Surface ultrastructure of the cochlear perilymphatic space[1]

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Introduction

The fine morphology of the surface architecture of the cochlear perilymphatic space has been clarified with some limitation following the advent of the transmission electron microscope (TEM) (Engström and Wersäll, 1958; Spoendlin and Balogh, 1963; Spoendlin, 1966; Iurato, 1967; Duvall and Rhodes, 1967). The preparation of tissue specimens for study with the TEM, however, is laborious and time-consuming, and is therefore prohibitive in studying the entire surface lining of the vast cochlear perilymphatic space.

A new type of electron microscope, the scanning electron microscope (SEM), has recently become available for the investigation of biological specimens (Hayes and Pease, 1968). The image obtained with the SEM is created by the emission of secondary electrons from the excited surface of the specimen, in contrast to the TEM in which the image is produced by the primary electrons passing through the specimen. The SEM, therefore, is an excellent tool for studying the fine surface morphology without the necessity of sectioning.

The surface architecture of the cochlear and vestibular sensory epithelia has been successfully examined using the SEM in our laboratory (Lim, 1969; and Lim and Lane, 1969a, b). This communication is a continuation of that already reported and deals with the ultrastructure of the surface covering of the perilymphatic space of the cochlear duct. The examination of the surface topography of the cochlear duct lining is vital to the understanding of the dynamics of inner ear fluids.

Materials and methods

Twenty adult guinea pigs were used in this study. Preparation of the cochlea was done in the manner described in a previous article (Lim, 1969). Temporal bones were fixed in 1 per cent osmic acid in phosphate buffer and dissected while immersed in 70 per cent alcohol. The dissected portions of the inner ears were air-dried and coated with gold in a vacuum evaporator on a rotary stage, and were examined with the scanning electron microscope (Cambridge Stereoscan) using magnifications ranging

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from 20 to 20,000 ×. Stereoscopic photographs were extensively used to determine accurately the depth of any depressed structures and their proper orientation.

Results

The perilymphatic space can be divided into the scala vestibuli and scala tympani within the bony cochlear duct. These will be discussed separately.

A. *Wall of scala vestibuli.*

The scala vestibuli is defined by the vestibular wall of Reissner's membrane and the endothelium-like simple squamous cells covering the spiral ligament, spiral limbus and the perilymphatic bony wall (Fig. 1). The points where Reissner's membrane makes attachment are supported by the connective-tissue type of cells of the spiral ligament and the spiral limbus. The vestibular crest area extends from the insertion of Reissner's membrane immediately above the stria vascularis to the thin bony plates which divide the cochlear turns. The limbal area refers to the cell covering of the upper surface of the osseous spiral lamina demarcated by the limbal attachment of Reissner's membrane and the roof-like bony plate separating the turns.

The vestibular crest area, immediately above the stria vascularis, is covered by thin squamous epithelium near the insertion of Reissner's membrane (Fig. 2). This area, particularly in the lower turns where the upper margin of the spiral ligament thins out, shows a band-like zone, parallel to the insertion of Reissner's membrane. It is formed by the underlying connective tissue of the spiral ligament. This zone is widest in the lower turns and becomes narrower as the helix ascends. The upper margin of this zone, where the spiral ligament is thinner, shows multiple "holes" and micropores which are at times mesh-like (Fig. 3a). Quite frequently the naked spiral ligament can be seen in this region (Fig. 3b). Where the spiral ligament disappears, the contour of the rough surface of the underlying bone can be observed beneath the thin squamous cells (Fig. 4).

The surface covering of the limbal side of the perilymphatic space is also covered with a simple squamous type of epithelium but the micropores or holes, which are frequently noted in the vestibular crest side, are rarely found (Fig. 5).

The squamous cells on the vestibular side of Reissner's membrane are much larger than those of the endolymphatic side. The latter are more closely packed and show a typical polyhedral shape (Fig. 6a). The cell processes of the vestibular squamous cells (mesenchymal epithelial cells) quite often overlap so that their cell margins are less clearly defined (Fig. 6b). Oftentimes, granular structures, which were interpreted

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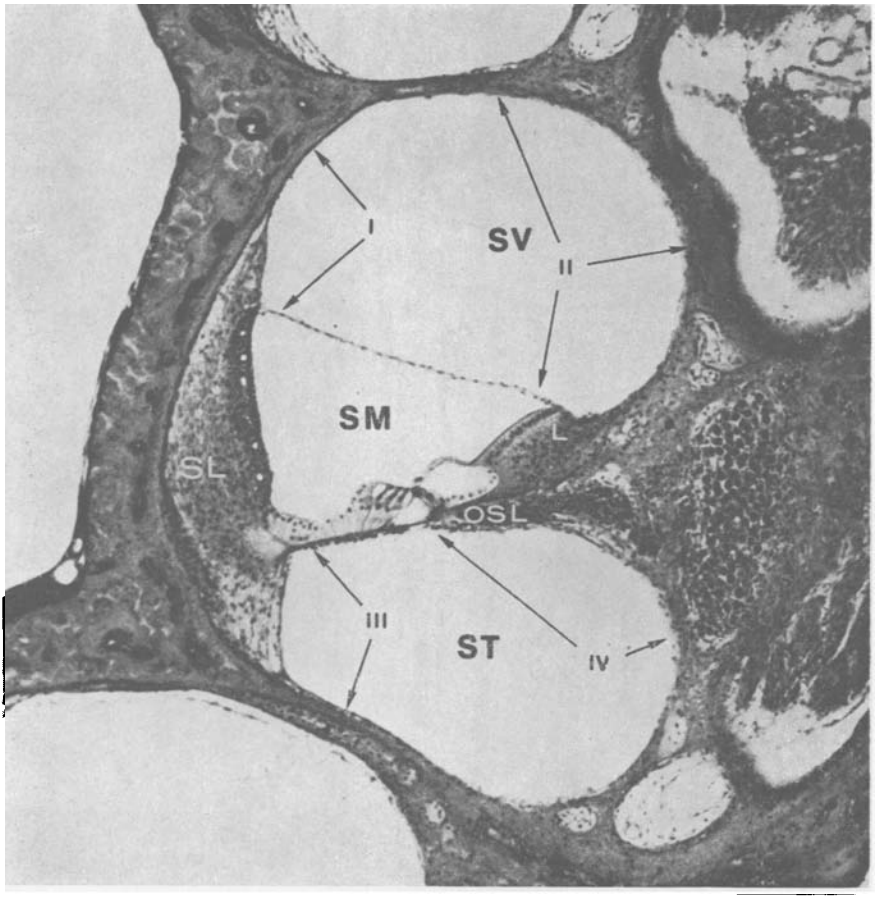


FIG. 1.

