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INTRACELLULAR AND EXTRACELLULAR ELEMENTAL COMPOSITION OF THE ENDOLYMPHATIC SAC STUDIED BY X-RAY MICROANALYSIS

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Abstract

X-ray microanalysis was performed along with light microscopy (LM) on rapidly frozen and cryo-sectioned endolymphatic sac tissues of adult guinea pigs, to determine the elemental composition of the different cell types in this tissue as well as the content of the sac lumen. The morphological preservation and spatial resolution of cryo-sectioned endolymphatic sac was found adequate for the identification of the different cell types of the sac in the transmission electron microscope. Further cell type identification was performed by comparing scanning transmission electron microscopy images with LM images on adjacent serial sections. X-ray microanalysis demonstrated differences between epithelial and sub-epithelial cells in the intracellular concentrations of sodium, chlorine, potassium, calcium, phosphorus and sulphur. Measurements performed in the lumen of the endolymphatic sac showed elevated sodium and decreased potassium levels as compared with the known levels of these elements in cochlear or vestibular endolymph. High phosphorus and sulphur levels were also found in the endolymph of the sac. Other morphological and analytical findings on the luminal content point out that otoconial destruction and cleaning of the endolymph from the cell debris and other products such as lipids and proteins take place in the endolymphatic sac. Our results suggest that the endolymphatic sac participates in fluid absorption (osmoregulation), ion transport and otoconial destruction. The data support the longitudinal flow theory of the endolymph.

Key Words: Endolymphatic sac, microanalysis, ion transport, otoconial degradation.

Introduction

Since the discovery of the endolymphatic sac by Cotugano in 1761, many investigators have been studying its structure and function (Siebenmann 1919, Guild 1927, Portmann 1927, Lundquist 1965, Teichmann et al. 1964, Allen 1964, Kimura and Schuknecht 1965, Adington 1967, 1968, Harada and Gaafar 1976, Shindler 1979, Rask-Andersen and Stahle 1980, Yamane et al. 1984). Morphological studies led to speculations on the function of the sac, including a role in endolymph resorption, endolymph secretion, osmotic regulation, otoconial destruction and phagocytosis. However, direct functional evidence has not been provided, since the surgical access to the sac in experimental animals is complicated and not easily achieved without affecting the continuity of endolymphatic space.

The morphology of the endolymphatic sac was described in several publications where tissue was prepared using different fixatives, dehydration agents and embedding media, which resulted in a different appearance of the structures described (Lundquist 1965, 1976).

Techniques involving fluid withdrawal by micropipeting have been used in the studies of kidney (Roinel and de Rouffignac, 1982) and inner ear fluids (Smith et al. 1954, Silverstein 1966, Bosher and Warren 1968). However, these studies did not facilitate simultaneous measurements of elemental contents of both fluid and surrounding tissues. Thus, in situ microanalysis seems to be the method of choice for obtaining reliable measurements of elemental content of fluids and cellular tissue components.

The goal of this study was to measure the elemental content of the endolymphatic sac and its epithelium with X-ray microanalytical methods. In the present study, we avoided chemical fixation and used a preparation technique involving cryo-fixation followed by cryo-sectioning and analysis of freeze-dried material. This technique has been shown to be suitable for analysis of tissues and of small fluid filled spaces. For instance, the technique has been applied

earlier on embryonic brain (Wróblewski et al., 1984), the intestinal content of the earthworm (Wróblewski et al., 1979), the content of the capsule of intrafusal muscle (Wróblewski et al., 1982) and the embryonic and mature inner ear (Anniko et al. 1982; 1987, Anniko and Wróblewski, 1980 a,b; 1981 a,b; 1983 a,b; 1986 a,b; 1988; 1989; Bone and Ryan 1980; Hunter-Duvar et al. 1981, Ryan et al. 1978; 1979; 1980; Ryan and Woolf 1983; Wroblewski 1989) and the follicular cells and colloid in the follicular lumen of thyroid (Wroblewski et al. 1991).

Materials and Methods

Freeze-fixation

The animals in this study, 3 adult pigmented guinea pigs (approximately 250 gr.) were decapitated and their endolymphatic sacs (one from each animal) were excised and mounted on silver pins using OCT compound (Tissue Tek Co.) and cryo-fixed by plunging the tissue into liquid Freon 22 (-171°C) cooled by liquid nitrogen. Since the quality of the cryo-sections is to a large extent dependent on the cryo-fixation, this method of fast freezing is essential to minimize ice crystal formation during freezing.

Cryo-sectioning

The silver pins with the frozen samples were mounted on cryostat holders with OCT compound, which contained no contaminating elements detectable by conventional energy dispersive X-ray microanalysis. The sacs were sectioned perpendicularly to their long axis.

In order to collect sufficient data for accurate statistics, a considerable number of cells from several samples had to be analyzed. We therefore used semithin cryo-sections, that are easily produced in large quantities. Such sections have successfully been used in several studies (Wróblewski et al. 1982, Wróblewski 1989, Wróblewski et al. 1991). In addition, X-ray microanalytical data were correlated to histochemical data obtained on adjacent serial cryo-sections as described in Wróblewski et al. (1978).

