Arch Otorhinolaryngol (1987) 244:284–287

Archives of
Oto-Rhino-
Laryngology

Ultrastructure of the endolymphatic sac in the mongolian gerbil*

M. Barbara[1], H. Rask-Andersen[2], and D. Bagger-Sjöbäck[3]

[1] ENT Department, University "La Sapienza", Rome, Italy
[2] ENT Department, Akademiska Hospital, Uppsala, Sweden
[3] ENT Department, Karolinska Hospital, Stockholm, Sweden

Summary. We describe our findings in an ultrastructural study of the endolymphatic sac of the mongolian gerbil. In conjunction with its specific renal physiology, enabling this animal to withstand long-term water deprivation, we have used our findings to hypothesize the existence of a local monitoring system within the endolymphatic space. The presence of elastic tissue in the subepithelial space of the endolymphatic sac could explain the mechanism through which this structure equilibrates endolymphatic pressure changes. Finally, we propose that a secretion of water-retaining macromolecules may act through osmotic forces to modulate inner ear fluid.

Key words: Mongolian gerbil – Endolymphatic sac – Transmission electron microscopy – Elastic fibers

Introduction

The endolymphatic duct (ED) and sac (ES) have been considered previously to be a relatively inactive part of the membranous labyrinth. In comparing their function with the auditory and vestibular sensory organs, they have been mostly regarded as a reservoir for waste products coming mainly from these other parts of the inner ear.

In past years, some investigators have suggested that the ED and ES actually have important biological functions. Among these, a pressure regulatory role has been proposed for the entire endolymphatic system [1, 6]. In fact, this portion of the membranous labyrinth is unique as it usually appears to constitute a non-rigid, compliable compartment which could possibly assimilate small internal pressure variations.

Recently, Friberg et al. [4] suggested that the ES might act as an active pressure regulator through secretion of water-retaining macromolecules into its lumen. This would enable the sac to both decrease and increase the endolymphatic hydrostatic pressure either by a secretory or an absorptive mechanism. We recently examined the general morphology of the ED and ES of a desert rodent, the mongolian gerbil *(Meriones unguiculatus)* [3]. In this animal species, the ED and ES are entirely covered by bone and, therefore, no real compliable extraosseous portion exists. In this case, a fine internal mechanism able to cope with the regulation of the hydrostatic pressure within the endolymphatic compartment would seem to be important. Our studies demonstrated the presence of a homogeneous substance almost completely filling the lumen of the gerbilline ES, together with the presumed existence of elastic tissue in its subepithelial layer. This finding directed us to look for more detailed information through an ultrastructural study of the gerbilline ES.

Materials and methods

Mature mongolian gerbils were used in this study. After the animals had been sacrificed by decapitation, the temporal bones were immediately removed and fixed in a solution containing 2% glutaraldehyde in phosphate buffer and 0.1 *M* Na-EDTA as decalcifying agent (pH 7.4). The solution was renewed every other day for 14 days. The specimens were postfixed in 1% buffered osmium tetroxide, dehydrated in graded alcohols, and embedded in Agar-100 resin. Ultrathin sections were cut, mounted on copper grids, stained with lead citrate

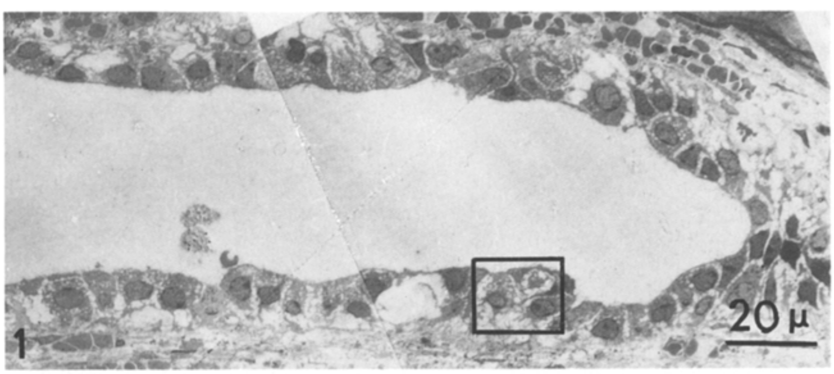
Offprint requests to: M. Barbara, Department of Oto-Rhino-Laryngology, University "La Sapienza", 00185 Rome, Italy
* Supported by grants from the Swedish Medical Research Council N. 00720-03908 and from the Centro Nazionale delle Ricerche, Italy

and uranyl acetate, and finally observed and photographed in a Philips 400 transmission electron microscope.

