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Studies on the Structure and Innervation of the Sensory Epithelium of the Cristae Ampullares in the Guinea Pig

A Light and Electron Microscopic Investigation

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JAN WERSÄLL

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BOKTRYCKERI AKTIEBOLAG

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To my Wife

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PREFACE

The investigation reported here was conducted under the supervision of Professor Gösta Häggqvist, Head of the Department of Histology, and Dr. Fritiof S. Sjöstrand, Associate Professor at the Department of Anatomy, Karolinska Institutet.

My chief, Professor Häggqvist, gave me invaluable support in my work and watched the progress of the investigation with keen interest.

Dr. Sjöstrand, with his wide knowledge of preparative techniques and electron microscopy, greatly facilitated these studies. He gave me very useful advice with regard to the disposition of the work and showed a never-failing interest in the diverse problems that arose during the course of the investigation.

Professor Ture Petrén, Head of the Department of Anatomy, very kindly placed the resources of his institution at my disposal.

Professor Carl Gustaf Bernhard discussed the physiologic aspects of my investigation and helped me with constructive criticism and good advice.

Dr. Hans Engström, Docent of Otology at the University of Gothenburg, was the first to call my attention to the many unsolved problems associated with the structure and function of the membranous labyrinth. It was he, too, who prompted me to study the fine structure of the vestibular apparatus, and his interest in my work was most inspiring.

The drawings in this paper are the work of Mr. A. Abele and Miss Maj Berghman, to whose skill are due the artistic merits they may possess. Miss Berghman also did admirable work in printing most of the photographs.

The technical part of the histologic work was done by Mrs. Benita Strunke.

Mr. T. Westerberg was very helpful in taking the photomicrographs.

Mr. Stanley Vernon translated the paper into English.

To all the above, and to my friends at the Departments of Histology and Anatomy who, with their advice and criticism in many useful discussions, were instrumental in the preparation of this paper, I wish to express my deep indebtedness and appreciation.

The investigation was made possible by grants from the Swedish Government and from the Swedish Society for Medical Research.

Stockholm March, 1956.

Jan Wersäll.

GENERAL INTRODUCTION

Since the early nineteenth century an immense amount of research work has been done on the structure and physiology of the ear. In numerous investigations the various components of the ear have been exhaustively studied with the use of classical histologic methods, fixing and staining procedures suited for the light microscope. Very important observations have been made with regard, for instance, to the innervation and structure of the various sensory epithelia of the membranous labyrinth. However, the limited resolving power of the light microscope has prevented any far-reaching studies of the fine structure of sensory cells and nerve endings therein. The rapid development of the electrophysiologic technique in recent years has therefore resulted in a hiatus between our physiologic and our histologic knowledge in this field. (Katsuki *et al.*, 1954; Davis *et al.*, 1950; Békésy, 1953, 1954; Tasaki, 1954.) The electron microscope, permitting of ultrastructural analysis, has nevertheless opened up new avenues for investigations of those structures that border on or are below the resolving power of the light microscope. In a number of investigations on the fine structure of the organ of Corti a method was devised for utilization of the electron microscope in studies of the structure of the inner ear (Engström, Sjöstrand & Wersäll, 1952, 1953; Engström & Wersäll, 1953, a, b; Wersäll, 1954; Engström & Sjöstrand, 1954). In the investigation reported here this method has been elaborated and employed in studying the sensory epithelium of the cristae ampullares. As a background to this investigation the innervation and structure of the crista epithelium was studied in ordinary microscopic specimens fixed and stained by classical methods.

The purpose of the investigation was to elucidate the peripheral innervation of the sensory epithelium of the cristae ampullares in the guinea-pig with special reference to the structure of nerve endings therein, as well as the fine architecture of cells and cell structures.

In recent years guinea-pigs have been extensively used in physiologic studies of the labyrinth. For this reason, and because the various components of the vestibular apparatus are fairly readily accessible in the guinea-pig, that species was used in the present investigation too.

Since the preparation required for electron microscopic examination of the membranous labyrinth calls for detailed knowledge of the anatomy of the guinea-pig ear, this paper also comprises a review of that subject.

CHAPTER I

MATERIAL AND METHODS

Experimental Animals

The animals used were guinea-pigs of varying age and a minimum body weight of 150 grams. The material consisted of more than one hundred ears from seventy animals. Specimens from 25 of these guinea-pigs were employed for routine anatomic and histologic studies. Specimens from 20 were stained for nerve study by the method reported below, and 75 ampullae from 20 animals were examined with the electron microscope. Six posterior ampullar nerves from five animals were analysed with respect to the diameter of the myelinated fibers.

Fixation of the Membranous Labyrinth

Numerous fixatives for the inner ear have been reported in the literature. Werner (1936, 1940) recommended the fixative that had been suggested by Held (1902) and Wittmaack (1903)—potassium bichromate–formol–acetic acid solution—as one of the most appropriate. Werner (1936) investigated various mixtures of these substances with respect to the influence, on the inner ear tissues, of different concentrations and osmotic pressures of the fixing solution. In so doing, he took into account, among other things, Sjöbring's (1900) requirement that the fixing solution must be "isotonic with the protoplasm". He found that isotonic solutions of "*kaformacet*" had very little fixing power; and in 1940 he advocated "Wittmaack's labor"—a mixture of potassium bichromate, formol, and acetic acid in which the freezing point depression was 2.1 and the pH was 3.2—as the most suitable mixture. Investigators as early as Retzius (1881 a) and Kaiser (1891) observed that excellent fixation of sensory epithelium could be obtained with 0.5–1 per cent osmium tetroxide solution. This was verified by Kolmer (1927). Due to the difficulty of staining specimens fixed with osmium tetroxide solution, that fixative has been little used, however, for examination of the labyrinth. The electron microscopic studies of different tissues that have been reported in recent years, have nevertheless demonstrated to an increasing degree the advantages of osmium tetroxide solution over other known fixatives. In this connection Palade (1952) pointed out that the pH of the solution should be equal to that of the blood—i.e., 7.2–7.4—and that major variations produced marked changes in the ultrastructural details of the cells. Sjöstrand (1953 a) demonstrated that osmium tetroxide solution provided better fixation when rendered isotonic with the blood. Meticulous investigations into the effect on epithelial cells of the pH and osmotic pressure of the fixing solution, and of postmortal changes, were presented by Rhodin (1954) and Zetterqvist (1956). Rhodin (1954) found that the volume of the mitochondria in the tubuli cells of the mouse kidney tended to increase when

he used hypotonic fixing solutions and to decrease when he used hypertonic solutions.

Zetterqvist (1956) did not find any variation in the diameters of the mitochondria in cells from mouse jejunum fixed in solutions of varying tonicity. A deviation in the value for the osmotic pressure of the fixative from that of the blood and tissues was shown to influence the water content of the cell and the diameter of the "cytoplasmic vesicles" but not the other structures of the cell.

Both Rhodin (1954) and Zetterqvist (1956) found better preservation of the tissues after fixation in 1 per cent veronal acetate buffered isotonic osmium tetroxide solutions with pH 7.2 than in acid fixatives.

Postmortal changes in renal epithelium were demonstrated by Rhodin 5–15 minutes after death, and in mouse jejunum by Zetterqvist within five minutes after death. Changes were observed first in the mitochondria, where the ground substance and inner membranes were already destroyed after five minutes (Zetterqvist).

In the present investigation, all specimens the ultrastructure of which is described here were fixed in 1 per cent veronal acetate buffered osmium tetroxide solution *ad modum* Palade (1952), modified by Sjöstrand (1953 a). Some of them were fixed at + 1 to + 2°C; others at + 17 to + 18°C. No definite difference was demonstrable in the ultrastructure of the cells in these two groups.

A number of specimens were tentatively fixed in osmium tetroxide solution at pH 4.2–4.5, with good results. This finding is consistent with Werner's (1936) observations of the effect of fixatives with low pH values on the cells of the membranous labyrinth.

None of the specimens used for analysis of the ultrastructure were fixed later than five minutes after death.

Preparation for Light Microscopy

For satisfactory fixation of the sensory epithelium of the inner ear the fixative must be injected either directly into the blood stream (Werner, 1940) or into the endolymph or perilymph after rapid mobilization of the inner ear. In this investigation the latter technique was employed.

A suitable procedure for preparation is the following. After decapitation of the animal, the head is divided in the middle line. The mandible and adherent soft tissues are cut away at the articulation with the temporal bone. The petrous bone, which in the guinea-pig is attached to the temporal bone only by loose connective tissues, is dissected from adjacent bone and tissues, and the bulla tympanica then opened by a wide incision. Using a fine needle, the stapes is lifted from its attachment in the oval window; the lateral bony septum above the vestibule is broken away, and the apical coil and the basal coil of the cochlea are opened. The vestibule is then carefully irrigated with fixatives via a fine glass pipet, after which the entire specimen is immersed in fixative. Care must be taken to ensure that no air bubbles form in the cochlea or vestibule that may prevent penetration of the fixative into the labyrinth.

The conventional method used in histologic examinations of the inner ear, with fixation, decalcification and embedding of the entire petrous bone at the membranous labyrinth, has many shortcomings. For instance, the decalcifying fluids hitherto employed have a directly deleterious effect on the tissues; and this has made difficult or impossible any observations of the fine cellular structures in the labyrinth. Due to the complicated architecture of that organ it is extremely difficult, when embedding by this method, to get an appropriate orientation of the specimen, with accurate lines of section in relation to the various parts of the labyrinth.

Engström, Sjöstrand & Wersäll, in a series of investigations on the fine structure of the organ of Corti, devised a technique better suited for studies of the fine structure of the cells in the labyrinth. With this technique different portions of the labyrinth are dissected free from surrounding bone after fixation, and then embedded, so that decalcification is avoided. This method was employed in the electron microscopic studies in the present investigation, and has been used for cytologic studies by light microscope. For studies of the innervation of the crista epithelium, the specimens were treated *ad modum* Palmgren (1948) by fixation in Bouing's fluid, decalcification in a solution of formic acid and sodium formate, embedding in paraffin, and gold-silver impregnation *ad modum* Palmgren of slices 10–40 μ thick. For more general histo-anatomical studies the specimens were treated *ad modum* Werner (1940); i.e., fixed in "Wittmaack labor", decalcified in HNO_3, embedded in paraffin, and stained.

In determination of calibers of myelinated fibers in the posterior ampullar nerve of the guinea-pig, the following method was employed.

After decapitation of the animals the vestibule was opened and the specimens immersed in buffered 1 per cent osmium tetroxide solution isotonic with blood, where they were fixed for one hour. The posterior ampullar nerves were then dissected free and fixed for a further 23 hours in the same solution. The nerves were embedded in paraffin and cut into slices 5 μ thick, efforts being made to cut at right angles to the long axis of the fibers. One section peripheral to the ganglion cells embedded in the nerve was selected from each nerve, after which the entire section was photomicrographed at an enlargement of 1000 diameters, twelve photographs being taken of each nerve. After these photomicrographs had been collated, all nerve fibers were measured under coincident control in a light microscope.

Preparation for Electron Microscopy

For the electron microscopic studies the petrous bone was dissected free by the method described above; 1 per cent isotonic osmium tetroxide solution buffered to pH 7.2–7.4 (Palade 1952, modified by Sjöstrand, 1953 a) was injected into the vestibule, and the specimens then immersed in the same fixative. The specimens were fixed within 2–4 minutes after decapitation of the animals. When they had been immersed for four hours, the ampullae with their nerves were dissected from the surrounding bone, dehydrated and embedded in a mixture of n-butyl and methyl acrylate *ad modum* Newman, Borysko and Swerdlov (1949). Sectioning was done by a method which Sjöstrand (1953 e, 1954) devised for cutting ultra-thin slices, using a Sjöstrand ultramicrotome with a circular movement and a thermal expansion specimen feed. The cutting edge consisted of highly polished Schick razor blades. The sections were floated on the surface of a 20 per cent ethyl alcohol

solution, then assembled on specimen grids with 200 meshes per square millimeter, covered with a thin film of formvar. They were examined in the electron microscope without the methacrylate being dissolved.

An RCA EMU 2c electron microscope was employed according to the routine at this laboratory. It had a standard compensated objective pole piece having an objective aperture of 50 μ, with three apertures in the projector lens, and a reduced condensor lens aperture.

The electron micrographs had a magnification varying from 1600 to 17,000 diameters, and were then photographically enlarged up to a maximum total magnification of 200,000 diameters.

Embedding Technique

Light Microscopy

Innervation
Fixation in Bouing's solution for 3–5 days.
Seventy per cent alcohol, 24 hours.
Decalcification in a 1:1 solution of formic acid and sodium formate, 2–4 days.
Dehydration, embedding in paraffin.
Sectioning, slices 10–40 μ thick.
Staining, silver-gold impregnation *ad modum Palmgren* (1948).

General histologic studies
Fixation in "Wittmaack labor", 3 days (Werner, 1940).
Decalcification in 5 per cent HNO_3, 14 days.
Dehydration and embedding in paraffin.
Sectioning, slices 5–8 μ thick.
Staining, iron choloride hematoxylin, regressive method *ad modum* Häggqvist.

Cytology and cell morphology
Fixation in 1 per cent buffered blood-isotonic osmium tetroxide solution, pH 7.2–7.4, 6 hours.
Dissection of ampullae from surrounding bone, dehydration and embedding in plastic (see embedding for electron microscopy).
Sectioning, serial sections 0.2–3 μ thick, with a Reichert sliding microtome and Spencer 821 with razor-blade cutters.
Mounting in glycerin and studying in phase contrast microscope.

Determination of calibers of nerve fibers
Fixation in 1 per cent buffered isotonic osmium tetroxide solution, 24 hours.
Rinsing in Tyrode's solution and preparation, 1 hour.
Dehydration.
Embedding in paraffin.
Sectioning—transverse sections of the nerve.
Photomicrography at magnification of 1000 diameters.

Electron Microscopy

Fixation in 1 per cent buffered isotonic osmium tetroxide solution, pH 7.2–7.4 (Palade, 1952; Sjöstrand, 1953 a), 0.34M *ad modum* Rhodin, 1954; 4 hours.
Rinsing in Tyrode's solution and dissection of ampullae from surrounding bone, 1 hr.

Dehydration.
70 per cent ethyl alcohol, 3 hrs. or overnight.
96 per cent ethyl alcohol, 2 hrs.
Absolute ethyl alcohol, 2 hrs.

Embedding.
1:1 solution of absolute alcohol and *n*-butylmethacrylate, ½ hr.
Methacrylate solution (one part meta-methylacrylate and 19 parts *n*-butyl methacrylate 1 per cent benzoyl peroxide), 1 hr.
Immersion in polymerized methacrylate solution of syrupy consistency, and polymerization at 37°C, about 24 hrs.

Sectioning.
Cutting of pyramid about 0.5 mm high with top surface area of about 0.01 mm^2 and top angle of 45°.
Sectioning in Sjöstrand's ultramicrotome with razor-blade cutters (Sjöstrand 1953 e, 1954). Specimen feed 200–400 Å.

CHAPTER II

GROSS ANATOMY OF THE GUINEA-PIG EAR

Introduction

Prominent among a large number of investigations on the anatomy of the ear from the late eighteenth century is Antonio Scarpa's work, *Anatomicae disquisitiones de auditu et olfactu.* Scarpa (1789) studied the inner ear in teleostei (bony fishes) and man, and in the latter he described the two "stone sacs", the membranous semicircular ducts and the latter's position in a system of canals in the bone. He found that all semicircular canals in man communicated with the upper and more tubiform of the "stone sacs", and that each semicircular canal had a dilated part at its junction therewith. To each semicircular canal ran a nerve branch. This latter ramified peripherally in a "septum" traversing the ampulla of the semicircular canal and dividing it into two communicating halves. Above this septum he found a mass, within which he surmised that the peripheral nerve endings were situated.

Steifsand (1835) found that the transverse septum in the ampullae formed, at its attachments in the ampullar wall on either side, a semilunar thickening which occupied a substantial part of the ampullar wall. He called this formation the planum semilunatum.