Semi-thin sections (2-6 μm) were cut at -30°C, mounted on Formvar film coated graphite plates (specimen holders) with a centrally drilled hole and freeze-dried in the cryostat overnight. In order to facilitate orientation and identification of the various cell types, cryo-sections adjacent to those used for X-ray microanalysis were stained with hematoxylin-eosin. LM images of these sections were projected on a video screen. The LM image could be rotated so that the orientation corresponded to the transmission electron microscopy (TEM) or scanning transmission electron microscopy (STEM) image of the section to be analyzed (Wróblewski et al. 1978, Wroblewski 1989).

TEM and X-ray microanalysis

Freeze-dried cryo-sections were analyzed in a JEOL 1200CX electron microscope with a scanning attachment. Energy dispersive X-ray microanalysis was carried out with a Tracor 5500 analytical system at an accelerating voltage of 100 kV. Both conventional TEM and STEM images (bright field) were obtained. During all observations, the cold finger and the cold trap were used.

The secondary image mode was used to analyze otoconia in the endolymphatic sac to check if analyzed surfaces were devoid of precipitates from the endolymph.

For quantitative analysis, the background level was determined by a non-linear interpolation routine, and P/B values were determined by taking the ratio between the characteristic intensity and the background intensity in the same energy region. Absolute concentrations of elements were obtained by comparing the relative peak intensities in tissues and in gelatin standards (Wroblewski et al. 1983). The present study is based on 110 analyses, half of which were performed on the luminal content. Statistical significance was addressed by Student's t-test; $p<0.01$ was accepted as significant.

Results

Morphological observations

In LM images, the distal and medial portions of the sac appeared similar. Typically, a distinctive narrow luminal space was surrounded by a layer of simple epithelium (Fig. 1a-b). In the distal portion of the sac the lumen was usually sub-divided into several branches. The luminal space contained clear fluid in some cases, but often included dense material which may have been cellular debris or otoconia in degenerative stages. The epithelium was surrounded by regular connective tissue with various fibrillary structures and connective tissue nuclei. There was a difference in density between lucid sub-epithelial connective tissue and the outermost layers of connective tissue (close to the dura) which was dense. Although it was possible to distinguish between the epithelial and non-epithelial elements of the tissue, it was not possible to distinguish between light and dark epithelial cells within the sections viewed in TEM or the adjacent sections observed with LM.

In the semi-thin cryo-sections (2-6μm) viewed in electron microscope, different types of cells in the endolymphatic sac could be distinguished (Fig. 1c-d). Analyzed areas were within the intermediate and distal parts of the sac. The electron density of the epithelial cells forming a 10 μm thick electron dense rim was different from that of the sub-epithelial cells of connective tissue which were electron lucid. Further identification of different cell types was done by comparison of LM and STEM images (Fig. 1a-d).

TEM micrographs of the sac revealed gross morphology similar to that observed with LM. The luminal contents were more distinctive and appeared

