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The Limbus Spiralis and Its Relationship to the Developing Tectorial Membrane in the Cochlear Duct of the Guinea Pig Fetus*

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Summary. The development of the interdental cells of the limbus spiralis and of the inner spiral sulcus cells as well as the formation of the mesenchymal teeth of Huschke are described during fetal life up to the day of birth in the guinea pig. Additionally, the changes of the developing tectorial membrane are studied. The ultrastructural observations allow the conclusion that during fetal development at least a considerable part of the material of the tectorial membrane is secreted by the interdental cells of the limbus spiralis.

Key words: Guinea pig fetus – Cochlea – Limbus spiralis – Interdental cells – Tectorial membrane.

Introduction

Continuing our studies on the development of the organ of Corti (Thorn, 1972, 1975) and of the greater epithelial ridge (Thorn et al., 1977, 1978), we examined the changes of the limbus spiralis and its relationship to the developing tectorial membrane during fetal life. For the present study we used exactly dateable guinea pig fetuses (*Pirbright*-albino-stem) at stages from 34 to 63 days of fetal development as well as new-born guinea pigs. In this species duration of pregnancy is 58 to 72 days. The cochlea of the guinea pig has $4^1/_2$ turns. In the 34 day old guinea pig fetus, the cochlear duct forms four turns. In the 36th day of fetal life, the formation of the turns is completed. Differentiation of the epithelium of the cochlear duct begins at the basal turn and proceeds gradually towards the apex.

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Materials and Methods

New-born guinea pigs were anesthetized with nembutal injected intraperitoneally. The fetuses were removed by Caesarian section under nembutal-ether-anesthesia of the mother. The new-born animals and the fetuses were decapitated. The cochlea on both sides was approached by opening the bulla tympanica. The stapes was removed and the round window membrane was opened to permit access of fixing solutions to the perilymphatic spaces. In very young fetuses the cochlea was opened in the basal turn by removing a part of the thin cartilaginous outer wall. Furthermore, each cochlea was opened at the apex by making a small hole in the cartilaginous (later bony) shell. Then the cochlea was separated from the temporal bone and fixed in glutaraldehyde (6.25%, phosphate buffer pH 7.4) for 12 h up to 3 days at 4° C. After washing in phosphate buffer, postfixation in osmiumtetroxide solution (1%, veronalacetate buffer pH 7.2) for 2 h at 4° C, washing in veronalacetate buffer, dehydration in alcohol, transfer into propyleneoxide and embedding in epon (Luft, 1961). Ultra-thin sections and semi-thin sections were made with an ultramicrotome LKB "Ultrotome I" or Reichert "OM 3", respectively. The ultra-thin sections were stained with uranylacetate and lead citrate and studied in a Siemens electron microscope "Elmiskop I" or in a Zeiss electron microscope "EM 10". The semi-thin sections were stained with paraphenylenediamine (Estable-Puig et al., 1965) or with toluidin blue and studied in a Zeiss photomicroscope II with phase contrast.

Results

Light Microscopy

In young fetuses (34th day of development) the cross-sections of the fourth turn of the cochlear duct show an approximately oval lumen. The lateral wall, the future stria vascularis, and the upper wall, the future Reissner's membrane, possess a low prismatic epithelium, whereas the basal wall shows a thickening of the epithelium at the site of differentiation of the organ of Corti and the inner spiral sulcus. Medially (= near the modiolus), this high columnar epithelium is covered by the developing tectorial membrane. The cochlear duct is surrounded by mesenchymal tissue.

At the same stage of fetal development (34th day) cross-sections of the third turn of the cochlear duct show a triangular configuration and the borders between the three walls become more distinct. At the medial side of the high epithelium in the basal part of the cochlear duct, densely lying mesenchymal cells form the anlage of the limbus spiralis (Fig. 1). This anlage is covered by a low prismatic epithelium, the future interdental cells. The tectorial membrane extends from this epithelium to the developing organ of Corti. On the 36th day (3rd turn) the high epithelium at the basal side of the cochlear duct forms two ridges: medially, the greater epithelial ridge and laterally (= near the outer wall of the cochlea), the lesser epithelial ridge.

The teeth of Huschke start to develop between the 37th and 42nd days of fetal life. They become more distinct in the subsequent days of development (Fig. 2).

On the 48th day the inner spiral sulcus is formed in the medial part of the greater ridge by cytolysis of supporting cells. Up to the 52nd day the inner spiral sulcus widens in the lateral direction, and the limbus spiralis obtains its definitive lateral covering by a low prismatic epithelium, which forms the wall of the inner spiral sulcus.

