Ultra-Structural Study of the Lateral Portion of the Auditory Sensorial Organ Using a Decalcification-Free Method

Iván Méndez-Benegassi, Almudena Trinidad, José Ramón García-Berrocal, Cristina Martín, Rodrigo Martínez-Monedero, José María Verdaguer, and Rafael Ramírez-Camacho
Grupo de Investigación Otológica, Servicio de Otorrinolaringología, Hospital Universitario Puerta de Hierro, Universidad Autónoma de Madrid, Madrid, Spain

Objective: To explain the development of a new personal technique to study the spiral ligament and stria vascularis in Guinea pig cochleae by obtaining sample tissue without decalcification and to assess its validity for electron microscopy analysis.
Material and method: Samples were taken from 5 female Guinea pigs weighing 200-250 g and were fixed in glutaraldehyde and paraformaldehyde for analysis of the spiral ligament and stria vascularis ultrastructure by transmission electron microscopy.
Results: All of the ultrastructure components in the spiral ligament and stria vascularis could be examined without the need for decalcification.
Conclusions: Our method to obtain and analyze samples of cochlea side wall is valid, easy and faster.

Key words: Stria vascularis. Spiral ligament. Fibrocyte. Transmission electron microscopy. Ultrastructure. Inner ear.

Estudio ultraestructural de la porción lateral del órgano sensorial auditivo mediante un método sin descalcificación
Objetivo: Explicar el desarrollo de una técnica personal de estudio de la estría vascular y ligamento espiral de la cóclea en cobaya mediante la obtención de la muestra sin necesidad de descalcificación y valorar su validez para su análisis con microscopio electrónico.
Material y método: Se obtuvieron muestras de 5 hembras de cobaya de 200-250 g que se fijaron con glutaraldehído y paraformaldehído, y se analizó la ultraestructura del ligamento espiral y la estría vascular obtenidos mediante microscopio electrónico de transmisión.
Resultados: Se pudo observar todos los componentes ultraestructurales del ligamento espiral y la estría vascular sin necesidad de descalcificar.
Conclusiones: Nuestro método de obtención de las muestras es una forma válida, sencilla y más rápida que otros métodos para la obtención y el estudio de la porción lateral de la cóclea.

Palabras clave: Estría vascular. Ligamento espiral. Fibrocito. Microscopio electrónico de transmisión. Ultraestructura del oído interno.

INTRODUCTION

Inner ear pathologies represent the greatest challenge posed to ENT specialists at the start of the 21st century. While the second half of the 20th century saw advances in the medical and surgical treatment of the middle ear and its related cavities, success with cochlear implants has aroused interest in an awareness of the pathology of the inner ear. Hearing loss of genetic origin, alterations in hearing secondary to endogenous and exogenous intoxications, presbyacusia and the effect of exposure to noise on the ears have been the challenges posed after the first description of the reversibility of certain types of sensorial hearing loss.[1,2]

After a phase in which imaging techniques of the inner ear[3-7] have been updated, this prior experience is now the basis for positing physiopathological experimentation correlating the ultra-structural findings with the aggression mechanisms. The possibility of integrating morphological, functional, immunohistochemical[8,9] and analytical data implies a multiple approach to conditions of great clinical

The present paper has been partly funded under the Health Research Fund Project, code FIS P1050673 and a Ministry of Education and Science grant, code MECCT2005-08593BQU.

Correspondence: Dr. I. Méndez-Benegassi Silva.
Grupo de Investigación Otológica. Servicio de Otorrinolaringología.
Hospital Universitario Puerta de Hierro.
San Martín de Porres, 4. 28035 Madrid. España.
E-mail: ibenegassi@hotmail.com

Received April 10, 2008.
Accepted for publication May 5, 2008.

interest, with mechanisms of action that allow the re-interpretation of well-known phenomena.

The stria vascularis and the spiral ligament are structures that are gaining in interest in recent years. Their function in the conservation of electrolytic balance, basically in the regulation of potassium, is of great importance when it comes to explaining lesions, reversible or otherwise, in the organ of Corti.

Procedures intended for the morphological study of the inner ear have presented limitations in terms of the need for a special histological technique. The immediate overlapping of structures with very different consistencies (bone in the otic capsule, delicate structures in the membranous labyrinth, nerve, and endolabyrinthine liquids) is the reason why the processing of specimens requires special skill in order to avoid the destruction of the study tissues. Insufficient decalcification may give rise to a non-decalcified bone fragment that does not tear the soft tissues, as well as the possible deterioration of the blade used. This fact is intensified when performing ultra-structural transmission techniques, in which the specimen must be sculpted to obtain semi-fine slices allowing the choice of the area desired in order to submit them to electron microscopy.