In the latter half of the nineteenth century new types of microscopes served to improve the histologic technique, thus facilitating studies of specimens at higher magnifications. From that time we have numerous important observations on the anatomy and histology of the inner ear which, in great part, constitute the basis of our present physiologic knowledge in this field. These investigations were substantially founded on Schultze's pioneering studies of the labyrinth in the shark and ray. Schultze (1858) found that the "transverse ampullar septa" in those species of fish were lined with a specialized epithelium within which the peripheral nerve fibers extended. He called this "septum" the crista acustica—a designation that has since been used in a large number of papers.

The numerous workers who, in the eighteen-seventies to 'nineties, conducted histologic investigations of the inner ear include Odenius (1867), Hasse (1867, 1870), Retzius (1881 a, b, 1884), and Kaiser (1891), whose papers will be discussed later on.

Elaboration of fixation and staining methods, notably various forms of silver impregnation, resulted in major advances, especially with regard to studies of the innervation in different parts of the inner ear (Bielschowsky & Brühl, 1907; Cajal, 1908; Lorente de Nó, 1925; Poljak, 1927). Fixation methods suitable for the inner ear were reported by Held (1902) and Wittmaack (1903), among others. Werner (1936) meticulously investigated the effect of fixatives on the labyrinth.

Retzius, in his papers *Das Gehörorgan der Wirbeltiere* (1881 b, 1884), made an important contribution to our knowledge of the comparative anatomy of the inner ear. Further work in this field was done by de Burlet (1934), among others.

An excellent study on the labyrinthine structure in the bat was presented by Iwata in 1924. Kolmer (1923, 1927), Held (1926) and Engström (1951 b) described in detail the normal anatomy of the human inner ear. In recent years Bast and Anson (1949) have studied, in an extensive investigation, the development and anatomy of the temporal bone and its relation to the membranous labyrinth. Vilstrup (1950) investigated the semicircular canals in *Acanthias vulgaris,* and discussed the structure of the ampullae and sensory epithelium therein with respect to their function. Descriptions of the anatomy of the ear in the guinea-pig were reported by Kolmer (1931) and Werner (1940).

Although a large number of investigations dealing with the anatomy of the middle and inner ear are available, of which only a few have been mentioned here, it seems justified to present an outline of the location of the vestibular apparatus in the petrous bone, since the special anatomy of the guinea-pig ear has been treated somewhat briefly in earlier investigations. This description is based on personal observations made at preparation of more than 200 guinea-pig ears.

Some Aspects of the Anatomy of the Middle Ear

The inferior portion of the middle ear cavity is dilated, in some rodents, into a semispherical space, the bulla tympanica, which has a relatively thin bony wall.

In this cavity the cochlea bulges in with its apical coil, the greater part of the middle coil, and large parts of the basal coil. The oval window forms the base of a fairly deep recess in the middle ear, the walls of which are formed by the thin bony septa enclosing, respectively, the facial nerve canal, the tensor tympani canal, and the basal coil of the cochlea, as well as by the round window. (Fig. 1.)

The tympanic portion of the Eustachian tube runs in a bony groove below and anterior to the cochlea. This groove extends towards the antero-inferior tip of the petrous bone, where it continues in the form of a bony canal. From the inferior border of this groove an osseous membrane extends to the summit of the cochlea.

In the guinea-pig, like the rabbit, the auditory ossicles have a peculiar appearance. The incus and malleus are united, embryonally, with a synchondrosis, which in the adult animal is ossified. The ossified zone can nevertheless be distinguished even in old animals. The bulk of the fused incus and malleus lies in an epitympanic recess above the superior margin of the drum membrane. The short process of the incus is attached to the posterior wall of the recess by means of a fan-shaped radiating ligament. The long process communicates, via a joint having a narrow joint space and highly developed capsule, with the head of the stapes. The long process of the malleus forms, at right angles to the drum membrane, an oval membrane the lateral border of which is reinforced to form a bony plate lying in the plane of the drum membrane, and attached thereto by dense connective tissue fibers. The footplate of the stapes forms, with the border of the oval window, a smooth joint having a very thin joint capsule. (Fig. 1.)

Midway between the two limbs of the stapes the oval window recess is traversed by a bony bridge, which contains the rudiments of the stapedial artery.

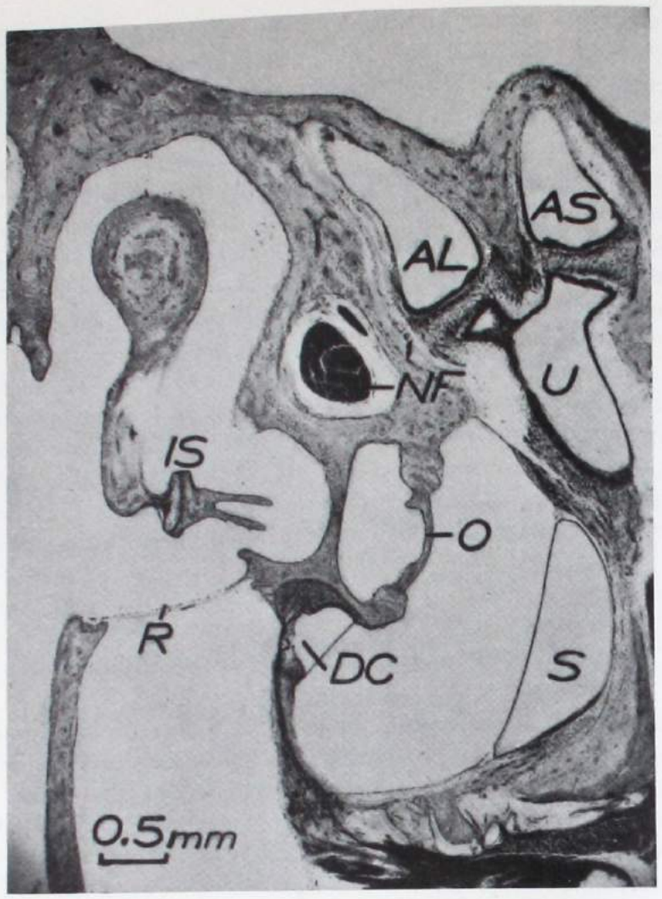

Fig. 1. Section through middle and inner ear of guinea-pig. IS, auditory ossicles with articulation of incus and stapes. The stapes is cut obliquely, so that only a part of one limb and the footplate in the oval window can be seen (O). Note the bony trabecula traversing the oval window recess. R, round window with secondary tympanic membrane; DC, basal coil of cochlea with cochlear duct; NF, facial nerve; U, utricle; S, saccule; AS, ampulla superior; AL, ampulla lateralis. Photomicrograph, 20 ×.

The facial nerve canal has its internal meatus on that aspect of the petrous bone which faces the cerebellum, immediately anterior to the entrance of the superior vestibular nerve. From here the canal runs laterad at the level of the upper side of the third cochlear coil, then curves round the underside of the lateral ampulla, deviates outward and backward, runs below the middle of the lateral semicircular canal and opens posterior to the external auditory canal. In a part of its course the canal with its enveloping bone bulges like a crest into the tympanic cavity from the superior portion of the latter's medial wall.

The stapedial muscle runs from a connective tissue septum in the midportion of the facial nerve canal, separated from the nerve only by that septum. It passes through an opening in the canal wall into the middle ear, and is attached to a process on the posterior part of the stapes head.

The Inner Ear

The membranous labyrinth consists of a system of thin-walled sacs and ducts filled with a clear fluid, the endolymph. This system is contained in canals in the petrous bone which are only partially filled by the membranous ducts. The perilymphatic space between the walls of the bony canals and the membranous labyrinth, is filled with a fluid called perilymph, which differs chemically from the endolymph (Smith, Lowry & Mei Ling Wu, 1954).

Centrally in the bony labyrinth is a long, rounded cavity, the vestibule, from which the semicircular canals and cochlea run. The lateral wall of the vestibule is occupied, in part, by the oval foramen and the stapes footplate; it consists otherwise of a very thin bony membrane, which can be readily dissected out to expose the whole of the vestibule.

The vestibule is divided, in the guinea-pig, into a smaller superior and a larger inferior portion by a connective tissue membrane, the "boundary membrane", extending from the lateral wall to the utricle. This membrane divides the labyrinth into a superior and an inferior part. The superior portion includes the utricle, semicircular canals, endolymphatic duct and sac with the surrounding perilymphatic space; the inferior portion is formed by the cochlear duct, the saccule, and the lower parts of the perilymphatic space. The "boundary membrane" was described by de Burlet (1920), who also found that the membranous labyrinth in the superior portion unites with surrounding periosteum via connective tissue fibers, whereas these latter are absent in the inferior part. It is difficult to decide, from sections or direct preparations, whether any communication exists between the perilymphatic space in the superior and inferior portions. According to de Burlet, no such communication is present.

The guinea-pig utricle consists of a sac of irregular shape which extends longitudinally through the upper part of the vestibule. The utricle proper is a short, somewhat flattened tube of regular caliber. (Fig. 2.) Anteriorly it dilates to form the utricular recess which contains the macula utriculi, while its posterior portion merges into another dilated part, the posterior sinus. The total length of the utricle is about 4 mm and its width 0.8–2 mm. The macula utriculi projects from the antero-medial wall of the vestibule in the form of a heart-shaped connective tissue plate clothed with epithelium and slightly concave upward. The greater part of the macula rests on a bony projection from the vestibular wall, and on the anterior portion of the "boundary membrane", which in this part of the vestibule extends directly from the lateral to the medial wall. The surface of the macula utriculi is clothed with sensory epithelium, which in turn is lined with a statoconium membrane. From the main surface, sensory epithelium extends to the antero-medial wall of the utricle and forms a small epithelial surface at an obtuse angle with the main surface.

According to de Burlet and de Haas (1923), the main surface of the macula utriculi forms an angle of 97 degrees with that of the homolateral macula sacculi. The macula utriculi on one side forms an angle of 160 degrees with the contralateral macula utriculi, whereas the maculae sacculi on either side form an anteriorly open angle of 60 degrees with each other.

A number of workers have studied the position of the semicircular canals in different species of animals. They have proposed varying horizontal planes and

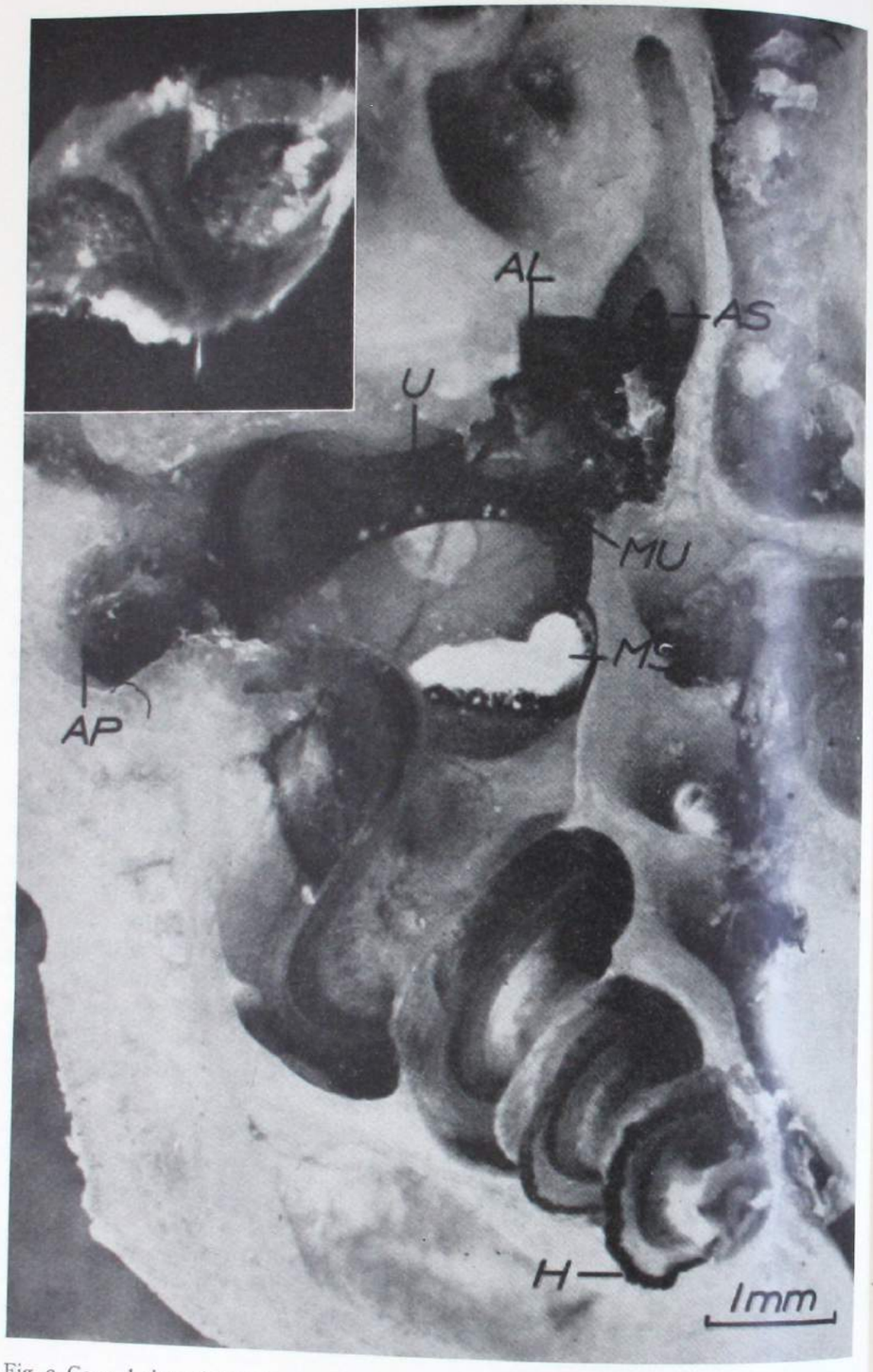

Fig. 2. General view of right inner ear of guinea-pig from the lateral side and somewhat below. The bone has been dissected away from the cochlea, vestibule, ampullae and parts of the semicircular canals. AS, ampulla superior; AL, ampulla lateralis; AP, ampulla posterior; MS, macula sacculi; MU, macula utriculi; H, Hensen cells in the organ of Corti. Preparation fixed in osmium tetroxide. Photomicrograph, 20 ×.
The inset shows the ampulla posterior, the summit of which has been opened, revealing the saddle-shape of the crista ampullaris. Photomicrograph, 50 ×.

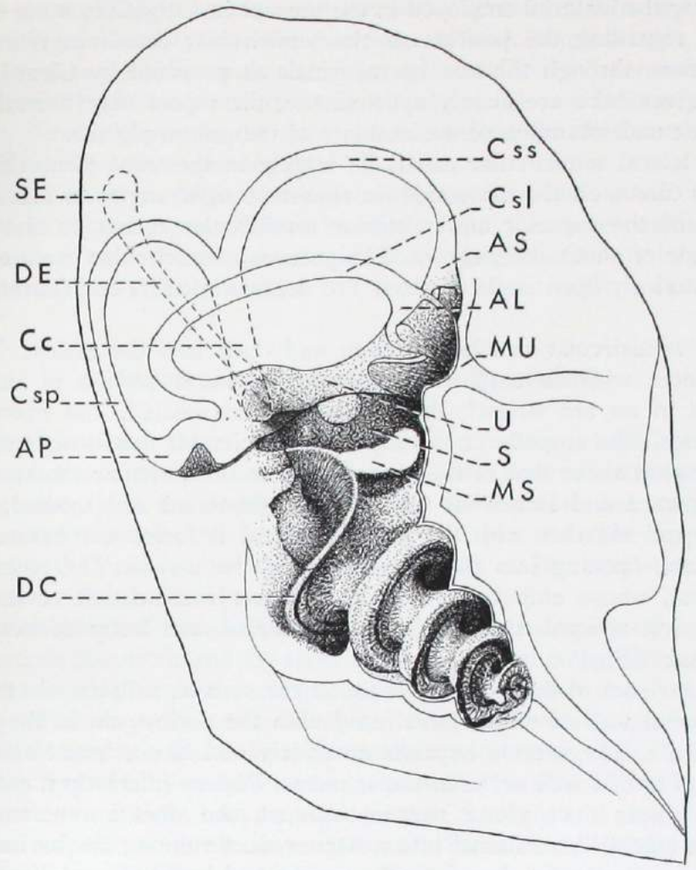

Fig. 3. Semi-schematic drawing of the membranous labyrinth in the guinea-pig. DE, endolymphatic duct; SE, endolymphatic sac; Css, superior semicircular canal; Csl, lateral semicircular canal; Ssp, posterior semicircular canal; Cc, Crus commune; U, utricle; S, saccule; DC, cochlear duct.

points of departure for calculation of the position of those canals in space. If, as pointed out by Girard (1910), the calculations were based on a plane through the horizontal canals on either side, a horizontal plane would be obtained that would be valid for all vertebrates regardless of the anatomy of the cranium in other respects.

