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Cochlear Structure in the Dolphin, *Lagenorhynchus obliquidens*

(ganglion cells/Pacific white-sided dolphin)

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ABSTRACT **The cochleas of five specimens of the Pacific white-sided dolphin, *Lagenorhynchus obliquidens*, that had been fixed by intravital perfusion, embedded in celloidin, and sectioned in a continuous series, were studied with particular attention to the numbers and distribution of hair cells and ganglion cells. The number of inner hair cells is estimated as 3272 and the number of outer hair cells is estimated as 12,899, for a total of 16,171 cells. The ganglion-cell population is estimated as 50,412 after correction for cell splitting in the sectioning process.**

In a series of experiments on sound conduction in the dolphin ear (1), the electrophysiological observations were followed by perfusion of the dolphin for a subsequent histological examination of the cochleas. Five specimens of the bottlenosed dolphin *Tursiops truncatus* and four specimens of the Pacific white-sided dolphin *Lagenorhynchus obliquidens* were perfused. Results have already been presented on the general morphology of the cochlea in *Tursiops*, with data on the dimensions of the basilar membrane and the number of hair cells and ganglion cells in this species (2–4). The present report deals with *Lagenorhynchus*, and gives particular attention to the form of the cochlea and the number and distribution of the hair cells and ganglion cells.

METHOD

Our histological procedure, as finally worked out, was described in the first report on *Tursiops*, and required about 1 year from the initial fixation through the various stages of decalcification, dehydration, and embedding in celloidin to the final stages of sectioning and staining.

Six ears were studied: four of them were sectioned in a plane parallel to the modiolar axis and two were sectioned in a plane perpendicular to this axis. The parallel plane was roughly vertical with respect to the head in three dolphins, and roughly horizontal in one; these are the usual planes of sectioning for mammalian ears and are useful for the general study of inner ear structure.

These cochleas were graphically reconstructed by Guild's method (5), which shows the form of the cochlear spiral and the length of the basilar membrane. These reconstructions were used further as a basis for measurements of the width of the basilar membrane by a method developed in principle by Guild and worked out for more general application by Wever (6).

Sectioning in the plane perpendicular to the modiolar axis gives views of the cochlear spiral that are always oblique, and for most of the structures are somewhat difficult to interpret. For an examination of hair-cell patterns, however, this orientation has many advantages, and our two series were prepared with this application in mind. The hair cells are cut transversely, and show up clearly in their row arrangements in relation to the supporting structures. Such oblique sections are suitable also for a study of the ganglion cells. With this plane of sectioning, the reconstruction method of Guild is not applicable, and a more tedious method had to be used. Each fifth section was projected upon tracing paper and the main features of the spiral structure were drawn in outline. Then these views were combined, with attention to suitable landmarks, to give the total picture.

In one of the specimens we obtained an estimate of the size of the ganglion-cell population by determining the ganglionic areas and then finding the relation between area and number of cells by counting under the microscope at ×200 magnification in several regions throughout the cochlea. We counted cell bodies, as in this specimen the nuclei and nucleoli were only faintly stained.

The density of innervation was found to be fairly uniform except at the two ends of the cochlea; at these ends direct counting was performed in all the sections. Through the middle of the cochlea, where the changes were gradual, the counting was performed in alternate sections, and intermediate values were obtained by interpolation.

RESULTS

In general morphology, the cochlea of *Lagenorhynchus* is closely similar to that of *Tursiops* (2). The form is that of a flat spiral of just under two turns in *Lagenorhynchus*, as compared with a spiral of a little over two turns in *Tursiops*. The principal differences noted were in the size of the Claudius cells and external sulcus cells, the number of Boettcher cells, and the form of the Deiters cells. The drawing of Fig. 1 represents a radial section at a point 14 mm from the basal end, and shows some of the features to be described.