A light microscopic photograph illustrating the areas examined by the electron microscope. SV—scala vestibuli, ST—scala tympani, SM—scala media, SL—spiral ligament, OSL—osseous spiral lamina, L—spiral limbus, I—area covering the spiral ligament on the scala vestibuli side; II—the area covering the spiral limbus, the upper-surface of the osseous spiral lamina and a portion of the bony plate dividing the cochlear turns; III—the area covering the spiral ligament on the scala tympani side; IV—the under-surface of the osseous spiral lamina.

as cell organelles, can be observed underneath collapsed cytoplasm, and the silhouette of the nucleus can be identified. Micro-separations of the cell margins which provide free communicating channels for perilymph observed in transmission electron microscopy were not positively identified. Microvilli which are well developed on the scala media side, are not observed on the scala vestibuli side.

B. *Wall of scala tympani*

The surface of the scala tympani is composed of the lower portion of

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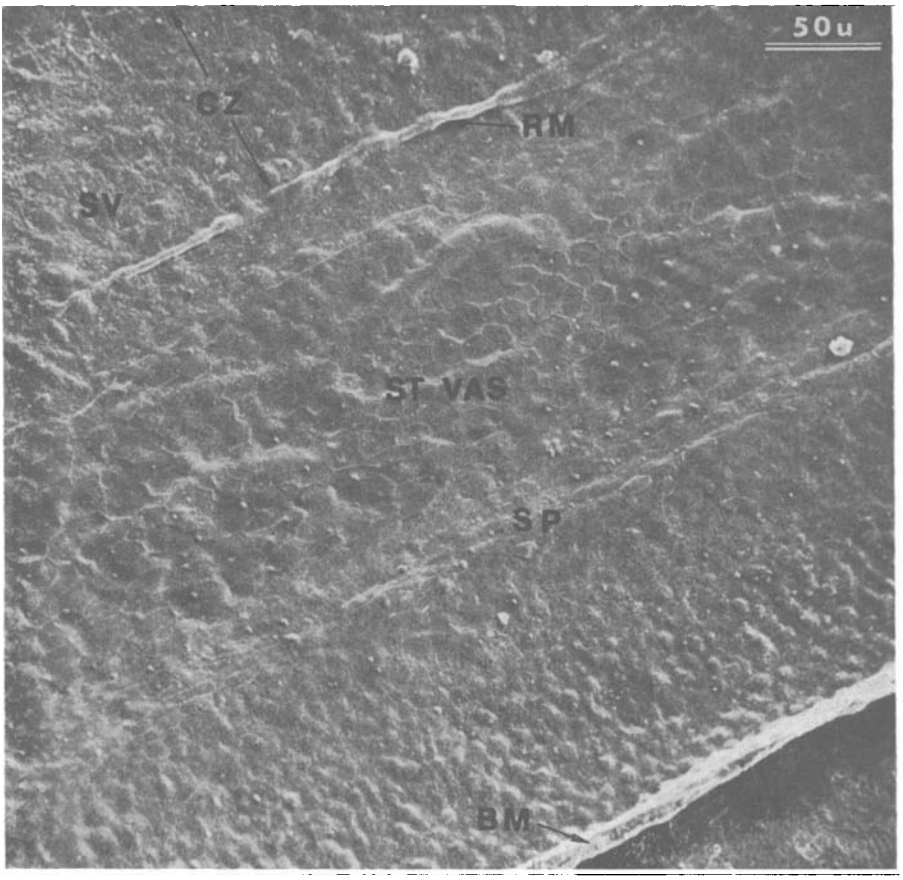


FIG. 2.

A scanning electron photomicrograph illustrating the stria vascularis and adjacent areas. Lateral surface of the scala vestibuli immediately above Reissner's membrane is covered with a layer of compact mesenchymal epithelial cells (CZ). Above this zone of compact cells, the micropores become evident. On the other hand, the lateral surface of the scala tympani demonstrates numerous micropores immediately beneath the basilar membrane. RM—Reissner's membrane; St Vas—stria vascularis; BM—basilar membrane; SV—scala vestibuli; ST—scala tympani. This picture originally appeared in *Trans. Am. Acad. Ophthal. and Otolaryng.*, **73,** 222, 1969.

the spiral ligament, the basilar membrane, the under-surface of the osseous spiral lamina and thin endothelium-like squamous cells lining the bony wall (Fig. 1).

The surface of the lower portion of the spiral ligament was covered with simple squamous cells similar to that of the vestibular crest but with abundant micropores and holes (Fig. 7). Frequently the deeper part of the mesh-like spiral ligament could be observed through these holes, suggesting the free communication of perilymph between the spiral ligament and scala tympani. As we move further away from the spiral

