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# Ontogenesis of rat cochlea. A quantitative study of the organ of Corti

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**Abstract**

A systematic quantitative set of data concerning the organ of Corti in developing Sprague-Dawley rats at intervals from 18 days of gestation to 10 days after birth (DAB) is provided in this study. Using phalloidin staining, the total number of inner and outer hair cells, the whole length of cochlea, as well as the diameter of inner and outer hair cells and the intercellular space between inner hair cells were determined in order to analyze the quantitative change of inner and outer hair cells during development and to explore some roles of the factors regulating the growth of cochlea. The results show that: (1) The length of cochlea approached its adult size by 7DAB. (2) The growth of the extreme part of the apex was responsible for the delayed elongation of the cochlea. (3) Growth in the cochlear length mainly results from an increase of cell diameter tempered by a decrease of intercellular space. (4) The adult size of inner and outer hair cells was obtained by 7–14DAB. (5) The final number of inner and outer hair cells was reached at 3DAB and remained constant through adulthood. No significant hair cell overproduction and cell death were observed during ontogenesis of the cochlea. The negligible importance of overproduction and missing hair cells during hair cell differentiation suggest that there is a precise regulation phenomenon for producing the right spatial organization of the organ of Corti.

*Keywords:* Hair cell; Development; Spiral lamina growth

## 1. Introduction

During early ontogenesis, the cochlea undergoes conspicuous modifications through tissue and neurotrophic interactions. Later, after the early differentiation of hair cells from progenitor cells it is interesting to observe a regular arrangement of the sensory epithelium where inner hair cells (IHCs) and outer hair cells (OHCs) with their supporting cells are well organized spatially [12,18,28,31]. The first differentiation of auditory hair cells in the rat observed by phalloidin staining and scanning microscope begins before birth, at 18 days of gestation (DG) for IHCs and at 20 DG for OHCs [31,42]. Hair cells in mammals seem to originate from progenitor cells present in what is called Kölliker's organ [18,31]. Despite several studies on the quantitative aspects of cochlear development [6,7,12,14,32,39] none have included the fetal period. However, it is known that hair cells differentiate before birth in rodent species studied so far [18,31,35], so problems related to hair cell differentiation and development may have been overlooked. For instance, is there an overproduction of hair cells during the earliest stage of hair cell differentiation as observed for cochlear cells during early developmental ages [33,40], followed by a stage of cell death as seen in some regions of the brain [24]. This mechanism might play a role in the formation of the inner ear and the regular geometric arrangement of the sensory epithelium. Cell death was observed during early rat otocyst ontogenesis [21] and fetal human otocyst [27] as well in the stato-acoustic ganglion in the rat [34] and the chicken [4]. However, none of these investigations reported if cell death occurred in hair cells. Hair cell death may be relevant to recent observations of supernumerary hair cells observed in rat fetal cochlea in vitro [1,2].

At the same time it was of interest to study the kinetics of hair cell ontogenesis. When and where do hair cells start to differentiate? We already know in the rat when hair cells start to differentiate from progenitor cells [31], although we do not know where they first appear. The cochlea is a tridimensional organ with a complex spatial organization reflected in hair cell ontogenesis. Hair cells and related cells do not start to differentiate at the same

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time in all regions of the cochlea [5,16,17,20,30]. Another relevant question was related to factors responsible for the growth of the cochlear length during cochlear ontogenesis, where several factors can be involved [12]. Of special concern was the possible overproduction of hair cells during early differentiation and their subsequent death during development.

In this study we addressed the above questions by using phalloidin staining which is a specific marker for F-actin [9] making possible the visualization of the apical pole of hair cells in surface preparations. It was possible by this technique to observe the first sign of hair cell differentiation from Kölliker's organ cells [31].

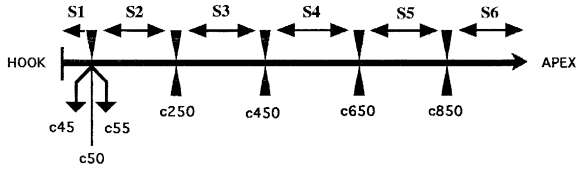



Fig. 1. Schematic diagram showing the position of IHCs chosen as marker along the cochlear partition. The first marker was the fiftieth IHC named c50, given that the count started from the first IHC at the beginning of the hook. c250 was the next 200th IHC after c50; c450 the next 200th IHC after c250; and the same with c650 and c850. c45 and c55 correspond to five IHCs before and after c50 used for quantitative analysis of hair cell growth. Each segment (S) corresponds to the interval between each cell location (horizontal arrows), for exemple, segment S2 refers to the interval between c51 and c250.