The superior semicircular canal in the guinea-pig not only forms its semicircular curve in the vertical plane, but a slightly S-shaped curvature in the horizontal plane, which fact makes an exact determination of the angles between the different semicircular canals extremely difficult. However, an approximate determination can be made with respect to the main plane of the canal.

Kristensson (1954) reported that the lateral semicircular canals in the guinea-pig are, with the head in its resting position, horizontal, and are parallel with a plane through the external meatus and the supraorbital margin.

According to Bodechtel (1930), the superior and posterior semicircular canals meet at an angle of 115 degrees.

In studying the material employed in the present investigation, some calculations were made regarding the position of the semicircular canals in relation to the horizontal plane through the two lateral canals as proposed by Girard. Although the angles given here are merely approximate, the report may nevertheless contribute to our understanding of the anatomy of the guinea-pig ear.

The two lateral semicircular canals lie largely in the same plane. The vertical semicircular canals on the same side lie almost at right angles to this plane. The main plane of the superior and posterior semicircular canals on the same side form an angle of about 100 degrees. The posterior semicircular canal on one side forms a posteriorly open angle of about 110 degrees with its contralateral counterpart.

All three semicircular canals run from and open into the utricle. The lateral one commences with an ampulla in the supero-lateral portion of the utricular recess, runs in an arc laterad, then posteriad and mediad, and opens into the posterior sinus. The ampulla of the superior semicircular canal originates anterior to and somewhat above that of the lateral canal in the utricular recess. From here it curves upward and somewhat mediad, then posteriad and mediad, and lastly downward; and together with the posterior canal it forms a common stem, the crus commune, opening into the mid-portion of the utricle. The posterior semicircular canal, whose ampulla constitutes a direct continuation of the posterior sinus, runs first upward, then mediad and anteriad, and lastly downward to the crus commune. (Figs. 2, 3.)

In the lower part of the vestibule is found the saccule, a flattened sac the lower part and medial wall of which have fused with the periosteum in the lower part of the vestibule. The saccule expands anteriorly and has a broad attachment to the lower and medial wall of the utricular recess. Postero-inferiorly it communicates with the cochlear duct via a narrow channel, the ductus reuniens. Postero-superiorly it tapers like a funnel into a narrow duct running on the medial aspect of the utricle, unites with a duct from the utricle, and forms the endolymphatic duct.

The inferior part of the medial wall of the sacculus is composed of a kidney-shaped sensory epithelium zone, the macula sacculi. This rests, throughout its extent, on the lower portion of the vestibular wall, separated from the bone only by a slight thickening of the periosteum, through which nerve fibers pass to various parts of the macula. The epithelial surface, like that in the macula utriculi, is lined with a thin statoconium membrane. (Fig. 2.)

The endolymphatic duct leaves the vestibule immediately after the union of the two channels from the saccule and utricle, and runs upward and backward via the vestibular aqueduct, to terminate in the endolymphatic sac, below the dura in close relation to the transverse dural sinus. The vestibular aqueduct is separated by only a thin bony membrane from the superior sinus at its origin in the vestibule, but then diverges somewhat from the superior sinus and runs more mediad and posteriad. According to Guild (1920), the endolymphatic sac often partly covers the transverse dural sinus.

The endolymphatic duct and sac, as demonstrated in a series of excellent studies by Bast, Anson and coworkers, contains several regions of morphologic and physiologic significance.

The first part of the endolymphatic duct proper is dilated to a varying degree. This part is generally described as the sinus (Bast & Anson, 1949). Entering

the aqueductus vestibuli it narrows and forms the isthmus, a thin part of the duct which widens immediately under the dura into the endolymphatic sac.

The proximal part of the sac has a very rugose wall, which becomes smooth in the sac proper. The latter forms about two-thirds of the sac. It expands from the rugose part, flattened between the dura and the periosteum. It has a slightly oval form and ends with a short pointed tip.

The cochlear duct in the guinea-pig comprises four and a half coils in the cochlea, but in other respects differs but little from the corresponding organ in other mammals. The cochlea, as mentioned above, is enclosed only by a thin bony shell bulging into the bulla tympanica.

Innervation of the Sensory Epithelia

The inner meatuses of three canals are located in the medial wall of the petrous bone. Through the largest of these openings, located postero-inferiorly in relation to the other two, pass the cochlear nerve and the inferior main branch of the vestibular nerve towards the respective sensory epithelia. The upper anterior opening forms the entrance for the superior branch of the vestibular nerve; the anterior opening, for the facial nerve.

The superior branch of the vestibular nerve passes antero-superiorly and somewhat laterally towards the utricle and the superior and lateral ampullae. Here it first gives off a branch that ramifies in the macula utriculi, then passes towards the ampullae, immediately before the entrances to which it divides into two separate branches. The inferior branch passes a short distance together with the cochlear nerve directly laterally, then divides into an anterior and a posterior branch. The latter—posterior ampullar nerve—runs in its own canal posteriorly, laterally and superiorly towards the posterior ampulla, while the anterior branch immediately divides and radiates into the macula sacculi. The cochlear nerve first passes laterally, gives off a small branch towards the macula sacculi, then curves abruptly antero-inferiorly towards the modiolus, where it divides into branches towards the spiral ganglion in the various coils of the cochlea.

The vestibular ganglion cells in the guinea-pig are partly collected in two ganglia, one in each of the superior and inferior branches immediately before these perforate the petrous bone. A large proportion of the ganglion cells, however, are situated between the nerve fibers of the superior and inferior branches and the first portion of the posterior ampullar nerve. The last-named branch often contains, therefore, ganglion cells in one half of its course.

Aspects of Preparation

The ampullae are readily localized when the walls of the numerous pneumatic cells in the bone have been broken away. The bony canal round the semicircular ducts then bulges partly into the air-filled cavity and is readily accessible. In mobilizing the lateral and superior ampullae it is advisable first to remove the facial nerve so that the entire canal can be opened. The lateral semicircular canal is then opened and the bone removed towards the ampulla with the aid of a fine

needle. The semilunar pigmented zone at the transition between semicircular canal and ampulla is easy to distinguish even in non-fixed specimens. By exercising some care it is then possible to lift off the thin bony shell covering the ampulla, dissect the lateral ampullar nerve from its bony canal, carefully insert a needle between the superior and lateral ampullae, and lift out the latter one. After this, it is a simple matter to remove the superior ampulla. It may often be easier to lift the two ampullae out together, and then to separate them.

The nerve branch of the posterior ampulla runs in a separate canal in the postero-inferior wall of the vestibule. It is covered by thicker bone than the nerve branches of the lateral and superior ampullae, but often has to be dissected free before the posterior ampulla can be removed intact. Thus the posterior ampulla is generally more difficult to remove than the other two ampullae.

CHAPTER III

GENERAL STRUCTURE AND INNERVATION OF THE CRISTA EPITHELIUM

Introduction

The inner wall of the membranous labyrinth which develops from the primitive otocyst is enveloped, during embryonal life, by highly cellular mesenchymal tissue. From the epithelial layer of the otocyst is differentiated, a layer of squamous endothelium-like cells, which in the adult animal are found in the greater part of the vestibular apparatus. From this epithelium, characteristic types of sensory epithelium and supporting cell elements are differentiated in strictly localized areas; namely, the three cristae ampullares, the macula sacculi and macula utriculi, and the organ of Corti in the cochlear duct. The zone contiguous to the sensory epithelium also has, as a rule, a somewhat highly differentiated, often cylindrical to cubical epithelium. In the further development a characteristic transformation occurs in the mesenchyma enveloping the otocyst. In this process large confluent cavities containing perilymph arise. In a thin layer lining these cavities, connective tissue cells of mesothelial character develop. We accordingly find in large portions of the utricle and saccule an outer lining of such mesothelial cells.

In the region round the semicircular canals can be observed a pronounced rarefaction of the cellular elements, and here too are formed large confluent fluid-filled cavities, permeated by spiderweb-like connective tissue trabeculae. In a transverse section through the semicircular canal it will be found that the inside of the membranous duct consists of endothelial cells with a thickness of about 3–5 μ, which are bounded outwardly by a basement membrane having a very homogeneous structure. A similar structure is found in the saccule and utricle. Outside the basement membrane is found a thin connective tissue layer, though some parts of it, notably in the vicinity of the sensory epithelium regions, may be substantially thicker. The perilymphatic space surrounding the membranous labyrinth is bounded outwardly by a thin layer of squamous connective tissue cells.

The structure of the regions where sensory epithelium occurs, fundamentally diverges from other parts of the membranous labyrinth. In the present investigation detailed studies were made of the structure and innervation of the sensory epithelium of the cristae ampullares. The ampullae and cristae ampullares were chiefly used as objects of study in view of the relative ease with which they could be dissected out and prepared for electron microscopic and light microscopic studies. Concurrent investigations of the epithelium of the macula sacculi and macula utriculi showed, however, that major similarities in principle exist between the different types of sensory epithelium in the vestibular apparatus. Preliminary reports on the fine structure of the cristae ampullares have already been presented by Wersäll (1954), and on the macula utriculi by Wersäll, Engström & Hjort (1954).

History

Through the pioneering works of Scarpa (1789), Steifsand (1835), Schultze (1858) and others, we know today that the ampullae in the semicircular canals are partially divided by a transverse connective tissue crest, clothed with sensory epithelium having highly differentiated epithelial cells—the crista ampullaris. Schultze claimed to have distinguished three different types of cells in this epithelium in the shark and the ray, and described, "cylindric cells, fiber cells and basal cells". On the surface of the epithelium he detected a large number of sensory hairs, but could not decide from which cells they originated. Both Scarpa and Schultze observed nerve fibers in the crista ampullaris. Schultze also described myelin sheaths in these fibers, and showed that the latter, before entering the epithelium, lost both the neurilemma and the myelin sheath. Schultze's original observations were made largely in fish, but Odenius (1867) was able to demonstrate corresponding cells and cellular elements in man. From the mid-nineteenth century we have innumerable reports on the structure of the sensory epithelium. Among them, the observations of Hasse (1867) are of considerable interest, for in birds he detected only two types of cells, one type, in his opinion, being in direct communication with the innervating nerve fibers. Retzius, in a series of papers from 1881, 1884, and 1892 described the structure of the sensory epithelium of the inner ear in a large number of animal species. Like Hasse, he found that the sensory epithelium was composed of two types of cells, which he designated as fiber cells and hair cells. He found, too, that the nerve fibers in the epithelium divided into a large number of fibrils that surrounded the base of the hair cells and were probably in communication with the cytoplasm.

Kaiser (1891) reported an important investigation on the histology of the vestibular apparatus. After detailed studies he found, like Retzius before him, that fixatives containing osmium were exceptionally good for preserving the sensory epithelium of the inner ear. Kaiser's investigations showed that osmium tetroxide solution in a concentration of 0.5–1 per cent ensured satisfactory fixation of the sensory epithelium. He presented a very clear and accurate description of the peripheral nerve fibers' communication with the sensory cells. Since his observations have a very close bearing on the present investigation, a few lines from his paper will be quoted here: "The axis cylinder after entering the epithelium divides into several branches, each of which forms a calyx round a hair cell, in which the latter lies like the egg in an egg cup. The calyx is the direct continuation of the axis cylinder which, in osmium-treated specimens, usually contains granules, which are present in great abundance." Summarizing, he wrote: "In my opinion the axis cylinder does not resolve into separate fibrils, but merely ramifies. The nerve calyx consists of the same hyaline ground substance as the axis cylinder, in which granules are stored." Kaiser's observations were verified in 1892 by Niemack, who used methylene blue staining for nerve impregnation. The investigations of Kaiser and Niemack were vehemently criticized, however, by Held (1902) and Retzius (1905), among others. Held asserted that Kaiser's and Niemack's description of the nerve calyces was implausible and that the latter consisted of densely packed granulated terminal branches, forming a dense network round the hair cells. In Retzius' opinion the authors in question had failed to understand the true fibrillar structure of the nerve calyces. In an investigation of 1892, Retzius, using Golgi's

silver impregnation method, demonstrated in mice a nerve plexus with free nerve endings in the sensory epithelium of the crista ampullaris.

Kolmer (1904), who conducted an extensive investigation into the intracellular fibrillar structures, regarded the hair cells as true nerve cells having intracellular neurofibrillar reticula in continuity with the neurofibrils of the vestibular nerve fibers. Bielschowsky and Brühl (1907) found rings that stained with silver in the lower parts of the sensory cells, and nerve branches that terminated with fine nerve end branches at the sensory cells.

Cajal (1908), in very important investigations, demonstrated two types of nerve fibers in the crista ampullaris: "colossal fibers" that ran to hair cells near the summit of the crista, where each "colossal fiber" branched into two or three nerve calyces enveloping a like number of hair cells, and fine fibers innervating the peripheral parts of the cristae. Lorente de Nó (1926) analyzed Cajal's observations and, on studying preparations silver-impregnated *ad modum* Cajal, was able to verify the latter's findings and also to enhance our knowledge of the innervation of the cristae. He distinguished, in the cristae, three zones with different types of innervation. One of these was a central zone supplied by relatively thick nerve fibers that branched into four or five fibrillar nerve calyces, each enclosing a sensory cell. Secondly, a more peripheral zone comprising the slope of the crista with more slender nerve fibers which, like the thicker fibers, formed nerve calyces but which were also mutually connected with collateral fibers. Lastly, there was a peripheral zone at the transition to undifferentiated epithelium that was innervated by fine nerve fibers ramifying into a large number of fibrils forming a plexus in the epithelium. From this plexus emerged fibers forming incomplete calyces round the peripheral sensory cells. Similar observations were made by Poljak (1927) in preparations silver impregnated *ad modum* Golgi.

The extension and structure of the crista epithelium was studied in detail by Vilstrup (1950), who used e.g. the freezing and drying method. He claimed to have demonstrated, in *Acanthias vulgaris,* a conspicuous asymmetry of the epithelial distribution in the individual cristae, and wrote that "the epithelium on the utricular crista side extends tongue-like downwards" in the superior and posterior ampullae, while the sensory epithelium in the crista of the lateral ampulla extended further downward on the canalicular side.

Structure of the Cristae Ampullares

The sensory epithelium in the semicircular ducts is collected into transverse crests—the cristae ampullares. The height of these crests corresponds to about one-third the diameter of the ampulla. The bulk of the crista is clothed by highly differentiated sensory epithelium, which changes peripherally to cylindric or cubical supporting epithelium. This latter epithelium, in turn, is distinctly delimited in relation to the remaining labyrinthine epithelium. The crista is often described as having a characteristic saddle shape. (Fig. 2.) At either end of it the ampullar wall has a semilunar zone clothed with supporting epithelium—the planum semilunatum, which was described by Steifsand as long ago as 1835. In the ampullae of the two superior semicircular ducts this formation is uniformly arranged on either side of the crista, but in the lateral ampulla it is best developed in the

superior ampullar wall. The periphery of the crista, like the utricle, contains an abundance of pigment cells, probably of mesodermal nature.

The connective tissue stroma of the crista consists of tissue which has relatively sparse cells, and within which a large number of nerve fibers run up to the epithelial layer. A zone about 2–3 μ thick in the connective tissue immediately beneath the epithelium is virtually devoid of nuclei, and for this reason has been designated as basement membrane by Retzius, Kolmer and others. The ultrastructural analysis has shown, however, that the connective tissue is separated from the epithelium by a membrane about 400 Å thick—the basement membrane—whereas the above-mentioned zone in the connective tissue stroma is not distinct from the rest of the connective tissue. The connective tissue stroma contains a network of fine capillaries, chiefly collected round the zone beneath the basement membrane.