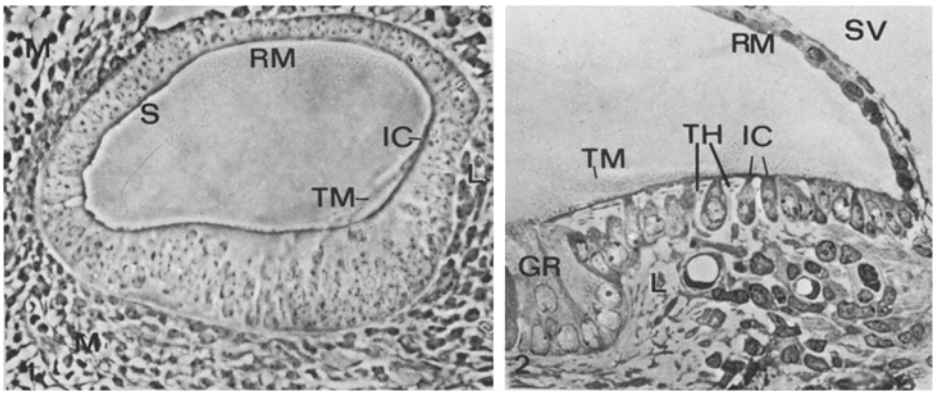


Fig. 1. Ductus cochlearis (34th day of development, 3rd turn). *L* anlage of the limbus spiralis, *IC* developing interdental cells, *TM* tectorial membrane, *RM* Reissner's membrane, *S* stria vascularis, *M* mesenchyme. Toluidin blue. Phase contrast. 250:1

Fig. 2. Limbus spiralis (48th day of development, 2nd turn). *L* limbus spiralis, *IC* interdental cells, *TH* teeth of Huschke, *TM* tectorial membrane, *GR* greater epithelial ridge, *RM* Reissner's membrane, *SV* scala vestibuli. Toluidin blue. Phase contrast. 320:1

Electron Microscopy

As seen by light microscopy, the limbus spiralis starts to differentiate on the 34th day of fetal life in the 3rd turn. An electron micrograph at low magnification of the limbus spiralis shows a low prismatic epithelium at the upper surface, the prospective interdental cells (Fig. 3). At the apical surface of the epithelial cells some microvilli are observed, and in the lateral portion small protrusions of the cytoplasm are seen (compare Fig. 4a). The lateral part of the epithelium is covered by the developing tectorial membrane, a thin layer of amorphous material including some vacuole-like spaces. The epithelium is separated from the underlying mesenchymal tissue by a thin basal lamina. The lateral cell membranes of the epithelial cells are intimately apposed. Narrow spaces between the plasmalemmata of neighbouring epithelial cells are observed only in the basal part of the epithelium. The epithelial cells are interconnected by gap junctions just beneath the apical surface. The nuclei of the epithelial cells lie in the basal part of the cytoplasm. Most nuclei are oval and slightly cleaved; only a few nuclei have a spherical shape. The cytoplasm is rich in organelles such as mitochondria of the crista-type, granular endoplasmic reticulum and free ribosomes. A prominent Golgi-apparatus is found in the supranuclear cytoplasm. Between the Golgi-complex and the apical cell membrane small vesicles are seen, and some of these vesicles fuse with the plasmalemma and open into the amorphous matrix of the tectorial membrane (compare Fig. 4b).

On the 37th day the second turn of the cochlear duct shows widening of the intercellular clefts between the developing interdental cells, their apical parts remaining in close contact, interconnected by gap junctions. The intercellular