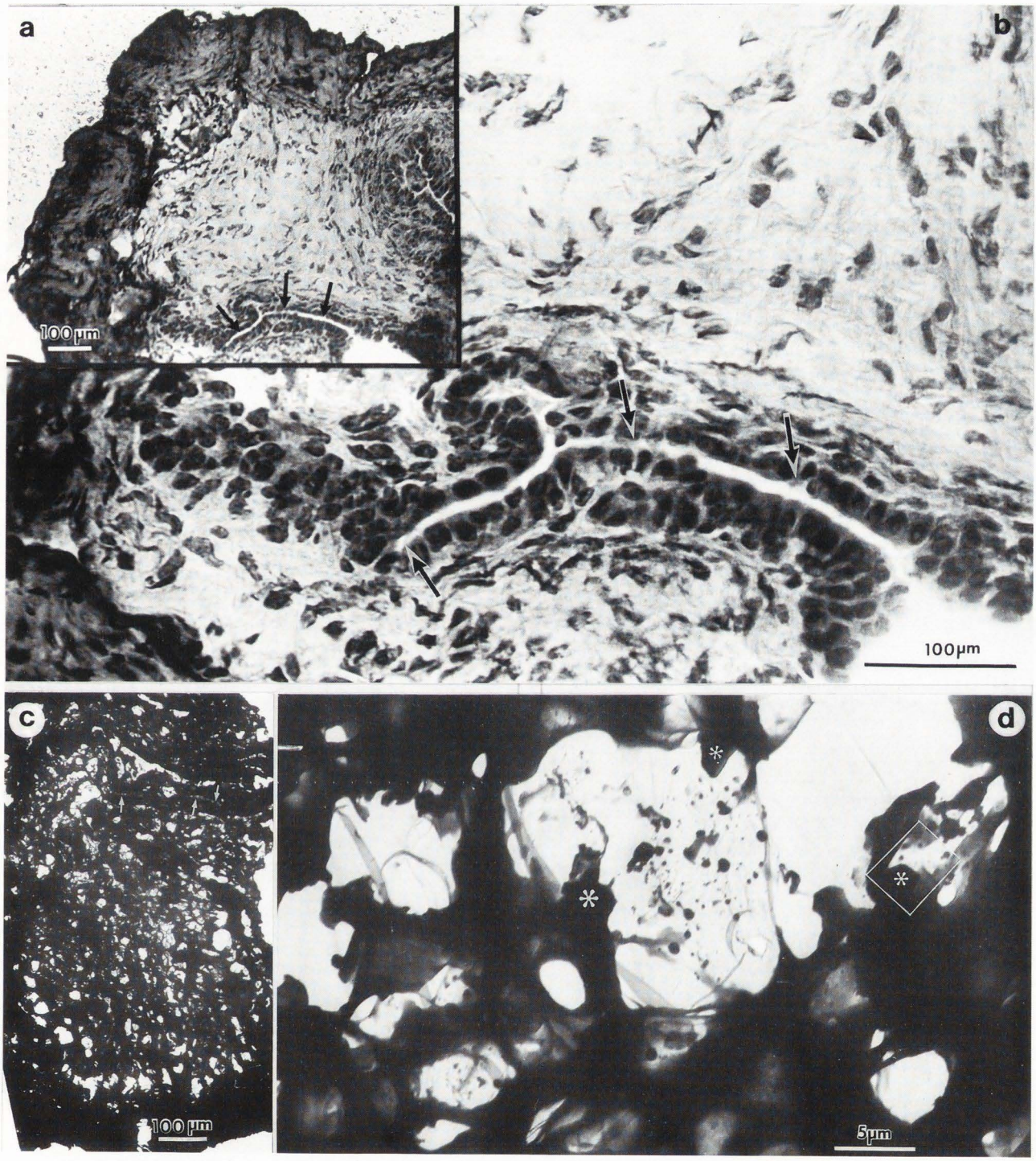

Fig. 1a-d. Light microscopical images (**a-b**) and transmission electron micrographs (**c-d**) of the endolymphatic sac. The luminal space (arrows in **a**, **b** and **c**) is narrow and surrounded by a layer of simple epithelium (**a-b**). The density of epithelial cells is different from that of the subepithelial connective tissue. When running elemental analysis it was also possible to identify different cell types by comparison of LM and TEM images. The luminal space **c** with fluid residues which remained after freeze-drying show small crystalline material, round dense droplets and various dense material which may represent cells or bigger cellular debris. Characteristic otoconia (asterisk) are often present (**d**).

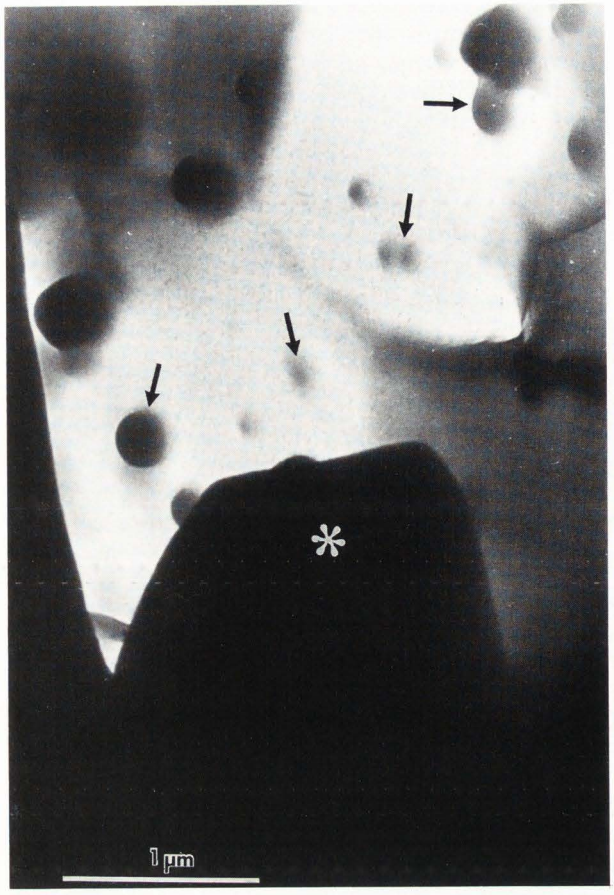

Fig. 2 High magnification of the luminal content of the endolymphatic sac. Distinctive droplets (arrows) of various density, several amorphous aggregates and the large otoconia (asterisk) are present.

as small and big crystals, droplets and several amorphous aggregates (Fig. 1 and 2). The size and morphology of the large crystals are similar to that of saccular and utricular otoconia. Although the otoconia in the sac were similar in shape to those in the otoconial membranes, the contours appeared slightly rounded so that the very sharp edges of normal otoconia were lost. The use of semithick cryosections allowed us to analyze the content of the lumen of the endolymphatic sac. We could distinguish areas with detached cells, cellular debris, protein-like condensations and crystalline material (Fig. 1 and 2). The appearance of the residues of the endolymphatic sac fluid was less distinct presumably because of the high content of protein and lipids. The content of the endolymphatic sac displayed different freezing and drying properties compared to the content of the cochlear and vestibular part.