## 2. Materials and methods

The animals used in this study were Sprague-Dawley rats free of middle ear infection. Three animals for each of eight age groups were used encompassing the fetal, postnatal and adult ages, i.e.: gestation day 18 (18DG), gestation day 20 (20DG). Newborn (N), 3 days after birth (3DAB), 5DAB, 7DAB, 14DAB and three months (A). Days of vaginal plug were counted as day 0.

The microsurgical dissection procedure was done as follows. After completely removing the bony wall of the cochlea, the stria vascularis and the Reissner's as well as tectorial membranes were removed. The sensory epithelium with the Kölliker's organ, when present and the spiral ganglion were carefully removed in order to obtain the complete spiral lamina, including the entire apex and the hook as shown in Fig. 2. The cochlea was cut into three pieces in order to get a flat preparation for microscopic observation. The phalloidin staining was done as previously reported [38]. Briefly, using the 96 well culture plate, the pieces of cochleae were immersed in fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS)) for 1 h. After washing, the cochlear pieces then were incubated in 33 nM phalloidin in PBS for 1 h in the dark at room temperature. After mounting, the slide was viewed by a fluorescence microscope.

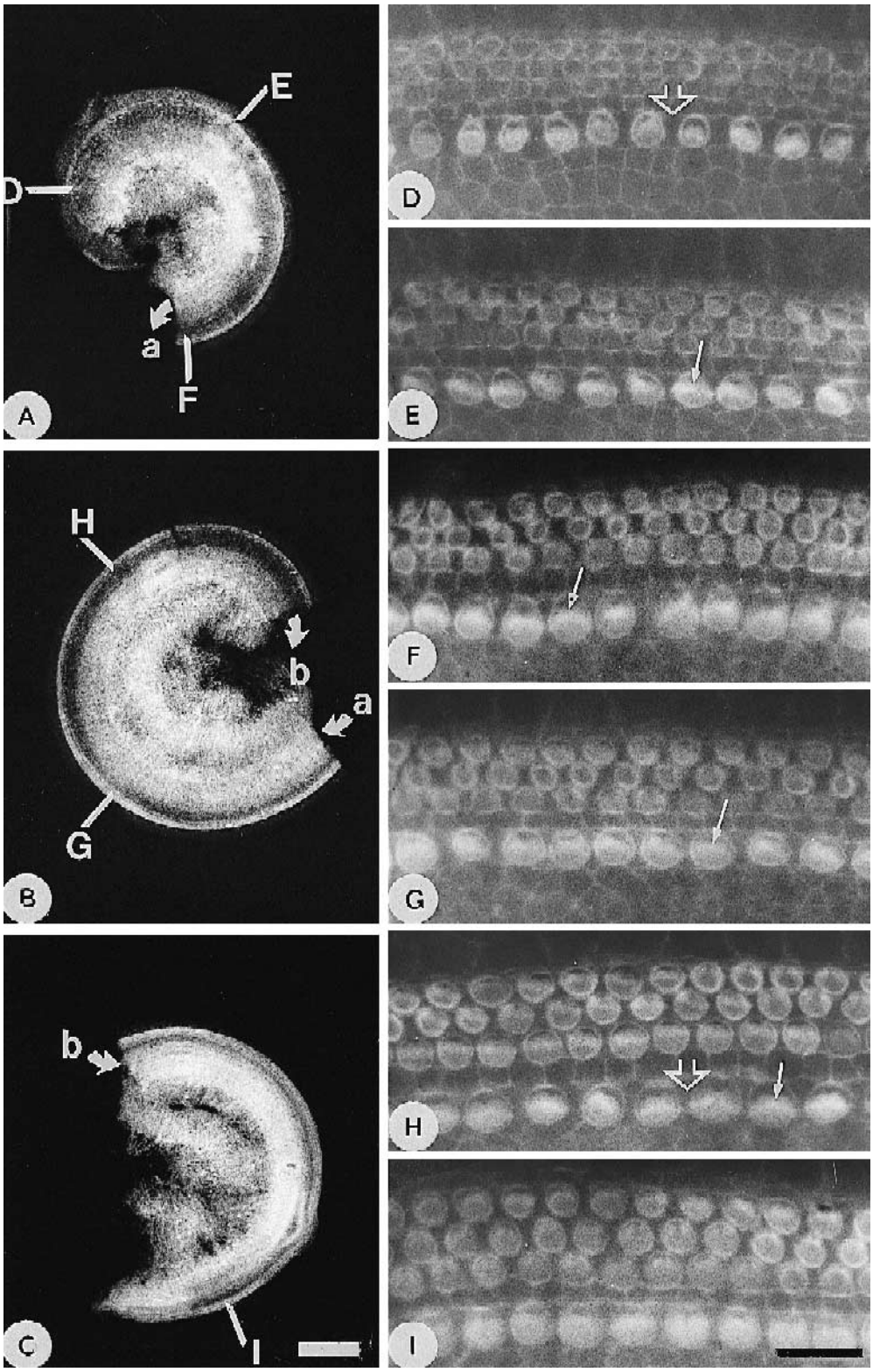
The length of the cochlear partition was measured at the level of IHCs, under direct microscope observation with a calibrated reticle, from the beginning of the apex to the end of the hook and compared with the measurement made by computer with an image analysis station from Biocom. In the youngest animals where IHCs had not yet appeared in the portion of the apex, the length was measured from the most apical extremity of the IHC row to the most apical part of the spiral lamina. This was made possible because a differential organization of the presumptive sensory epithelium was revealed by phalloidin staining. The mean value of length $\pm$ one SEM were computed from the measures obtained from three cochleae. The numbers of IHCs and OHCs were counted from the beginning of the apex to the end of the hook. Cells were counted as hair cells when they first differentiated from cells from Kölliker's organ. They were recognized by a round ring of phalloidin staining lining the plasma membrane [31]. Measurements of the diameter of hair cells along selected locations of the cochlear partition were based on the position number of hair cells and not on distance or percentage of the cochlear length. This time-consuming method was chosen because we wanted to follow the growth of hair cells from the same hair cell location across animals at different ages. We used IHCs as the marker because they appear first during ontogenesis and they show a very regular spatial organization during development. The first selected position was designated to be the fiftieth IHC, named c50, which was counted starting from the beginning of the hook (Fig. 1). We chose the hair cells from the hook as reference because they were present at the earliest age investigated, i.e. 18DG and are well differentiated in all following ages. After the first selected location, the next position was the 200th IHC after the location c50, so the second position was named c250, and the third cells chosen were 200 cells away from c250 and was c450, and so on for the following positions, c650, c850 as shown in Fig. 1.