As in *Tursiops*, the Claudius and external sulcus cells grade into one another without any marked distinction, especially in the basal turn. These cells in *Lagenorhynchus* do not reach the great size in the basal region that they do in *Tursiops*. Their heights near the basal end of the cochlea were about 110 μm, as compared with 145 μm in the corresponding region in *Tursiops*. The heights then diminish progressively to about 7 μm near the apical end, just as in *Tursiops*.

The number of Boettcher cells was about half that seen

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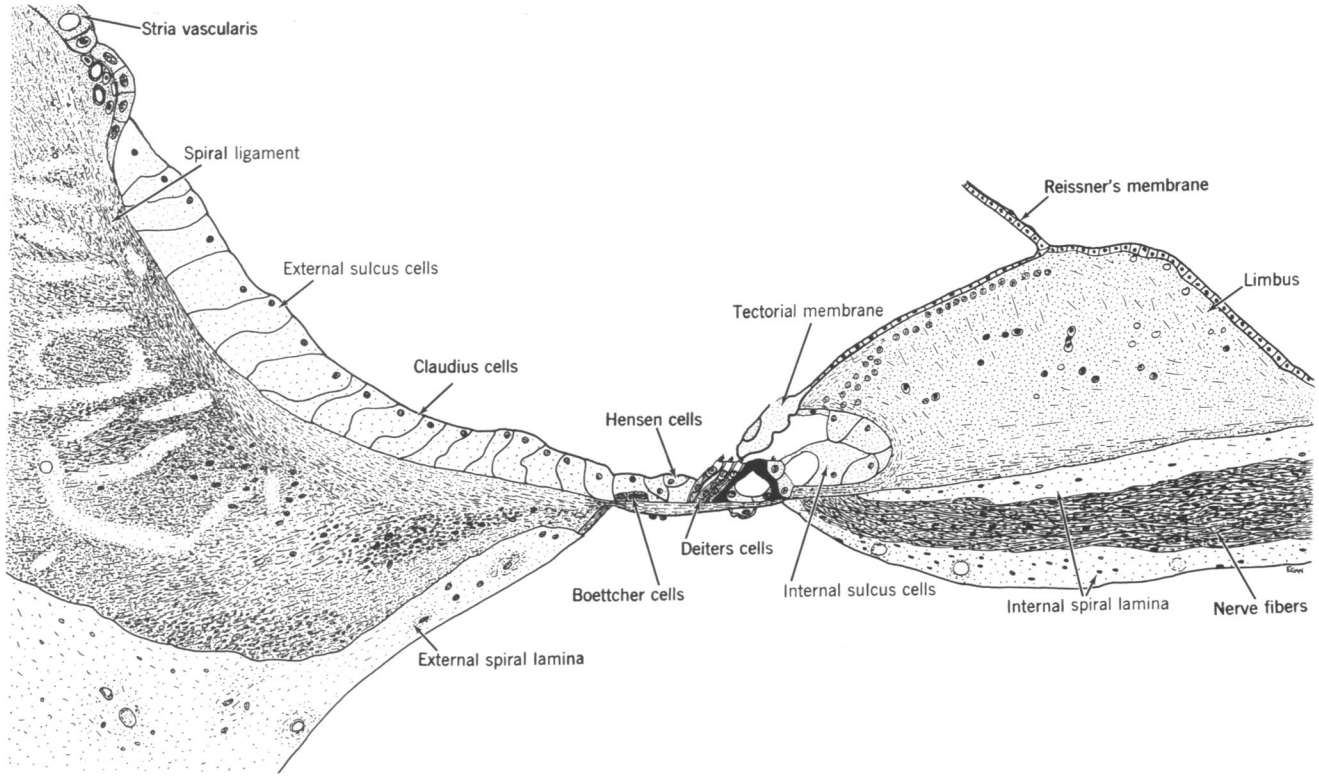

FIG. 1. A cross section of the organ of Corti in the dolphin *Lagenorhynchus obliquidens*, at a point 14 mm from the basal end of the cochlea. Scale ×200.

in *Tursiops* cochlea. No cells were found near the basal end, and only a single cell appeared about 5 mm up the spiral. Then the number of cells rose to 2 or 3 in the next 9 mm, and slowly increased to a maximum of 8 or 9 in the lower part of the apical turn. For the most part these cells are arranged in a single row, as they are arranged in other mammals, but in contrast to the double-row arrangement found in the middle of the *Tursiops* cochlea. However, in a few instances a single cell was seen resting on top of the regular row, giving a suggestion of the double-row pattern. Beyond the middle of the apical turn, these cells were no longer seen.