While scanning electron microscopy (SEM) does not require decalcification of the specimens but rather the extraction of the side wall of the cochlear spiras, as it solely offers a surface view, transmission electron microscopy (TEM) does require decalcification of the walls of the cochlea and the modiolus prior to the performance of the semi-fine slices.

The goal of the present paper is to present a technique, developed by one of our group (RRC), for the extraction of the structures alongside the organ of Corti (spiral ligament and stria vascularis) without the need to subject the cochlea to decalcification, by applying manoeuvres from the preparation of specimens for SEM to the obtention of processed tissues for TEM.

MATERIAL AND METHOD

Animals

Five female albino guinea pigs weighing 200-250 g were included and the regulations on protection of laboratory animals were followed in line with Royal Decree 1201 dated October 21, 2005 (Official Gazette n.º 252).

Anaesthesia/Intravital Perfusion

After intraperitoneal injection of ketamine (100 mg/kg) and Valium® (5 mg/kg), intracardiac-intraaortic perfusion was performed, with lavage of the blood volume by means of 300 mL of saline solution at 0.9% and intravital fixing, with a volume of fixing solution 1.5 times the animal's weight, comprising glutaraldehyde at 2.5% and paraformaldehyde at 1% in a 0.1 mol phosphate buffer (PB or Sorensen's buffer) with a pH of 7.4 administered by means of a Masterflex peristaltic perfusion pump (Cole-Palmer, Illinois, United States) for 7-10 min.

Dissection of the Cochlea

After separating the head from the body, the skullcap is opened up along its midline and both temporal bones are extracted to expose the contents of the tympanic ampulla (Figure 1A). Subsequently, using an otological surgery microscope, the intracochlear perfusion of 0.5-1 mL of fixing solution is performed with the plastic sheath of an Abbocath® nº 25 needle, through the oval window, after extraction of the stirrup and perforation of the round window with an insulin needle, in order to achieve circulation of the fixing solution through the ramps of the cochlea.

Extraction of the Specimen from the Membranous Labyrinth

Under microscopic control and using an insulin needle, an orifice is made at the apex of the cochlea with gentle rotating movements, similar to the creation of the safety hole in the footplate during stapedectomy.

The tip of the needle must penetrate into the otic capsule but not enter the underlying spaces in order to avoid damaging the membranous labyrinth. From the orifice created, the nearby bone is gradually fractured by means of delicate movements from the inside out to reveal the contents of the tympanic, middle and vestibular ramps, normally hidden by the outer face of the spiral ligament running like a banister and preventing the direct sighting of the organ of Corti (Figure 1B). The spiral ligament and the stria vascularis adhered to the inner face must removed for the SEM of the organ of Corti. The extraction, as extensive as possible, of the spiral ligament and stria vascularis as a whole using an insulin needle and/or thin clockmaker's pincers, allows their ultra-structural analysis by means of TEM (Figures 1C and 1D).

Processing the Specimens

Fixation

Once the specimen has been obtained, its fixation continues by immersion in an Eppendorf tube with the same fixing solution for 1 h at 4°C. In 2 cases, they were stored for 24 h without any differences being found. The specimen was then washed with 0.1 mol PB at a pH of 7.4 using 3 washes lasting 10 min each.

Osmification

A 2-hour wash in osmium tetroxide at 2% in 0.1 mol filtered Sorensen's buffer, followed by two 10-minute washes in Sorensen's buffer with intermittent stirring.

Cold Dehydration

This is done by means of several stages in gradually stronger ethanol (up to 100%) and subsequently a 15 min stage in propylene oxide at room temperature.

Embedding in Durcupan®-Type Araldite Epoxy Resin

The specimen is kept overnight in propylene oxide resin and is then inserted in pure resin (for 4 h) and finally the resin is polymerized for 48 h in an oven at 60°C. During the first state of the inclusion, since the resin is still not completely