Results

The epithelial lining of the ES appears smooth in the proximal and distal parts, but somewhat more rugose in the intermediate portion. The majority of the epithelial cells are cuboidal or wedge-shaped, but some flat cells can also be observed (Figs. 1, 2). The light and dark cells display features similar to those found in other species, whereas the granular cells are predominant, and are more or less packed with membrane-bound vesicles that are approximately 0.5 μm in size. These structures thus give the epithelium a general areolar appearance and seem to be specific for this animal species. In the granular cells the vesicles can be found both supranuclearly and infranuclearly, hence giving the cells a "Swiss-cheese" appearance. The vesicles generally contain a granular or floccular material.

The epithelial cells are not infrequently separated by clear intercellular spaces of varying sizes and shapes (Fig. 2). These lateral intercellular spaces (LIS) constitute large areas within the epithelium. They are in general quite wide all the way up to the tight junctions, separating them from the endolymphatic compartment. The LIS are particularly wide in



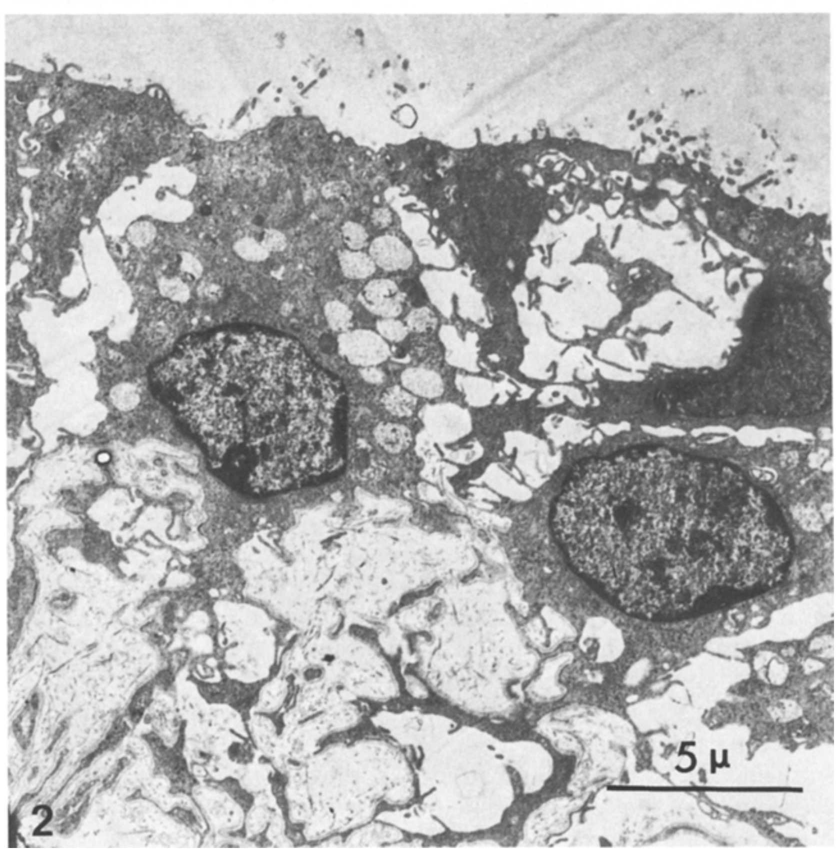


Fig. 1. Low-power electron micrograph of a cross-section of the intermediate portion of the gerbilline endolymphatic sac (ES). The epithelial surface appears slightly rugose and the majority of the epithelial cells are either cuboidal or wedge-shaped. Flat epithelial cells can also be recognized

Fig. 2. High-power micrograph of the epithelium of the gerbilline ES, showing epithelial cells rich in membrane-bound vesicles. Clear areas or lateral intercellular spaces (LIS) separate individual epithelial cells. At some places LIS are wide, with only tight junctions separating them from the lumen. The basal lamina runs irregularly and often projects between cellular invaginations