The Deiters cells do not undergo the systematic changes in size and orientation that they do in *Tursiops*, but throughout the cochlea these cells are rather thick and sturdy, and are fairly well lined up with the hair cells that they support.

The length of the cochlea as shown in a Guild type of reconstruction in two ears of the same dolphin was 24.8 mm for the right side and 26.6 mm for the left side. In three other specimens sectioned parallel to the modiolar axis, the length in each case was 28.5, 32.5, and 35.0 mm. The variations in these lengths are no doubt due in part to individual differences and, to some degree, to errors of measurement. In dolphins, as in many other mammals, the basal end of the cochlea curls around in a hook that ends nearly at right angles to the main course of the spiral, and this hook may make up about 10% of the total length. In the Guild type of reconstruction, the hook is likely to be foreshortened, and this effect accounts in part for the shorter measurements reported for the two ears of the first animal. The reconstruction as described for the sections cut perpendicular to the modiolar axis is subject to measurement error also, but probably represents the basal hook more adequately.

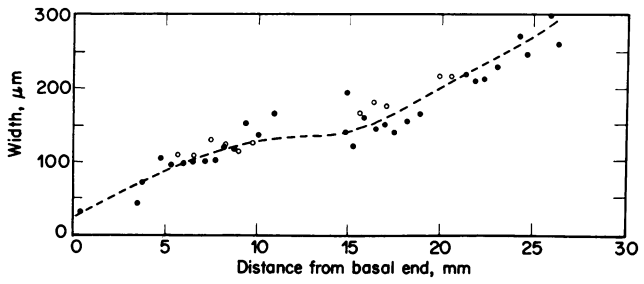

FIG. 2. The width of the basilar membrane in the two ears of a specimen of *Lagenorhynchus obliquidens*. *Open circles* represent the right ear and *filled circles* represent the left ear.

The two ears of the same dolphin were used for measurements of the width of the basilar membrane; the results are shown in Fig. 2. As will be noted, the basilar membrane varies in width about 11-fold from basal to apical ends.

The reconstruction of one of the cochleas sectioned perpendicular to the modiolar axis gave the spiral shown in Fig. 3. The *circles* indicate points at which the organ of Corti was cut across; these points were connected by the solid line to obtain the form of the structure. In the region from 14 to 25 mm from the basal end, the line departs from a regular spiral, and a broken line has been drawn in to indicate a true spiral course. We cannot find anything in the preparation of the sections that would account for the distortion of cochlear shape; hence, we regard it as a growth anomaly. In two other cochleas of this species of dolphin in which the form was studied, the cochlea resembled a regular spiral except for two variations, a somewhat gradual bulge in an apical di-

rection in the lower part of the basal turn and a terminal hook that has already been described.

Distances along the cochlear spiral are shown in millimeters from the basal end. In our working copy of this diagram, section numbers were indicated at frequent points along the spiral, which made it easy to locate any desired region and to designate features in terms of their position along the cochlea. Our main use of this diagram was in a study of the hair-cell patterns.

In those places where a section cuts across the organ of Corti and its hair cells, the picture is like that of Figs. 4 and 5. Shown from above downward are the single row of inner hair cells, the heads of the arches of Corti with the extended processes of the outer pillar cells, the three rows of outer hair cells, the Hensen cells, and finally the Claudius cells. In the photomicrograph, it is often difficult to distinguish the Hensen cells from the outer hair cells, but in microscopic examination there are differences of level and of staining that usually make the distinction clear. In Fig. 5, *small arrows* point to two outer hair cells that are displaced from their usual position in the third row.