The diameter of hair cells was measured directly under microscopic observation at $\times 1000$ with a calibrated reti-

Fig. 2. Micrographs of surface preparations of a newborn cochlea labeled by phalloidin. A, B and C correspond to the three pieces of the spiral lamina obtained after dissection of the cochlea. A: apex, B: middle, C: base. For each piece one to three micrographs at higher magnification (micrographs on the right side) were taken several hundreds of $\mu$m apart (white marks with a letter corresponding to micrographs on the right side) in order to show the variation of hair cell differentiation along the cochlear partition. One can observe the difference in the diameter of the apical hair cells and intercellular spaces between different regions (open arrows). Focus was made at the level of the cuticular plate. Stereocilia can be guessed by the stronger staining on the apical pole of hair cells, especially IHCs (arrows). A–C: bar = 400 $\mu$m; D–I: bar = 20 $\mu$m.

cle, from the largest width of the apical cap. Five cells were measured on the basal and apical sides of the chosen cell, for 11 hair cells for each cochlea, i.e., a total of 33 from three cochleae. The same measurements were performed for OHCs from the first row as described for IHCs.

The cochlear partition was divided into several segments according to previous locations as established by IHC count. Each segment was measured by direct observation under the microscope and computed from three cochleae for each age. Segments correspond to the interval



between each designated cell location, e.g. segment 2 refers to the interval between c51 and c250, segment 3 corresponds to the interval between c251 and c450, etc... (Fig. 1). Each segment refers to 200 hair cells, except segment 1 which corresponds to 50 hair cells between location c1 to c50. For comparison purposes with other segmenst, segment 1 was multiplied by 4. The width of the intercellular space was obtained from ten spaces between 11 IHCs at each location, 5 intercellular spaces on the basal side of the chosen cell and 5 spaces on the apical side. The mean value of 30 intercellular spaces was computed from measurements obtained from three cochleae.

Comparisons across ages or positions were performed using ANOVA and Student-Fisher's procedures based on *t*-test computations.

