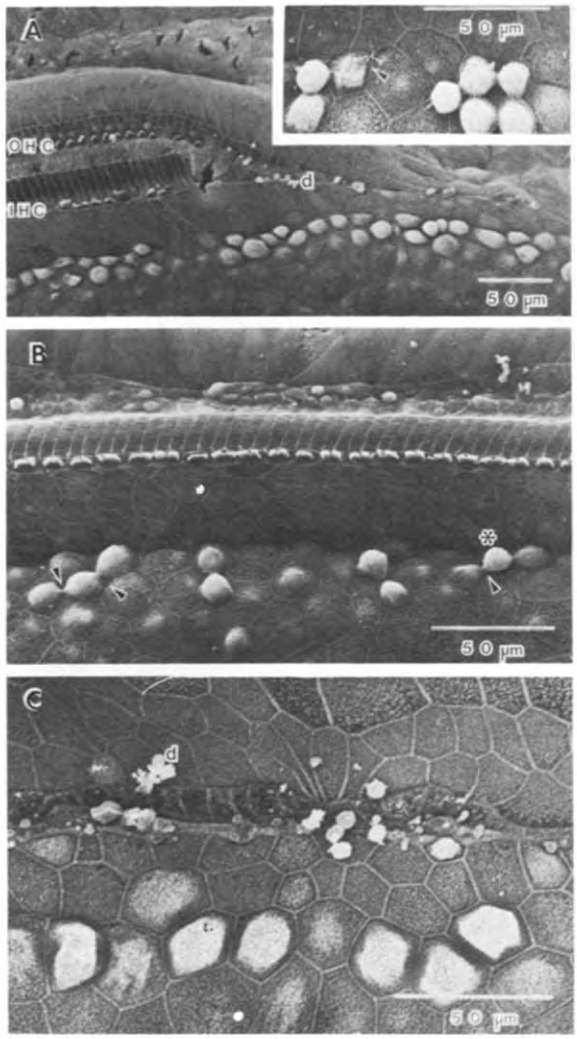

Fig. 10. Examples of the inner sulcus cells taken from a 30-day survival specimen. (A) Example of the prominent surface distension of the ISC in the area of the primary lesion. These cells have an abnormally high density of microvilli. d, debris. Insert: higher resolution of the distended ISC shows indications of surface interactions as discussed in Fig. 6. ▶, filamentous debris. (B) Similar to plate A, but taken from an area where the IHC appear relatively normal, and scar tissue has generally sealed over the OHC loss. Note the prominent surface interactions (▶) among the distended cell surfaces. *, a cell of unknown origin. (C) Similar to the above, but taken from a region where there is a complete loss of sensory and supporting elements.

inner sulcus cell extensions. In all three areas, the distensions of ISC with very dense microvilli growths are evident.

As part of this study, we also sectioned the endolymphatic sac in an effort to discover if the sac plays a significant role in the elimination of large particles of debris of cochlear origin. Fig. 11 illustrates two sections of the endolymphatic sac. The specimens were embedded in spurr epoxy resin cut at 1 μm and stained with Methylene Blue