In the present investigation it was notably the sensory epithelium and the peripheral nerve branches of the cristae ampullares that were studied.

Structure of the Sensory Epithelium

The cristae ampullares in the guinea-pig show some asymmetry with regard to the extension of the sensory epithelium on their surfaces. In the superior and posterior ampullae the sensory epithelium is found to extend further down the utricular than the canalicular surface of the crista. (Fig. 4.) In the lateral ampulla, however, the epithelium is equally distributed on both sides of the crista.

These observations, insofar as the superior and posterior ampullae are concerned, accord with Vilstrup's description of the extension of the sensory epithelium in the cristae ampullares of *Acanthias vulgaris*. He found, however, that in the lateral ampulla of those animals the epithelium extended further downward on the canalicular than on the utricular surface—a finding that has not been duplicated in the guinea-pig, where complete symmetry exists in that crista ampullaris.

The sensory epithelium has a thickness of about 40 μ. It is separated from the connective tissue by a basement membrane around 400 Å thick, which is perforated only by nerve fibers that run from the connective tissue up into the epithelium and ramify there.

The sensory epithelium, as earlier reported by Retzius (1881), Held (1902), Kolmer (1911, 1927) and others, is composed of two main types of cells: hair (sensory) cells and supporting cells. In the pioneering investigations on the innervation of the crista epithelium that were conducted by Kaiser, Cajal, Poljak and Lorente de Nó, the close relation of the hair cells to the terminal branches of the vestibular nerve in the ampullae was established. Both Cajal, Lorente de Nó and Poljak, as pointed out earlier, observed different types of nerve endings in the central and the peripheral parts of the cristae. None of those workers, however, described any differing types of hair cells in the epithelium. Iwasa (1925) alone has observed, besides the commonly described bottle-shaped hair cells, "a few modified hair cells, mostly resembling goblet cells". He did not, however, describe them in detail.

In the present investigation were observed, in both the light microscopic and electron microscopic studies, two types of hair cells that distinctly differed from each other both in structure and innervation. These cell types will be designated,

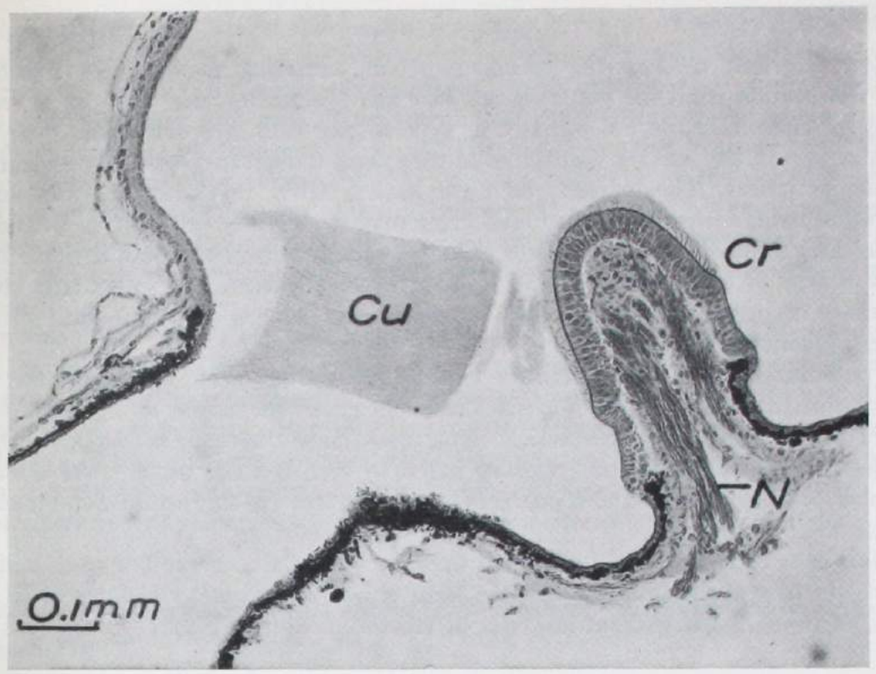

Fig. 4. Section through the superior ampulla and crista ampullaris (Cr). The cupula was dislodged at preparation of the specimen, yet has retained its normal shape (Cu). Note the division of the nerve fibers into two bundles (N). Preparation fixed in osmium tetroxide and embedded in metacrylate. Phase contrast photomicrograph, 100 ×.

in the following, as hair cells of type I and hair cells of type II. Their general structure will be seen from the schematic representation in Fig. 9.

Hair Cells of Type I

These cells have the form of bottles with rounded bases and short necks (Fig. 10.) They extend from the epithelial surface about two-thirds the thickness of the epithelium. They are bounded apically by a cuticular plate from which about fifty sensory hairs arise. The nucleus is almost spherical and located in the broad basal part of the cell. The Golgi apparatus is localized to a zone in the supranuclear part near the nucleus. In the infranuclear portion is a zone within which, in silver impregnated preparations, a silver staining plaque or ring can be observed (Bielschowsky & Brühl, 1907). The corresponding area of the cell shows, at electron microscopic examination, a system of ultrastructural membranes enclosing thin cleftlike spaces in the cytoplasm.

Each hair cell of type I is innervated by a nerve calyx enveloping like a shell the entire cell except for its most distal part, which lies between the surrounding supporting cells. Immediately beneath the cell membrane, in the zone of contact between hair cell and supporting cells, there is a density in the cytoplasm—the terminal bar. Hair cells of type I are found throughout the sensory epithelium of the crista except for a narrow zone contiguous to the peripheral cylindric epithelium. They are densest, however, in the central parts of the crista and decrease in number towards the periphery.

Hair Cells of Type II

Cylindric hair cells in the crista epithelium, differing distinctly in structure and innervation from the bottle-shaped hair cells, were first described by Wersäll (1954). These cells, here designated as type II hair cells, are distributed over the greater part of the sensory epithelium of the crista, though they tend to concentrate in the periphery. The majority of them have a fairly regular cylindrical shape with a rounded lower part. They are longer than the sensory cells of type I, and in some cases extend to the immediate vicinity of the basement membrane. Some hair cells of type II nevertheless show a more irregular structure, the lower part being narrower than the upper part and, in some cases, having only one-third of the latter's diameter.

The intracellular localization of the nucleus varies from cell to cell. There are, for instance, hair cells with the nucleus located in either the upper or the middle or lower parts. From the dense cuticle, lying immediately beneath the upper surface of the cell, emerge about fifty sensory hairs. In each cell can be observed a small number of mitochondria and a distinct Golgi apparatus situated in the supranuclear part.

Hair cells of type II are innervated by a number of fine nerve branches joining the lower part of the cell, either in the form of bud-shaped nerve endings or in the form of nerve loops without any special terminals. (Figs. 9, 17.)

Supporting Cells

The supporting cells are present throughout the sensory epithelium and exceed the hair cells in number. They reach from the basement membrane to the surface of the epithelium. They are devoid of cuticles and sensory hairs. The apical surface, which often bulges somewhat, has a sparse number of microvilli. The nucleus, situated in the basal part, is oval and contains one or, more commonly, two very dense nucleoli. In the distal part of the cell is a ring of dense substance at the same level as the terminal bars of the hair cells. In surface sections these supporting rings from several cells form a network of dense trabeculae, termed the reticular membrane. The Golgi apparatus is found in the apical part of the cell. Virtually the whole of the apical part and the middle part are otherwise filled by densely packed granules having a diameter of 0.2–0.3 μ.

Innervation of the Sensory Epithelium

Introduction

From the physiologic point of view the distribution of nerve fibers of varying caliber in the crista is a question of great importance. The excellent investigations conducted by Cajal (1908) and Lorente de Nó (1926) into the innervation of the crista indicated the existence of thick, medium and fine nerve fibers, innervating different parts of the crista epithelium. These authors did not, however, report any analyses of calibers of nerve fibers in the peripheral branches of the vestibular nerve. Nor did Kolmer (1927)—who wrote that the crista was innervated by fine (1–2 μ) and thicker (3 μ) fibers—present any distribution curve for nerve fibers of varying caliber.

Rasmussen (1940) found the human vestibular nerve to show a preponderance of nerve fibers about 10 μ in diameter. Engström & Rexed (1948) employed Häggqvist's method for nerve fiber analysis in an investigation of the cochlear and vestibular nerves in man. They found that 85 per cent of the fibers in the vestibular nerve had diameters of 2–9 μ. The mean of their measurements from two posterior ampullar nerves showed that 71.5 per cent of the relevant fibers had diameters of 3–5 μ; 22 per cent were thicker than 5 μ, and 6.5 per cent thinner than 3 μ. They found, in the posterior ampullar nerve, a greater number of fine fibers and a smaller number of thicker fibers than in the vestibular nerve as a whole.

In the present work the myelinated fibers of the posterior ampullar nerve in the guinea-pig were analyzed with respect to caliber. The distribution of nerve fibers of varying calibers was also studied in both osmium impregnated and silver impregnated preparations. For this purpose the crista ampullaris was sectioned both parallel with the base of the crista and at right angles thereto, so that a good idea was obtained of the course of the thicker and finer fibers in the crista. The light microscopic findings were implemented by electron microscopic studies.

Quantitative Analysis of Fiber Calibers in the Posterior Ampullar Nerve of the Guinea-Pig

Material.—This consisted of six posterior ampullar nerves from five animals having body weights of 250–350 grams.

Results.—The mean number of myelinated nerve fibers in the nerves studied was 1178. Of the nerve fibers, 66.1 per cent had diameters of 3–5 μ, 24.8 per cent diameters of 1–2 μ, and 9.1 per cent diameters of 6–9 μ. (Fig. 5. Table I.)

Discussion.—The systematic analysis of fiber calibers in different nerves was introduced by Häggqvist (1936). In a large number of investigations conducted by Häggqvist and his associates, measurements were chiefly made of nerves fixed in formaldehyde and stained by the method of Alzheimer, Mann and Häggqvist. Rexed (1944) showed, however, that this method is associated with considerable shrinkage, whereas impregnation with osmium tetroxide gives a picture closer to the native state. The latter method has accordingly proved to constitute more suitable treatment for fiber analysis than does the method of Alzheimer, Mann and Häggqvist. When, moreover, the specimen is treated with osmium tetroxide before preparation, the darkened nerve, contrasting sharply with the surrounding bone, will be easier to recognize—an important consideration here, because the posterior ampullar nerve is extremely slender and difficult to detect. A drawback of osmium tetroxide as a fixative and staining agent is its fairly poor penetrating power. However, the minute caliber of the posterior ampullar nerve ensures satisfactory impregnation, and the method employed should provide a fairly reliable idea of the caliber distribution of its fibers.

Each of the three semicircular canals in the vestibular apparatus of the guinea-pig has its own nerve, but only the nerve of the posterior ampulla is of such length and course as to permit accurate transverse sections. Comparisons of nerve branches to the various ampullae after special staining did not, however, show any demonstrable difference in fiber calibers; hence the fiber distribution in the

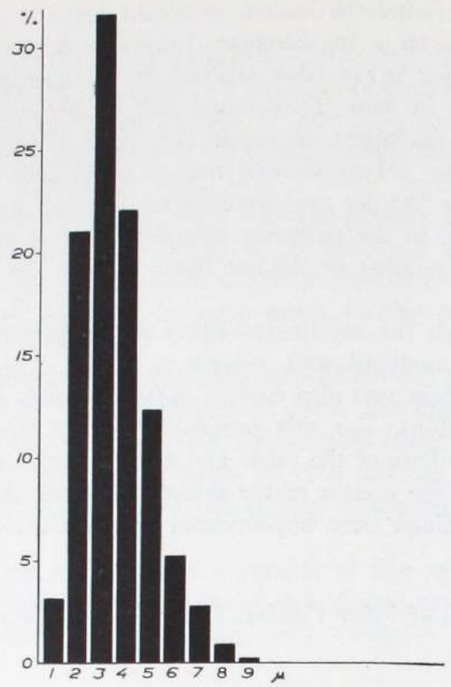

Fig. 5. Caliber distribution of posterior ampullar nerve from guinea-pig.

posterior ampullar nerve was assumed to be characteristic of the other two ampullae too.

The caliber analysis that was undertaken here yielded results which, in the main, were consistent with those reported by Rexed and Engström in humans. The whole of the posterior ampullar nerve accordingly consists of rather fine fibers. Most of the latter have diameters of 3–5 μ; a smaller proportion, 1–2 μ, and only a few exceeding 5 μ. No fibers with diameters exceeding 9 μ were observed in this investigation. The number of thin fibers was higher in the present material than in that reported by Engström & Rexed.

Table I. *Caliber distribution of the myelinated nerve fibres of posterior ampullar nerves from guinea-pigs.*

Animal No.	Per cent of total number of fibers in each nerve									Number of fibers
	1 μ	2 μ	3 μ	4 μ	5 μ	6 μ	7 μ	8 μ	9 μ	
1	2.6	18.4	32.0	26.8	14.5	3.2	2.0	0.5		841
2	3.9	20.0	29.3	23.2	12.1	6.6	3.7	1.0	0.4	1 032
3	2.2	14.8	28.1	25.1	15.2	7.6	4.9	1.8	0.3	1 194
4_1	6.2	27.9	32.3	17.4	8.8	4.5	2.1	0.6	0.1	1 210
4_2	3.1	27.3	32.9	18.1	11.1	4.2	2.3	0.9	0.2	1 278
5	1.0	21.4	34.5	22.5	12.8	4.8	2.0	0.9	0.1	1 511
Mean	3.17	21.63	31.52	22.18	12.42	5.15	2.83	0.95	0.18	1 178

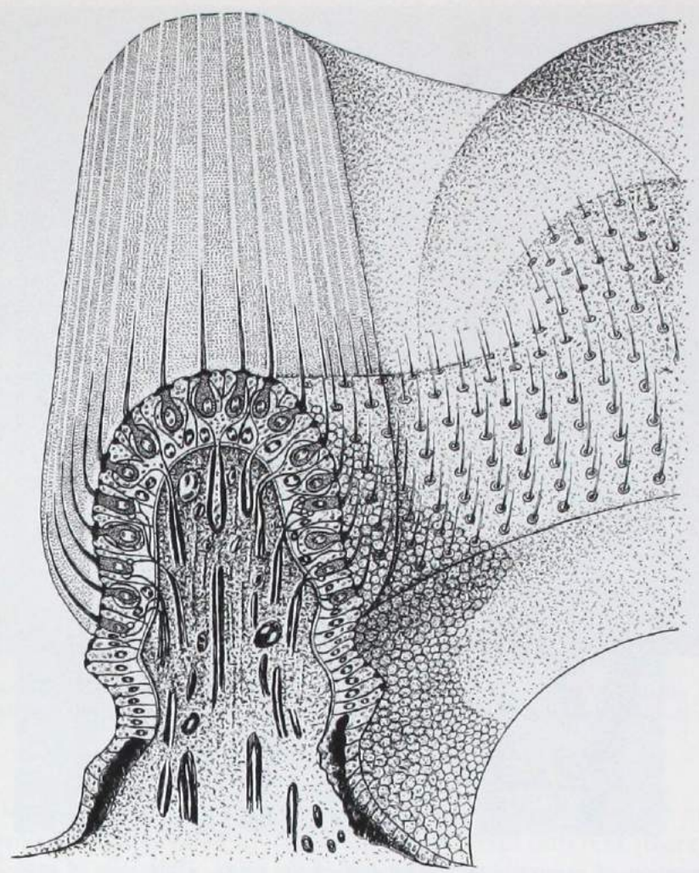

Fig. 6. Schematic drawing of one half of a crista ampullaris, showing innervation of its epithelium. Thick nerve fibers forming nerve calyces round type I hair cells at the summit of the crista; medium caliber fibers innervating type I hair cells on the slope of the crista; medium caliber and fine nerve fibers forming a nerve plexus innervating hair cells of type II. The sensory hairs pass from the hair cells into fine canals in the cupula, which is separated from the epithelium by a narrow subcupular space.