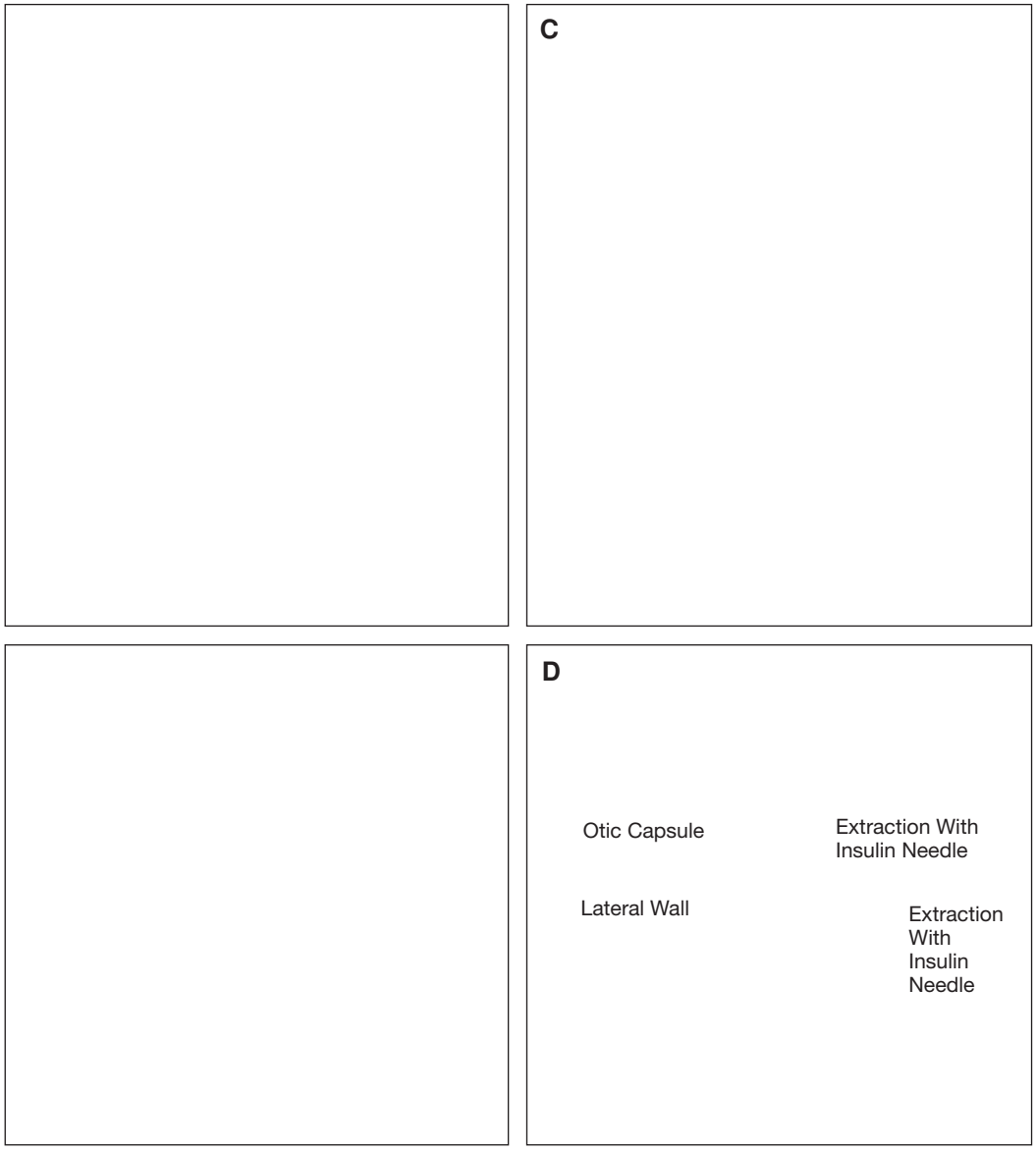

Figure 1. Methodology for extraction of the lateral portion of the cochlea in adult guinea pig. A: opening of the tympanic ampulla with gouge tweezers from its anteroinferior aspect; note the relief of the cochlea at the rear. B: the otic capsule is extracted with the aid of an insulin needle and clockmaker's pincers under microscopic control. C: panoramic view of the membranous content of the cochlea; the organ of Corti is hidden from sight by the spiral ligament attached to the stria vascularis (long arrow), which is more medial and therefore not visible and will be extracted for ultrastructural processing by means of an insulin needle drawn from its tip; the short arrow indicates a spira with the lateral wall already removed. D: diagram of the extraction of the lateral wall.

solid, the specimen is aligned and re-aligned so that the tissue has the correct slicing angle.

Sections

Using a Leyca ultramicrotome (Reychert Ultracut S model), the first semi-fine slices are made with a 0.5 µm glass blade and stained with toluidine blue for their visualization using an optical microscope and so confirm the integrity of the sample and its correct alignment. Next, a series of ultra-fine 50 nm slices are made with a Diatome diamond blade. The specimens are deposited on single hole nickel grids, coated with a pioloform film to improve the amount of specimen viewed, since, although it is true that it may worsen the focusing of the electron microscope and there is a greater risk of the electron beam perforating the slices and the film, it avoids a large part of the tissue being hidden behind the bars of the grid. The contrast of the sections is then made with uranyl acetate saturated in methanol by placing each grid on a drop of reagent, in parafilm on a Petri dish that, during the contrast process, will be covered with an opaque lid to prevent precipitation of the uranyl acetate. The contrast time with the reagent will be 5 min. The grid is rinsed with distilled water in 5 stages and it is then contrasted with Reynolds lead citrate, proceeding in the same way as with uranyl acetate. In this case, drops of caustic soda are placed around the drop of reagent to prevent the lead from precipitating with the CO_2. It is recommended to filter the reagent to prevent precipitates, as well as to ensure that both solutions have been correctly stored and sealed to prevent artefacts. The contrast time with the reagent will be 10 min. The grid is rinsed with distilled water in 5 stages.