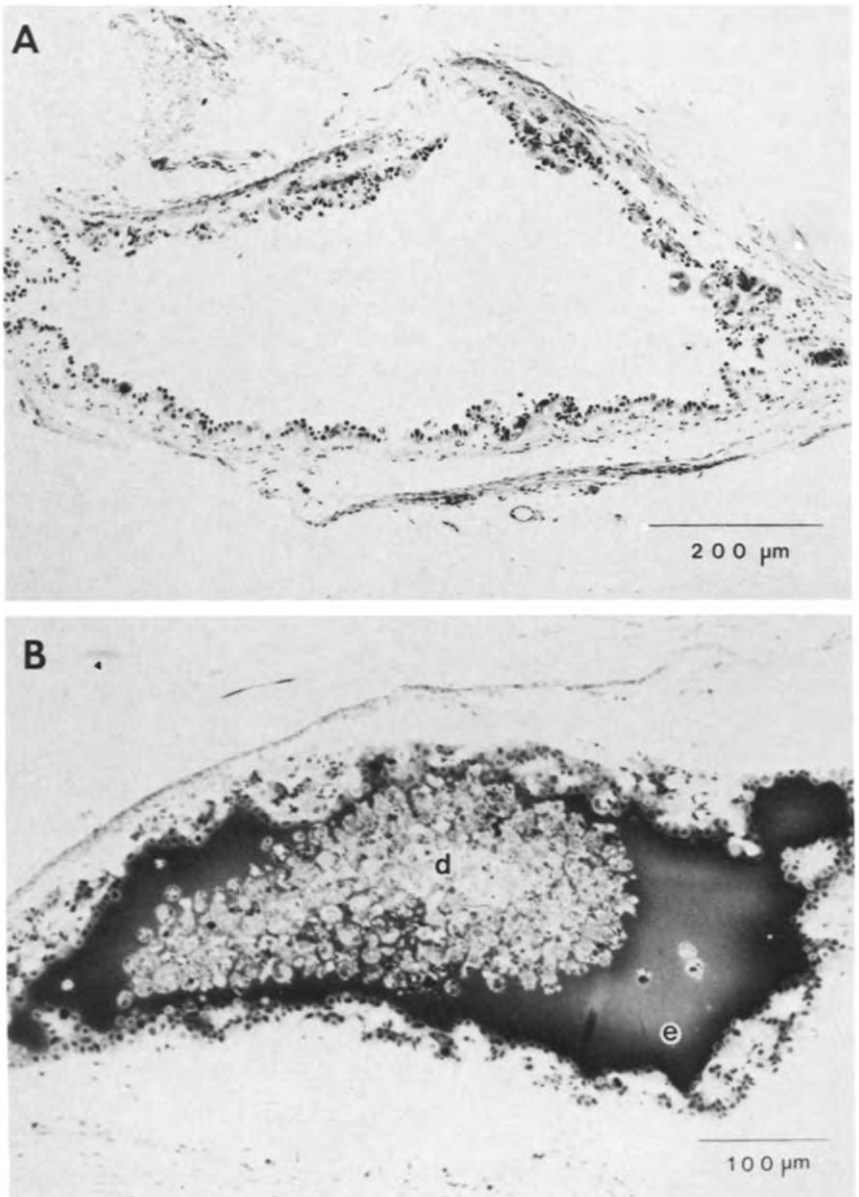

Fig. 11. (A) 1-μm stained section of the rugose portion of the endolymphatic sac from a 30-day survival animal. Note the lack of stain in the lumen, and the relatively normal appearing epithelial lining. (B) Similar section of the endolymphatic sac taken from a chinchilla that had been treated with an ototoxic drug (see text) that destroyed much of the vestibular neuroepithelia. Note the debris (d)-filled lumen and the dark staining (e) endolymph.

and Azure II. The upper plate A taken from the rugose portion of the sac is from a noise-exposed animal killed after 30 days. The lumen of the sac is clear and for all practical purposes, the section is not different from that of a control animal. By way of comparison, the sacs from animals sacrificed at 0, 5 and 10 days post-exposure were also sectioned. Their appearance at the light microscopic level was identical to the section in Fig. 11A. Fig. 11B illustrates a similar section of an endolymphatic sac taken from an animal killed 4 days after injection of 0.5 cc of Cortisporin otic suspension into the middle ear cavity. The drug severely damaged the cochlea as well as the vestibular labyrinth [39]. The lumen of this sac is densely filled with debris (d) and the endolymph (e) stains darkly. In both these animals, the cochleas sustained a similar degree of sensory cell damage on the organ of Corti.

Discussion

We have shown that in a situation in which the organ of Corti is severely traumatized by noise, such that as much as 7 mm of Corti's organ is torn loose from the basilar membrane, the cells of the inner sulcus respond to the altered biochemical milieu of the endolymph by a prolific elaboration of surface membrane, zeiosis and the development of numerous pseudopodia and microvilli. On the basis of our longitudinal study, this surface reaction appears to peak at approximately 10 days after trauma. However, changes in the microvilli were noted immediately after trauma, and severely distended inner sulcus cells with a higher than normal density of microvilli on their endolymphatic surface were still apparent 30 days after exposure. While it may be coincidental, Bohne [3] has also reported that the greatest number of macrophages were seen in her noise-damaged specimens between 12 and 16 days post-exposure. This period of phagocytic activity coincides with our observation that the ISC also appear to be most surface active at this time.