## 3. Results

At 18DG on a surface preparation from the basal cochlea it was possible to recognize a single row of IHCs, although not well differentiated, and three rows of cells that may correspond to future OHCs, owing to their rounded apical shape, compared to neighboring cells. Later at 20DG, OHCs were more differentiated, although a large difference existed between turns of the cochlea, as can be observed later, at birth (Fig. 2). In fact, in the basal turn, hair cells were well differentiated with their cuticular plates and their stereocilia, although in the upper part of the cochlea at the apex OHCs were only outlined by staining restricted to the plasma membrane (Fig. 2A). In the following ages, hair cells grew in diameter and become more differentiated with adult shaped cuticular plate and stereocilia. Detailed qualitative observations of hair cell development studied by phalloidin staining have been published elsewhere [31].

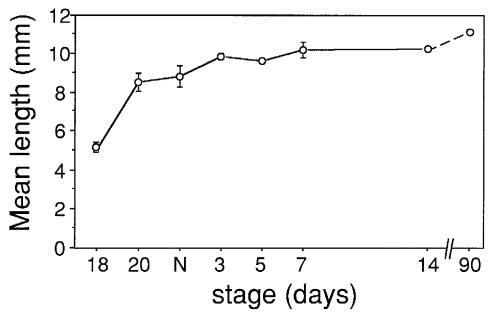



Fig. 3. Development of the spiral lamina mean length of cochleae at different ages of development. Note the rapidly increasing length from 18DG to 20DG. After birth the mean length of the cochlear partition continues gradually, growing until 7DAB. At this last age the length was close to the adult one. 18: embryo age of 18DG; 20: embryo age of 20DG; N: newborn; 3: 3DAB; 5: 5DAB; 7: 7DAB; 14: 14DAB; 90: 3 month old. Symbols will be the same for the following figures. Each data point represents the mean total length ± one SEM computed from three cochleae. The error bars do not show up for each datum because they are too small.

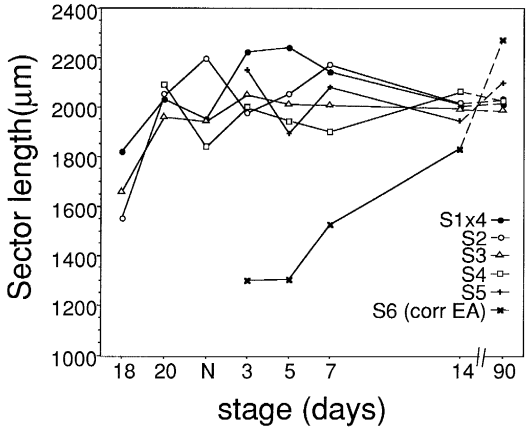



Fig. 4. Modification of the mean length during development for six cochlear segments. Note that the segment 6 (extreme apical segment: EA), corresponds to the distance calculated from c 850 (see Fig. 1) to the end of the apex. It is the latest region where hair cells differentiate but presents the fastest growth. Growth of basal segments (1, 2 and 3) between 18DG and 20DG is rapid, whereas small discrepancies are observed between later ages. Each datum point represents the mean length of each segment computed from three cochleae. The length of segment 1 is multiplied by 4 in order to be comparable to other segments which included 200 cells. For the same reason the length of segment 6 was corrected.

### *3.1. Supernumerary and missing hair cells*

Usually, supernumerary hair cells are present as an extra row of OHCs in addition to the usual 3 rows. Extra hair cells were seen at 3DAB, 7DAB, 14DAB and adult ages. At 3DAB the number of extra hair cells was restricted to OHCs and was no more than 5 to 7 cells in the whole cochlea and 1 to 3 cells for other ages.

Cells were considered as missing when phalangeal scars and/or a gap in the normal geometric array was present. A few missing cells were found in postnatal and adult ages, most of them restricted to OHCs. For example, in one adult cochlea 27 OHCs and no IHC were missing. Except for the adult age that presented the highest number of missing cells. On average, only 2 or 3 OHCs were absent for an entire cochlea.

### *3.2. Increase of the cochlear partition*

The length of the cochlear partition measured by direct microscope observation was very similar to that measured by computer, and the difference between these two measurements never differed by more than 0.9% to 1% (average difference not significant, $P > 0.05$). At 18DG the cochlea was less then 6 mm in length (Fig. 3). Between 18DG and birth the length increased rapidly from $5.3 \pm 0.3$ mm to $8.5 \pm 0.8$ mm. After birth growth continued gradually until 7DAB when it reached $10.2 \pm 0.6$ mm. At 7DAB the length of the cochlea was close to the adult value $10.9 \pm 0.1$ mm. The length of the cochlear partition showed a statistical difference with ages below 7DAB compared to the adult ($0.001 < P < 0.05$).