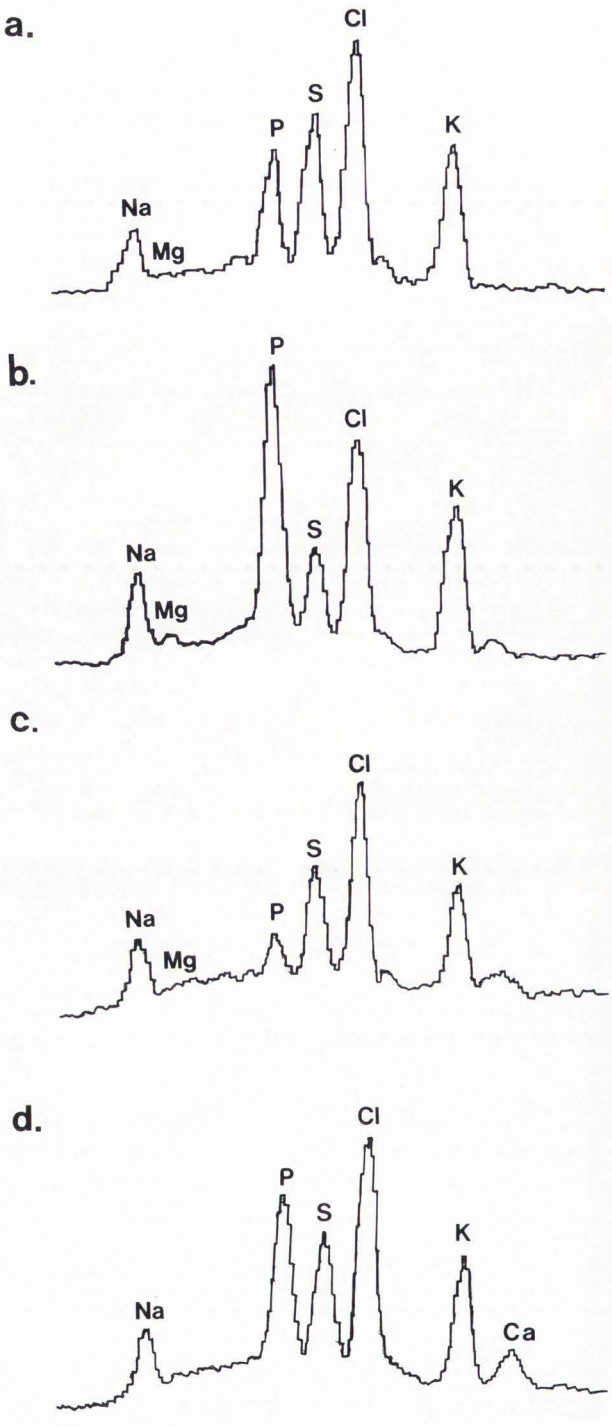

Fig. 3 a-d. Characteristic elemental spectra obtained from the cryosections of the endolymphatic sac. **a**) from small crystals representing endolymph showing high Na, Mg , P and S content among the for cochlear endolymph characteristic Cl and K. **b**) from epithelial cells of the sac, **c**) from subepithelial connective tissue and **d**) from outermost layers of connective tissue.

Energy dispersive X-ray microanalysis

Three cell layers, (a) epithelial cells of the sac, (b) connective tissue underlying the sac epithelium and (c) connective tissue in the periphery of the sac were identified in the TEM and in the corresponding hematoxylin eosin stained sections viewed in the LM.

Cellular debris, amorphous condensations and crystalline material were found in the endolymphatic sac lumen. Small crystalline material represented endolymph and revealed sodium, chlorine and potassium (Fig. 3a). Compared with cochlear and vestibular endolymph, the endolymph of the sac contained lower potassium and high sodium levels. Phosphorus, magnesium and sulphur were also present. In amorphous condensations, elements like phosphorus, magnesium, sulphur, sodium, chlorine and potassium were present.

The larger crystals were otoconial material, based on their shape (Fig. 2 and 3) and elemental contents. Specifically, the high level of calcium strongly indicated the otoconial origin of these crystals (Fig. 4). In addition to calcium, phosphorus was the main element present in the otoconia (Fig. 4a). Other elements typical for sac endolymph were also present in the otoconial crystals. Some crystals of otoconial size included, besides calcium, sulphur instead of phosphorus (Fig. 4b). Analyses of the outermost layers of connective tissue which had an electron dense appearance had a higher elemental content of phosphorus and sulphur and lower content of elements representing mobile ions than adjacent electron lucid connective tissue. Typical elemental spectra from different tissues of the sac are presented in Fig. 3 and quantitative data in Fig. 5.

In the distal part of the sac epithelium, the sodium and phosphorus levels were significantly higher while the levels of potassium and calcium were lower ($p<0.01$) than in the medial part (Fig. 5).