Ordinarily, and more especially in the basal half of the cochlea, the arrangement of outer hair cells is very regular. Occasionally a cell is missing altogether. Fairly often, and increasingly so beyond the middle of the cochlea, a few extra outer hair cells may be found, suggesting a fourth row.

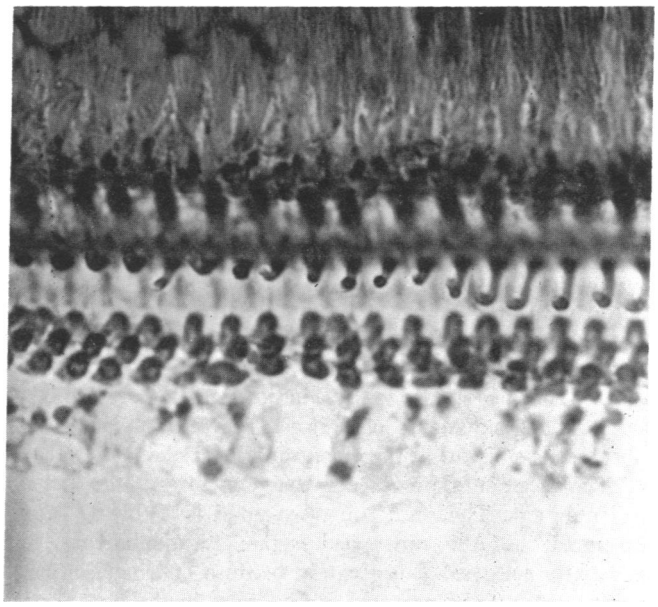

FIG. 4. Photomicrograph of a region of the cochlea showing the hair-cells and some of the supporting structures.

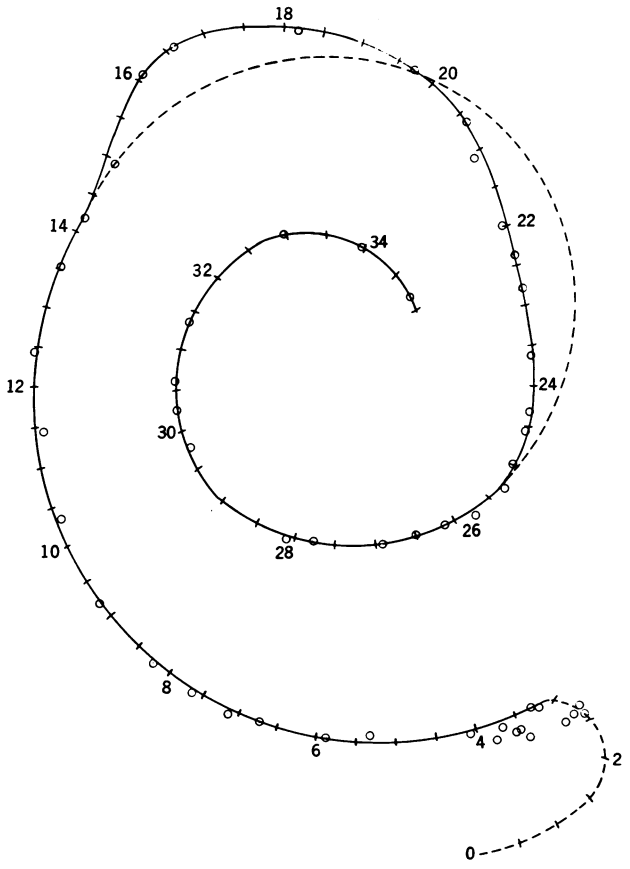

FIG. 3. Graphic reconstruction of a cochlea of *Lagenorhynchus obliquidens* sectioned in a plane perpendicular to the modiolar axis. The *circles* represent points at which the organ of Corti was cut across. At the basal end, the terminal part of the hook as drawn leaves the plotted points; actually this portion should extend at right angles to the plane of the paper, away from the observer; it has the proper length.