Since the cochlea continued growing in length until 7DAB, the interesting question is which part of the cochlear partition presents the fastest growing rate? From Fig. 4, we can compare the growth of the different segments of the basal turn. Segments 2 and 3 increased from 18DG to adult (1552 $\mu$m to 2028 $\mu$m for segment 2 with a mean rate of 14.8 $\mu$m/day; 1661 $\mu$m to 2025 $\mu$m for segment 3 with a rate of 11.3 $\mu$m/day). This rapid growth was observed before birth, and can reach up to 200 $\mu$m/day. The fastest growing region after birth was the extreme apical part of the cochlea after the c850 location that presents a delayed growth compared to other segments (from an average of 1257 $\mu$m at 3DAB to 2274 $\mu$m in the adult). Interestingly enough, apical segments (4 and 5) presented a more or less adult size at the beginning of their differentiation.

### 3.3. Ontogenesis of inner hair cells

The number of IHCs increased rapidly during the period before birth, from less than 550 at 18DG to around 900 at birth (Fig. 5). From 3DAB on, the number of IHCs was stable for all older ages. The mean number of IHCs at 18DG was $530 \pm 54$, which was statistically different from 20DG ($P = 0.001$) and N ($P < 0.01$). In the adult age the number of IHCs reached the mean value of $1009 \pm 13$, but there were no significant differences between postnatal ages and the adult ($P > 0.05$). The number of IHCs thus remained stable from 3DAB to adulthood.

Growth of the apical diameter of IHCs gradually increased from 18DG (4.5 $\mu$m $\pm$ 0.7) to 14DAB (10 $\mu$m $\pm$ 0.6). Comparing cell size from different locations along the cochlear partition (Fig. 1) shows that, at birth, the diameter of IHCs was larger at the hook compared with the apex. The apical IHCs differentiated later; however, they sharply increased in diameter and caught up with cells from the hook (Fig. 6A). Comparing the change of cell diameter between c50 to c650, significant differences were found for all ages including the adult ($P < 0.001$). Additionally, it can be seen (Fig. 6A) that the growth of IHCs was not synchronous in all five locations of the cochlear partition studied. The cells located at c450 never reached the size of basal or apical hair cells even in the adult. In

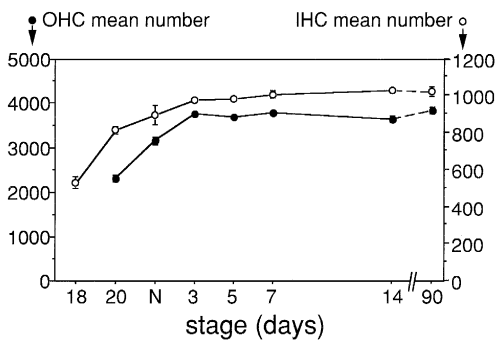



Fig. 5. Total mean number of IHCs and OHCs at different ages of development. Note that before 3DAB the number of IHCs and OHCs increased rapidly. After this age the number of the hair cells was stable throughout adulthood. Each data point represents the mean count of 31 IHCs and OHCs $\pm$ 1 S.E.M. computed from three cochleae. The error bars do not show up for each datum because they are too small.