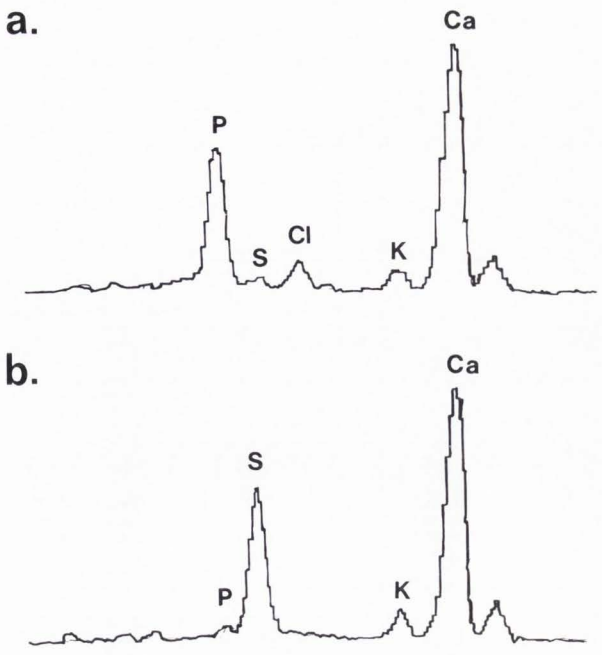

Fig. 4 a-b. Characteristic elemental spectra obtained from otoconia in the lumen of the endolymphatic sac. In (**a**) addition to the calcium peak, typical for otoconia in maculae, also Na, Mg, P, S, Cl and K are present. In a few otoconia besides Ca, a high concentration of S was detected (**b**).

Table 1

The elements present in the endolymph along the membranous labyrinth at various points as determined by means of X-ray microanalysis

	Na	Mg	P	S	Cl	K	Ca
Cochlea					+	+	
Vestibular organ					+	+	
Endolymphatic duct	+				+	+	
Endolymphatic sac	+	+	+	+	+	+	+

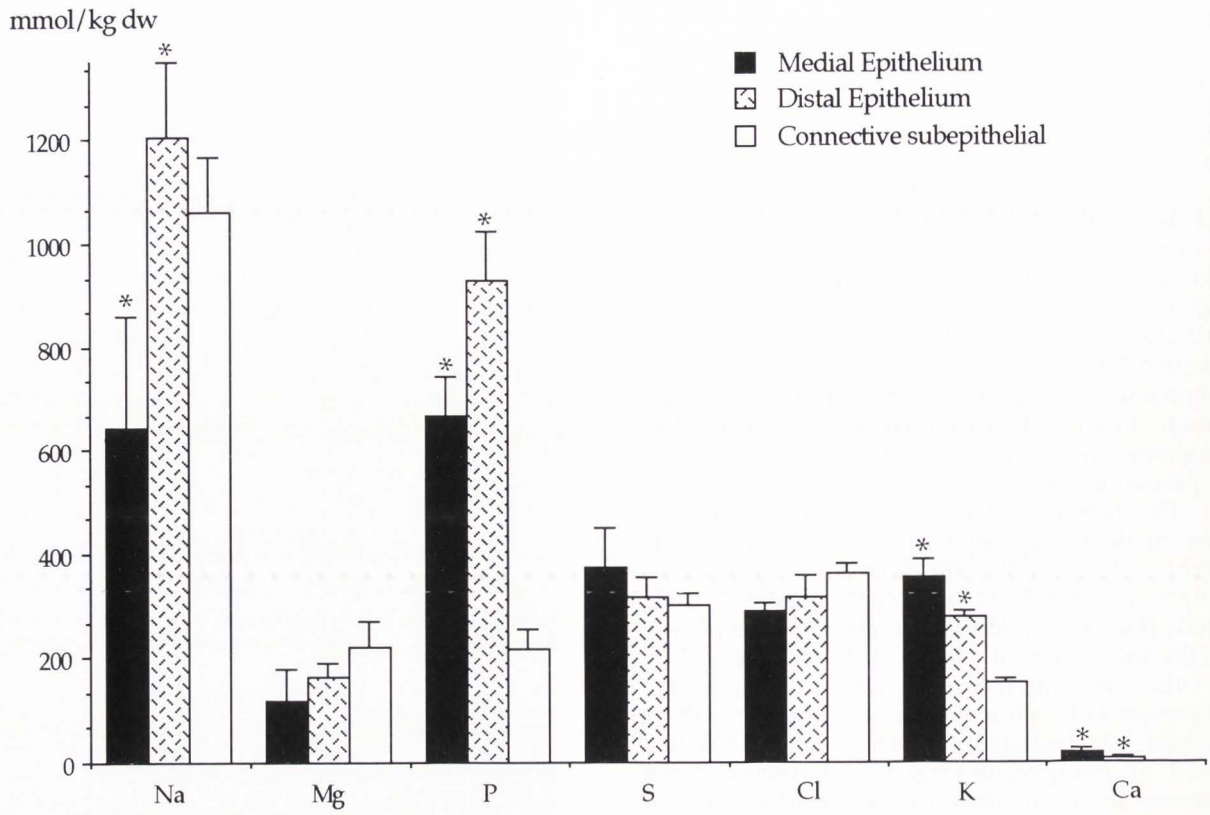

Fig. 5. Absolute concentrations (mmol/kg dw) of sodium (Na), magnesium (Mg), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K) and calcium (Ca) in epithelial and subepithelial connective tissue in medial and distal portion of the endolymphatic sac. Mean and standard deviations are given. An asterisk (*) denotes a significant difference between medial epithelium and distal epithelium ($p<0.01$).

