CONTRIBUTIONS TO SENSORY PHYSIOLOGY

Edited by William D. Neff

VOLUME 1

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WILLIAM D. NEFF

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Preface

The publication of *Contributions to Sensory Physiology* was undertaken with two principal objectives in mind: (1) to bring together reports of current research on all of the sensory systems and (2) to provide an opportunity for the scientist studying a sensory system to give a detailed account of a series of experiments or to present, at some length, a theory about the physiological basis of sensation. It is not the intent of *Contributions* to present review articles. Authors have been asked to write about their own research findings and theoretical notions and to review the work of others only as it seems suitable for the interpretation of results and theoretical discussion.

As the contents of this first volume suggest, sensory physiology has been given a broad definition—it includes the range from microscopic anatomy to psychophysics. The anatomist has been urged to speculate about the functional significance of his discoveries regarding structure; the psychophysicist has also been encouraged to consider the physiological mechanisms that might explain the findings of his experiments.

Additional volumes of *Contributions to Sensory Physiology* will appear at intervals of approximately one year. It is the hope of the editor and publisher that this series will provide better communication among those who study sensory systems and that it will also be a valuable source of information for scientists from other fields who occasionally seek a representative sample of research that is being done in this important area of physiology rather than just a summary.

WILLIAM D. NEFF

November 1964

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Vision in Intermittent Light

H. PIÉRON

Cellular Pattern, Nerve Structures, and Fluid Spaces of the Organ of Corti[1]

HANS ENGSTRÖM,[2] HARLOW W. ADES,[3] and JOSEPH E. HAWKINS, JR.[2,4]

I. INTRODUCTION

The foundation of our present concepts of the complex cytoarchitecture of the cochlea in man and higher animals was laid in the late 19th century by Retzius, Held, and others. Out of that fruitful period in European cytology came the lucid descriptions and the unsurpassed drawings which have remained the accepted standard of our knowledge of cochlear structure almost to the present day. In the first half of the present century considerable attention has been given to the cochlea, but most of this has been along functional lines, based on studies of electrophysiology, wave mechanics, and psychophysics. These studies have yielded abundant amounts of new information, forging ahead of

[1] This study was supported in part by the Office of Naval Research (Contract No. 62558-263), the National Institutes of Health (Grant No. OH-93) and the Swedish Medical Research Council.

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the available morphological information and thus raising important questions that can be answered only by new analytical approaches to the fine structure of the inner ear.

In response to this situation, several groups of investigators, beginning in the last decade, have applied modern techniques of structural analysis to the inner ear. These have included phase contrast microscopy, electron microscopy, autoradiography, histochemistry, and quantitative microchemical methods. They have already added to, and changed in some respects, the hitherto unsurpassed descriptions and illustrations of Retzius, Held, and others of the early period. They have also extended and enriched the earlier observations which were based on light microscopy alone.

Much of the newer work has concerned itself with ultrastructure of various cochlear cell types. It has now become necessary to reconsider the cytoarchitecture of the cochlea in relation to other recent findings. This paper will describe and illustrate the cellular pattern of the neuroepithelium, giving special attention to the sensory cells, neural structures, and fluid spaces of the organ of Corti. It will also illustrate that the simultaneous full use of light, phase contrast, and electron microscopy yields a composite whole which is much greater than the sum of its independently employed components.

II. TECHNIQUE

Most descriptions of cochlear structure and cytoarchitecture are based on sections of fixed and decalcified specimens cut axially through the modiolus. The standard illustration shows a radial section of the organ of Corti with one inner hair cell and three outer hair cells, surrounded by their supporting structures. By studying a large number of serial sections it is possible to get a general impression of the cells throughout the cochlea and to make a graphic reconstruction. In practice such reconstructions are usually based on an inspection of every tenth serial section, and the condition of the cells in between is inferred by interpolation. This method has been used by a number of investigators (Guild, 1921; Guild *et al.*, 1931; Wever and Smith, 1944; Wever and Neff, 1947; and Schuknecht *et al.*, 1951; Schuknecht, 1953) to assess cellular changes caused by acoustic trauma, by toxic agents, or by other means.