Finally, the specimens were analyzed with a JEOL transmission electron microscope, JEM 1010 80-100 KV model (Tokyo, Japan), fitted with a Gatan Byoscan (California, United States) digital camera to capture images on a Macintosh computer using Micrograph Digital 3.2 software.

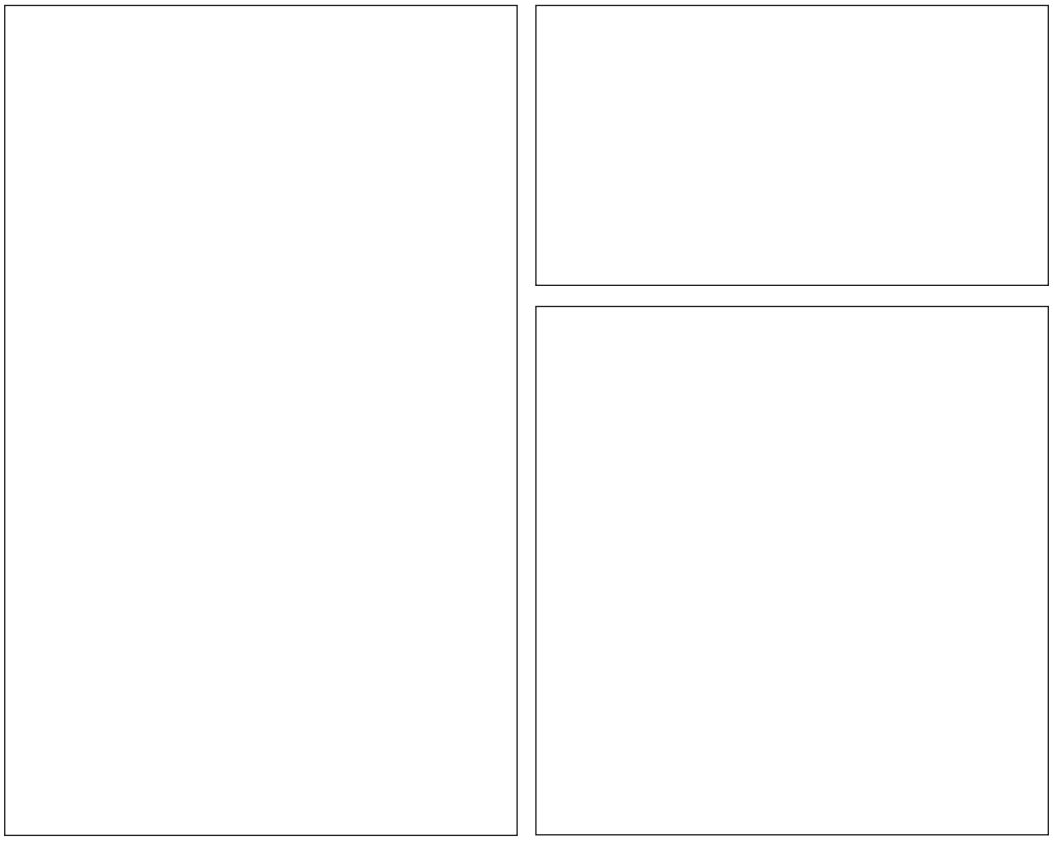

Figure 2. A: view of the lateral portion of the cochlea studied by means of a scanning electron microscope and arrangement of the different fibrocytes and the stria vascularis (SV). B: view of a type I fibrocyte by means of a transmission electron microscope (×5000). C: vessel (VA) of the spiral prominence (PE) surrounded by type II fibrocytes (FII) (×6000).

RESULTS

Gross

Of the 5 animals included in this preliminary study, it was possible to obtain sufficiently large fragments (about 3 mm long) of the side wall of the cochlear spiras in 4 of them for inclusion in the protocol for carrying out the TEM techniques. In the other animal, the first in the series, the side portion was included in the bone of the otic capsule, so it was impossible to recover enough tissue without producing tissue damage. The appearance of vascularization with haematic content in one of the cochleae, on the surface of the spiral ligament, did not correlate with microscopic data leading us to think of worse fixation due to a lack of penetration of fixing agent, although keeping the specimens in fixation for an hour after extraction has probably helped.

Microscopic

We managed to obtain images from the membranes, the cytoplasmic contents, the nucleus and the intercellular binding and relationship in 3 animals, whereas tissue disaggregation data and incorrect fixation appeared in one, making its study impossible. The different characteristics of the fibrocytes could be determined on the basis of their location, the greater or lesser mitochondrial content and the cell types and arrangement in the external sulcus, the spiral prominence and the stria vascularis (Figure 2).