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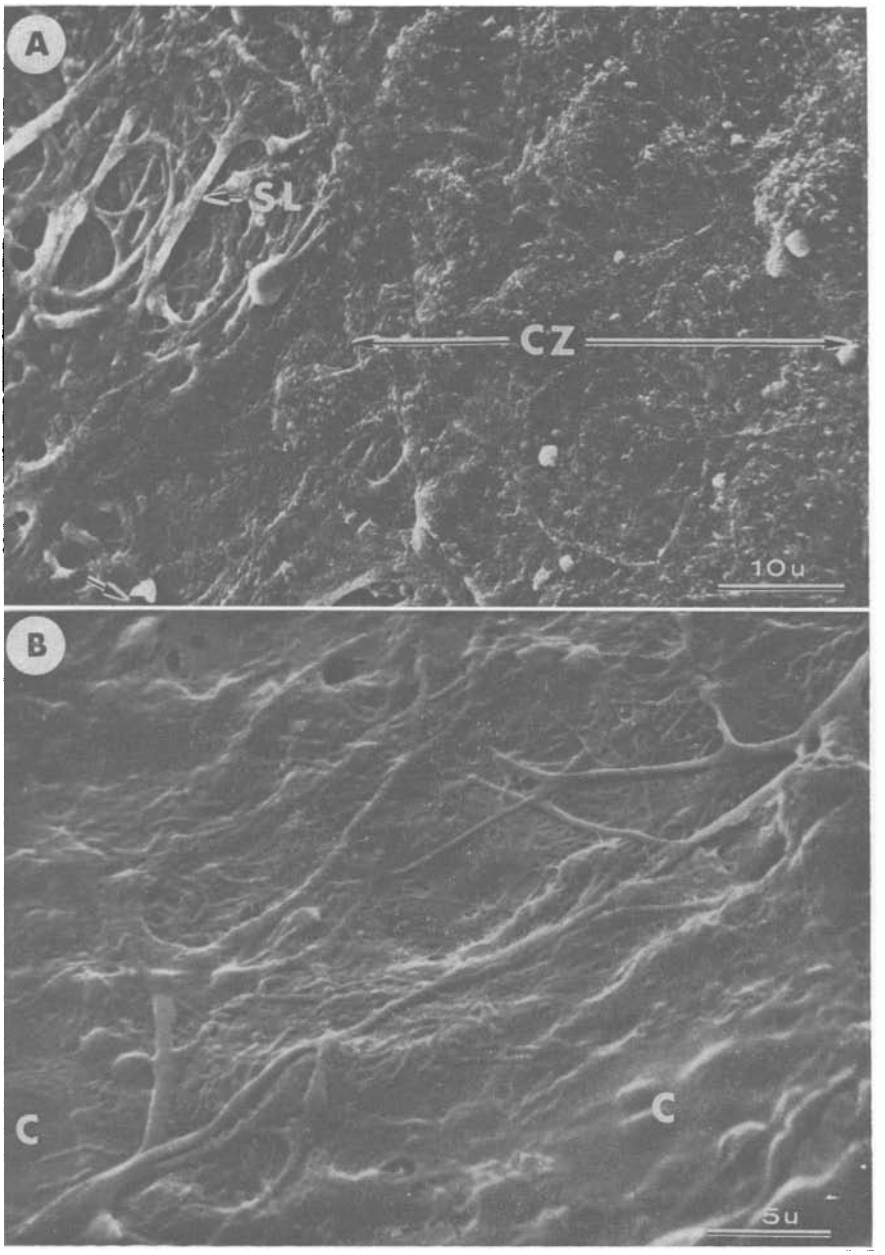


FIG. 3.

(a). A close-up view of the transitional zone where the cells are loosely attached showing a deep area of the exposed spiral ligament (SL). The cells observed in the compact zone (CZ) show the typical polyhedral cell arrangement. This micrograph also appeared in *Trans. Am. Acad. Ophthal. and Otolaryng.*, **73,** 222, 1969.

(b). Same region as in (a) showing some areas covered with squamous cells (C) in the apical turn. Some areas are completely devoid of this covering of squamous cells exposing the deeper part of the spiral ligament.

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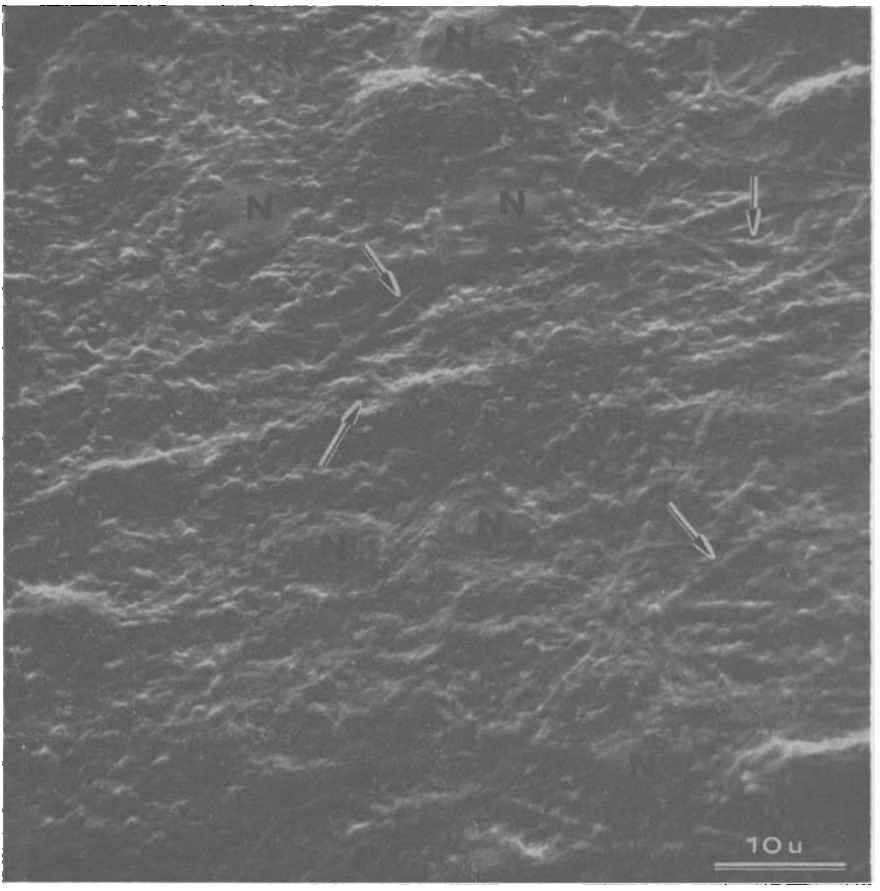


FIG. 4.

The area covering the lateral surface of the scala vestibuli. Flat squamous cells are shown and seem to make direct contact with the bony surface in such a way that the rough surface of the underlying bone can be appreciated. Some fibrotic bands underneath the cell surfaces are also seen (arrows). There were no appreciable micropores which could be observed on the under-surface of the spiral ligament. N—nucleus.

ligament the epithelium becomes thinner and the micropores and holes decrease in number, eventually disappearing, until the rough imprint of underlying bone can be observed beneath the thin squamous cells (Fig. 8).