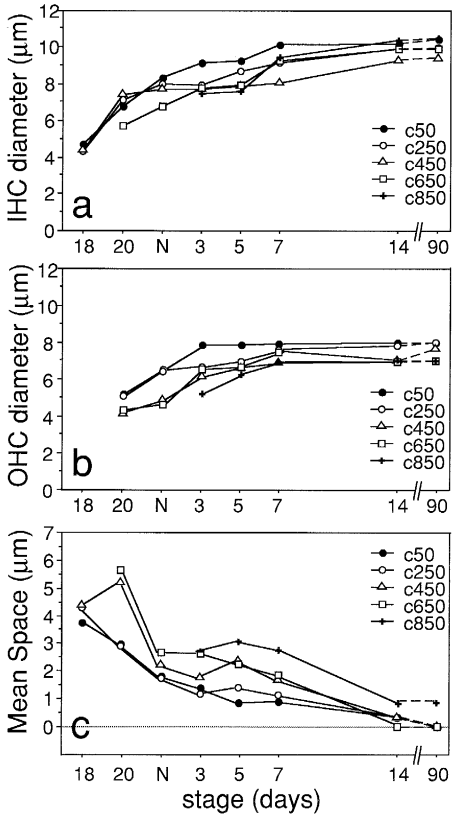



Fig. 6. A-Mean diameter of IHCs for five selected locations along the cochlear partition: c50, c250, c450, c650 and c850 as shown in Fig. 1 in relation to development. The diameters of IHCs are larger in the hook (c50) than that in other locations ($P < 0.001$) for each age studied except ages before birth and segment 5 after 7DAB. During development the diameter of IHCs from the apex steeply increased and caught up with the basal cell despite their later differentiation. In the basal turn the IHCs were the first to reach adult size by 7DAB and there was no statistical difference between 7DAB and the older ages ($P > 0.05$), except that IHCs from the c450 segment are smaller in size. The diameter of IHCs from the apex (c850) was smaller than the diameter of cells from the hook (c50) before 7DAB ($P < 0.0001$). At 14DAB IHCs from the apex caught up to the size of IHCs from the base. IHC measurements from the c850 segment were not studied for the newborn age because for two cochleae they were not well differentiated. Each data point represents the mean value from 33 IHCs of three coch B-The diameter of OHCs from the hook was much larger than those from the apex at 3DAB. The OHCs from the hook (c50) were the first to reach adult size at 3DAB. The diameter of apical OHCs (c850) never caught up with the size of cells from the base (c50) ($P < 0.0001$). Each data point represents the mean diameter from 33 OHCs of 3 cochleae. C-Modification of spaces between IHCs during development. The space was smaller in the hook region (c50) and in the first turn (c250) compared with other regions and presented a regular decrease with development. Between 3DAB and 7DAB the kinetics of decreasing space slows down and space reached adult values after 14DAB. The spaces between IHCs in the apical cochlea (c50) are still larger than those for the other regions ($P < 0.0001$) in the adult age. Each datum point represents the mean space obtained from 30 measurements.

the hook region (c50) the IHCs reached their adult size by day 7, around one week earlier than the other segments of the cochlea, which reached an adult size by 14DAB.

### 3.4. Ontogenesis of outer hair cells

The onset of OHC differentiation lags behind that of IHC. In fact, there is no clear sign of OHC development at 18DG. The OHCs first appeared at 20DG, their average number was $2315 \pm 140$. It then increased rapidly until 3DAB (Fig. 6B). The total mean values of cells from the three rows of OHCs at birth and 3DAB are 3157 cells $\pm 140$ and 3756 cells $\pm 82$, respectively, then the number slightly increased to 3853 cells $\pm 58$ toward the adult age. The differences between the adult and 20DG ($P = 0.003$), or adult and newborn ($P = 0.01$) were statistically significant, but there was no significant change between 3DAB and older ages, showing that the final number of OHCs was reached by 3DAB and remained stable thereafter.

The diameter of the apical part of OHCs increased from 20DG ($4.0\ \mu m \pm 0.7$) to adult ($7.5\ \mu m \pm 0.5$). The growth pattern of OHCs was similar to the pattern observed for IHCs. For each age studied including adult, the OHC diameter for locations c50 and c850 was significantly different ($P < 0.0001$). The diameter of OHCs was larger for basal than apical cells (Fig. 6B). Comparing the growth between locations, cells from the c50 location were the first to reach adult-like size by 3DAB, around 4 days before the other parts of the cochlea. There was no significant difference in the other four locations between 7DAB and 14DAB with the adult.

### 3.5. Modifications of intercellular space

Parallel to the change in hair cell diameter, a change of the intercellular space was observed. The space between IHCs from 18DG to adult decreased progressively (Fig. 6C). The intercellular space between IHCs was smaller in the basal part (c50 and c250) than for the apical and decreased from $3.7\ \mu m \pm 0.2$ at 18DG to $1.8\ \mu m \pm 0.1$ in the newborn to unmeasurable space between cells in the adult from ligth microscope observations. The IHC space in the c450, c650 and c850 locations were larger than that in the base (c50 and c250). During development, the decreasing width of the space between IHCs in the apex (c850) presented the same trend as that observed in the base, but the space remained larger even in the adult (Fig. 6C).

### 3.6. Cell concentration

Since changes in cell diameter and intercellular space were observed, it may be expected to find modifications in cell concentration in different regions of the cochlea. For IHCs, cell concentration is lower for basal and apical segments, which represent the two extreme positions along the cochlear partition. In contrast a larger concentration is found in the intermediate segments. As for IHCs, OHCs presented a more or less constant concentration during development for each segment studied, although the basal segment has a lower concentration of OHCs throughout the ontogenesis.