The ultrastructural observation of the cell components in the lateral portion of the cochlea allowed identification of the 3 cell layers in the stria vascularis (Figure 3B), namely the marginal, intermediate and basal cells with inclusion of some vessels. Adjacent to this is the spiral ligament comprising a fibrous mesh with 5 types of fibrocytes currently given great functional importance. While in the past researchers have preferred to pay more attention to the sensorial elements of the organ of Corti, special attention is currently being given to the non-sensorial epithelial cells[10] and the mesenchymal cells located in the limbus and the spiral ligament, the fibrocytes of which have been differentiated into various types by aspect, location (Figure 2A) and the expression of markers indicating functional specialization[11] (Table).

We were thus able to observe in our preparations that the type I fibrocytes are lateral to the stria vascularis with a spindle-like appearance and very scant mitochondria and cytoplasmic structures (Figure 2B); the type II fibrocytes, just below the first ones, are on and adjacent to the spiral prominence (Figure 2C); type V fibrocytes can be found on the upper or apical tip of the spiral ligament, above the stria vascularis and close to the vestibular ramp. These 2 types (II and V) are the ones revealing the greatest evidence of functional differentiation so far known. Type II fibrocytes are the ones clearly showing the greatest density of mitochondria and interdigital cytoplasmic prolongations, circumstances respectively indicative of great metabolic activity and exchange. Type III fibrocytes are located most laterally and are found closest to and in parallel with the circumference of the otic capsule. They do not always appear

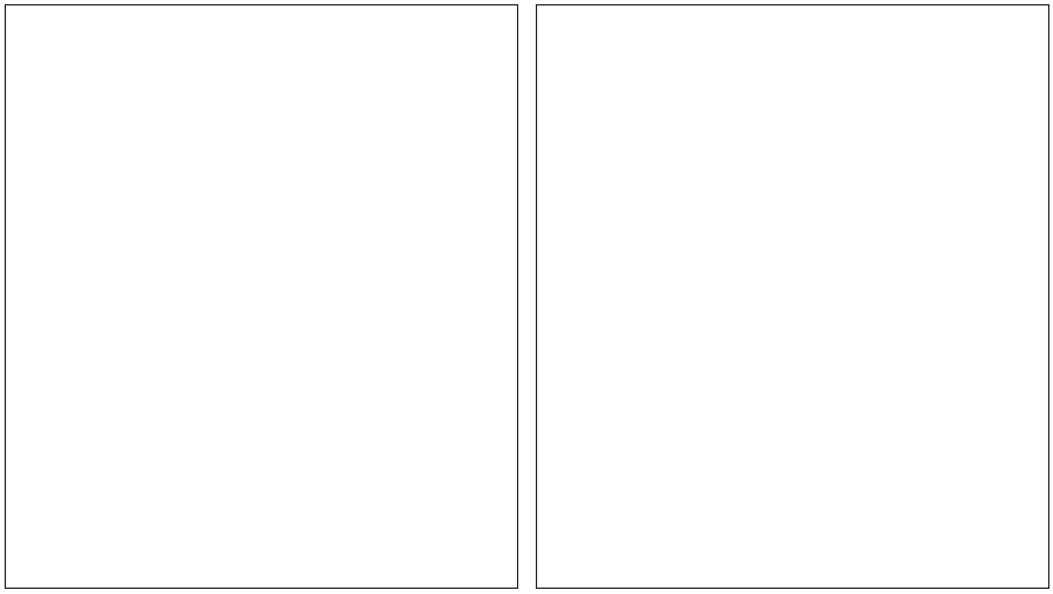

Figure 3. A: intercellular joins between tight-junction marginal cells of the stria vascularis (arrow head) and anchor joins (desmosomes, long arrows) (TEM, ×8000). B: stria vascularis (EV) and spiral ligament (LE) (TEM, ×1200).

in all spiras, generally in the basal ones, and are only found in the medial-inferior region of the spiral ligament. Type IV fibrocytes, lateral to the insertion of the basilar membrane, are thought to participate also in the support for the cochlear content and dampen the mechanical tensions induced by sound. Its appearance is mostly spindle-like with intermediate length prolongations.[12]

Using TEM, it has also been possible to observe the kinds of bonds between such cells as desmosomes and "tight junctions" (Figure 3A), ie, occlusive areas in which glycoproteins and adhesion bridges keep together the membranes of 2 cells to form a virtual barrier impermeable to fluids ("gap junctions") between type I, II, and V fibrocytes and the intermediate cells of the stria vascularis that provide communication between the cells[13] and are of fundamental importance in the recirculation of potassium and the intercellular circulation of ions and water, and thus in the control and maintenance of ionic concentrations of the endolymphatic liquid, the key to allow action potentials in the ciliated cells of the organ of Corti[14] (Figure 4).