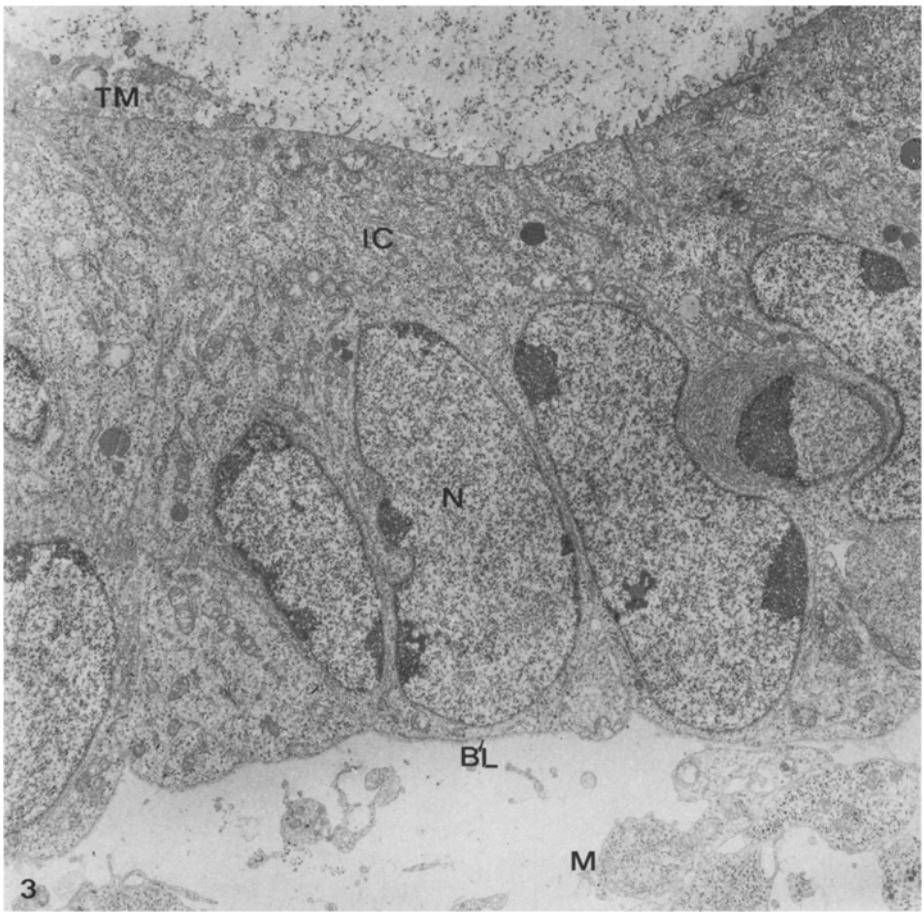


Fig. 3. Apical part of the limbus spiralis (34th day of development, 3rd turn). *IC* interdental cell, *N* nucleus of an interdental cell, *TM* tectorial membrane, *BL* basal lamina, *M* mesenchyme. Uranylacetate – lead citrate. 4,100:1

spaces, the future teeth of Huschke, contain some small bundles of collagen fibrils in an amorphous ground substance, being in connection with the underlying connective tissue. Between the plasma membrane of the epithelial cells and the connective tissue a meander-shaped basal lamina is interposed. The tectorial membrane now contains thin filaments in its upper part in addition to the amorphous matrix near the apical surface of the epithelial cells.

During the following days of fetal development the distance between the lateral plasmalemmata of neighbouring epithelial cells increases, whereas the apices of the cells remain in close contact. The apex of each interdental cell forms a thin horizontal plate or phalanx. The epithelial cells thus assume an amphora-like shape (Fig. 5). Between the cylindric cell bodies small borders of connective tissue, the teeth of Huschke, are interposed, apically being covered by the horizontal plates