## 4. Discussion

In an early qualitative study [31] it was shown that the staining of the plasma membrane of hair cells by phalloidin is the first sign of differentiation from Kölliker's organ cells. The next step corresponds to the appearance of the cuticular plate followed by the stereocilia and by the typical V or W spatial organization of stereocilia that may take several days after birth to develop. This early study did not address the following question. Do the cells of the sensory epithelium during the course of this early development go through remodeling after a stage of hair cell overproduction?

### 4.1. Supernumerary and hair cell death

From our present observations it is interesting to observe at the earliest stage of hair cell differentiation the regular geometrical arrangement of the sensory epithelium, with one row of IHCs and 3 rows of OHCs. Since we did not observe significant supernumerary hair cells nor any hair cell missing in young ages during ontogenesis of the cochlea, we suggest that progenitor cells are committed to become hair cells very early during ontogenesis. This commitment would take place before cells start their differentiation, at least as we have observed by phalloidin staining, that corresponds more or less to what has been observed by optic microscopy in the rat [23].

Cell death is a widespread phenomenon occurring during normal development of vertebrates and corresponds to morphogenetic cell death as put forward by Glücksmann [10]. Later, during histogenesis, (i.e. remodeling of tissues), cells are produced in excess and a large percentage die [11,41]. For example, cell death has been observed for the rat retina, where about 50% of ganglion cells die during the first weeks after birth [15]. In sensory systems, cell death has been observed in the retina [36] and in the olfactory epithelium [25]. In the peripheral auditory system, decrease in cell numbers has been observed in the rat spiral ganglion innervating the sensory epithelium [34] as a late event during ontogenesis. In the cochlear nucleus, conspicuous cell death occur between 12 and 14DG in the mouse [22] just before hair cell differentiation. Overproduction of cells in the embryonic cochlea is known [27] and may be followed by programmed cell death as observed in the rat cochlea between 12 to 16DG [13,21]. However, no information is available at the present time concerning overproduction and subsequent hair cell death,

despite the fact that terminal mitosis is known to occur just before hair cell differentiation [33].

Our observation presents important consequences with regard to the supernumerary hair cells observed in vitro [1,2]. This suggests that supernumerary cells observed from fetal explants do not come from an original overproduction of hair cells, but rather from a transdifferentiation of cells that were not supposed to become hair cells in vivo. This is supported by explantations made at 3DAB, at an age when all hair cells are present; despite hair cell differentiation at this age, numerous supernumerary hair cells were observed after several days in vitro [8]. What is the trigger signal that may induce transdifferentiation of some cells? One possibility among others is that intercellular environmental factors may be responsible through lateral inhibitory cues between cells mediated by cell adhesion molecules [26,27,29].

The almost negligible occurrence of supernumerary hair cells and missing hair cell during the earliest age of hair cell differentiation strongly suggests that there is a very precise and tight regulation process present before the first sign of hair cell differentiation for producing the correct spatial and geometrical organization of the organ of Corti. This observation may not be general to all mammals since, in some mouse strains and in humans, supernumerary hair cells form much more commonly (unpublished observations).

### *4.2. Growth of the cochlear partition*

The cochlea has reached its final size by 7DAB (Fig. 3). Most of the growth process occurred mainly before birth. However, in another rodent species studied recently, elongation was mainly present after birth, although prenatal growth was not studied for this species [12]. This difference is probably related to variations in gestation period. Several factors may be responsible for the increasing size of the cochlea: addition of new hair cells, increasing size of hair cells. Do all these factors contribute to the growing length of the cochlea? Do they contribute to the growth only at a specific stage of development?

In the early age investigated, i.e. 18DG, the apical turn was present but was not completely developed as suggested by McRobert [23], whereas all turns were said to be completely present at 20DG. This difference may be explained by the fact that the sensory epithelium of the apical cochlea at 18DG was not differentiated and so has not reached its final size.

The main growth of the cochlea occurs before birth, between 18 and 20DG for the basal and intermediate segments (Fig. 4) and well after birth for the extreme apical part of the cochlear partition. The elongation at the extreme apex after birth accounts for most of the residual late increase of cochlear length occurring after 7DAB (Fig. 4). Complex combination of three processes seem to occur during ontogenesis with regard to cochlear elongation: 1, increasing hair cell diameter, 2, elongation of the extreme apical part of the cochlea, 3, decreasing intercellular space. The first two phenomena may be in part offset by the decreasing intercellular space between hair cells (Fig. 6C). This was confirmed when comparing cell concentration for several segments over the cochlear partition. The cell concentration, despite changes in hair cell diameter and other modifications remained stable throughout development. Our conclusion joins those of Kaltenbach and Falzarano, [11] whose studies in the hamster showed that elongation was not produced by increasing cell number. In the rat, the adult number of hair cells was reached at 3DAB, before the final growth of the cochlea was completed at 7DAB, i. e. several days before the final growth of hair cells (Fig. 6). The above conclusion may apply to the rat after 3DAB when the cochlea has reached its full complement of hair cells. Before this age, growth in size of new hair cells may be an important factor for cochlear elongation, since they are larger than Kölliker' cells.