Distribution of Nerve Fibers of Varying Caliber in the Crista Ampullaris

Observations.—No appreciable difference was found in the innervation of the crista epithelium in the posterior, superior and lateral ampullae.

The nerve fibers in each crista ampullaris pass through the connective-tissue stroma up to the sensory epithelium in two bundles, innervating, respectively, the canalicular and the utricular side of the crista. These two bundles of nerve fibers are separated by a central connective-tissue zone running in the direction of the long axis of the crista and dividing the latter, with respect to innervation, into an utricular and a canalicular part. (Fig. 4.)

According to Lorente de Nó (1926) this division is total in the rat. The same is not the case in the guinea-pig, where single nerve fibers pass from the fasciculus on one side of the crista to the epithelium on the other. In the ampullar nerve itself no distinct division of the fibers into two similar bundles was demonstrable.

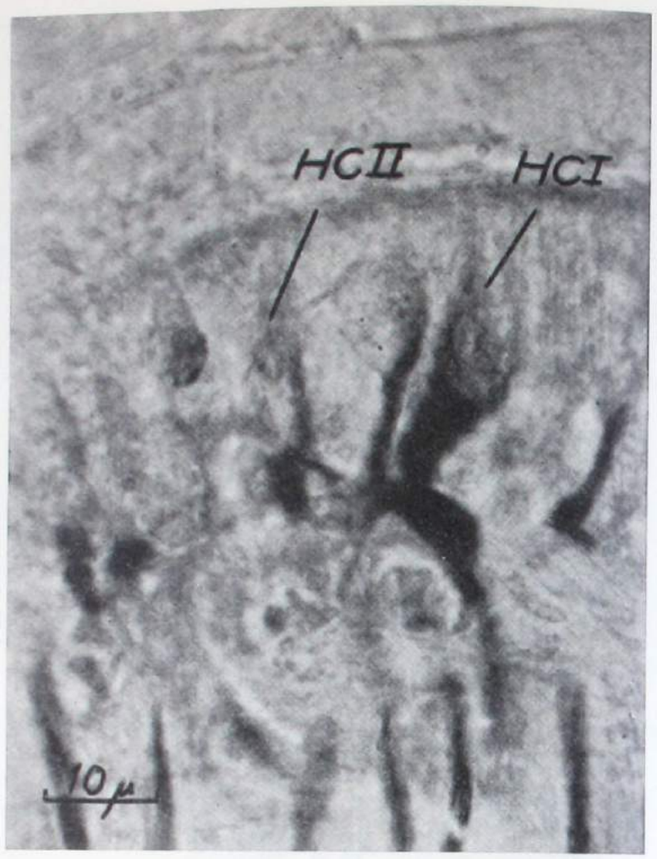

Fig. 7. Innervation of hair cells at the summit of the crista. Hair cells of type I (HC I) are enclosed by a nerve calyx, but those of type II (HC II) are innervated by finer branches. Preparation fixed in Bouing's solution and silver impregnated *ad modum* Palmgren. Photomicrograph, 1500 ×.

Nerve fibers of varying diameter, as pointed out by Cajal (1908) and Lorente de Nó (1926), have a characteristic distribution in each crista ampullaris.

The present investigation showed that the thickest nerve fibers (6–9 μ) in the guinea-pig run to the sensory epithelium clothing the summit of the crista. Here each fiber gives off a small number (usually three or four) of branches, each of which forms a nerve calyx round a type I hair cell. These calyces enclose the hair cells like a continuous shell, and, contrary to the findings reported by Retzius, Lorente de Nó and others, are not composed of fibrillar end branches.

Nerve fibers of medium caliber (3–5 μ) are found throughout the crista. The end branches of these fibers thus innervate hair cells on both the summit and the slope of the crista. Each fiber divides into a larger number of branches than do the thick fibers, and accordingly innervate a greater number of hair cells. (Figs. 6–9.)

Some of the medium-caliber fibers form nerve calyces innervating hair cells of type I; others, together with the finest fibers (1–2 μ), form a highly ramified plexus on the slope of the crista that supplies the hair cells of type II. These

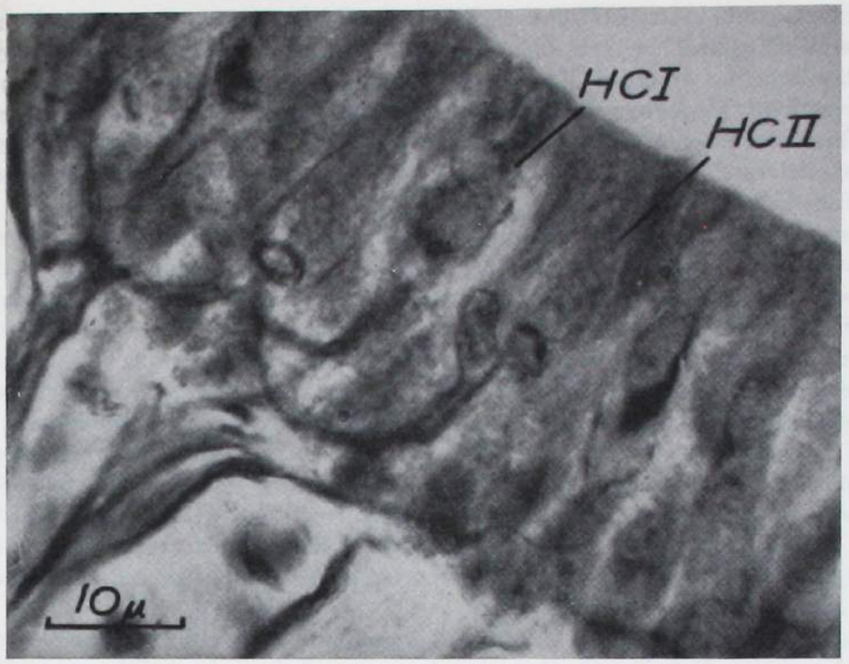

Fig. 8. Innervation of hair cells on the slope of the crista. Note the extremely fine fibers innervating cells of type II. Fixation in Bouing's solution; silver impregnation *ad modum* Palmgren. Photomicrograph, 1800 ×.

latter are innervated, as already pointed out, by a number of nerve end branches. Each cell is usually innervated by branches from several different fibers, and several hair cells, often at relatively great distances from each other, are innervated by the same fiber.

Discussion

The investigations into the innervation of the cristae that were conducted notably by Cajal and Lorente de Nó, using various silver staining methods, have constituted the basis for the studies and theories of recent years concerning the function of the semicircular canals (Steinhausen, 1927, 1933; Loewenstein & Sand, 1940; Dohlman, 1941; Gernand, 1949; Hallpike & Hood, 1953).

However, the various silver impregnation methods, as pointed out by Kolmer (1927) yield only incomplete pictures of the innervation and the relationship between nerve fibers and sensory cells in the cristae ampullares. The silver impregnation methods of Cajal, Golgi and Bielschowsky-Agduhr generally impregnate only relatively few of the peripheral end branches, and usually it is very difficult in these preparations to determine the relationship between sensory cells and nerve endings. This particularly emerges in the discussion on the true structure of the nerve calyces. The view propounded by Retzius, Cajal, Lorente de Nó and others, that the nerve calyces are basket-like formations of intertwined peripheral end branches, has thus conflicted with the observations of Kaiser and Niemack that

the calyx actually consists of a shell forming a direct continuation of the axon and enveloping the hair cell. Opinions have likewise diverged with regard to the question whether or not a direct communication exists between sensory cells and nerve endings. Moreover, the finer nerve fibers in the crista that were first observed by Cajal and, later, carefully described by Lorente de Nó, and their relationship to the sensory epithelium, have not been possible to elucidate with the use of silver staining methods.

Nor did the silver staining method of Palmgren (1948), that was employed in the present investigation, give complete staining of all nerve endings in the sensory epithelium of adult animals. However, comparative light microscopic studies of silver impregnated preparations embedded in paraffin, and others fixed in osmium and embedded in plastic, together with electron microscopic studies of the crista epithelium and its nerve branches, yield far more information on the innervation of the crista epithelium than can be obtained by studying silver-impregnated material alone. It has thus been possible, as will be seen from the aforegoing, to elucidate both the structure of the nerve calyces and the innervating characteristics of the finer nerve fibers. Further, a type of hair cell not previously described has been demonstrated; namely, hair cells of type II, whose innervating characteristics totally differ from those of the earlier reported bottle-shaped hair cells, here termed hair cells of type I.

Due to the difference, in principle, between the innervating characteristics of type I and type II hair cells, these two types are likely to have different functions in the sensory epithelium of the cristae ampullares.

Stimulation of one type I hair cell will thus produce impulses only in one nerve ending, the nerve calyx, and only in one nerve fiber. Stimulation of one type II hair cell, on the other hand, will produce impulses in several nerve endings and also in more than one nerve fiber.

Each nerve fiber innervating hair cells of type I represents only a few hair cells localized to a limited field in the sensory epithelium, whereas each fiber innervating hair cells of type II represents several hair cells located at various distances from each other.

The thickest nerve fibers represent hair cells of type I situated in the summit of the crista, but the thinnest fibers come from hair cells of type II on the slope of the crista.

Having regard to all these facts, the type I hair cell seems to represent a higher degree of sensitivity than does the hair cell of type II. However, the difference in innervation between the two types of hair cells may also have other physiological implications.

If, for example, efferent fibers exist in the cristae ampullares, they probably run to hair cells of type II. Only these latter have several nerve endings, some of which may conceivably be of efferent nature, whereas the nerve calyces enveloping type I hair cells merely permit of direct conduction from hair cell to calyx. The presence of such efferent fibers in the epithelium does not seem to be altogether improbable, having regard to the fact that efferent fibers have been demonstrated in the organ of Corti (Rasmussen, 1942, 1953; Fernandez, 1951; Portmann & Portmann, 1951; Portmann 1952). Recently Petroff (1955) also published results of experiments with sectioning of the eighth nerve that might point to the existence of thin efferent fibers in the vestibular nerve.

The theories outlined above merely provide some indication of the possibilities which two types of hair cells open up in our attempts to elucidate the functions of the cristae ampullares.

However, only by continued electrophysiologic studies of the epithelium and the innervating fibers will we be able fully to understand the significance of the different receptors in the crista epithelium.

CHAPTER IV

FINE STRUCTURE OF THE CELLS OF THE SENSORY EPITHELIUM IN THE CRISTAE AMPULLARES

Introduction

The histologic literature contains but few references to the structure of the various components of the hair cells in the cristae ampullares. Retzius (1884) found granular structures in the cytoplasm, but gave no details of their appearance. Iwasa (1924), who observed "plastosomes and vacuoles" in the cytoplasm of the hair cells, reported that the plastosomes mostly had the form of rods and filaments and showed a very irregular arrangement. Kolmer (1927) wrote that the protoplasm of the hair cell in fresh condition was fairly homogeneous, but shortly after death a slightly granular appearance arose, probably due to swelling of the plastosomes. Kolmer (1908) had observed, about 3 μ beneath the upper surface of the hair cells in the dolphin, an assembly of finely granular fibers, taking silver stain, which in his opinion corresponded to Golgi's internal reticular apparatus. Similar formations were also described by Kawano (1922) in the hen, rabbit and mouse. A network of silver staining fibrils, permeating the entire hair cell and isolated from the Golgi network, was demonstrated by Kolmer (1904, 1927). He regarded these as neurofibrils and assumed them to be in communication with those of the innervating nerve fibers. Tello (1931) considered, however, that these fibrils were quite independent of the nerve fibers.

The electron microscopic investigations of recent years have revolutionized our knowledge of the fine structure of various types of cells. Meticulous analysis of different tissues has shown that the cell is composed of a far more complicated system of organelles than the light microscope has been able to reveal. Many organelles with similar structures are found in a large number of different types of cells. Among these cell components are mitochondria of characteristic structure (Sjöstrand, 1953 b); a system of membranes and vacuoles of specific type, constituting Golgi's apparatus (Dalton & Felix, 1953, 1954; Sjöstrand & Hanzon, 1954); and intracellular membranes of varying type and distribution. These structures have, in different types of cells, an organization and localization characteristic for the particular type.

The above-mentioned organization of organelles is found notably in highly differentiated cells, such as the sensory receptors in the retina (Sjöstrand, 1953 a, c) and the organ of Corti (Engström & Wersäll, 1953 a, b; Engström, Sjöstrand, Wersäll, 1954, 1956), or various types of glandular cells, as for instance the exocrine cells of the pancreas (Sjöstrand & Hanzon, 1954).

In an earlier paper (Wersäll, 1954) a brief report was presented regarding the structure of the sensory epithelium of the crista ampullaris in the guinea-pig. The present investigation comprises further studies thereof with special reference to the fine structure of cells and nerve endings as well as the cupula.

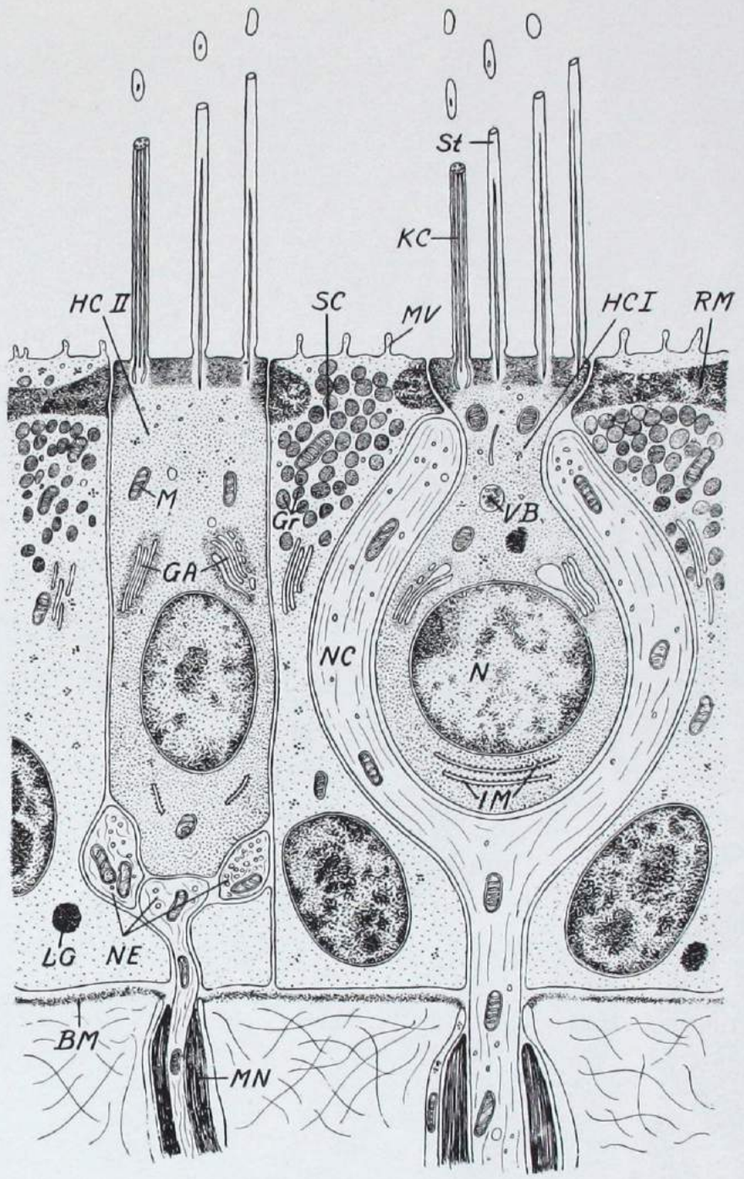

Fig. 9. Highly schematic drawing of a section through the sensory epithelium of the crista ampullaris, showing the ultrastructural architecture of cells and nerve endings. HC I, hair cell of type I; HC II, hair cell of type II; SC, supporting cell; St, stereocilia; KC, kinocilia; N, nucleus; GA, Golgiapparatus; IM, intracellular membrane system; VB, vesicular body; NC, nerve calyx; RM, reticular membrane; M, mitochondrium; NE, nerve endings; BM, basement membrane; MN, myelinated nerve; LG, lipid granule; MV, microvilli.