For more than ten years we have been developing new techniques for preparing specimens from the inner ear for examination under the electron microscope. In making these preparations the experimenter also gets, as a bonus, an excellent view of the entire membranous labyrinth at low magnifications (Fig. 1). Even with an ordinary preparation microscope, using a magnification no greater than 80×, one can see all

FIG. 1. Survey picture of the cochlea showing different cochlear coils. From the top coil a small portion (arrows) has been taken out for study.

the major features of the normal structure and make a reasonably reliable estimate of the extent of major damage in a pathological specimen. Detailed analysis using higher magnification can be made on fresh, stained or unstained segments of the organ of Corti dissected free and mounted. Surface preparations of similar type were used extensively by

Retzius (1884) and the method has recently been revived by Neubert (1952), Beck (1956), Vinnikov and Titova (1961) and others, especially for histochemical analysis and intravital staining.

Phase contrast microscopy has been used in this laboratory for several years in the study of the organ of Corti and other structures of the inner ear. Initially it was used with low magnification in the study of larger pieces of tissue especially when it was desirable to dissect out small groups of cells for examination under higher magnification; however, the organ of Corti is actually so thin that it is also feasible to use oil immersion objectives (50 to 100×) without any special dissection. By focusing up and down at different levels, one can, as it were, use the microscope to obtain a series of horizontal "sections" through the specimen. In this way the microscope can be focused first on a plane through the tectorial membrane, then on another plane through the hairs at the surface of the sensory cells, then through the cuticular plates, the region of the nuclei, and so on down to the nether surface of the basilar membrane, all at a magnification of 100 to 1000×. At low magnification it is possible to study a long segment of the organ of Corti in a single specimen. In a guinea pig, for example, a good preparation of this type may extend to half a turn or more even from the basal coil. In this way each of more than 1000 sensory cells can be studied, counted, and plotted individually in its normal, orderly relation to all the rest.

The method has been developed and standardized for pathological studies so that both the inner and the outer hair cells of an experimental animal can be systematically divided into groups and numbered. Our phase contrast microscope is fitted with an inexpensive 6 × 6 cm rollfilm camera, with which we routinely photograph interesting specimens, often with excellent results. The method is easy, reliable, and quick. Pictures which have been made in this way are shown in Figs. 2–7 and 9. After the structure has been studied as a whole, a detailed analysis can be made of individual cells removed from the preparation. The material can also be prepared so as to permit further study with the electron microscope.

In addition to making surface preparations, we have followed the usual methods for light- and electron-microscopic examination of the inner ear. To a great extent we have also used sections cut from specimens embedded in plastic for phase contrast microscopy. This is also an excellent method for studying the organ of Corti, and it is especially well suited for the vestibular sensory epithelia. For the analysis of normal structure and of experimental lesions the method of plastic embedding, sectioning (even freehand) and examination by phase contrast is superior to the conventional method of decalcification, celloidin embedding

and staining, with its many delays and uncertainties. This is not to say that the celloidin technique is to be abandoned. By selectively using the various methods in combination, i.e., surface preparations, plastic embedding and celloidin, and by taking full advantage of each of the various microscopic procedures, it is possible to get a much better idea of the normal structure of the inner ear and of the changes occurring in various pathological conditions than with any one method alone.