A question which can be asked is which cochlear region starts to reach adult-like morphological aspects? It is generally assumed that the final age of structural maturation begins at the base of the cochlea in a variety of species [3,33,37,39]. However, for other authors, such as Lorente de No [19] in the mouse, Larsell et al. [16] in the opossum, Romand and Romand [30] in the cat and Burda [6] in the rat, the late phase of maturation begins in the mid-first turn and develops toward both directions, the apex and the basal parts of the cochlea. From the present observations in the rat cochlea, it seems that when comparing growth of the apical surface of hair cells in different locations, both types of hair cells from the hook (c50) reach their adult size first (Fig. 6). It may be inferred that they are the first to differentiate. Which corresponds to what we have observed: cells from the hook and the first turn were first to start their differentiation from the Kölliker's organ. However, at this age it is difficult to state, based on anatomical criteria, which precise region of the basal cochlea is more mature when observing the apical poles of IHCs. Moreover, hair cells from the apical turn (c850) start to differentiate later and reach adult value with a delay, corresponding to the base-apex development.

### *4.3. Hair cell growth*

Hair cell growth occurs steadily from 18DG to 14DAB for IHCs and from 20DG up to 7DAB for OHCs. A question which can be asked is whether hair cells grow in diameter at the same rate in different regions of the cochlear partition? Our observation on the comparison of growth between regions was not done on the percentage of the cochlear length, because if different regions of the cochlea grow at different rates, then the different percentages used by Kaltenbach and Falzarano [12] for defining regions along the cochlea during ontogenesis cannot corre-

spond to the same location across different ages of development. Therefore in order to avoid this problem we used markers corresponding to a fixed number of IHCs along the cochlear partition. These markers are particularly useful, since it has been inferred [12] that the relative position of hair cells is preserved during cochlear growth.

The diameter of IHCs from the first turn (c450) is significantly smaller than those from other regions in the adult (Fig. 6A), especially after 7DAB. Therefore, one could expect to have a higher cell concentration for this region. This conclusion corresponds with those of Burda [6] who found in the same strain of rat a maximum IHC concentration at around 3 mm from the beginning of the hook, which corresponds approximately to the segment c450.

OHC diameters from the apical turn (c650–c850) were significantly smaller than those from the base (c50–c250) throughout development (Fig. 6B). This observation did not fit well with results from Burda [6], who found an opposite trend in the adult OHC triad. The OHC triad was defined as the radial width of a strip occupied by cuticular plates of 3 rows of OHCs [6]. In Burda's work the mean width of the triad decreased by half from apex to base in the adult cochlea. This difference might be explained, in part, by the fact that the width of the triad included the apical pole of Dieters cells. Thus, intercellular space between OHCs may explain discrepancies between our results and those in previous study [6].

In comparing growth of both types of hair cells, it was observed that OHCs reached adult-like size before IHCs. The same trend was observed by scanning electron microscopy studies on the apical pole of hair cells, where OHCs were the first to reach adult-like appearance [17,42].

In the rat when looking at the onset of hair cell differentiation, the kinetics of cochlear maturation seems to correspond more to a straight hook-apical development, which is consistent with a recent structural finding in the hamster [12]. However, other anatomical findings contradict this conclusion in that the length of the OHCs and width of OHC triad present a first adult-like development localized in the middle region of the rat cochlea [32], which corresponds to the cochlear region where the maximum concentration of hair cells was found [6]. We face two different hypotheses for the rat. Does the rat cochlea present a straightforward development from base to apex or does it show an early maturation in the mid-first turn followed by differentiation that progresses toward the hook and the apex? One possible explanation that could in part fit both hypotheses is that a region where the cell differentiation was delayed when compared with the hook, may have faster developmental kinetics and will reach adult-like configuration before the other cochlear regions. However, more precise anatomical criteria, such as those obtained by electron microscopic observations along with quantitative studies may help to elucidate the kinetics of cochlear development of different regions of the cochlea.

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