The observations made in the present study would implicate the inner sulcus cells in the endocytosis of debris. It would not be surprising to learn that the cells of the inner sulcus are involved in endocytotic activity since virtually all animal cells are capable of endocytosing a large spectrum of materials [33,34]. What is surprising, however, is the amount of membrane that can develop on the surface of the inner sulcus cells. There is indirect evidence in the literature [9] that the elaboration of surface membranes via folds, pseudopodia or villi is not only related to endocytosis. Endocytosis of debris, as inferred from these micrographs, can only be determined with certainty when the endocytic vacuoles or phagosomes can be identified by transmission electron microscopy. Nevertheless, the surface activity of the ISC does point to endocytosis as an important event. Furthermore, the micrographs from both the normal and noise-affected ISC often exhibit a density of microvilli which is very 'patchy' (e.g., Figs. 1C and 4A). Such bare spots on a cell surface with a 'normally' uniform population of villi may be indicative of the completion of an endocytotic event [25,27,32, 37] or some other surface-related phenomena such as exocytosis or cell movements. In general, Porter et al. [27] have indicated that there is a causal relation between events on the cell surface as seen in its membrane morphology and the internal cell processes.

The findings of this study provide some clues regarding the role of the ISC in the normal physiology of the cochlea. There is evidence that the inner sulcus area may be isolated from the endolymph and may, in fact, contain a fluid of different chemical composition [16–18,20]. The extremely labile nature of the ISC may indicate that these cells have the internal machinery necessary to actively clear the fluids of the internal sulcus of various ions or macromolecules, thus possibly providing the subtectorial membrane regions of the organ of Corti with a specialized fluid environment. The ISC were not the only cells that reacted to the trauma by elaborating surface membrane. Occasional Deiters cells, Claudius cells and Hensen cells could be found with proliferation of microvilli. However, on these epithelial cells, there was considerably less surface activity. In noise-damaged chick cochleas, Saunders and Tilney [28] mention that great numbers of microvilli rapidly appear on the reticular surface soon after trauma. A conflicting finding has been reported by Thorne et al. [35]. They showed that initially the Deiters and the pillar cells were usually devoid of micro-

villi. However, by 7–14 days after exposure the number of microvilli on the supporting cells was increased, particularly in areas of hair cell loss. Once again, a time of approximately 10 days post-exposure is linked to increased surface activity of the epithelial cells in the area of a cochlear lesion. It is interesting to compare our Fig. 2B–C with similar micrographs of Thorne et al. [35]. In this figure the inner pillar cells from an animal killed 5 days after exposure (Fig. 2B) show a non-uniform villi distribution which is densest near the IHC and thins out considerably toward the OHC; while in Fig. 2C taken from a 10-day post-exposure animal, the inner pillar cell heads and cell junctions are virtually devoid of villi, as in the Thorne et al. [35] micrographs. However, this is the area of the lesion which is in the process of being covered by the thin veil of Claudius cell membrane.

It would appear that cells such as the Claudius cells are capable of spreading their membranes to an astonishing degree, as evidenced in Fig. 3. This thin veil of advancing cell membrane appears to cover considerable quantities of damaged organ of Corti, essentially trapping this material between its own thin epithelial sheet and the underlying layers of connective tissues. Conceivably this material can then be disposed of by phagocytic cells of the reticulo-endothelial system.

It further appears that the advancing front of the Claudius cell membrane is selective as to the cellular elements that it covers. In a situation where only OHC and supporting elements are destroyed, scar formation, or more accurately re-epithelialization, frequently terminates at the outer pillar cells indicating that perhaps the more centrally located cellular elements are still viable, or that their surface still retains an appropriate biochemical composition. However, in a situation such as that seen in Figs. 2C and 3, the pillar heads which upon casual inspection appear normal, are seen in fact to be devoid of villi; also the pillar cell junctions which normally are densely populated with villi are now deficient; thus, the morphology of the pillar head surface has dramatically changed. The surface of these particular pillar cells appears 'willing' to accept the epithelial veil of the Claudius cell while the line of IHC appears to be where this epithelial advance ceases. This raises the possibility that certain surface receptors on viable cells may act to guide the advance of the re-epithelialization of the damaged organ of Corti. The extensive spreading of Claudius cell membrane further raises some interesting questions as to the mechanisms involved in triggering the spreading phenomena; building the extra membrane; sources for the energy requirements; and internal mechanisms presumably associated with triggering the development of an actin–myosin system for providing for the movement necessary to cover debris located somewhat distant from the normal location of the Claudius cells. Elsbach [8] has indicated that there is ample evidence to support the hypothesis that a contractile apparatus resembling that of skeletal muscle is instrumental in the mechanical work associated with such membrane motions. A number of studies [9,15,27] have shown that the extra membrane required for cell spreading is available in the blebs and microvilli of the cell surface and that actin filaments within the microvilli which are membrane-attached may provide for the redistribution of surface membrane when needed. The process of villi formation and utilization is a very dynamic event. The villi are very labile, and rapidly emerge from the cell surface and with equal rapidity can disappear [10,11]. The examples shown in Fig. 2 represent extreme cases of noise damage, i.e., the basilar membrane has been nearly denuded of sensory elements; however, as regards the severity of damage in other situations where only OHC are damaged, supporting cells are left intact, and a 'scar' is formed along the reticular lamina, it is conceivable that the extra growth of villi seen on the Deiters cell heads [28] is the cell's response to the immediate need for additional membrane for 'scar formation' through a cell-spreading mechanism.