III. CYTOARCHITECTURE AND STRUCTURE OF THE COCHLEAR HAIR CELLS

A. Cytoarchitecture

The sensory cells of the cochlea in higher mammals are arranged as a single row of inner hair cells and three or four rows of outer hair cells. They form a remarkably regular geometrical mosaic, which is seen to admirable advantage in phase contrast studies of the organ of Corti. The pattern is enhanced by the membrana reticularis, which is made up of the compact edges of the phalanges of Deiters' cells and the narrow cuticular reinforcement at the periphery of the hair cells. The tonofibrils of the Deiters' cells radiate outward in the phalangeal plates and end in the membrana reticularis. The inner hair cells form a very regular and even row paralleling the tunnel of Corti, and the stereocilia on their surfaces form an almost continuous, slightly irregular stripe from the base of the cochlea to the apex, as can be seen beautifully in phase contrast. The inner hair cells are separated from each other at the surface by a small phalanx. Both inner and outer hair cells show distinct variations in size and appearance from one turn of the cochlea to another. Surface views of outer hair cells from different turns of the guinea pig cochlea are shown in Figs. 2–5. The pictures reveal these variations far better than can be done in words. The differences between the hair cells of the first, second, and third rows in the same coil also become clear from these pictures. Of great interest is the gradual change in the arrangement of the hairs on the outer hair cells from base to apex. At the base the angle formed by the outer limbs of the W pattern is 120° to 130°; it decreases continuously to about 70° at the apex.

In practically all of the guinea pig cochleas studied thus far, the regularity of the haircells has been most striking, but Retzius (1884) has pointed out significant irregularities in the human cochlea, and we have now started a study of the cytoarchitecture of the cochlea in man. It is evident that the regularity that is normally found in the arrangement of hair cells and supporting cells in the guinea pig is markedly changed if the cochlea is exposed to toxic agents or acoustic trauma.