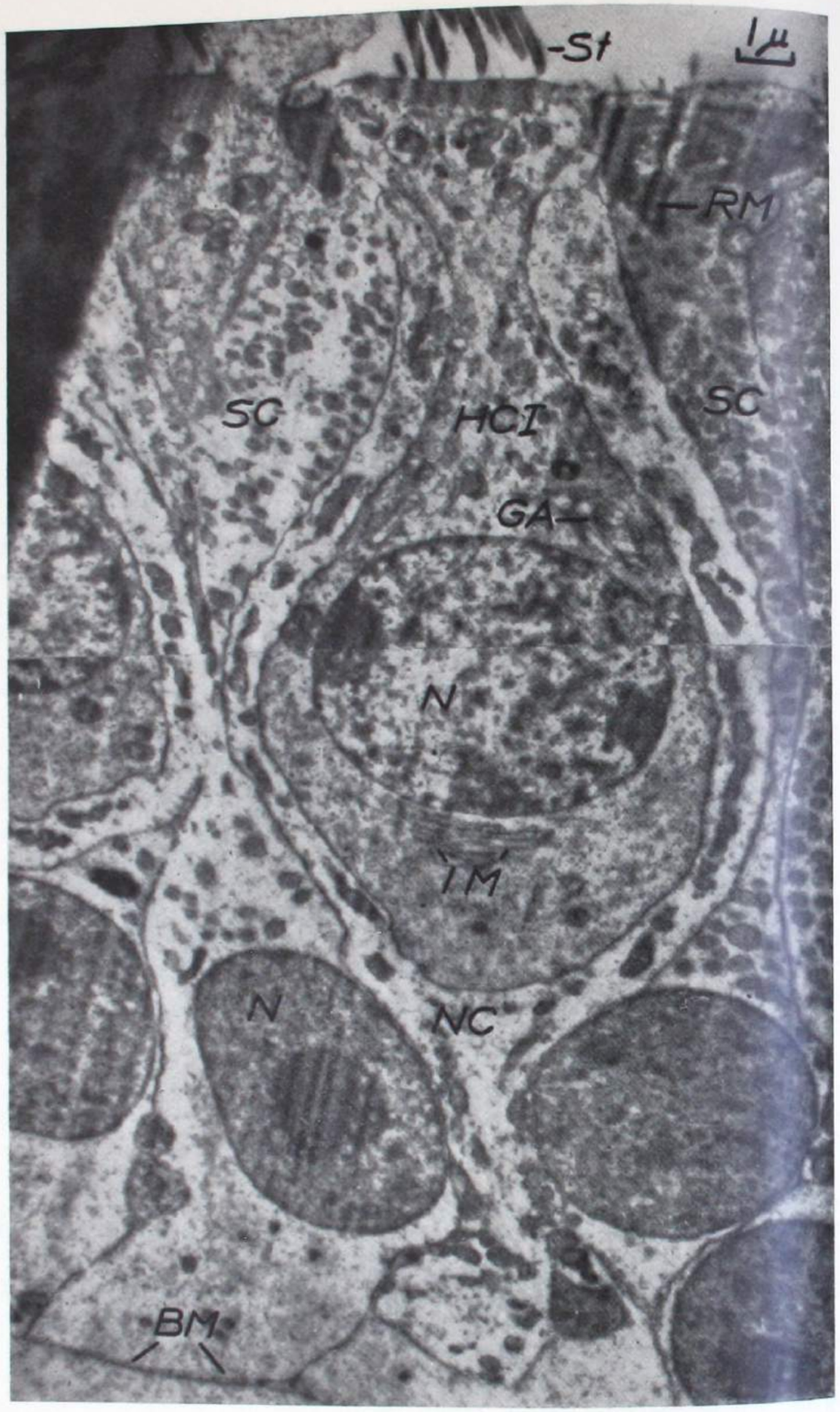

Fig. 10. Section through the sensory epithelium of the crista ampullaris, showing the structure and innervation of a type I hair cell (HC I) and surrounding supporting cells (SC). N, nucleus; St, stereocilia; GA, Golgiapparatus; IM, intracellular membranes; NC, nerve calyx; RM, reticular membrane; BM, basement membrane. Electronmicrograph, 8000 ×.

Hair Cells of Type I

Hair cells of type I, as pointed out in the foregoing, are bottle-shaped with a fairly broad rounded basal portion, in which the nucleus is located. Each cell contains a number of mitochondria, a Golgi apparatus, and a characteristic system of thin, flat spaces, each of which is enclosed by a thin membrane. (Figs. 9–12.) The cell is bounded apically by a thick cuticle and has a nerve calyx enclosing the greater part of the cell. About fifty sensory hairs arise from the upper surface of the cell. The apical portion of the hair cell lies between the adjacent supporting cells.

Cytoplasm

The ground cytoplasm of type I hair cells appears to be composed of a homogeneous substance in which are dispersed a large number of irregular opaque particles having diameters around 100 Å. These particles are generally assembled in groups with diameters of approximately 500 Å. A smaller number of dense granules having diameters of 200–400 Å are embedded in the ground cytoplasm. Most cells also contain a small number of opaque granules with diameters around 0.3 μ, presumably lipoid granules, and a spherical structure about 0.3 μ in diameter, containing a large number of vesicular formations with diameters of approximately 300 Å. This structure probably corresponds to the "vacuole-containing body" observed in the nerve cell by Palay and Palade (1955), and in the cylindric cells in the epithelium of mouse jejunum by Zetterqvist (1956). (Figs. 10–12.)

The cytoplasm is bounded peripherally by a continuous plasma membrane in which can be observed an opaque layer about 60 Å thick. (Table II.)

In the region of contact between hair cell and adjacent supporting cells the cytoplasm is denser immediately contiguous to the plasma membrane of the hair cell than in other parts of it. This zone corresponds to previously described terminal bars (Kolmer, 1927).

Table II. *The dimensions of the plasma membranes separating the hair cell of type I from the nerve calyx. Each animal is represented by one cell. Each figure represents the mean value of ten measurements.*

Animal No.	Total thickness in Å	The light space between the two opaque layers in Å	Calculated thickness of one opaque layer in Å
1	290	166	62
2	234	134	50
3	309	189	60
4	262	158	52
5	359	204	75
Mean	291	170	60

Intracellular Membranes

The infranuclear part of the type I hair cell contains varying numbers (three to ten) of irregular flattened spaces in the cytoplasm, each of which is bounded by

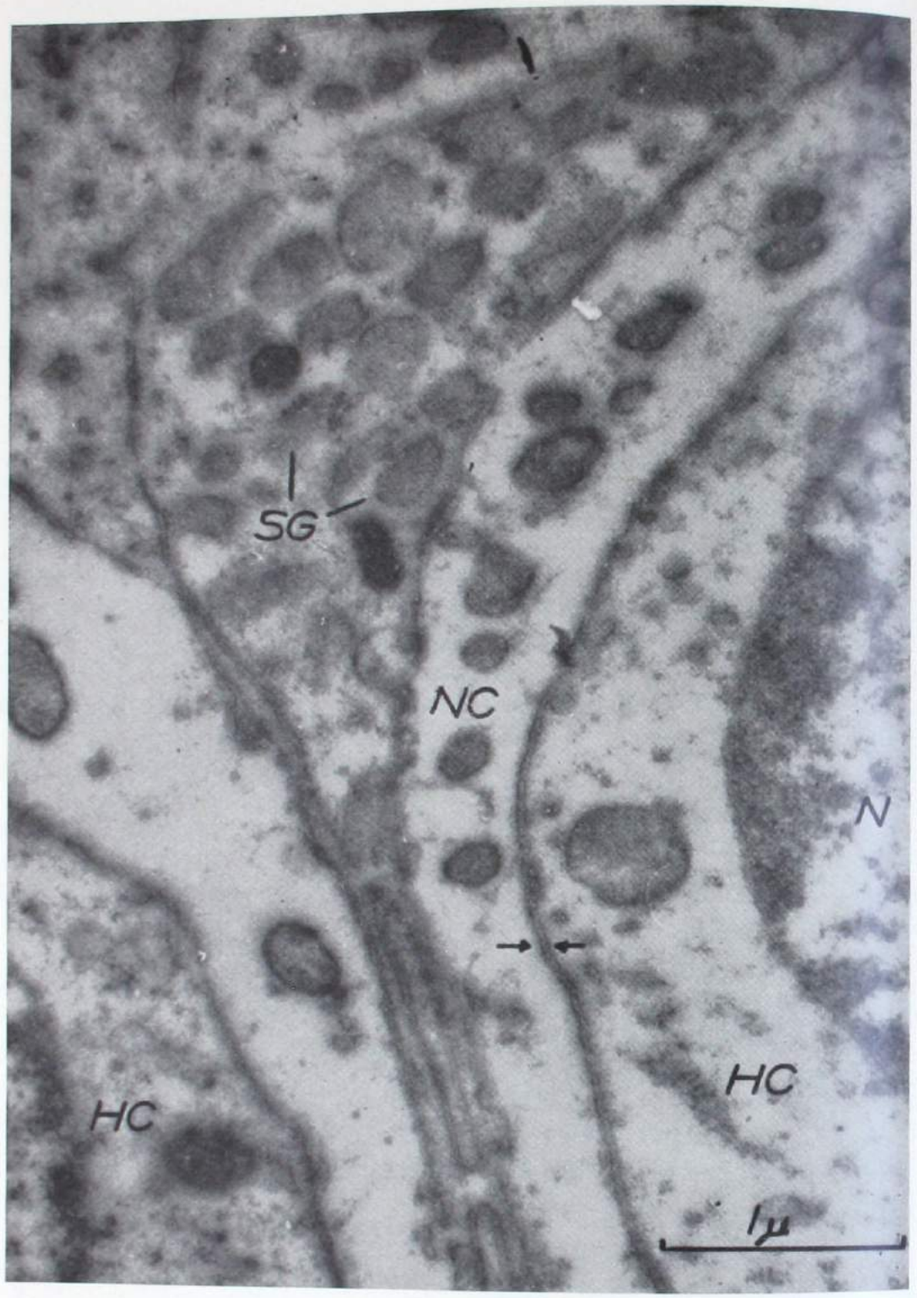

Fig. 11. Transverse section through two hair cells of type I and intermediate supporting cells. Note the two opaque lines with a lighter intermediate layer which separate a hair cell (HC) from its nerve calyx (NC). N, nucleus; SG, supporting cell granules. Electronmicrograph, 33,000 ×.

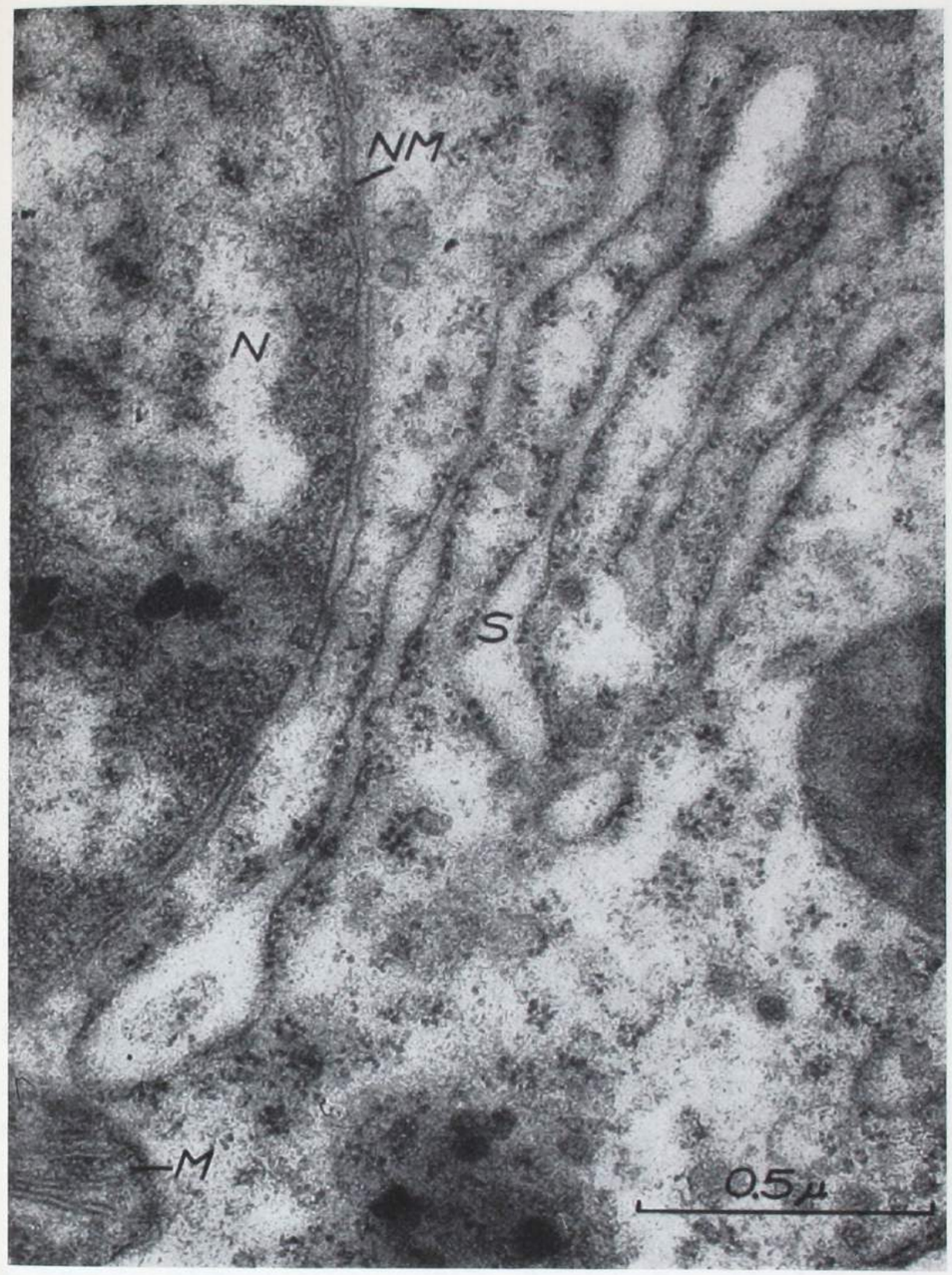

Fig. 12. System of intracellular membranes surrounding narrow spaces in the cytoplasm (S) in the infranuclear part of a type I hair cell. To the surface of the membrane facing away from the space are attached small granules. N, nucleus; NM, nuclear membrane; M, mitochondrium. Electronmicrograph, 65,000 ×.

Table III. *Thickness of the opaque layer of the intracellular membranes in hair cells of type I (alpha-cytomembranes). Each animal is represented by one cell. Each figure represents the mean value of ten measurements.*

Animal No.	Thickness of the opaque layer in one alpha-cyto-membrane in Å
1	58
2	49
3	46
4	45
5	42
Mean	48

an opaque layer or membrane about 50 Å thick. (Table III.) To the surface of the membrane facing away from the space are attached a large number of irregularly formed opaque particles with diameters around 100 Å.

The spaces vary from 200 to 400 Å in thickness. With their enclosing membranes they usually lie parallel with each other, frequently being arranged almost at right angles to the long axis of the cell, with relatively constant intervals between adjacent membranes. They accordingly form a circumscribed system of opaque and semi-opaque layers in the cytoplasm, with characteristic structure and localization in the cell. (Fig. 12.)

The Golgi Apparatus

The Golgi apparatus in hair cells is divided into several zones located in the supranuclear part and chiefly assembled in the region near the nucleus.

Each zone contains a system of opaque membranes bounding thin, slit-like semi-opaque spaces, which are often dilated to vacuoles of varying size. The distances between adjacent membranes in the same zone are fairly constant. The mebranes are smooth and, at this resolving power, have no detectable attached granules.

The different zones of the Golgi apparatus often show, in the periphery, a number of irregular vesicular formations with diameters of 200–300 Å. The ground substance surrounding the Golgi membranes appears to be denser, in electronographs, than the rest of the cytoplasm. However, no sharply defined border or membrane demarcating the Golgi zone from the surrounding cytoplasm has been observed. (Figs. 10, 13.)