The process of cell spreading and endocytosis may be related in more than simply a superficial way. When one considers the mechanisms that trigger these events (i.e., cell spreading and prolific microvilli growth), a number of possibilities exist. During the severe mechanical trauma that takes place on the organ of Corti, the cytoplasmic contents of various cells are discharged into the endolymph. Further, in the initial post-exposure time (< 5 days), autolysosomal digestion probably proceeds at an accelerated rate. Both these processes

would have the effect of filling the endolymph with various macromolecules, especially lipids, lipoproteins and glycoproteins. Studies by Margolis and Bergelson [21], Chen et al. [4], and others [22,38] have shown that various macromolecules, notably lipids and glucose, can exert a very strong influence on the development of microvilli. Lipids, for example, can induce a tremendous increase in the population of villi in cell cultures. Fusion of cell membranes with liposomes is thought to be one of the mechanisms responsible for this 'lipid-induced' growth of microvilli, which can at times become excessive (e.g., Fig. 4C). Such a lipid incorporation into the cell surface membrane is accompanied by a reversible or temporary change in the viscosity of the cell membrane [2] (i.e., the membrane is more fluid, e.g. in Fig. 7).

It is clear from the illustrated micrographs as well as from the literature, that the phagocytosing cell is prepared ahead of time for the particles that it eventually ingests, indicating that factors other than actual contact between the cell and the particle are in operation to generate the internal and surface morphology and membrane properties that are best designed for capturing debris. A variety of factors which act as primary messengers to alert the cell membrane have been studied [34]. New chemical components in the endolymph from the damaged organ of Corti may be instrumental in providing these messengers.

The pseudopodia of the ISC appear to engulf the foreign body by conforming to its shape and, in a descriptive sense, flowing over the particle. This can clearly be seen in Fig. 7. Closely opposed pseudopodia overlap and appear to fuse and eventually a single membrane seems to cover the foreign particle. When one observes the debris that is scattered in the scala media, it often appears as an irregular, filamentous or multifaceted mass of material (e.g., Fig. 5A); however, when entangled in the pseudopodia of the ISC, the particles all appear smooth and (relatively) uniformly spherical (e.g., Fig. 7). It is these 'spherical' masses that the pseudopodia begin to envelop by a process of membrane spreading and perhaps fusion. The sphering of particles destined for ingestion by an endocytosing cell has been observed as a 'rapid' sequel to the adherence of the particle to the endocytotic cell surface. This sphering phenomenon has been well documented in the work of Tizard and Holmes [36] and Orenstein and Shelton [25]. These authors further refer to the 'flow' of macrophage membrane (lowered membrane viscosity) [2] around the particle being ingested (e.g., Fig. 7 – insert). They discuss concerns for the requirement of a reduction in interfacial energy to allow this type of wetting phenomenon to occur. The micrographs which these authors have published display a striking resemblance to the processes observed in the inner sulcus, and in particular, to the details presented in Fig. 7. Thus, on the basis of the foregoing evidence, it may be that the inner sulcus and not the outer sulcus should have the distinction of being referred to as the "sewer of the inner ear" [29].

Finally, our survey of endolymphatic sacs from animals with survival times of up to 30 days yielded essentially negative results. At the light microscopic level it was not possible to distinguish between control and experimental specimens. Thus, under the experimental conditions of this study, we found no evidence that the sac plays a role in disposal of large amounts of cellular debris of cochlear origin.

In summary, we have attempted in this paper to draw attention to a variety of interesting events that take place in the noise-damaged cochlea, among some of the less 'interesting' cell types of the cochlea. Hopefully these micrographs will help focus attention on the possible significance of some of the less 'impressive' cell types whose sole reason for existence has traditionally been thought to provide passive or mechanical support for the sensory neural elements of the organ of Corti.

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