The sodium content in the distal epithelial part was of the same magnitude as in the connective sub-epithelial tissue. The chlorine level in the medial portion of the sac was very similar to that in the distal part. The elemental content of the sub-epithelial connective tissue was the same in the medial and distal part showing typical Gaussian distribution for all elements.

The occurrence of sodium, magnesium, phosphorus, sulphur, chlorine, potassium and calcium along the membranous labyrinth are shown in Table 1. The endolymph in the vestibular and cochlear part contained mainly chlorine and potassium with some trace elements. In the endolymphatic duct and sac other elements were present. Sodium and magnesium, normally absent in the endolymph, are present at rather high concentrations. Besides elements representing mobile ions, sulphur and phosphorus are also frequently found in luminal residues of endolymph in the sac. When we analyzed endolymph in the cochlear or vestibular portions, residues appeared more uniform than in the sac.

Discussion

The elemental analysis of the endolymphatic space revealed that the chemical composition in the distal and medial part of the endolymphatic sac was different from that in other parts of the membranous labyrinth and contained various residues. Endolymph in the sac contained besides chlorine and potassium - characteristic for cochlear and vestibular endolymph - also sodium, phosphorus, and sulphur. This supports suggestions by Kimura and Schuknecht (1965) that the endolymphatic sac is a site of endolymph resorption. Our X-ray microanalytical observations of the fluid spaces in the cochlea, vestibule (Anniko and Wroblewski 1980a; 1986a) and endolymphatic sac endolymph (present study) are in agreement with the slow longitudinal flow theory (Guild 1927), suggesting that a slow flow of endolymph along the membranous labyrinth removes high molecular-weight waste products and cellular debris from the cochlea and vestibular organ.

The composition of the sac epithelium and the sub-epithelial layers of connective tissue appeared similar to epithelia and connective tissues in other organs.

In amorphous condensations in the lumen of the sac, elements like phosphorus, magnesium, sulphur, sodium, chlorine and potassium were present. This indicates that the analyzed material was composed of elements with an atomic number below that of sodium, which is the lightest element detectable. It is likely that this material represents carbohydrates, proteins and lipids. Irregular appearance of the residues in the lumen of the sac was likely due to the protein, carbohydrates and fat content in the endolymph in this portion of the membranous labyrinth. In the cochlear and utricular part, however, the residues appeared more uniform than in the sac.

Otoconial material was found in the sac, with size and shape similar but not identical to otoconia in the vestibular organs. However, otoconia found in the endolymphatic sac lumen exhibited elements absent in the utricular or saccular otoconia. Nevertheless, calcium was the main component in otoconia found in all these anatomical sites .

Much smaller otoconia (0.5-1μm) have been found in the endolymphatic sac of the fetal guinea pig (Imoto et al. 1983). These otoconia were very small, 0.5-1 μm in comparison to otoconia with our study. Yamane et al. (1984) found similar small otoconia as well as giant otoconia in 30 days old fetuses. Dark cells of the utricle dissolve otoconia dislodged by streptomycin sulphate treatment, as previously observed by Lim (1973). Otoconia may also be dissolved by the non-sensory epithelium of the saccule. None of the dislodged otoconia were found in the endolymphatic sac. Rask-Andersen et al. (1984) reported otoconia-like bodies in the human endolymphatic duct.

Increased phosphorus content in the sac otoconia was also found in maculae of Dancer mice mutants (Anniko et al. 1988) and in humans where phosphatization of otoconia is likely to occur with aging (Anniko et al. 1984). Only minor changes were found in different Shaker mice mutants (Anniko and Wroblewski 1983b). Anniko et al. (1984) showed that fetal and early postnatal otoconia contain higher amounts of elements other than calcium which was interpreted as a sign of immaturity. Pathological changes in otoconia following streptomycin exposure have been reported by Harada and Sugimoto (1977) who showed that degenerating otoconia became decalcified near the dark cell epithelium. Ross et al. (1976) described otoconia as non static structures as their morphology changes during their life span which can be directly related to elemental findings by Anniko et al. (1984) and to changes in morphology and elemental composition found in endolymphatic sac otoconia in present study.

The complex morphology of the membranous labyrinth requires precise and sensitive sampling and analysis of its ionic content. Microanalytical techniques such as X-ray microanalysis and laser microprobe mass analysis (LAMMA) seem to provide reliable chemical analysis of the luminal content of the endolymphatic sac. Additional information concerning the luminal contents can be obtained by cytochemical, autoradiographical and immunohistochemical methods. In early studies (Siebenmann 1919; Guild 1927; Lundquist 1965; Teichmann et al. 1964) the presence of stainable substance in the lumen of the sac was documented. Using more selective histochemical staining the substance in the lumen was found to contain acid glycoproteins (Teichmann et al. 1964; Marovitz et al. 1972). It is likely that this material may partly originate from secretory activity of the cells in the sac (Boettcher 1871; Erwall et al. 1989), but it is possible that the material might also originate from degradation of cellular material within the sac. Phagocytosis by both epithelial cells and free cells of endolymphatic sac was demonstrated experimentally by Andersen (1948) and Lundquist (1965). Rask-Andersen and Stahle (1980) described lymphocyte-macrophage interactions in the distal part of the sac.

