## Morphology of Hensen's cells

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#### INTRODUCTION

Hensen's cells form a layer of tall cells arranged in several rows which contact the basilar membrane of the cochlea. They are arranged in Corti's organ in an area delimited by the phalangeal process of the third row of supporting cells and by Claudius' cells (Hensen, 1863). Subsequent descriptions (Kolmer, 1927; Hallpike, 1936; Vinnikov & Titova, 1964; Kimura, 1975) have clarified some structural characteristics including the existence of lipid droplets in the cytoplasm, but no data relating to the functional significance of Hensen's cells have been published up to the present time.

#### MATERIAL AND METHODS

Ten young (250–300 g) healthy guinea-pigs were used after checking that the Preyer reflex and electrocochleographic recordings were normal. All the animals were anaesthetized with Nembutal. The cochleae were fixed with 2.5 % glutaral-dehyde (buffer Sorensen pH 7, 440 mOsm/1) injected through the oval window.

Specimens for transmission electron microscopy were fixed in ice cold glutaraldehyde and post-fixed in 2 % osmium tetroxide and 0.5 % uranyl acetate in accordance with the method of Karnovsky (1967). Tissues were dehydrated with acetone, embedded in Araldite, sectioned in an LKB-III Ultratome and observed in a Hitachi HU-12A instrument (75 kV) after lead citrate counterstaining.

Cochleae for scanning electron microscopy were fixed in slightly warmed glutaraldehyde and post-fixed and dehydrated as for TEM, after the bone had been removed with a drill. Dessication with  $CO_2$  was carried out in a Hitachi HCP-1 critical point dryer. Gold-palladium sputter-coated samples were studied in a Hitachi HHS-2R scanning electron microscope operating at 15 kV.

Specimens for light microscopy were fixed in Lillie's buffered formaldehyde. Surface preparations were stained with osmium tetroxide, red oil-O or Sudan III.

#### RESULTS

The shape of Hensen's cells was not the same in all areas of the cochlea. In the lower or basal coil of the cochlea a single layer of cells, of cuboidal form in sections and with elongated apical surface (Figs. 1, 2), formed a smooth area without any entumescences except for the projection of the cellular nuclei (Fig. 2, arrow). The cytoplasm was of low electron density and contained very few organelles except for some dispersed mitochondria (Fig. 1).

In the apical coil of the cochlea the apical pole of Hensen's cells was completely

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Fig. 1. Hensen's cells at the basal turn. The cytoplasm is almost devoid of organelles. Microvilli and apical cell junctions are prominent. TEM.  $\times 4000$ .

Fig. 2. Hensen's cells in the basal coil. Nuclear reliefs (arrow) and microvilli can be seen. SEM.  $\times\,2800.$ 

Fig. 3. Hensen's cells at the apex showing circular pits (arrow). The shape of the first row of cells resembles the basal coil, but the lateral rows are expanded. SEM.  $\times 2600$ .

Fig. 4. Hensen's cells at the apex. Lipid droplets nearly fill the cytoplasm. TEM.  $\times 2400$ .

different and formed a surface on which a line of cells, of globular appearance, was conspicuous (Fig. 3). All of the surface formed by Hensen's cells showed small depressions on the enlarged free poles (Fig. 3). The cytoplasm of the cells situated at this level was a little more dense than in cells situated in more basal coils, and contained more organelles (compare Figs. 1 and 4), while spaces of low electron density, which usually occupied large areas of the cells (Fig. 4), were consistently observed. These areas were rounded in shape and lacked a limiting membrane (Fig. 4): they have been taken to represent lipid droplets.

The application of specific techniques for staining fats was very positive in the apical and negative in the basal coil for the whole band of Hensen's cells (Figs. 5, 6). The second and third turns showed a progressive increase in the lipid content above the level formed basally.

The depressions in the enlarged Hensen's cells of the apical coils revealed interesting morphological details when studied with the scanning electron microscope. On some occasions slight central depressions were seen, the microvilli being absent from the depression (Fig. 7). Other cells showed partial loss of the plasma membrane, so that hollows were formed which could be associated with clotted homogeneous material (Fig. 8) or with remains of plasma membrane (Fig. 9). Most frequently, however, they appeared as smooth rounded openings (Fig. 10), thus suggesting that the large, ragged perforations (Fig. 9) were initial stages of the hollow formation process in which material was extruded into the endolymph.

### DISCUSSION

Lipid droplets are a well known feature of Hensen's cells (Hallpike, 1936; Vinnikov & Titova, 1965; Kimura, 1975). The distribution of these droplets suggests that they may be related in some way to the auditory process, since their spiral arrangement (Figs. 5, 6) is, broadly speaking, parallel to that of the total distribution established by Bekesy (1943). In fact, it is generally accepted that the vibration of Corti's organ in the apex is different from the vibration in the basal coil. In this respect it is interesting to comment on the possible connection between the two facts.

It is known (Kimura, 1966), that the tectorial membrane is attached by its lateral end to Hensen's cells. The spatial arrangement of this anchorage may influence the connection of this membrane with the sensorial hairs. If the lateral point of attachment is above the level of Corti's tunnel, the distance between the cuticular plates and the tectorial membrane will be greater than in the opposite case. This would represent a factor of variability in the interaction of the two elements on which the initiation of electrogenesis depends (see Dallos, 1975) so that it is possible that the height of Hensen's cells may influence the process of stimulation in some way. This height is determined by the shape of the cells at the apical pole which, in turn, depends on the quantity of lipid that the cells contain.

A process of lipid expulsion which perhaps is indicated in Figures 7, 8, 9 and 10 might be considered as a mechanism of variation of the height of Hensen's cells and therefore as an additional factor of modulation in the interaction between the hairs and the tectorial membrane. On the other hand, the concentration between the lipid content and the position of the tectorial membranes must also be considered in relation to the data of Davis (1958), according to which Hensen's cells are situated in the area of maximum vibration of the basilar membrane. Therefore, the modula-



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tion in the transmission of mechanical energy previously referred to would be situated in an especially critical area, i.e. at the point where this transmission is maximal.

#### SUMMARY

The cochleae of ten guinea-pigs were studied by transmission electron microscopy, scanning electron microscopy and optical microscopy, using specific techniques for staining fats. The study of Hensen's cells showed the existence of prominent lipid droplets in the apex and the third coil of the cochlea. Similar images were not found in the basal coil. Lipids appear to be expelled into the endolymphatic space. The significance of these findings is discussed with respect to the geometry of the lateral anchorage of the tectorial membrane and to the possibility that Hensen's cells represent a modulation mechanism in the transmission of mechanical energy.

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Figs. 5–6. Light microscopy of Hensen's cell at the basal coil (Fig. 5) and apex (Fig. 6) after staining with Sudan III. There are no lipids in the basal turn but Hensen's cells of the apex are filled with sudanophilic droplets.  $\times$  270.

Figs. 7–10. SEM images of the secretory process of Hensen's cells at the apex and third coil. The first stage appears as a central depression, devoid of microvilli (Fig. 7), which seems to detach itself at one border (Fig. 8), releasing an amorphous material. In a later step the pit is completely open (Fig. 9), and later on smooth, rounded hollows appear, once the released material has dissolved itself in the endolymph. Fig. 7,  $\times$  9610; Fig. 8,  $\times$  9620; Fig. 9,  $\times$  9050; Fig. 10,  $\times$  9000.