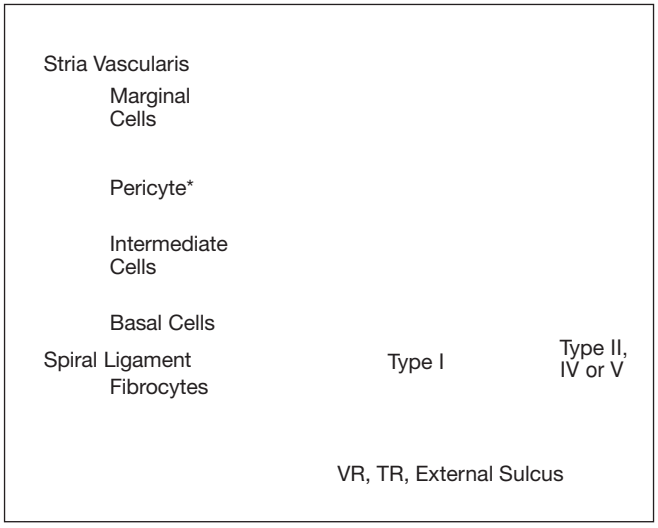

Figure 4. Diagram of the cell composition in the spiral ligament and the stria vascularis showing active (black arrows) and passive transport (grey arrows) of K^+. TR indicates tympanic ramp; VR, vestibular ramp.

DISCUSSION

The preparation of cochleae intended for observation by means of SEM involves a delicate process whereby the bony wall of the spiras is removed to reveal the inside of the medial ramp where the organ of Corti is housed. This procedure must be performed in the least traumatic way possible to preserve the delicate structures of the membranous labyrinth and must be extended to as many spiras as may be accessible, as various conditions have shown considerable topographical preference.

Despite this, the convexity of the spiral ligament represents a barrier for viewing the organ of Corti as it covers, in the form of continuous curtain, the lateral portion of the medial ramp. Although the processes of dehydration the cochlea is subjected to prior to metallization lead to the retraction of the tectorial membrane, disclosing the upper portion of the cuticular membrane housing the upper portions of the ciliated cells, with the characteristic image of ciliate plumes, the clear view of these is limited from a side-on angle by the presence of the spiral ligament.

The need to remove the spiral ligament and stria vascularis as a whole to facilitate the direct vision of the components in the organ of Corti also allows us to obtain a fragment of the lateral structures, currently under increasing attention from researchers of the inner ear.

There are mechanical difficulties to obtain complete shelling of the circumference of each spira but, without a doubt, the larger the number of ligament-stria complexes

Main Cell Types in the Side Wall of the Cochlea[a]

	Location	*Morphology*	*Function*
Stria vascularis			
Marginal cells	Endolymphatic surface (cochlear canal)	Apical microvellosities. Tight junctions. Numerous mitochondria. Basal digitations	Active K^+ transport to the endolymph from the perilymph (Na/K ATPase)
Intermediate cells	Between marginal and basal	Cytoplasmic processes towards marginal cells and vessels. Gap junctions	Passive K transport to the interstriatal space from basal cells and fibrocytes
Basal cells	External surface (on the spiral ligament)	Flattened spindle. Connexin +. Gap junctions with intermediate cells and fibrocytes. Tight junctions	Passive K^+ transport from fibrocytes (gap junctions) and perilymph (tight junctions)
Spiral ligament			
Type I fibrocytes	Under the stria vascularis. Ia: aligned in parallel, with dense intercellular bands of fibre; Ib: lower and less aligned	Ample contact with basal cells by gap junctions and limited contact with other type I cells. Ample cytoplasm with scant mitochondria	BK-channel dependent passive K^+ transport from type II, IV, and V fibrocytes
Type II fibrocytes	IIa: under the spiral prominence	Connexin +. Abundant mitochondria and increased plasma lemma with thin processes.	Active pumping of K^+ to endolymph from perilymph (Na/K ATPase) through external sulcus cells
	IIb: between root cells in the external sulcus and type I fibrocytes	Connexin +. Folds in pole close to roots of external sulcus cells. At the opposite pole, dendrites upward towards Ib fibrocytes. Lots of mitochondria	IIb with greater active transport than other type II fibrocytes
	IIc: deep under the epithelial of the external sulcus in the lower area	Connexin +. Thin processes giving a stellar outline. Lots of mitochondria	
Type III	Close to the bone in the deep lower area of the spiral ligament	Long, thin processes	
Type IV	Next to the perilymphatic surface of the TR. Vertical	Filamentous body, lengthened nucleus	Active pumping of K^+ to endolymph from perilymph of the TR (Na/K ATPase)
Type V	Next to the perilymphatic surface of the VR. Suprastriatal cells on Reissner's membrane, in parallel and separated by stroma	Narrow processes towards VR. Connexin +	Active pumping of K^+ to endolymph from perilymph of the VR (Na/K ATPase)