The under-surface of the basilar membrane has an interesting texture as seen with light microscopic examination of the surface preparation. There was a distinct fibrillar arrangement which was orientated radially. At the basilar crest, longitudinal fibres were observed. The hexagonal cell arrangement abruptly disappeared and became fibrillar in appearance (Fig. 9a). The large nuclei of the basilar membrane cells were seen. In

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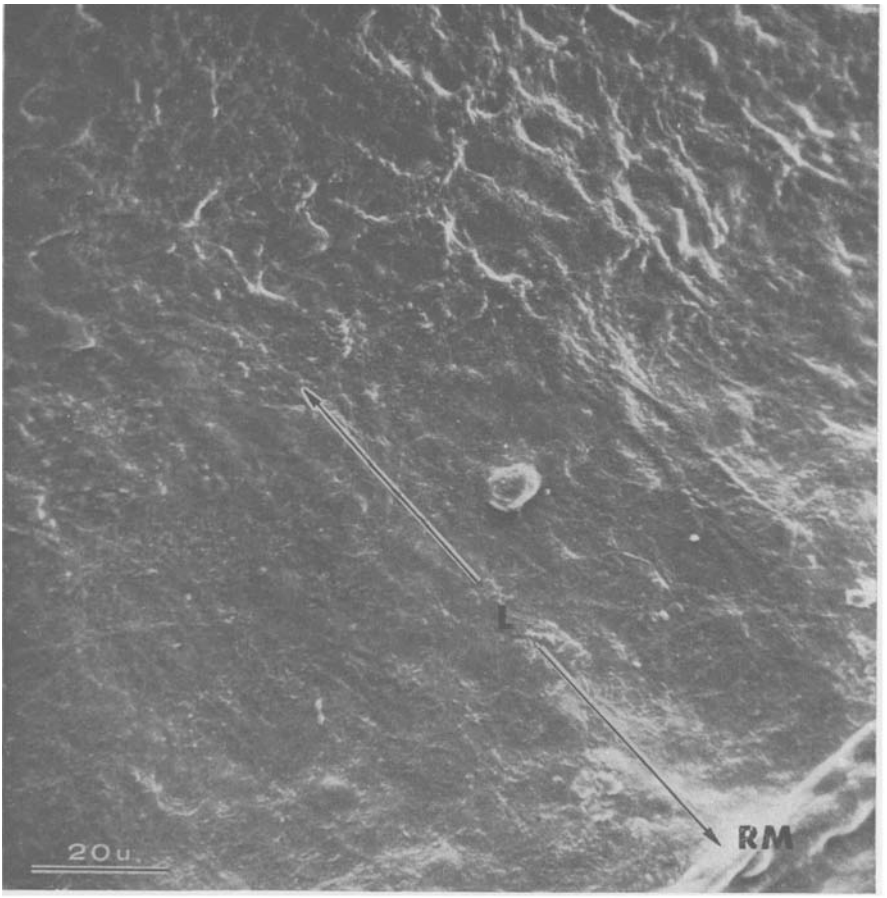


FIG. 5.

A survey scanning micrograph of the upper-surface of the osseous spiral lamina in the limbus. The spiral limbus appears to be covered by compact squamous cells (L). The upper-surface of the osseous spiral lamina is covered by cells similar to those described in Fig. 4. RM—Reissner's membrane.

one case, there was an area in the under-surface of the basilar membrane which appeared to be mesh-like, devoid of compact cell covering. In this area, the radial and longitudinal fibres of the basilar membrane could be seen at the basilar crest (Fig. 9b). In a few instances, where the basilar membrane was cracked due to drying, the two layers of bundles of the basilar membrane fibres were observed (Fig. 10a and b). This area was interpreted as the pars pectinata. When the basilar membrane was torn away, distinct fractured fibrils were seen, which agrees with the findings by light microscopy. Occasionally, the organ of Corti side of the basilar membrane was also observed and the point of attachment of the tono-filaments of the pillar cells were seen.

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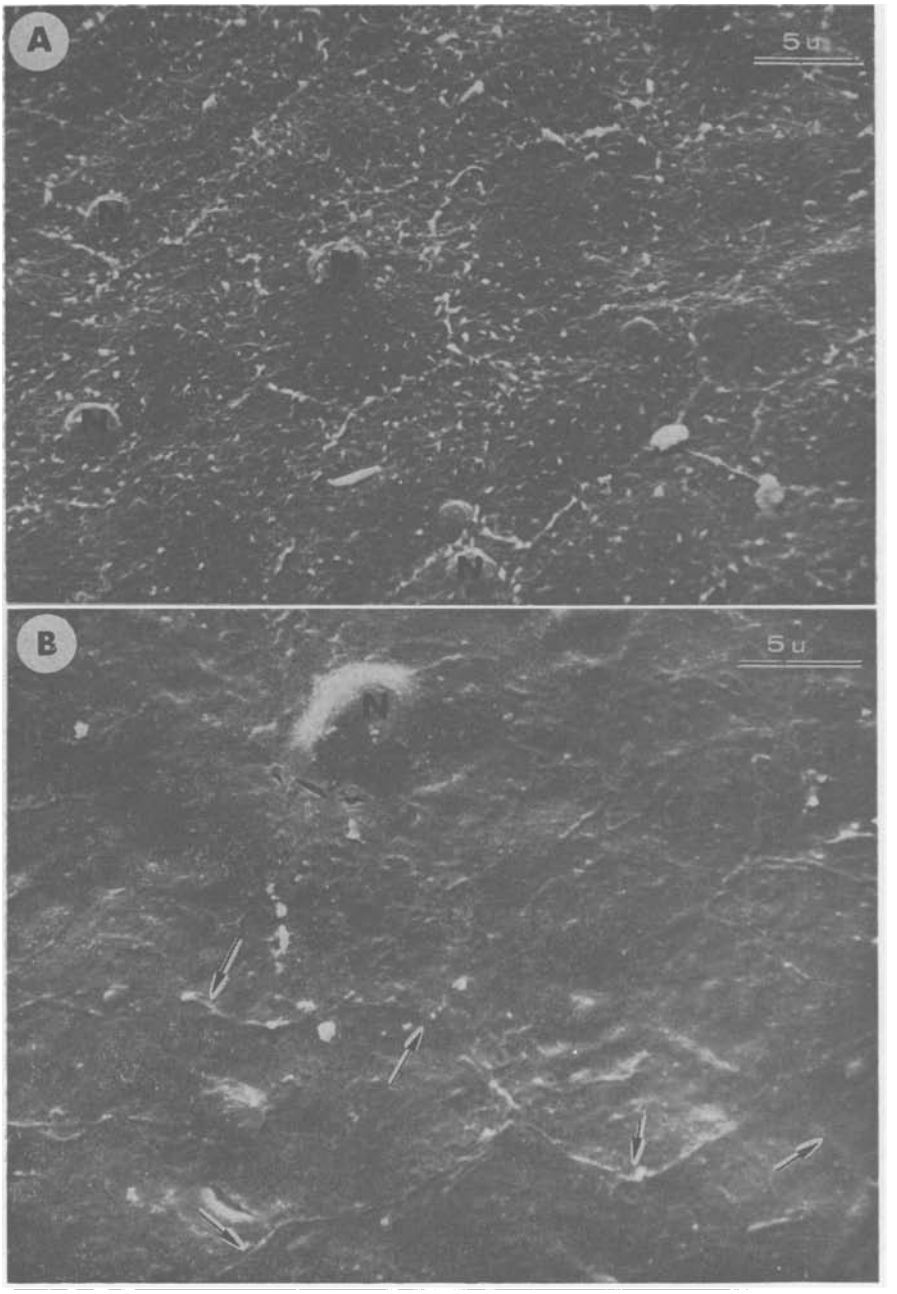