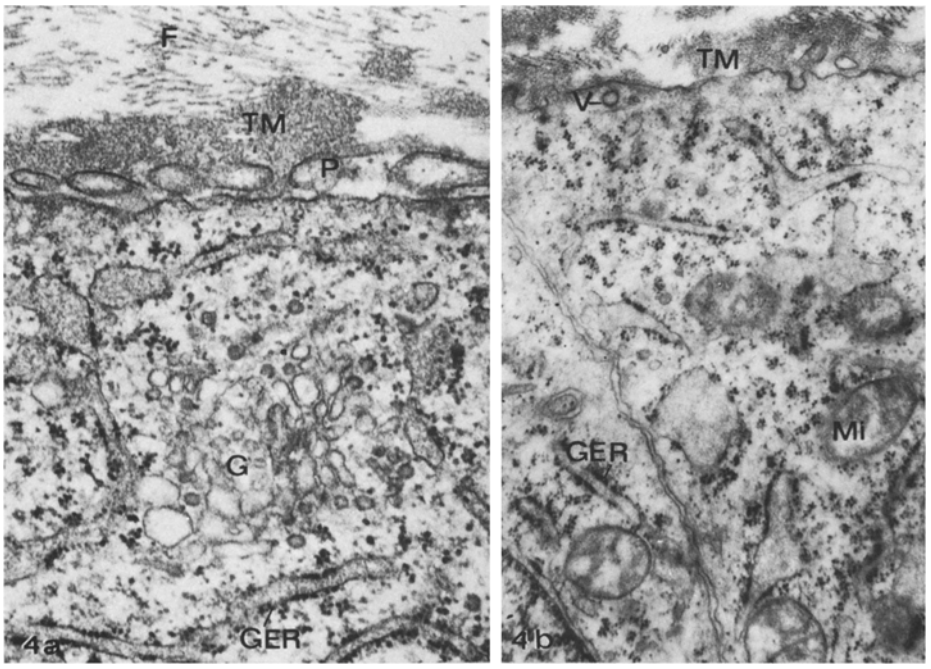


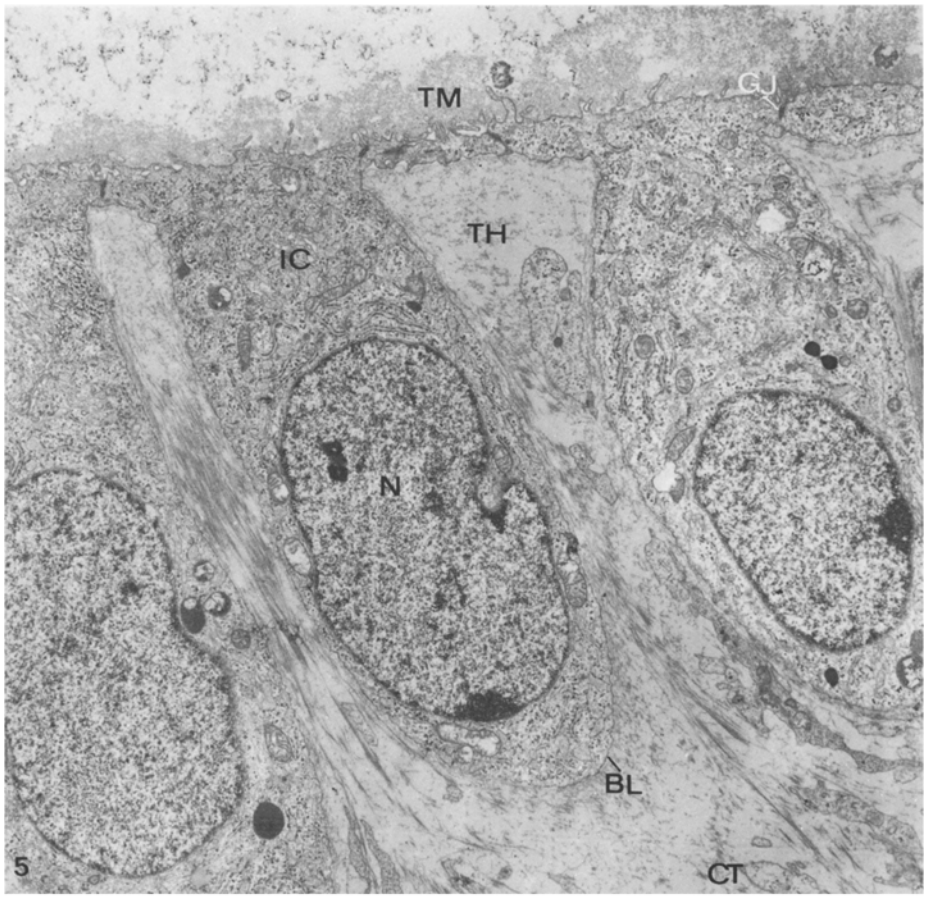
Fig. 4a and b. Apical surface of interdental cells (37th day of development, 2nd turn). *G* Golgi-apparatus, *GER* granular endoplasmic reticulum, *Mi* mitochondrion, *V* vesicle, *P* protrusion of the cytoplasm, *TM* tectorial membrane, *F* filaments in the upper part of the tectorial membrane. Uranylacetate – lead citrate. **a** 25,000:1, **b** 32,800:1

of the interdental cells. The interdental cells continue to be rich in organelles. Additionally, their cytoplasm now contains some irregularly shaped dark bodies and a few granulated vesicles.

Until the 48th day the tectorial membrane becomes thicker and richer in filaments. Up to this stage of development, at the lateral side of the limbus spiralis, the high epithelium of the greater ridge is situated at the corresponding site of the future inner spiral sulcus.

On the 48th day the inner spiral sulcus is formed rapidly by cytolysis of epithelial cells of the greater ridge. The vestibular lip of the limbus spiralis consists of the connective tissue of a tooth of Huschke, which is covered by the thin phalanxes of interdental cells. The tectorial membrane is attached to the horizontal phalanxes. One interdental cell faces the inner spiral sulcus just below the vestibular lip of the limbus spiralis. The basal part of the inner spiral sulcus is lined by the inner sulcus cells, which arise from the high columnar epithelial cells of the greater ridge by cytolysis of the apical cytoplasm. The inner sulcus cells have a cuboidal shape with a spherical nucleus and a clear cytoplasm, which contains only a few organelles. In the intercellular clefts between neighbouring inner sulcus cells, there are some microvilli projecting from the lateral surfaces of these cells.