Fluid samples obtained by micropipet penetration of the endolymphatic sac may suffer from sampling errors due to the constricted space and the varying viscosity of this lumen. For instance, when the sac endolymph is collected with a pipette and then prepared for analysis, only the fluid with the lowest viscosity collected and the resulting analysis gives incorrect results (Wroblewski unpublished observations).

Analysis of inner ear fluids by means of X-ray microanalysis was performed by Ryan et. al (1979, 1980) and Peterson et al. (1978), who used freeze-drying and dissection of fluids from different locations of cochlea and vestibular organ. Their results concerning the elemental content of the endolymph and perilymph are in good agreement with our data performed on freeze-dried cryosections. The type of preparation used by Ryan et al. can not be used in the analysis of fluid spaces of the endolymphatic sac due to the complicated morphology of the sac and its small diameter. Ryan et al. (1980) proposed that differences in morphological appearance of inner ear fluid residues can be related to differences in contents of ions, proteins and lipids which affect the final morphology of freeze-dried residues, and suggest that lipid and protein contents in the endolymphatic space are higher than in other fluid compartments. Rauch (1964) reported that the freezing points of endolymph and perilymph are different thus making feasible a method to separate frozen samples of cochlear fluid. His data are supported by the compositional differences between the cochlear fluids. No data appear to have been collected on the freezing point of the sac fluids. Administration of ethacrynic acid (Bosher et al. 1973; Wroblewski and Anniko 1986) resulted in an increase in sodium concentrations and

the decrease in potassium. Analyses by Wroblewski and Anniko (1986) showed in addition to changes in sodium and potassium also presence of phosphorus, sulphur and calcium in the scala media. Altered ionic composition was related to the crystallization pattern of cochlear endolymph with large variation of irregular appearance and distribution of crystalline residues at 30-60 min. after exposure. The changes, which normalized 2 hours following exposure, may have accounted for macromolecule content of the endolymph. The fate of the macromolecules was unknown, and it can be speculated that they have been cleared by endolymph flow or locally re-absorbed. Transport of macromolecules in the endolymphatic sac according to longitudinal flow theory was described by Manni and Kuijpers (1987) and Erwall et al. (1989).

The endolymph in cochlea, utricle and saccule is characterized by a low sodium and high potassium content. This unique extracellular fluid composition has to be maintained by active and energy dependent mechanisms. Changes in the composition of endolymph may affect hearing and balance. For instance, Ménières disease is thought to involve changes in inner ear fluid composition as well as pressure due to the diminished resorption of endolymph in the endolymphatic duct (Hallpike and Cairns 1938, Horner and Cazals 1991). Zenner (1986) showed that exposure of hair cells to high potassium concentrations causes reversible depolarization and contraction of the cochlear sensory cells.

Recent data on regenerative capability in the auditory system of chicks and vestibular system of chicks and mammals (Ryals and Rubel 1988; Corwin and Cotanche 1988; Raphael, 1992, 1993; Forge, et al. 1993, Warchol et al. 1993) raise the need for better understanding of the role of inner ear fluid during the regenerative process. For example, it is likely that diffusible factors might regulate the mitotic activity of supporting cells in the auditory epithelium of chicks exposed to noise (Raphael, 1993). In addition, early studies by Suga et al. (1970) showed direct effect of sound on endolymphatic sodium and potassium. Our present evidence for the longitudinal flow theory and new data on regenerative capability of cells in the inner ear, further emphasize the need to analyze sac fluid in different pathological conditions such as after acoustic trauma, irradiation, different genetic degenerative disorders and drug toxicity.

It has been documented by different analytical techniques in different species that endolymph characteristic content is maintained in most compartments of the membranous labyrinth. The calcium content has also been shown to vary, and was recently shown by Ninoyu et al. (1986a and b), to be almost two fold in the endolymphatic sac as compared with the other parts of membranous labyrinth. This is in agreement with our present results where a calcium peak was clearly seen in the spectra obtained from the sac lumen. In the present study the analysis of the endolymphatic sac lumen in semithick sections always showed sulfur and phosphorus in combination with sodium, chlorine, potassium and calcium. The presence of sulphur and phosphorus is probably due to the appearance of different macromolecules while salts represent ionic composition of endolymphatic sac endolymph. Macromolecules may originate from the vestibular and cochlear parts of inner ear or be a secretion product originating from the sac epithelium. Another source of macromolecules might be the material from free floating cells in the lumen.