Mitochondria

The mitochondria of the hair cells constitute readily classifiable organelles of characteristic structure. Each mitochondrium is delimited from the cytoplasm by an unbroken outer membrane. Running transversely across the mitochondria are a number of inner membranes embedded in a finely granulated intermediate substance. Both inner and outer membranes are composed of three layers; two opaque ones, separated by a less dense middle layer. The inner membranes of the mitochondria are usually virtually parallel with one another and extend without a

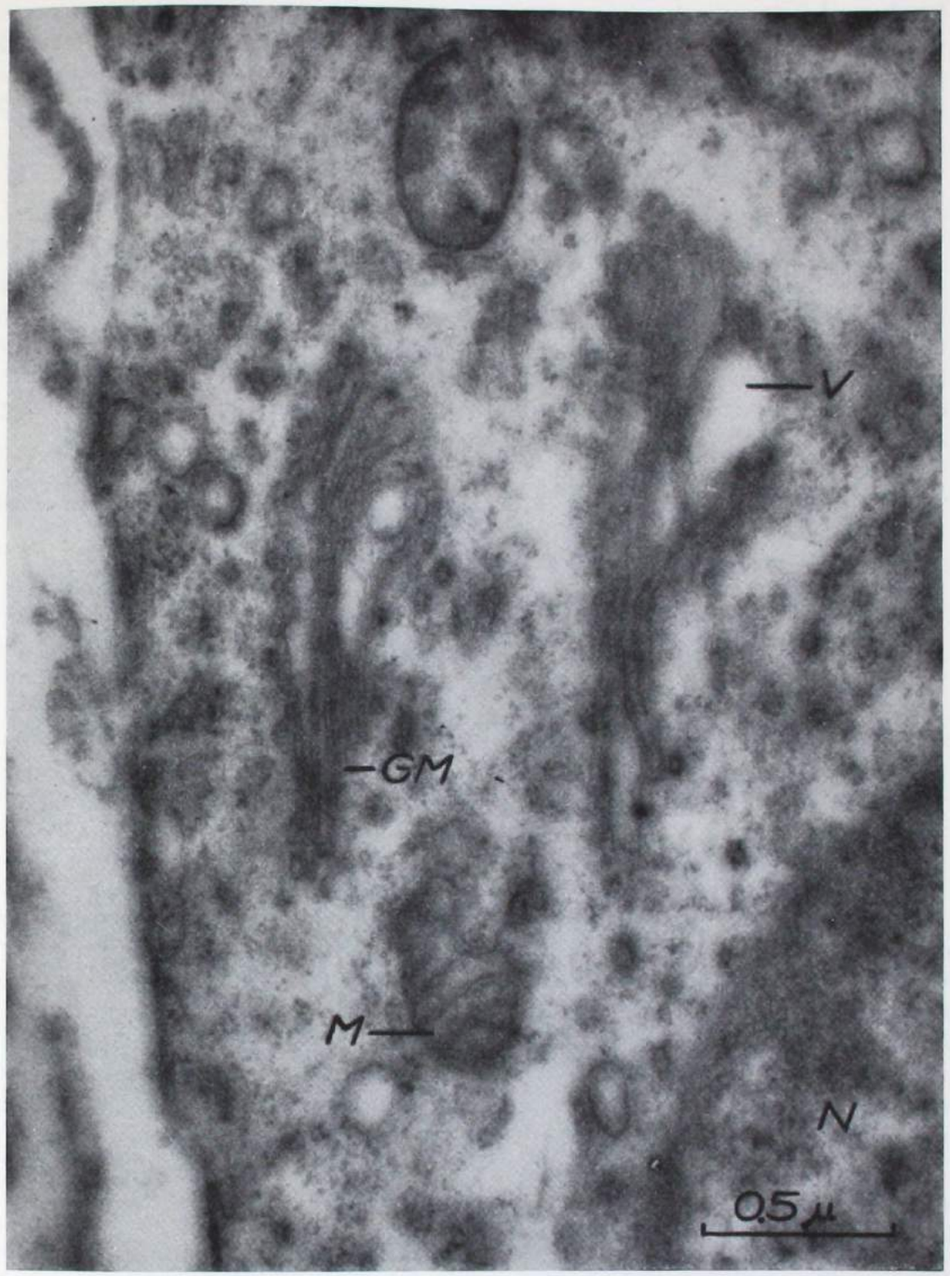

Fig. 13. Part of the Golgi apparatus of a type I hair cell, showing the Golgi membranes (GM), each of which encloses a narrow space in the cytoplasm. The membranes are smooth and have no granules attached to their surfaces. V, vacuole, a characteristic component of the Golgi apparatus; M, mitochondrium; N, nucleus. Electronmicrograph, 50,000 ×.

break across the mitochondrium. In this investigation occasional membranes were observed which extended only over a part of the mitochondrium. Even branched membranes were noted. The majority of inner membranes were quite separate from the outer membrane, though in some places communications were observed between the layers in inner and outer membranes. (Fig. 14.)

Nucleus

The nucleus of the hair cells is usually almost spherical, with a diameter of approximately 8 μ. It is bounded by a nuclear envelope consisting of two opaque layers with a thickness of around 50 Å, (Table IV) separated by a less dense layer approximately 150 Å thick. In sections at right angles to the surface of the nuclear membranes, discontinuities of the membrane were occasionally noted. It is uncertain, however, whether these discontinuities consist of genuine pores, connecting the cytoplasm with the nuclear substance.

Table IV. *The dimensions of the nuclear envelope of hair cells of type I. Each animal is represented by one cell. Each figure represents the mean value of ten measurements.*

Animal No.	Total thickness in Å	The light space between the two opaque layers in Å	Calculated thickness of one opaque layer in Å
1	166	86	40
2	247	131	58
3	296	168	64
4	244	148	48
5	262	142	60
Mean	243	135	54

Within the nucleus are observed irregular masses of an apparently homogeneous opaque substance in which are scattered small particles arranged in clumps or fibers. These masses are assembled in filaments of varying length, and also form a continuous layer of varying thickness on the inside of the nuclear membrane. Usually there is a nucleolus, a larger dark body apparently of the same structure as the dark masses in the rest of the nucleus. Intranuclear spaces not occupied by the opaque substance are filled with an apparently amorphous clear substance or liquid. (Fig. 12.)

Cuticles and Sensory Hairs

The apical boundary of the hair cell consists of a plasma membrane and, inside it, a cuticle varying between 0.2 and 0.5 μ in thickness. This latter is composed of dense, apparently amorphous ground substance containing a large number of small particles. From the free surface of the hair cells about fifty hairlike processes emerge, with a diameter of 0.2 μ and a length of about 40 μ. The majority of them have a stereocilial structure, though one process from each cell has a kinocilium-like structure. These two types of processes will be termed, in the following, stereocilia and kinocilia respectively.

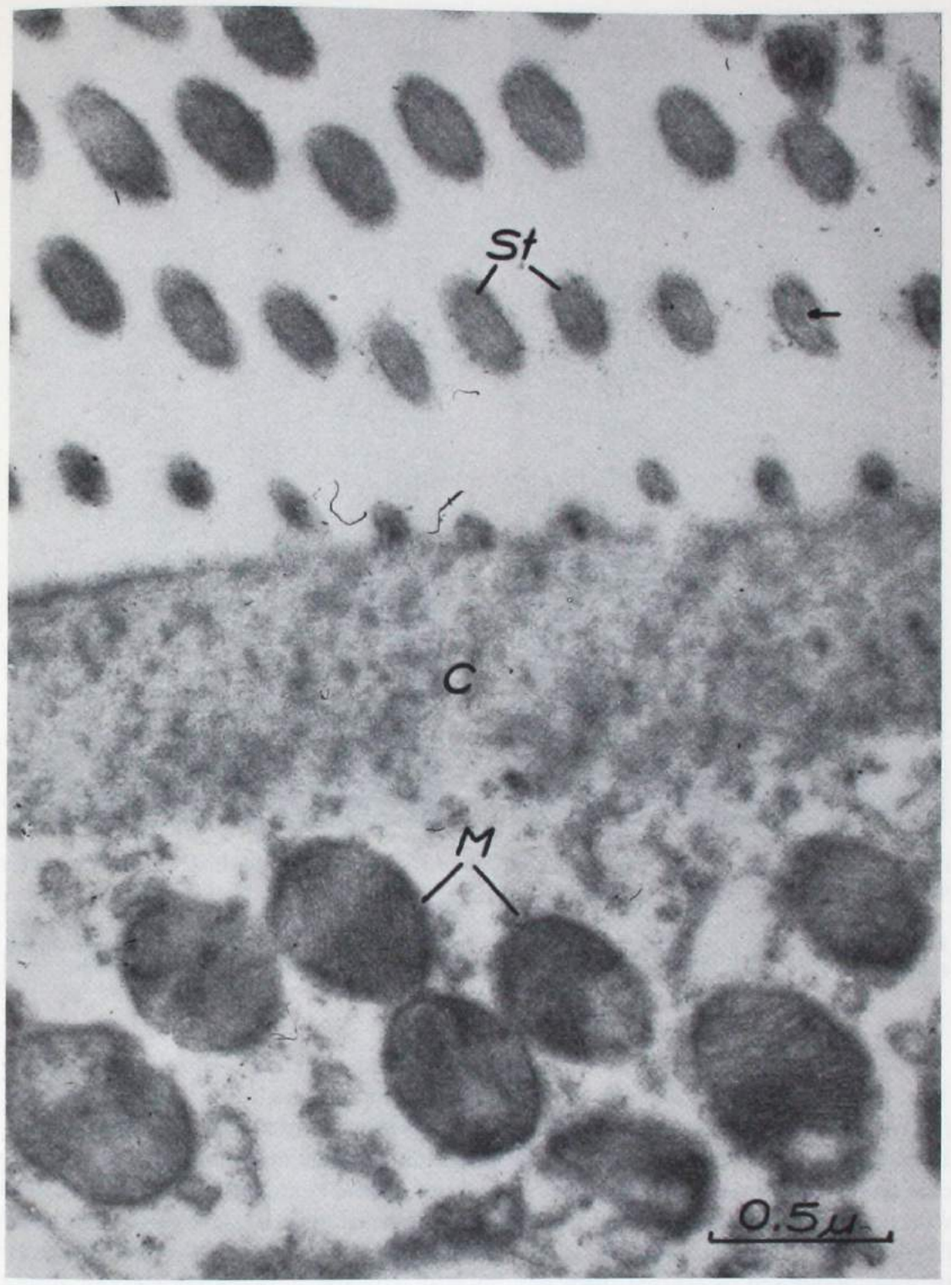

Fig. 14. Oblique section through the apical portion of a type I hair cell, with transversely cut stereocilia (St). The arrow indicates a stereocilium with a distinct axial fibril. The axial fibrils can be seen in the cilia that have been cut at the base but not in those cut further apically. The mitochondria (M) beneath the cuticle contain a large number of almost parallel inner membranes; C, cuticle. Electronmicrograph, 47,000 ×.

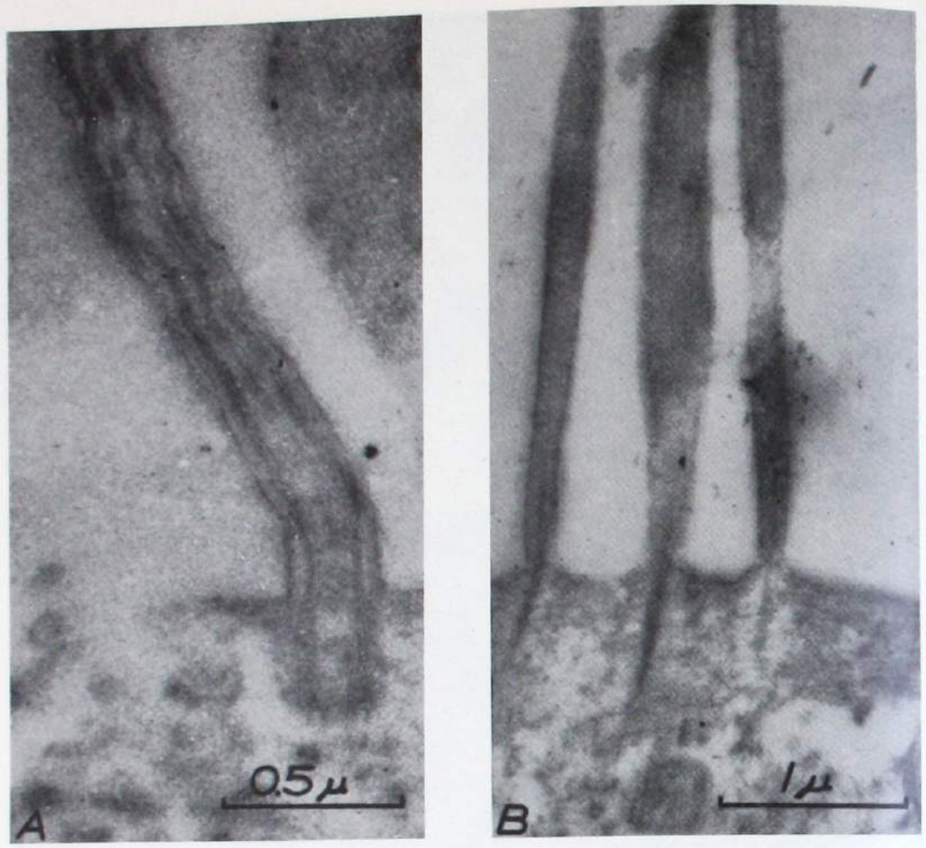

Fig. 15 A. Longitudinal section through a kinocilium. While the outer membrane of the cilium merges basally with the cell's plasma membrane, the intracellular part of the fibrils forms a basal corpuscle in the cuticle. Electronmicrograph, 50,000 ×.

Fig. 15 B. Showing stereocilia cut somewhat obliquely. The axial fibril in the stereocilium continues from the latter's extracellular portion into the cuticle, where it is surrounded by a narrow light zone. Electronmicrograph, 25,000 ×.

The *stereocilia* are composed of an extracellular and an intracellular portion. The basal part of the former portion consists of an axial fibril surrounded by finely granulated protoplasm, which in turn is bounded by a thin outer membrane. It was possible to observe axial fibrils for a distance of only about 4 μ along the cilia from the cell surface; their actual extension in the cilia could not be established with assurance. However, the apical portion of the cilia is devoid of any axial fibril. While the outer membrane of the cilia unites, basally, with the hair cell's plasma membrane, the axial fibril continues downward into the cuticle and forms the intracellular portion of the cilia. It is surrounded here by a lighter zone. Within this latter zone no structural features were demonstrable. In occasional cilia the axial fibril could be visualized through the whole of the cuticle. In no case, however, was any continuation detectable in the cytoplasm.

The *kinocilium* in each cell is closely consistent in structure with the kinocilia that have been observed in the trachea, the fallopian tube, and in a number of

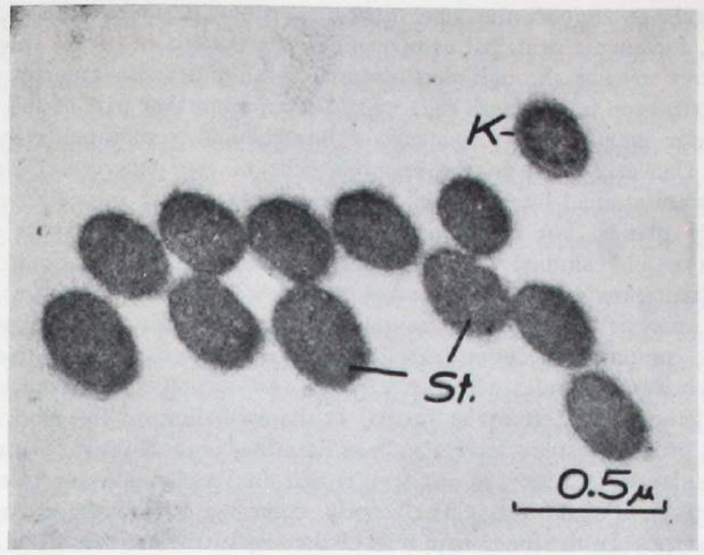

Fig. 16. Transverse section through a few stereocilia (St) and one kinocilium (K). Since the section line is through the apical part of the stereocilia, no axial fibrils can be seen therein. The kinocilium has a central fibrillar bundle of two single fibrils surrounded by nine double ones. The double structure in the peripheral fibrils does not emerge distinctly in this picture. Electronmicrograph, 40,000 ×.

unicellular organisms (Engström, 1951; Engström & Wersäll, 1952; Fawcet & Porter, 1954; Rhodin & Dohlman, 1956, and others). It is possible, as in the stereocilia, to distinguish an extracellular portion, the cilium proper, and an intracellular portion, the basal corpuscle. The extracellular part is composed of a central bundle of fibrils and protoplasm of fine structure, bounded by a thin membrane. The fibrillar bundle consists of two single central fibrils surrounded by nine double fibrils. The outer membrane unites basally with the plasma membrane of the cell, but the fibrils continue downward into the cuticle, and there form a budlike swelling, the basal corpuscle. (Figs. 14–16.)