In the cochlea that was studied in detail here, at the beginning of the lower apical half-turn, the extra cells became sufficient to constitute a veritable fourth row, at first somewhat sparingly represented, and then becoming complete and regular. This condition did not extend far, however; after a millimeter or so these fourth-row cells became sporadic once more and then disappeared altogether. Three rows of cells continued, with occasional irregularities, through the remainder of the apical turn, until near the end numerous omissions from the third row were observed. At the very end, only two rows of cells remained.

At numerous points along the cochlear spiral, a determination was made of the spacing of inner and outer hair cells along their spiral rows. This was done by measurement with a screw-micrometer ocular of the length occupied by a given number of cells (usually 10), and division of the length by 10 or the number of cells.

Retzius (7) and others estimated the number of cochlear hair cells at some convenient point in the cochlea and assumed

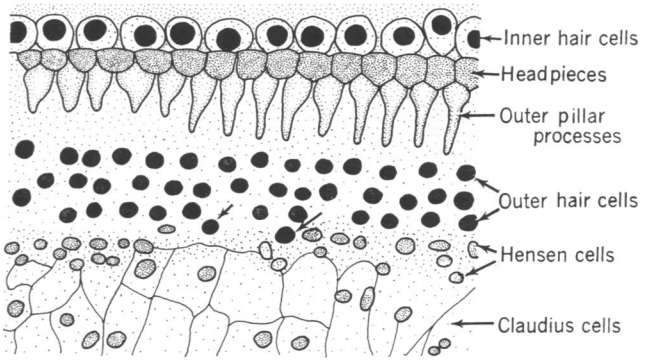

FIG. 5. A schematic drawing from a region of the cochlea near that shown in Fig. 4, to assist in identification of the elements.

that the distribution was uniform. This is not the case for the dolphin cochlea. The spacing is always greater for inner than for outer hair cells, and for both types it changes along the cochlea.

Our observations showed that for inner hair cells the spacing is greatest near the basal end, where it is around 12.7 μm, decreases rapidly through the lower basal half-turn to the middle of the upper basal half-turn, and then levels off around 9.5 μm. For the outer hair cells, the spacing decreases regularly from about 9.4 μm near the basal end to about 7.0 μm at the apical end. Satisfactory determinations could not be made in the first 4 mm of the basal end because of the twisting of the basal hook, but such observations as could be made indicated spacings similar to those in the 5- to 6-mm regions.

These measurements of hair-cell spacings, together with observations of row arrangement and lengths of the different cochlear segments, provided a basis for an estimate of hair-cell numbers. The results are presented in Table 1. It will be noted that the two apical segments are subdivided and the parts are treated separately because of irregularities in row structure: the presence of four rows in the first portion of the lower apical half-turn and the decline at the end of the upper apical region to two rows. This terminal decline reduced the number of rows in the last portion to an average of 2.5.

The total number of inner hair cells is 3272 and the number of outer hair cells is 12,899, for a grand total of 16,171 cells.

The second of the two specimens sectioned perpendicular to the modiolar axis was used for an estimate of the number of ganglion cells by the method already described. This procedure gave a total number of 88,442 ganglion cells before correction for cell splitting. Such a correction involves a consideration of section thickness (30 μm) and the average diameter of the ganglion cells (22.6 μm). By the use of Abercrombie's revision of Agduhr's formula (Konigsmark, ref. 8), the number of ganglion cells is reduced to 50,412.