FIG. 6.

(a). A scanning photomicrograph showing the scala media side of the Reissner's membrane. The exaggerated silhouette of nuclei (N) and distinct polyhedral cell margins are seen. The fine freckle-like structures are interpreted as microvilli.

(b). The surface of the scala vestibuli side of Reissner's membrane showing a large cell with less distinct cell margins than that of the media side. Small cracks near the nucleus (N) are interpreted as artifact. The cell surface is smooth and occasionally the silhouette of cell organelles can be observed. The area of recognizable cell margins can be seen in this micrograph (arrows).

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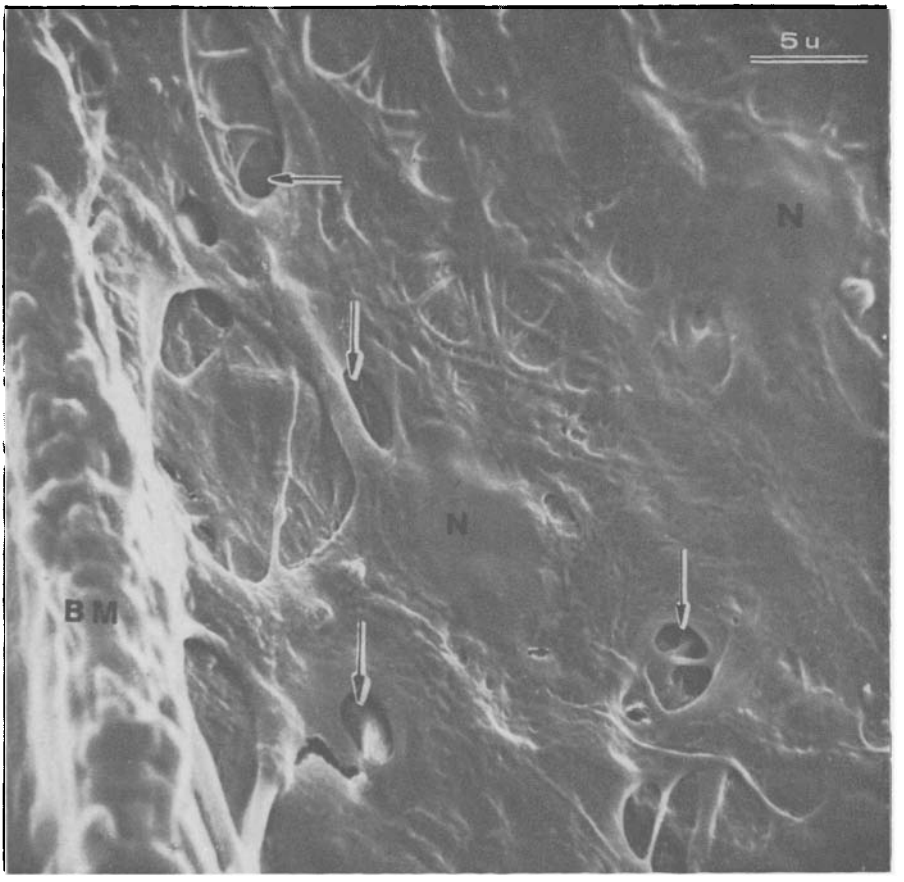


FIG. 7.

A scanning photomicrograph of the spiral ligament adjacent to the basilar membrane (BM) insertion showing rather loose cell coverings. The squamous epithelial cells possess abundant cell processes which overlap. Due to the incompleteness of this overlapping there appears numerous micropores (arrows) which expose the spiral ligaments. The silhouette of the nucleus of the epithelial cells can be seen (N).

The under-surface of the osseous spiral lamina is most interesting. Abundant micropores and holes covered the entire area in most of the specimens we examined (Fig. 11a and b). The occurrence of these holes varied considerably from animal to animal rather than with the cochlear turns in the same animal.

Discussion

The existence of Cortilymph, besides endolymph and perilymph was suggested initially by Wittmaack according to Rauch (1964) and also by Engström (1960). This concept was supported by the studies of Rauch (1960) and Tasaki *et al.* (1954) who favoured the idea that the Cortilymph is different from the endolymph but rather similar to the perilymph.