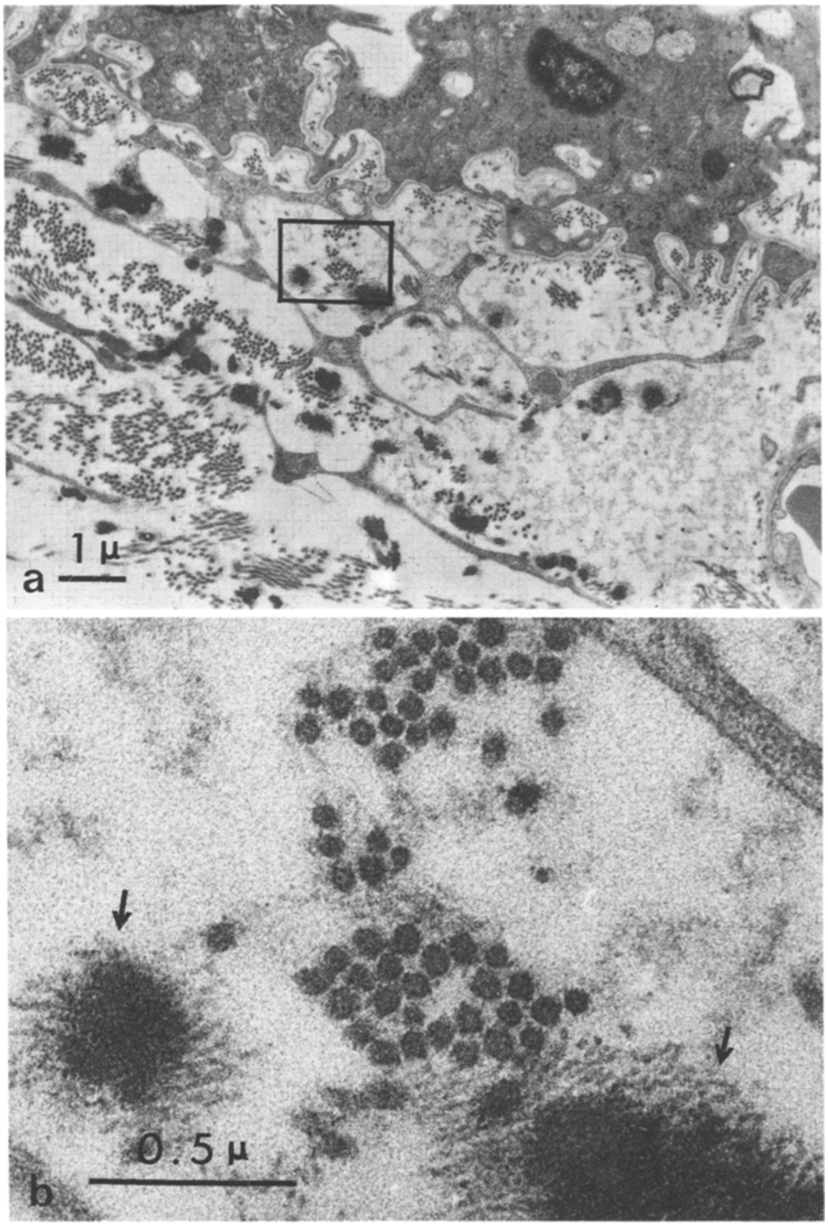


Fig. 3. a Electron micrograph of the subepithelial space of the intermediate portion of the gerbilline ES. A trabecular network is formed through the cellular ramifications of the fibrocytes. Fibrillar structures and a floccular substance can be observed among the cellular projections. The framed area is magnified in Fig. 3b. **b** Inset of Fig. 3a, showing the loose subepithelial tissue containing longitudinally oriented collagen fibrils. Bundles of thin fibrils *(arrow)* resembling elastic fibers are also observed

the lower portion of the epithelium towards the subepithelial space and show evidence for containing bundles of collagen fibrils in this region. At certain points the LIS widen into large lacunae that are separated from the lumen of the sac only by a thin bridge of epithelial cells.

The subepithelial tissue is generally loose and traversed by several small and a few larger blood vessels (Fig. 3a). The subepithelial space is separated from the basal portion of the epithelium by a thin, irregular basal lamina. It is sometimes split and projects between the cells, forming invaginations that are partially occupied by bundles of collagen fibrils (Fig. 3a). These fibrils in general seem to have a longitudinal orientation along the sac and dominate the subepithelial space. A finer type of fibril is also present, arranged in dense bundles or interspersed between the collagen fibrils in the subepithelial space. Morphologically, these fibrils resemble elastic tissue (Fig. 3b). The subepithelial space contains a large amount of a floccular substance and is divided into smaller compartments by fine cellular trabeculae emanating from fibroblasts situated beneath the basal lamina. Small blood vessels in the subepithelial space lie interspersed between these trabeculae. Besides occasional erythrocytes, only a few freely floating cells occur in the lumen of the ES. These cells seem to have phagocytic properties, as they display intracytoplasmic lysosomal-like bodies and a rough cell coat (or dendrites).