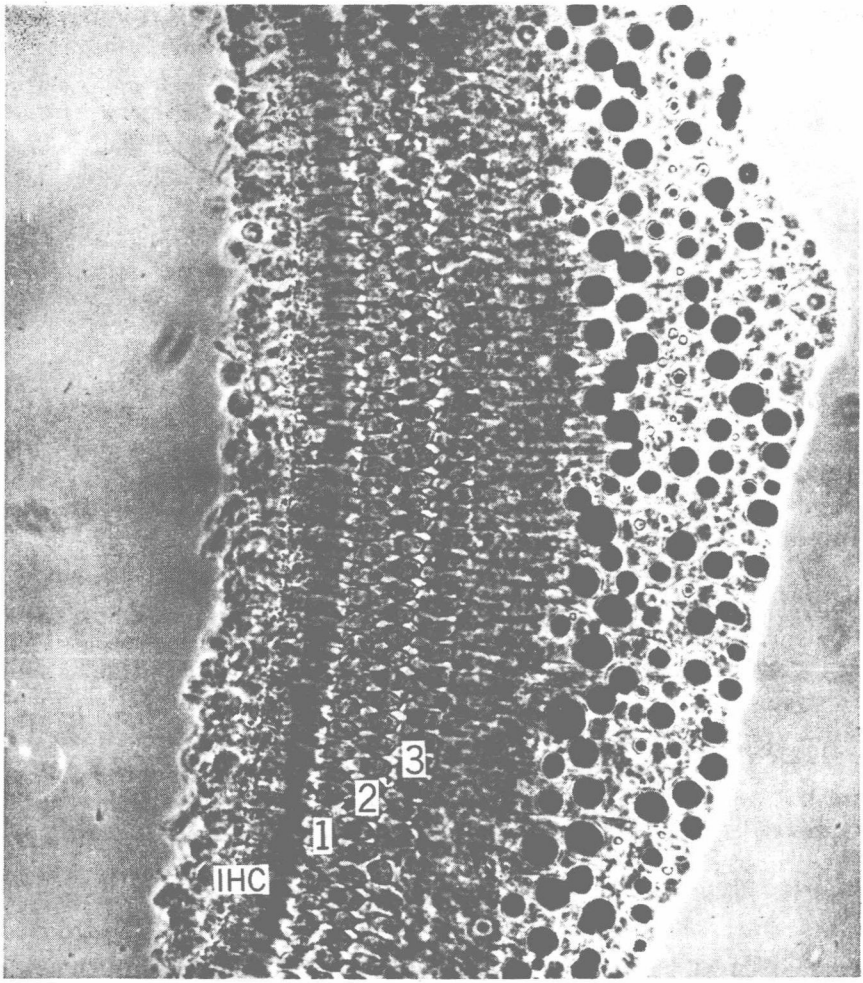

FIG. 2. Low power magnification of the organ of Corti from upper cochlear coil of a guinea pig. One row of inner hair cells (IHC) and three rows of outer hair cells (1, 2, 3) are seen. The dark dots to the right are lipid-like inclusions in the Hensen cells.

Experiments we have done with various ototoxic antibiotics (Hawkins and Engström, 1963) and with intense noise (Engström and Ades, 1964) show that as the sensory cells degenerate, irregularities and gaps appear in the geometrical pattern. In Figs. 6 and 7 the effects of kanamycin intoxication and of exposure to pistol shots are shown. In the normal cochlea, as Engström, Ades, and Hawkins (1962) and Flock *et al.* (1962) have described, the cilia of the hair cells show the characteristic, regular patterns already referred to. After damage by ototoxic

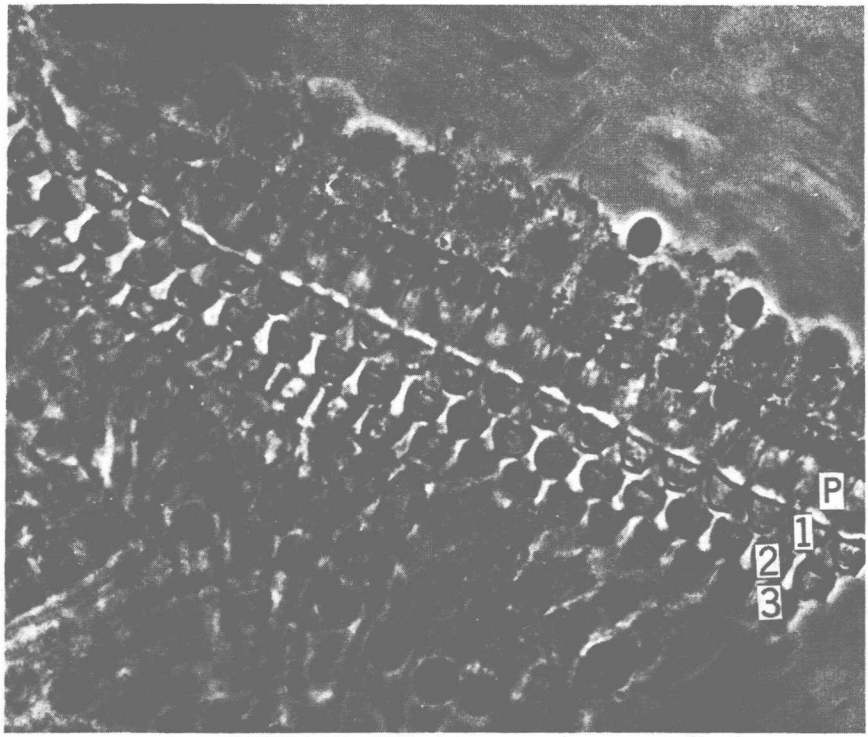

FIG. 3. Small portion of a basal cochlear coil of a guinea pig. 1, 2, and 3 represent the first, second, and third rows of outer hair cells. The single row of inner hair cells is seen above P.

agents or intense noise, the precision of their patterns is disturbed, and the hairs assume bizarre positions, before the cells degenerate. By recent studies we have observed a modified kinocilium, or a basal body, on all the cells of the organ of Corti in both animals and man.