There are several analytical studies of the sections of inner ear tissues performed by means of laser microprobe analysis (Amano et al. 1983, Meyer zum Gottesberge-Orsulakova and Kaufmann 1985). The Na/K ratio found in the epithelial layer of the endolymphatic sac was almost the same as in the vestibular hair cells and outer hair cells of the organ of Corti. A higher Na/K ratio was found in inner cochlear hair cells and marginal cells of the stria vascularis. Amano et al. (1983) found approximately 30 % higher Cl concentration in the cochlear endolymph compared to the sac. During anoxia, Cl levels increased in the endolymphatic sac. An almost 3 times higher K/Na ratio was found in the epithelial cells as compared to sub-epithelial cells of the endolymphatic sac. After injection of ethacrynic acid K^+ activity increased, but the K/Na ratio decreased. Presentation of the results in terms of K/Na ratio is not adequate in systems with differential Na/K concentrations. Elevated ratios might for example be a result of elevated Na or decreased K. In our study on the time dependent changes in stria cells following ethacrynic acid exposure (Wroblewski and Anniko 1986) sodium changes in marginal cells were inversely related to sodium fluctuations. Therefore the ratio model should only be used when one of the elements is stable or when presenting the elemental changes within the same cell type.

There are two systems involved in the formation of the endolymph (Kimura et al. 1982): the stria vascularis and the dark cell epithelium around the vestibular organs. Anniko et al. (1988) showed that both marginal cells of the stria vascularis, dark cell epithelium and the epithelium of endolymphatic sac have a high amount of cytokeratins as demonstrated by means of immunolabeling techniques. These cell types are thought to play active roles in ion/water transport (Kimura et al. 1982). Several morphological features in the endolymphatic sac seem to facilitate endolymph absorption; rugose epithelium with microvilli, fenestrated capillaries, venous vasculature and large perivascular spaces (Lim and Frelich 1981). There is however difference in the type of junctions found in the cells of membranous labyrinth. Epithelial cells of endolymphatic sac have ultrastructural characteristics

of leaky epithelium, allowing movement of fluids and ions, whereas the rest of membranous labyrinth contains sealing tight junctions (Jahnke 1975; Anniko and Wroblewski 1984). In the distal part of the sac epithelium, the sodium and phosphorus levels were statistically higher than in the medial part. The potassium and calcium levels were lower. The sodium content in the sac epithelial part was of the same magnitude as in the stria vascularis marginal cells exhibiting almost the same ratios between P/S, S/Na, Na/Cl, and K/Cl. The difference in potassium concentrations between epithelial cells and sub-epithelial connective tissue were higher in the medial part than in the distal part of the sac. The chlorine level was almost the same in the epithelium and connective tissue. Comparing ratios between different elements we found a consequent increase in ratios involving potassium. Thus, S/K, Na/K which increased between medial-, distal- epithelial cells and sub-epithelial connective tissue and K/Cl which decreased, confirming our findings concerning potassium distribution based on absolute concentration. These observations indicate the possible role of connective tissue in electrolyte and water transport as well as functional differences in epithelia of medial and distal portion of the sac.

One interest in studies of the endolymphatic sac is associated with its possible role in causing an endolymphatic hydrops. Juhn (1984) postulated that hypersecretion of endolymph, biochemical changes in perilymph and/or malfunction of the endolymphatic duct and sac are possible mechanisms responsible for the formation of endolymphatic hydrops.

Conclusions

The endolymph in the membranous labyrinth has a constant elemental composition in the cochlear and vestibular part, with high potassium and chlorine in a ratio > 1. The elemental content of endolymph in the duct and the endolymphatic sac was different from other areas, with sodium contents increased to the levels found in the perilymph. Combined with our morphological findings, the data suggest that the membranous labyrinth is ionically sealed in the cochlear and vestibular portions, whereas the epithelial cells of the endolymphatic sac are leaky. The differences in the ionic content in the epithelial cells in different portions of the sac may reflect specializations of ion and water transport. The sub-epithelial regions composed mostly of connective tissue, are known to have high water binding capacity and probably act as water content regulator creating an osmotic gradient. The findings on the luminal content suggest to us that otoconial destruction and cleaning of cell debris such as lipids and proteins take place in the endolymphatic sac. Our results suggest a role for the endolymphatic sac in fluid absorption, ion transport, and otoconial destruction. The data also support the longitudinal flow theory of the endolymph.

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Discussion with Reviewers

A Campos: Did you compare the elemental composition of mature otoconia from vestibular region and the degenerated otoconia of the endolymphatic sac within the same animal ?

Author: No. The elemental data concerning composition of vestibular otoconia are from animals taken at other occasions.

A Campos: How would you account for the high levels of Na in the endolymphatic sac epithelia and connective subepithelial tissue ? With different cryomethods, intracellular Na levels are usually lower.

Author: I agree that values for sodium are rather high both in the sac epithelium and in the connective subepithelial tissue. I compared them with data obtained on the stria vascularis cells (Wroblewski and Anniko 1986). The normal values for sodium in marginal and intermediate cells were in the same range. 1 hour after ethacrynic acid administration sodium concentrations in the guinea pig stria cells fall to 1/3 of the normal value. As our data are based on dry weight and we have no values concerning water content in the epithelial cells of the endolymphatic sac, it is difficult to speculate in final concentrations based on wet weight.

A Campos: How do you interpret the presence in the endolymphatic sac of a few otoconia with high concentrations of sulphur, which resemble more immature otoconia?

Author: I do not have any direct explanation to otoconia with high sulphur and calcium content. One should notice, however, that sulphur to calcium ratio in such otoconia is almost the same as in otoconia which contained phosphorus in addition to calcium.