[a]TR indicates tympanic ramp; VR, vestibular ramp. After Spicer et al[11] and Kikuchi et al[22] with modifications.

that can be harvested, the more information investigators will obtain about the cells under study. This factor is one of the critical points of the technique, especially in the basal spira, where the separation of the bone covering the spiral ligament must be done more carefully, if possible, so as not to separate the 2 attached structures, which would render the specimen useless, as we have the impression that they are more firmly attached to each other. Although no more than 2-3 mm of tissue are needed to be able to work relatively comfortably with the specimen and obtain results, the longer it is, the easier it will be to manipulate to align the tissue longitudinally when it is included in the resin block, from which the transverse slices will be obtained to let us study the different regions of the spiral ligament-stria vascularis combination. A poorly aligned sample or an incorrect slicing angle in the ultramicrotome would frustrate the study.

Tissue extraction might be thought to be highly traumatic, but we have not obtained any signs of this effect in comparison with decalcification methods that are therefore 1-2 weeks slower, although it is true that it is necessary to avoid harvesting the specimen with tweezers that might squash the tissue and it must be left resting on the back of the needle when pushed upwards during extraction.

Guinea pigs and gerbils are animals with cochleae that stick out into the lumen of tympanic ampulla, hence the preference for using these in the ultrastructural research of the inner ear. The larger number of cochlear spiras, in comparison with rats and even human ears, even included in the petrous bone, facilitates the manipulation intended to reveal the interior of the cochlea. The location of the oval and round windows allows procedures for the intracochlear perfusion of fixing fluids, thus adding safety and quality to

the fixation with respect to purely intracardiac perfusion, a fundamental fact bearing in mind that fixation is one of the essential factors to obtain a high-quality view of the ultrastructure when working with TEM.

Therefore, care must also be taken to ensure the use of appropriate concentrations and pH values for the fixing agents, as a lack of fixation, excessive acidity or impurities in the glutaraldehyde or paraformaldehyde may resemble (through oedema artefacts, intracellular and intercellular degeneration and de-structuring of the spiral ligament-stria vascularis complex) cochlear disease in specimens from healthy animals (verified by means of a quarantine period, adequate care and isolation, otoscopy and evoked auditory potentials), as we were able to confirm in one of the specimens. To this we must add the artefacts derived from contrast agents with uranyl and lead, which may make the specimen useless due to dark stains on it, or the use of badly sharpened cutting blades, so it is always necessary to verify the alignment and the quality of the specimen under an optical microscope. In addition, the beams of the electron microscope may heat the specimen, the resin and the film on the grid (as it is a single hole grid) and produce focusing difficulties, particularly with enlargements in excess of 8000 times, or even holes making the specimen unusable. This is why it is important to have good technicians both for inclusion and for the use of TEM and to prepare at least four grids per specimen.

The presence of the stapedial artery, running through the stirrup crures in rodents, is not important, as the specimen extraction procedure is effected after the animal is perfused and sacrificed, which rules out the possibility of bleeding.

The observation of histological preparations under an optical microscope with normal staining[15] or in preparations for immunohistochemistry,[8] surface,[16] SEM,[17] and TEM implies the possibility of complementing electrophysiological techniques in the study of a sensorial condition such as hearing loss, increasingly frequent among aged populations or those subjected to aggressions in the western world.

The lateral portion of the cochlea, especially the spiral ligament, is the target of ever greater interest in the community devoted to the physiopathological study of hearing and its alterations. A large number of articles[18] are currently appearing in connection with the functions of fibrocytes of the spiral ligament as structures with a decisive role in the electrolytic regulation of the hearing sensory reception complex.[19]

Fibrocytes express different proteins related to their functional activity in the exchange of ions and water through specific channels, carbonic anhydrases II and III,[20] as well as connexins 26, 30, and 31 to form gap junctions[19] (Table).

At the present time, a highly important role is beginning to be posited for fibrocytes in certain conditions. Thus, for example, certain kinds of age-related hearing loss reveal mutations in genes expressed by fibrocytes.[21] With our model for the extraction of the side wall of the cochlea, we have shown that, once the learning curve has been conquered and the technique fully implemented, it is possible to conduct ultrastructural studies into the organization of this kind of cell in the spiral ligament and the stria vascularis without the need for decalcification and to evaluate the existence of alterations in these structures and their cells in the various types of hearing loss.