Towards the end of fetal life, the content of cell organelles in the cytoplasm of the interdental cells diminishes. In the newborn guinea pig, the apical plasmalemma



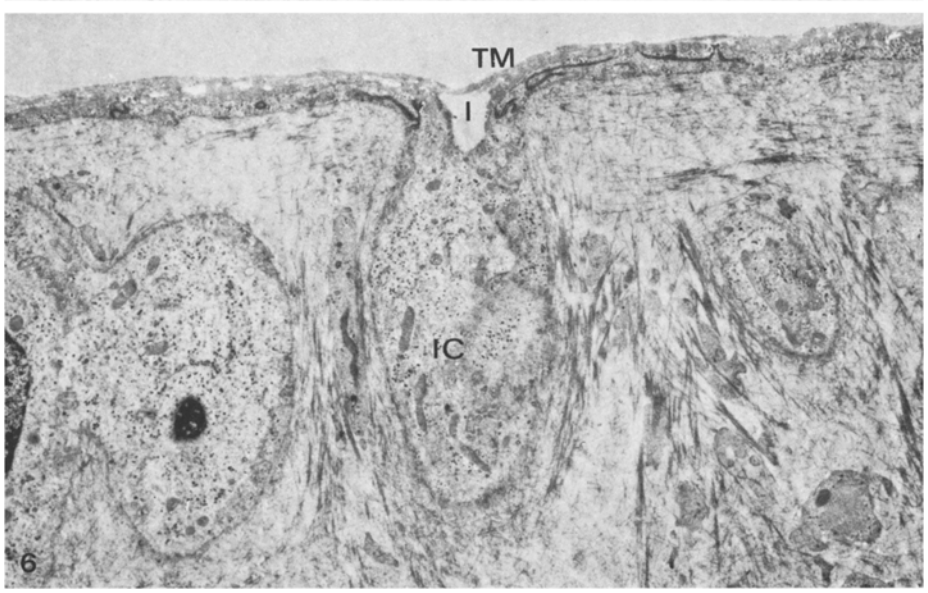


Fig. 5. Interdental cells and teeth of Huschke (48th day of development, 2nd turn). *IC* interdental cell, *N* nucleus of an interdental cell, *GJ* gap junction, *TM* tectorial membrane, *TH* tooth of Huschke, *BL* basal lamina, *CT* connective tissue. Uranylacetate-lead citrate. 52,000:1

Fig. 6. Interdental cells (new-born animal, 3rd turn). *IC* interdental cell with an invagination (*I*) of the apical plasmalemma. *TM* tectorial membrane. Uranylacetate-lead citrate. 4,000:1

of some interdental cells forms an invagination, which contains a conglomeration of an amorphous substance resembling that of the tectorial membrane (Fig. 6).

Discussion

Based on light microscopic observations, Held (1909: several mammals) and Weibel (1957: mouse) concluded that the developing interdental cells participate in the formation of the tectorial membrane. Iurato (1962) studied the interdental cells in adult, young and new-born rats with the electron microscope. He found a higher content of cell organelles in the young and new-born animals than in the adult ones, and supposed that these cells produce the filaments of the tectorial membrane.

Our electron microscopic findings suggest that the developing interdental cells secrete an amorphous material into the endolymph of the cochlear duct. In contact with the endolymph, this amorphous substance may be partially transformed into filaments. The developing interdental cells show typical features of a secretory function. Their cytoplasm contains abundant granular endoplasmic reticulum, a prominent Golgi-complex and small vesicles in the apical area. The content of these vesicles seems to be discharged by exocytosis, and to be incorporated into the amorphous substance of the tectorial membrane. Additionally, the small protrusions at the apical surface of the interdental cells appear to be expelled into the tectorial membrane. In the guinea pig, the fetus as well as the new-born animal, the thin amorphous lower layer of the tectorial membrane remains in close contact with the apical surface of the interdental cells. In the upper part of the tectorial membrane, numerous filaments are incorporated into the amorphous substance. At the site of the greater epithelial ridge, an intimate contact between the plasmalemma of microvilli and filaments of the tectorial membrane was observed (Thorn et al., 1977, 1978). At the site of the interdental cells, this kind of connection was not seen.

In the new-born guinea pig, we additionally find invaginations of the apical plasmalemma in some interdental cells. These invaginations contain some amorphous material resembling that of the tectorial membrane, as described in the adult guinea pig by Lim (1969, 1970). Our data support the view of Voldřich (1967), von Ilberg (1968), Lim (1969, 1970) and Arnold and Vosteen (1973) that the interdental cells of the limbus spiralis maintain the tectorial membrane during post-fetal life by secretion of an amorphous material.

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