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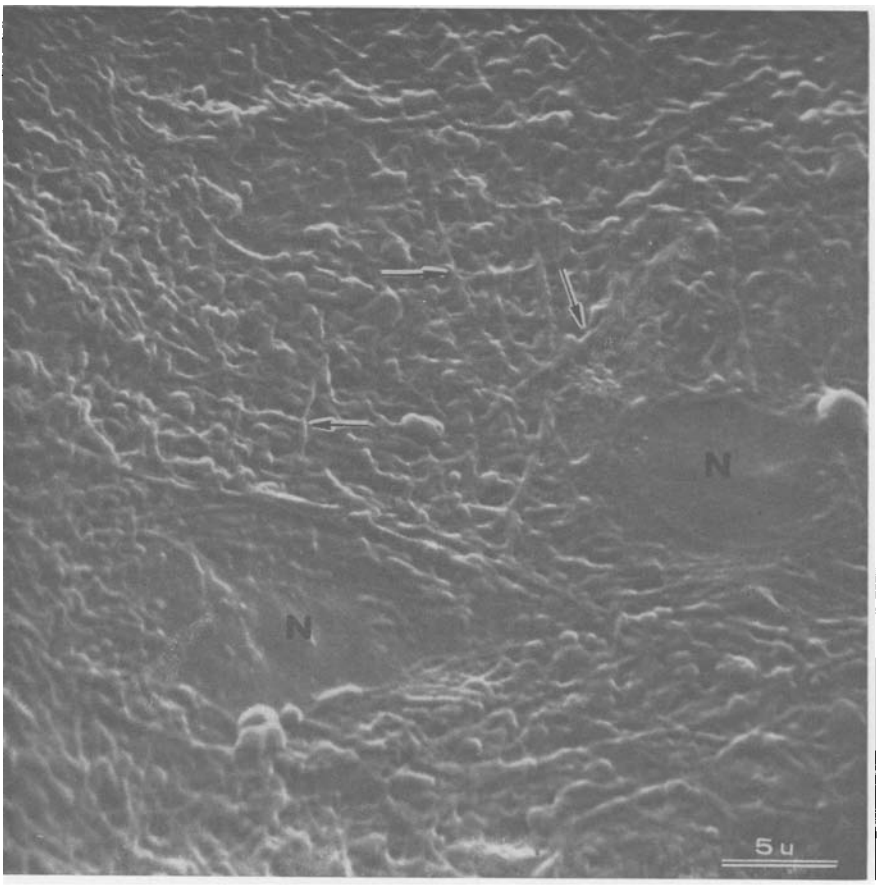


FIG. 8.

This survey scanning micrograph illustrates an extremely thin squamous type epithelium which covers the bony wall housing spiral ganglions in the scala tympani. The 'nuclei' (N) of the epithelial cells shown resemble pancakes. The abundant fibrillar arrangement (arrows) of the thinning-out spiral ligament can be observed. Many globular bodies, intermixed with fibrillar structures, are interpreted as the silhouette of the hard bony tissue of the otic capsule.

The electron microscopic examination of the cochlea by Engström (1955) and Spoendlin (1966) failed to demonstrate any channels which can permit free flow of the perilymph between the two chambers. Churchill, Schuknecht and Doran's histochemical study (1956) suggested that there might be microchannels which permit free communication of the perilymph between the scala tympani and Corti's tunnel. The openings of these channels (Schuknecht's canaliculae perforantes) are believed to be located on the under-surface of the osseous spiral lamina, halfway between the habenula perforata and the spiral ganglion. The recent study of Ilberg (1968a) demonstrated the free passage of thorium dioxide particles (40 Å) from

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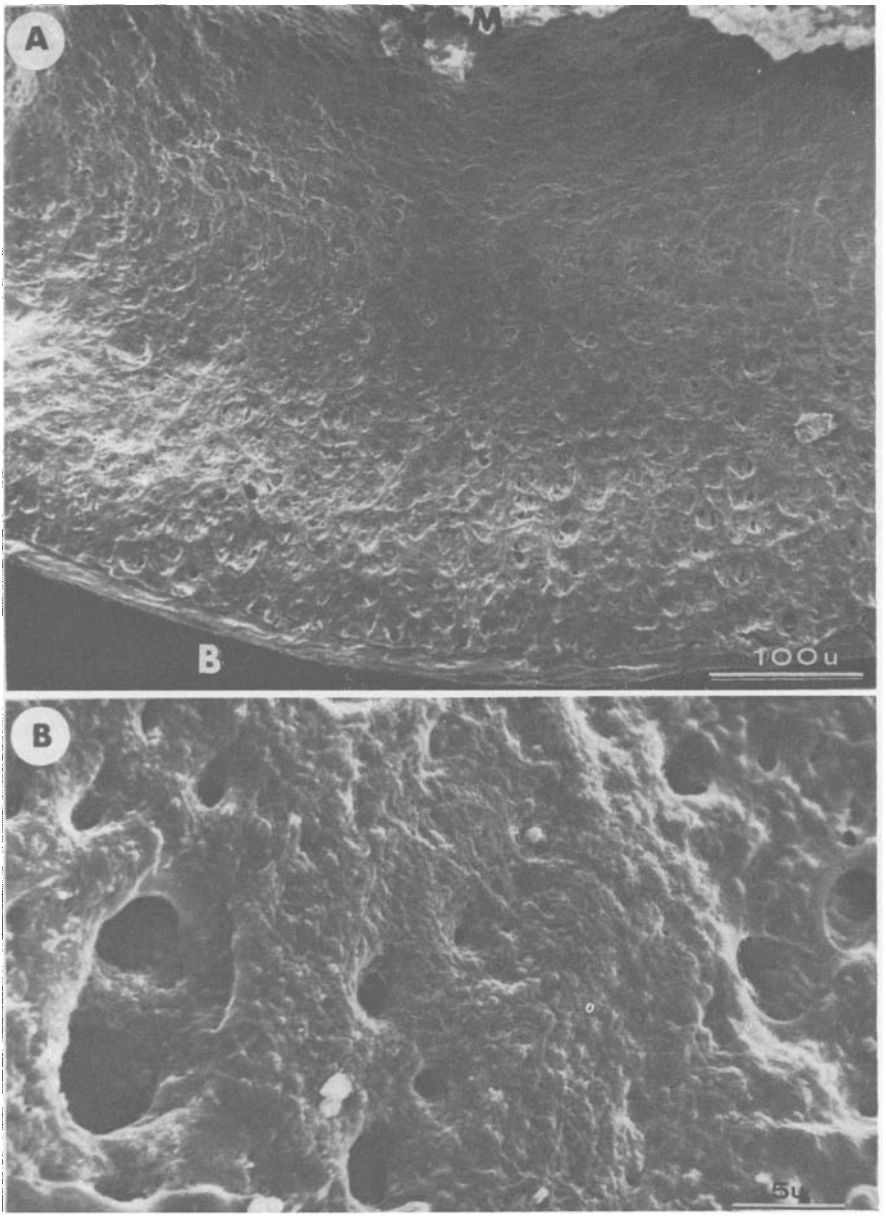


FIG. 9.

(a). A survey micrograph illustrating the under-surface of the basilar membrane which attaches to the spiral ligament (arrows). Regular protrusions seen on the surface of the membrane are interpreted as the nuclei of the basilar membrane cells. The zone near the attachment shows a rather smooth surface.