Discussion

The dynamics of the inner ear fluids have been discussed by several investigators. In 1976, Lundquist [7] proposed the so-called "dynamic flow theory", thus combining the two earlier main flow theories, which differ as to the possible site of reabsorption of the endolymph. The longitudinal flow theory, as postulated by Guild [5], suggests that the endolymph is secreted by the stria vascularis and reabsorbed in the endolymphatic sac. In contrast, Naftalin and Harrison [8] proposed a radial-flow theory, in which endolymph is thought to be reabsorbed in the same site as it is produced. In his "unitary theory", Lundquist [7] attributed specific roles to the different epithelial cell types found in the ES and used the guinea pig as an animal model. The light cell is extremely rich in microvilli and vacuoles and was presumed to be involved mainly in absorptive and pinocytotic activity. A phagocytic property was hypothesized for the dark cell on the basis of its often elongated cellular processes.

The ES of the mongolian gerbil is suitable for studies of inner ear fluid homeostasis, mainly as a result of its structural properties. In contrast to other species, the ES of the mongolian gerbil is entirely covered by bone, which means that there is no true extraosseous portion. This may have a functional application, since this portion has been suggested to have a pressure regulating potential. However, other internal mechanisms could intervene for this purpose. The presence of elastic fibers in the subepithelial space of the ES in man has already been described by Bagger-Sjöbäck et al. [2]. To our knowledge, such a rich amount of elastic tissue has not been mentioned in any earlier study on the ES. The unusually rich amount of elastic fibers could indicate that the gerbilline ES has elastic or compliant properties, which could be important for the regulation of pressure in the endolymphatic compartment. This could account for the reduction of harmful pressure variations around the delicate inner ear sensory structures.

Recently, Friberg et al. [4] and Rask-Andersen et al. [9] found signs of secretory activity in the ES. Such activity seems to be attributable to the so-called light cells. In animals subjected to surgical labyrinthectomy as well as in mouse mutants showing strial dysfunctions, the light cells of the ES seemed to secrete glycoproteins. These findings indicated that reduced endolymph production in the stria vascularis might result in a compensatory secretion of endolymph in the ES, raising the endolymphatic hydration or pressure.

Our studies of the mongolian gerbil have shown that many of the epithelial cells normally display secretory-like vesicles, which contain a floccular material of similar appearance as the substance normally occurring in the sac lumen. This would seem to indicate that the epithelium may have a secretory potential. In order to verify such a discharge of macromolecules out into the sac lumen, tracer or labelling studies are currently being performed. The possible mechanism resulting in an increased endolymphatic pressure may involve the secretion of macromolecules into the ES, leading to either an increased hydrostatic pressure or a secondary osmotic attraction of water. In the light of such a mechanism, the elastic fibers in the ES could be essential in order to assimilate or modulate pressure variations created by the secretion of water-retaining substances into the sac. This could mean that the gerbilline sac may act as an active pressure regulator by both absorbing and secreting endolymph through the epithelial layer.

Acknowledgement. We are grateful to Miss Anne-Marie Lundberg for skillful technical assistance.

References

1. Allen GW (1964) Endolymphatic sac and cochlear aqueduct. Arch Otolaryngol 79:322–327
2. Bagger-Sjöbäck D, Rask-Andersen H (1986) The permeability barrier of the endolymphatic sac. A hypothesis of fluid and electrolyte exchange based on freeze fracturing. Am J Otol 7:134–140
3. Barbara M, Rask-Andersen H, Bagger-Sjöbäck D (in press) Morphology of the endolymphatic duct and sac in the mongolian gerbil. Acta Otolaryngol (Stockh)
4. Friberg U, Wackym PA, Bagger-Sjöbäck D, Rask-Andersen H (1986) Effect of labyrinthectomy on the endolymphatic sac. A histological, ultrastructural and computer-aided morphometric investigation in the mouse. Acta Otolaryngol (Stockh) 101:172–182
5. Guild SR (1927) Observations upon the structure and the normal contents of the ductus and saccus endolymphaticus in the guinea-pig *(Cavia cobaya)*. Am J Anat 39:1–56
6. Kolmer W (1923) Mikroskopische Anatomie des nervösen Apparates des Ohres. In: Alexander G, Marburg O (Hrsg) Handbuch der Neurologie des Ohres, Vol 1. Urban und Schwarzenberg, Berlin, S 101-174
7. Lundquist P-G (1965) The endolymphatic duct and sac in the guinea-pig. An electron microscopic and experimental investigation. Acta Otolaryngol (Stockh) [Suppl] 201:1–108
8. Naftalin L, Harrison MS (1958) Circulation of labyrinthine fluids. J Laryngol 72:118
9. Rask-Andersen H, Steel K, Erwall K, Friberg U (1987) The endolymphatic sac in the mouse mutant with cochleo-saccular degeneration. Electrophysiological and ultrastructural correlations. Hear Res 26:177–190

Received December 30, 1986 / Accepted June 22, 1987