DISCUSSION

It is of interest to compare the features studied in *Lagenorhynchus* dolphin species with those studied in *Tursiops truncatus*. The number of cochlear turns in *Lagenorhynchus* is about 1.75 as compared with slightly over two turns in *Tursiops*. The basilar membrane in the ear of *Lagenorhynchus* is somewhat shorter than that of *Tursiops*. The width of the basilar membrane is of the same order of magnitude, with perhaps a significantly smaller range of variation, but both the 11-fold variation in *Lagenorhynchus* and the 14-fold variation in *Tursiops* are striking in comparison with the 6.25-fold variation in man, which suggests a considerable capability of frequency differentiation in the dolphin ear.

Though the general anatomy of the cochlea is closely similar in *Lagenorhynchus* and *Tursiops*, there are differences in the arrangement of the outer hair cells. In *Tursiops* the 3-row arrangement extends from the basal end well into the lower apical half-turn, then a 4-row pattern is observed that shows some irregularities but continues to the apical end. In *Lagenorhynchus*, the 4-row pattern appears earlier in the lower apical half-turn, but it prevails for only a short stretch. The 3-row arrangement recurs in the remaining part of this half-turn, continues over most of the upper apical region, and then finally deteriorates to a 2-row pattern.

In spite of a somewhat shorter cochlea and a less favorable row structure in the apical region, the number of hair cells in *Lagenorhynchus* is only a little smaller than in *Tursiops*, because of a closer spacing of the hair cells in *Lagenorhynchus*. The outer hair cells in the apical region, in particular, are tightly packed. The number of inner hair cells of 3272 comparies rather closely with the number of 3451 hair cells in *Tursiops*, and the number of outer hair cells of 12,899 is only moderately less than that of 13,933 for *Tursiops*.

For both species these numbers compare favorably with those given by Retzius (7) for the human ear: 3475 inner hair cells and 11,500 outer hair cells. It appears that these two dolphin species and man are on about the same level as regards the primary receptor elements of the cochlea.

The number of ganglion cells in *Lagenorhynchus* is 50,412 after correction for cell splitting, but it should be mentioned that the evidence as reviewed by Konigsmark (8) has shown that the splitting of cells and cell inclusions in the sectioning process is in fact less frequent than what is indicated by the simple geometry of the situation. These bodies are elastic and move laterally out of the path of the knife; hence, the application of the formula results in an overcorrection. A more accurate size of the ganglion-cell population in *Lagenorhynchus* is perhaps of the order of 60,000–70,000 cells. Even so, the number is significantly smaller than that in *Tursiops*, where the estimate was 104,400 before correction and 95,004 after correction for cell splitting. For both these species the numbers are considerably greater than man's complement of 30,500 ganglion cells.

If we consider the relation between ganglion cells and hair cells, using our best estimate as 65,000 for the ganglion-cell population in *Lagenorhynchus*, the ratio becomes 4:1, which

TABLE 1. *Number and spacing of hair cells*

Cochlear segment	Location (mm)	Segment length (μm)	Inner hair cells		Outer hair cells				Inner + outer
			Spacing (μm)	Number	Spacing (μm)	No. per row	Rows	Number	
Lower basal	0–16.0	16,000	12.1	1322	9.0	1777	3	5,331	6,653
Upper basal	16.0–26.0	10,000	9.8	1020	8.0	1250	3	3,750	4,770
Lower apical	26.0–27.5	1,500	9.5	158	7.5	200	4	800	958
	27.5–32.0	4,500	9.7	464	7.3	616	3	1,848	2,312
Upper apical	32.0–33.5	1,500	9.7	155	7.1	211	3	633	788
	33.5–35.0	1,500	9.8	153	7.0	215	2.5	537	690
			Totals	3272				12,899	16,171

is somewhat below the 5:1 ratio found in *Tursiops*. These ratios are both high in comparison with the 2:1 ratio in the human cochlea.

As was suggested for *Tursiops*, this high ratio of ganglion cells to hair cells may be regarded as aiding the representation of high-frequency information and of fine details of cochlear events to higher centers of the auditory nervous system, thereby assisting in the performance of the dolphin's echolocation mechanism.

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