(b). A higher magnification of the basilar membrane surface illustrating radial (R) and longitudinal (L) fibre arrangements. The mesenchymal cell covering is not complete in this region, revealing numerous micropores and the mesh-like structure of the fibrous membrane.

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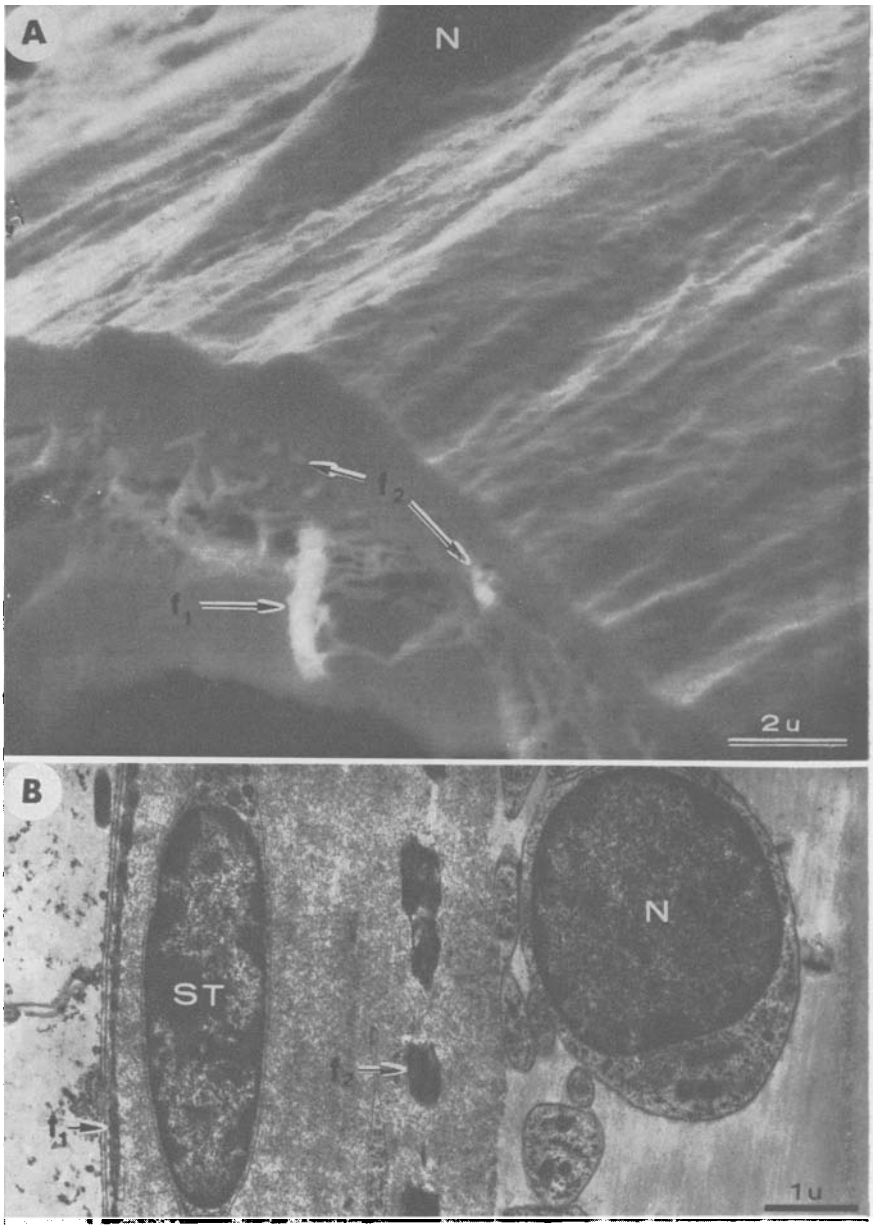


FIG. 10.

(a). This scanning photomicrograph illustrates the fractured surface of the basilar membrane (zona pectinata). Two layers (f1 and f2) of the fibrillar bundles can be seen. The protrusion on the surface of the membrane is interpreted as the nucleus of the basilar membrane cell. The basilar membrane, in this picture, is upside down showing the scala tympani side up. (b). A transmission micrograph of the same area illustrates two layers of fibres (f1 and f2) and two mesenchymal epithelial cells (N) in cross section, and a spindle-shaped stroma cell of the basilar membrane (ST).

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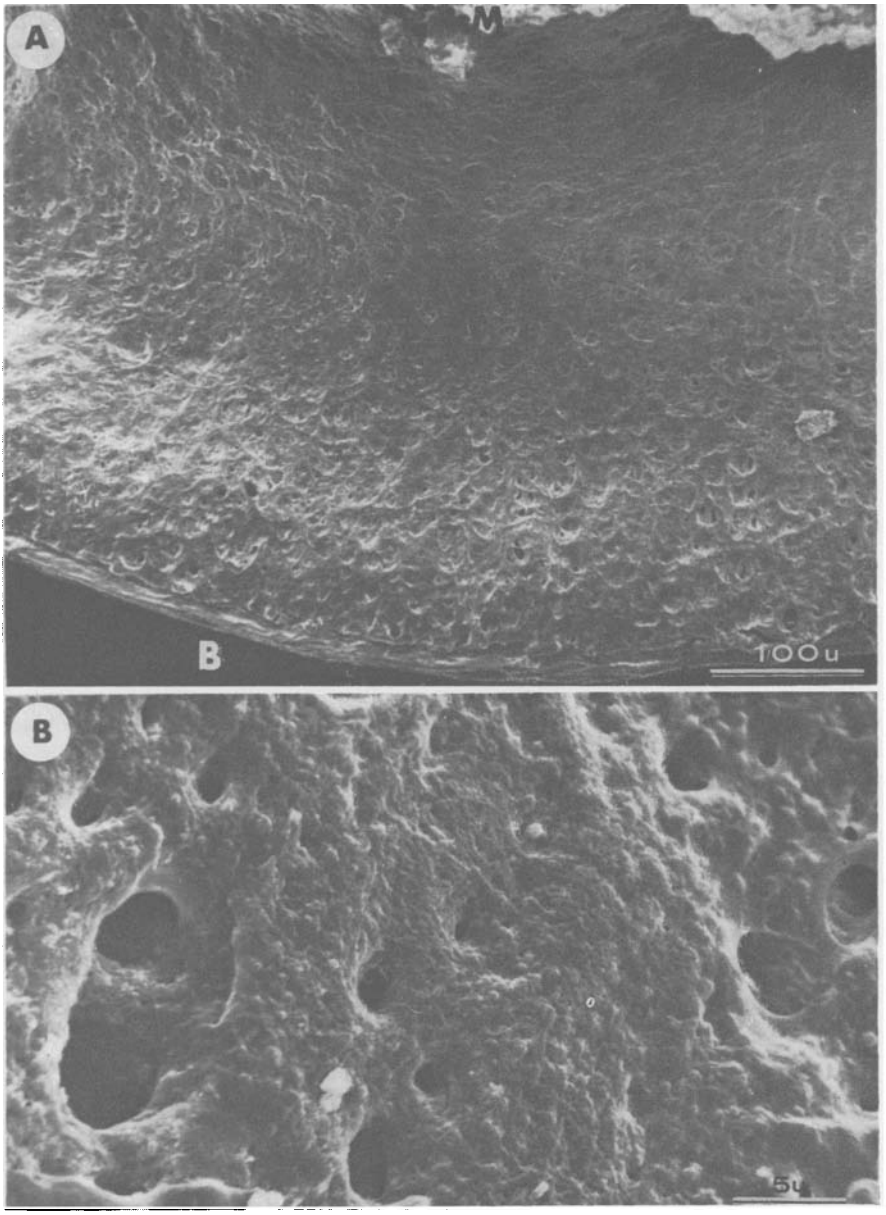