Acknowledgments

Our gratitude to Francisco R. Urbano and Covadonga Aguado at the transmission electron microscopy department at the SIDI of the Autonomous University in Madrid (UAM), as well as to Dr Miguel Garzón and Dr Esther del Cid-Pellicero at the Department of Anatomy, Histology and Neuroscience of the UAM for their inestimable assistance.

REFERENCES

1. McCabe BF. Autoimmune sensorineural hearing loss. Ann Otol Rhinol Laryngol. 1979;88:585-9.
2. García-Berrocal JR, Arellano B, Vargas JA, Trinidad A, Ramírez-Camacho R, Vergara J. Enfermedad inmunomediada del oído interno. Presentación de casos clínicos. Acta Otorrinolaringol Esp. 1997;48:565-70.
3. Engstrom H, Wersall J. The ultrastructural organization of the organ of Corti and of the vestibular sensory epithelia. Exp Cell Res. 1958;14 Suppl 5:460-92.
4. Ciges M, Quesada P. Estudio vital de la cóclea del cobaya. Acta Otorrinolaringol Iber Amer. 1970;21:45-63.
5. Ciges M, Quesada P, González M. Supravital study of the guinea pig cochlea referring especially to kanamycin labyrinthotoxy. Acta Otolaryngol. 1972;73:270-9.
6. Labella T, Morales J. Estudio ultraestructural de la estría "vascularis" del cobaya. Rev Esp Otoneurooftalmol Neurocir. 1976;34:217-25.
7. Sanchez-Fernández JM, Rivera-Parra JM. Study of the development of utricular and saccular maculae in man and rat. Am J Otol. 1983;5:44-55.
8. García-Berrocal JR, Nevado J, Ramírez-Camacho R, Sanz R, González-García JA, Sánchez-Rodríguez C, et al. The anticancer drug cisplatin induces an intrinsic apoptotic pathway inside the inner ear. Br J Pharmacol. 2007;152: 1012-20.
9. Bartolomé MV, López LM, Gil-Loyzaga P. Galectine-1 expression in cochleae of C57BL/6 mice during aging. Neuroreport. 2001;12:3107-10.
10. Ramírez-Camacho R, García-Berrocal JR, Buján J, Martín Marero A, Trinidad A. Supporting cells as a target of cisplatin-induced inner ear damage: therapeutic implications. Laryngoscope. 2004;114:533-7.
11. Spicer SS, Schulte BA. Differentiation of inner ear fibrocytes according to their ion transport related activity. Hear Res. 1991;56:53-64.
12. Kikuchi TR, Kimura S, Paul DL, Takasaka T, Adams JC. Gap junction system in the mammalian cochlea. Brain Res Rev. 2000;32:163-6.
13. Delprat B, Boulanger A, Wang J, Beaudoir V, Guitton MJ, Ventéo S, et al. Down regulation of otospiralin, a novel inner ear protein, causes inner ear degeneration and deafness. J Neurosci. 2002;22:1718-25.
14. Herson MM, Herson OW. Tension fibroblasts and the connective tissue matrix of the spiral ligament. Hear Res. 1988;35:237-58.
15. García-Berrocal JR, Vicente J, Arellano B, González F, Ramírez-Camacho R. Laberintitis experimental autoinmune. Evaluación de diversos modelos en cobaya. Acta Otorrinolaringol Esp. 1997;48:5-10.
16. Benito JI, Gil-Carcedo LM, Gayoso MJ. Evaluación morfológica del órgano de Corti del cobaya sometido a trauma acústico. I. Estudio de preparación de superficie. Acta Otorrinolaringol Esp. 1991;42:405-9.
17. Ramírez-Camacho R, García Berrocal JR, Trinidad A, Martín Marero A, Buján J. Actividad citotóxica coclear en animales de experimentación. Un estudio con microscopía electrónica de barrido. Acta Otorrinolaringol Esp. 2002;53: 538-42.
18. Delprat B, Boulanger A, Wang J, Beaudoir V, Guitton MJ, Ventéo S, et al. Down regulation of otospiralin, a novel inner ear protein, causes inner ear degeneration and deafness. J Neurosci. 2002;22:1718-25.
19. Delprat B, Ruel J, Guitton MJ, Hamard G, Lenoir M, Pujol R, et al. Deafness and cochlear fibrocyte alterations in mice deficient for the inner ear protein spiranile. Mol Cel Biol. 2005;25:847-53.
20. Spicer SS, Gratton MA, Schulte BA. Expression patterns of ion transport enzymes in spiral ligament fibrocytes change in relation to strial atrophy in the aged gerbil cochlea. Hear Res. 1997;111:93-102.
21. Wu T, Marcus DC. Age-related changes in cochlear endolymphatic potassium and potential in CD1 and CBA/CaJ mice. J Assoc Res Otolaryngol. 2003;4: 353-62.