B. Inner Hair Cells

The ultrastructure of the inner hair cells has been described by a number of different authors, but since there is still some uncertainty about many points, it seems worthwhile to describe the major features once more. The drawing in Fig. 8 shows the structure of an inner hair cell and its innervation. The upper surface of the cell is formed by a plasma membrane with the underlying cuticle, in which the roots of the stereocilia are fastened. The stereocilia have different lengths and form three or four slightly irregular lines. They are coarser and differ markedly in arrangement from those on the outer hair cells (Fig. 9). Each inner hair cell is provided with a single basal body placed at the side of

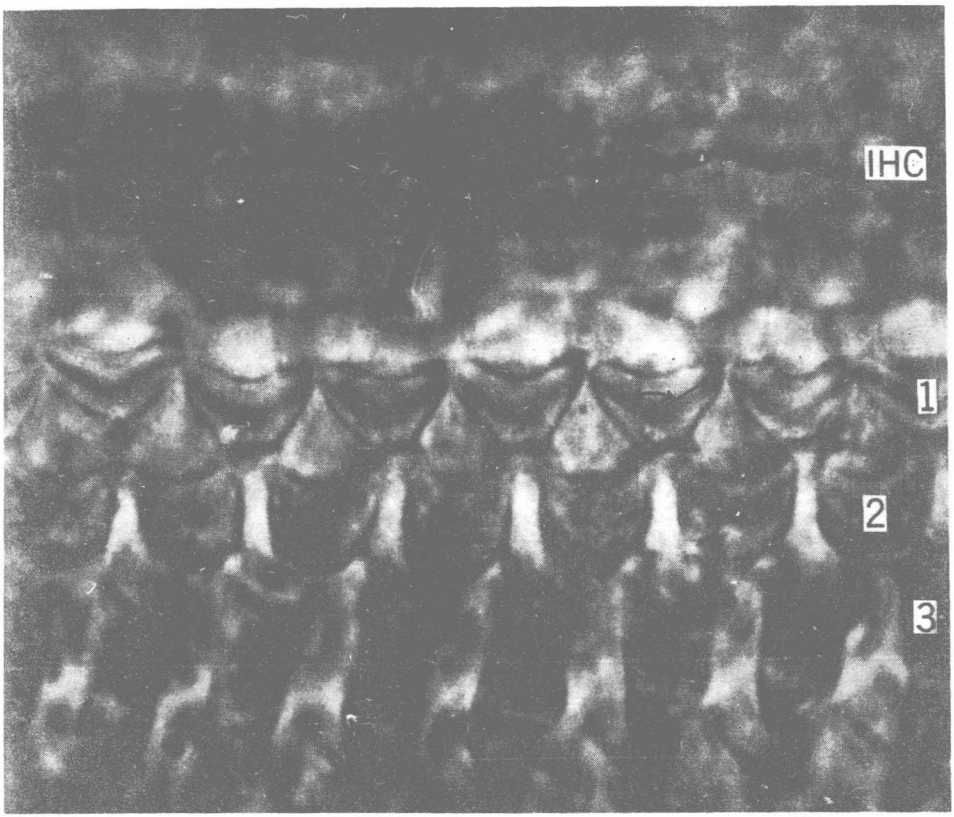

FIG. 4. Typical picture from the basal coil of a guinea pig cochlea. Observe the row of hairs belonging to inner hair cells (IHC) and the characteristic form of the surface of the outer hair cells (1, 2, 3) in this region. Observe also the wide angle formed by the W pattern on the hair cell.

the rows of stereocilia away from the modiolus. The inner hair cells have a very rich endoplasmatic reticulum (Figs. 10, 11), which appears mainly in the form of small tubelike profiles or cisterns, some of which are provided with osmiophilic particles and ribosomes along their outer surface.

The greater number of inner hair cells have a characteristic shape with a relatively slender upper portion terminating in the cuticular plate, a bent neck and a thicker lower cell body containing the nucleus. There is some variation in the relative diameter of the lower part of certain cells which are more slender than the average. While neighboring cells are generally similar in shape and size, it sometimes happens that two adjacent cells may, between them, include the extremes of this morpho-