FIG. 11.

(a). A survey scanning photomicrograph illustrating the under-surface of the osseous spiral lamina (scala tympani side). The top of the picture is the modiolar side (M) and the lower margin is the attachment of the basilar membrane (B). Observe the irregular surface with numerous micropores.

(b). A higher magnification photomicrograph of above. The picture shows numerous micropores. Unlike the spiral ligament side, no distinct fibres can be seen except for the fine fibrillar arrangement over the bone.

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the scala tympani into Corti's tunnel through the basilar membrane as well as through the habenula perforata. The present finding—the under-surface of the osseous spiral lamina revealing numerous micropores or holes which can permit free communication of perilymph—leads us to believe that the perilymph can easily pass through the under-surface of the osseous spiral lamina. Whether these holes continue into the aforementioned bony channels is not conclusive at this point.

Due to the numerous extracellular spaces found in the spiral ligament as demonstrated by electron microscopy, it is conceivable that the spiral ligament is bathed with perilymph (Spoendlin and Balogh, 1963; Takahashi and Kimura, 1970). The thorium dioxide study of Ilberg (1968b, 1968c) and also the study of Tonndorf *et al.* (1962) support the above concept. Electron microscopic examination of the squamous cells of the perilymphatic space has been rather sketchy; however, it is generally considered that they are of mesenchymal origin. Therefore, it is not surprising to find that the cell margins at times overlap and at other times make no contact at all. In the case of the vestibular lining of Reissner's membrane the cells seem to cover continuously the wide area of the membrane. So far we were unable to confirm the electron microscopic findings of the wide intercellular spaces among these cells reported by Hagiwara (1963) and Duvall and Rhodes (1967). However, the area covering the spiral ligament and the under-surface of the osseous spiral lamina showed numerous micropores or holes. These holes are thought to be due to the incomplete covering of the mesenchymal epithelial cells which possess multiple cell processes randomly overlapping. It is interesting to note that Davis (1958) observed no significant barrier to diffusion of fluid or to electric current flow between the scala tympani and the interior of the spiral ligament. His finding seems to be supported by my observation that the surface covering the spiral ligament at the scala tympani possessed numerous micropores, much more than the scala vestibuli side, which should permit free flow of perilymph.

The electron microscopic observation of the spiral ligament (Takahashi and Kimura) also revealed that the luminal surface of the spiral ligament, where it faces the scala vestibuli, is randomly covered by "mesenchymal epithelial cells" and cells similar to "fibrocytes", which are believed to be actively engaged in the inner ear fluid metabolism. Whereas Iurato (1967) described these cells as "connective cells" and others as "tympanic border cells". Immediately above Reissner's membrane where the spiral ligament faces the scala vestibuli, a more compact squamous cell covering was noted in my study.

Borghesan (1957) on the basis of rich vascularity found in this area (vasculo-epithelial zone) postulated the spiral limbus, facing the scala vestibuli, secretes perilymph. However, the micropores were not obvious on the perilymphatic surface of the spiral limbus.

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The ultrastructure of the basilar membrane has been carefully studied by many investigators (Iurato, 1962; Engström, 1955; Lawrence *et al.* 1961; and others) by electron microscopy. The scanning microscopic observation that the basilar membrane is composed of amorphous ground substance, fibrous layers and tympanic covering cells are in agreement with their findings. In the zona pectinata the fibrous layers are formed by two separate fibrillar bundles which are embedded in the ground substance. Whereas, in the zona tecta the ground substance is thinner than the fibrous layer which is formed by a single layer of fibrils.

Despite the numerous technical difficulties in examining the surface topography of the perilymphatic space, the scanning electron microscope procedure gave valuable information, particularly regarding the distribution of the micropores and the general arrangement of the epithelial surface. Unlike the endolymphatic surface of the cochlear duct, the perilymphatic space revealed numerous micropores or holes suggestive of a free communication of the perilymph with the underlying structures, such as the spiral ligament, under-surface of the osseous spiral lamina, under-surface of the basilar membrane and to a much lesser degree the spiral limbus.

Originally, the micropores of the perilymphatic surface were interpreted as an artifact. However, the consistency of the same finding in the scanning electron microscopic specimen and the transmission electron microscopic observations (Takahashi and Kimura, and others) lead us to believe that these holes are an anatomic reality.

Conclusion

The fine morphology of the surface architecture of the cochlear perilymphatic space was studied with a scanning electron microscope. The magnification ranged between 20–20,000×. A large part of the cochlear perilymphatic space was covered by cells resembling fibrocytes and mesenchymal epithelial cells. These cells possessed many long cell processes which often made contact with each other leaving many uncovered bare areas. These areas resembled holes or pores. The observation of these numerous holes or micropores supports the speculation that the cochlear perilymphatic covering allows free communication of the perilymph with the underlying structures, such as the spiral ligament, undersurface of the osseous spiral lamina and basilar membrane, and to a much lesser degree the spiral limbus.

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