Discussion

Cytoplasm.—Small opaque particles or granules, constituting a substantial component of the ground cytoplasm, have been demonstrated in many different types of cells. Sjöstrand (1953 c) observed a large number of black dots in the cytoplasm from rods in the retina of guinea-pigs. Granules with diameters of about 40 Å, forming dots 100 Å in diameter, were described by Sjöstrand & Rhodin (1953) in cells of the proximal tubules in the rat. In the opinion of Palade (1953), granules with diameters of 80–300 Å constitute an important part of the cytoplasm in most types of cells. Zetterqvist (1956) observed granules of irregular shape, 150 Å in size, forming clumps in the cytoplasm in the epithelium of the mouse jejunum.

The above findings, like those in the present investigation, suggest that the cytoplasm has a granular structure. The extremely regular distribution of the

granules tends to support the view that the opaque particles with diameters of 100–200 Å, forming a principal component in the cytoplasm of the fixed cell, are not structures arising through denaturation of an otherwise amorphous ground cytoplasm but, even in the fresh cell, constitute an important part of the cytoplasm.

Intracellular membranes.— Systems of intracellular membranes were first described by Dalton (1951) in the exocrine cells of the pancreas. Corresponding structures were studied by Bernhard and coworkers (1952) in the liver, pancreas and salivary glands, but were regarded as fibrils or canals in the cytoplasm. Sjöstrand (1953 b) studied the membranous systems in pancreas cells at higher resolution than previous workers and, together with Hanzon (1954 a)), made a meticulous analysis of the cytoplasm of the pancreas cells. This cytoplasm was found to be composed of concentrically arranged membranes 40 Å thick, on one side of which were granules with diameters of 140 Å. Single membranes of similar type were detected by Zetterqvist (1956) in the epithelium of the mouse jejunum. Membranes or duct systems have also been described in the thyroid (Monroe, 1953), in salivary glands (Gautier *et al.*, 1953), and in the hypophysis (Reinhardt & Farquar, 1953). Palade (1955 b) recently described membrane systems in 40 various cell types. In the inner hair cells of the organ of Corti has been observed a system of spaces bounded by membranes closely consistent, both in appearance and intracellular localization, with the membrane system existing in hair cells of type I.

Membranes with a thickness of about 50 Å, to one side of which were attached irregularly shaped opaque particles with diameters around 150 Å, were described by Sjöstrand (1956) as alpha-cytomembranes. According to Sjöstrand they represent a characteristic cellular component differing in structure from beta-cytomembranes, which consist of "tight folds of the cell membrane extending into the interior of the cell body", as found in the tubular cells of the kidney (Sjöstrand & Rhodin, 1953; Rhodin, 1954). They also differ morphologically from the type of membranes found in Golgi's apparatus, and designated by Sjöstrand (1956) as gamma-cytomembranes.

Although alpha-cytomembranes appear to be morphologically well-definable structures occurring in cells of many different types, they vary conspicuously in extension and localization. This is probably attributable to the functions of the different cells. It seems justified, therefore, to assume that the system found in type I hair cells, consisting of thin spaces enclosed by membranes to which are attached small opaque particles, constitute a structure characteristic of those cells and having a specific bearing on their function. The membranes have no connection with the nuclear membrane or plasma membrane of the cell as suggested by Palade (1955 b).

Golgi apparatus.—The *appareil reticulaire interne* which Golgi (1898) observed in nerve cells was originally described as a silver-staining network in the cytoplasm. The term "Golgi apparatus" has since been extended to comprise intracellular vacuoles and granules of extremely varying appearance and staining characteristics. Some workers (Baker, 1950; Bourne, 1950) have considered that the phenomena described as Golgi apparatus in various cells have actually consisted of artefacts arising through transformation of the cytoplasm at fixing and staining. In recent years a Golgi apparatus has nevertheless been demonstrated in living cells by Gatenby (1953) and Dalton & Felix (1953), among others; hence its existence as a true cell organelle may well be generally accepted today.

In the electron microscope, the Golgi apparatus was first observed by Dalton (1951, 1952) as an osmiophilic component of the cytoplasm associated with vacuoles that were assumed to contain an osmiophobic component.

Dalton & Felix (1953, 1954), in comparative studies of the Golgi apparatus by light microscope and electron microscope, observed an osmiophilic and an osmiophobic component with both microscopic methods. They found, too, that mitochondria and droplets "supravitally stainable with either methylene blue or neutral red in the epithelial cells of epididymis and duodenum from mouse had no morphologic relationship with the Golgi substance".

Sjöstrand & Hanzon (1954 b) studied the Golgi apparatus in the exocrine cells of the mouse pancreas at a resolving power better than 30 Å, and found it to be divided into several intracellular zones. Each zone consisted of a system of membranes with a thickness of 60 Å, arranged in pairs, each pair delimiting spaces or vacuoles; a fairly homogeneous ground substance, and granules of varying form and density. Corresponding structures have been demonstrated in the Golgi apparatus of cells in the proximal tubules of mice (Rhodin, 1954), in the duodenal epithelium (Zetterqvist, 1956), and in striated muscle cells of the same animal (Andersson, 1956), as well as in mast cells from mast-cell tumors (Bloom *et al.*, 1956).

Kolmer (1908, 1911) and Kawano (1922) described a reticular system of Golgi type in the supranuclear portion of the hair cell. The system of spaces and vacuoles, varying in size and bounded by membranes of characteristic type, that was observed, in corresponding zones, in the present investigation is consistent in structure with the Golgi apparatus in other types of cells. Although the connection between these structures and Golgi's *appareil reticulaire interne* is not clear, there has been described, in a large number of papers, a well-defined structure with small variations between cells of different types; and there appear to be substantial grounds for regarding this as Golgi's apparatus.

The zones which in this paper have been termed spaces in the cytoplasm, in connection with the description of the membrane systems, are probably zones containing a substance that is either destroyed or released during preparation, or has an ultrastructural architecture the components of which cannot be detected at this resolving power.

Mitochondria.—The existence of mitochondria in the hair cells, earlier reported by Retzius, Kolmer and Iwasa, was verified in this investigation.

An inner structure within the mitochondria was observed more or less coincidently by Palade and Sjöstrand. While Palade (1952) described the mitochondria as being enveloped by a single outer membrane penetrating, in the form of folds (cristae mitochondriae), into the mitochondrium substance from one or more sides, Sjöstrand (1953 b) found both that the outer membrane was double and that most of the inner membranes were quite separate from, and could not constitute folds of it. Similar observations were reported by Engström, Sjöstrand & Wersäll (1953), Rhodin (1954), and Zetterqvist (1956). Sjöstrand's observation of a double membrane enveloping the mitochondrium was verified by Palade (1953).

Due to the difficulty of obtaining a consecutive series of sections from the same mitochondrium, no complete reconstruction was possible, in the present study, of the structure of the mitochondria in the hair cells. Hence it could not be definitely established to what extent the inner membranes of each mitochondrium

are connected with each other or with the outer membrane. The mitochondrium apparently contains, however, both membranes that are more or less separate from the outer membrane and others in direct communication with it. The majority of inner membranes nevertheless run transversely across the entire mitochondrium and are in contact with the outer membrane at both ends, though without uniting with it.

Nuclear membranes.—A double nuclear membrane was described by Callan & Tomlin (1950) around the nuclei of amphibian oocytes. In mammals, the double nuclear membrane was first described by Hartmann (1952, 1953). He found the nuclear membrane in nerve cells of the central nervous system to be composed of two dense layers 80–120 Å thick, separated by a less dense layer with a thickness of 100–150 Å. Sjöstrand & Rhodin (1953) observed a similar structure in the nuclear membrane of cells from the proximal tubules of the mouse kidney. In further studies of the tubular cells in mice, Rhodin (1954) found, in tangential sections through the nuclear membrane, annular structures having a diameter of about 900 Å. Whether these consisted of pores in the nuclear membrane, resembling those described by Callan and Tomlin in the inner layer of the membrane, could not be established with assurance. In sectioned material, true pores penetrating the double nuclear membrane have been described in salivary glands and gut from *Chironomus* by Bahr & Behrman (1954), in the exocrine cells of the pancreas (Watson, 1954), in nerve cells (Palay & Palade, 1954), and in liver cells from rats (Fawcet, 1954). In the opinion of Watson (1955), pores in the nuclear envelope are fundamental features of all resting cells. Afzelius (1954) found that the nuclear membrane of the sea-urchin oocyte appeared as a double membrane with 40 to 80 "holes" per μ^2. These holes were found to be covered by a very thin single membrane and were surrounded by walls. Zetterqvist (1956) found discontinuities in the double nuclear membrane of epithelial cells from mouse gut, but was unable to state with assurance whether they consisted of true pores in the membrane.

It is possible that the occasional discontinuities in the nuclear membrane that have been observed in hair cells, consist of pores. But since no transverse sections of such pores in the form of rings or similar structures have been demonstrable, in tangential sections, this cannot be definitely established.

Cuticle and sensory hairs.—The structure and function of the sensory hairs in the various types of sensory epithelium of the inner ear have been extensively discussed in a number of papers on the structure of that organ. Schultze (1858) observed numerous "auditory hairs" arising from the upper surface of the cristae. Hasse (1867) found that these hairs arose from a special type of cell in close proximity to the nerve fibers of the epithelium. O. van der Stricht (1908) observed, on the surface of each hair cell in the sensory epithelium of the labyrinth, a number of fine fibrils held together by an intermediate substance, and together forming the sensory hair proper. Separated from this bundle and situated more towards the periphery was a flagellum united with the centriole of the hair cell. In the opinion of Held (1909), all epithelial cells lining the labyrinthine ducts had a flagellum arising from a centriole in the cell. According to Held's description this flagellum stains darker than other sensory hairs. These observations were confirmed by Kolmer (1927).

In the present investigation the observations made by van der Stricht, Held and

others were verified in studies, by phase contrast microscope, of sections of material fixed in osmium and embedded in methacrylate. In these preparations a hair or cilium that seemed denser than the other hairlike processes of the cell was observed in each hair cell, usually being located in the periphery of the bundle.

In the electron microscopic studies were observed, similarly, two distinctly different types of processes arising from the hair cells: one resembling stereocilia, the other kinocilia. The former process had the same structure as the cilia arising from the hair cells in the organ of Corti, the structure of which was described by Engström, Sjöstrand & Wersäll (1952, 1953) and Engström & Wersäll (1953 a, b). The kinocilium-like process that was present in each bundle differed markedly in structure from the stereocilia, and was consistent with the kinocilia that have earlier been described in various types of epithelium.

Ecker (1844), in direct studies of cristae ampullares from *Petromyson marinus*, found a mobile cilium on each sensory cell in the crista, the movements of which were detectable in the microscope. He described the movement as a bending of the tip followed by a lashing in which the basal part of the cell took little part. Mobile cilia were also observed by Bowen (1931) in the crista ampullaris of *Amieurus nebulosus*.

In attempts at direct studies of non-fixed cells in the cristae ampullares, I was unable to observe any movements in the sensory hairs arising from the cells. In view of the structural similarity existing between the kinocilia observed in the hair cells and the mobile cilia in other types of epithelium, it seems likely, however, that the first-named kinocilia may be mobile during some stage of development of the crista. Whether it has any bearing on the resting discharge in nerves coming from the crista ampullaris, as assumed by Ewald (1844), is not clear, Granit (1955) nevertheless found it unlikely that any mobile cilia on the hair cells could influence the electrical activity in the cristae ampullares.

Hair Cells of Type II

The cylindric hair cells in the sensory epithelium of the cristae ampullares, which are innervated by a number of peripheral nerve end branches have been designated, as mentioned in the foregoing, hair cells of type II.

The *cytoplasm* in these cells has the same ultrastructural characteristics as those in type I hair cells.

The cell is bounded apically by a cuticle, from which about fifty stereocilia and one kinocilium emerge, and is enveloped by a continuous plasma membrane. (Figs. 9, 17.)

The *Golgi apparatus* in type II hair cells appears to be divided into a greater number of zones than in the type I cell, but has the same ultrastructural components as the latter; i.e., Golgi lamellae enclosing light slit-like spaces, small vesicular formations, and a dense ground substance. The various zones in the Golgi apparatus are often scattered in the supranuclear portion of the cell, though with the main part assembled in the region of the nucleus.

No systems of alpha-cytomembranes (Sjöstrand, 1956) of the kind found in the type I hair cells were observed in hair cells of type II. The infranuclear portions of these cells showed, however, short, irregularly disposed granulated membranes

of similar type. The mitochondria were fairly sparse, spherical or rod-like, with typical inner structural features.

The type II hair cells are innervated, as pointed out above, by a number of fine nerve branches which terminate either as a short loop or as a bud-shaped dilation at the end of the nerve fiber, in contact with the base of the hair cell. The plasma membrane at this point of contact usually shows a shallow depression or groove in which the nerve ending rests. In some cases this depression may be so deep that the greater part of the nerve ending is enclosed by the plasma membrane of the hair cell. (Figs. 18, 22, 23.)

The nerve ending is bounded, like the hair cell, by a plasma membrane in which a thin opaque layer can be distinguished. This is separated from the opaque layer in the plasma membrane of the hair cell by a lighter layer 100–200 Å thick. In some cases two opaque layers were observed in hair cells within the zone of contact of the nerve ending.

Supporting Cells

The supporting cells in the sensory epithelium of the cristae ampullares surround sensory cells and nerve fibers. They are irregularly prismatic and extend from the basement membrane to the epithelial surface. (Figs. 10, 17.) The *nucleus* is oval and about 9 μ in height. It lies in the broad basal part of the cell, usually with its long axis coinciding with that of the cell. It is bounded by a nuclear membrane in which two denser layers can be distinguished, separated by a lighter space. Some discontinuities were demonstrable in the nuclear membrane. The nuclear substance, as in the hair cells, is composed of irregular masses of apparently amorphous intermediate substance, containing a large number of small opaque granules. Two very dense nucleoli are usually observed in each nucleus. These nucleoli are far more compact in their structure than those observed in the sensory cells. Similarly, the nuclear substance is far more densely packed than in the hair cells. (Fig. 19.)

A continuous plasma membrane envelops the cell.

On the surface facing the lumen of the ampulla there are a small number of microvilli about 0.2 μ long. The plasma membrane is otherwise smooth and without any major irregularities. Basally, the cell membrane is separated from the basement membrane by a space about 300 Å in width.

The *cytoplasm*, as in the sensory cells, is composed of clumps of granules and an amorphous intermediate substance. The upper part of the cell is filled by densely packed granules having diameters of 0.2–0.3 μ. These granules consist of very finely granulated substance enclosed by a single membrane. Occasional short mitochondria are observed between granules in the upper part of the cell.

In the uppermost portion of the supporting cell is a ring of dense substance constituting a part of the supporting apparatus of the epithelium, the *reticular membrane*. This ring has a triangular transverse section with a height of 0.5–1 μ. Like the cuticle of the supporting cells, it is composed of a dense, apparently

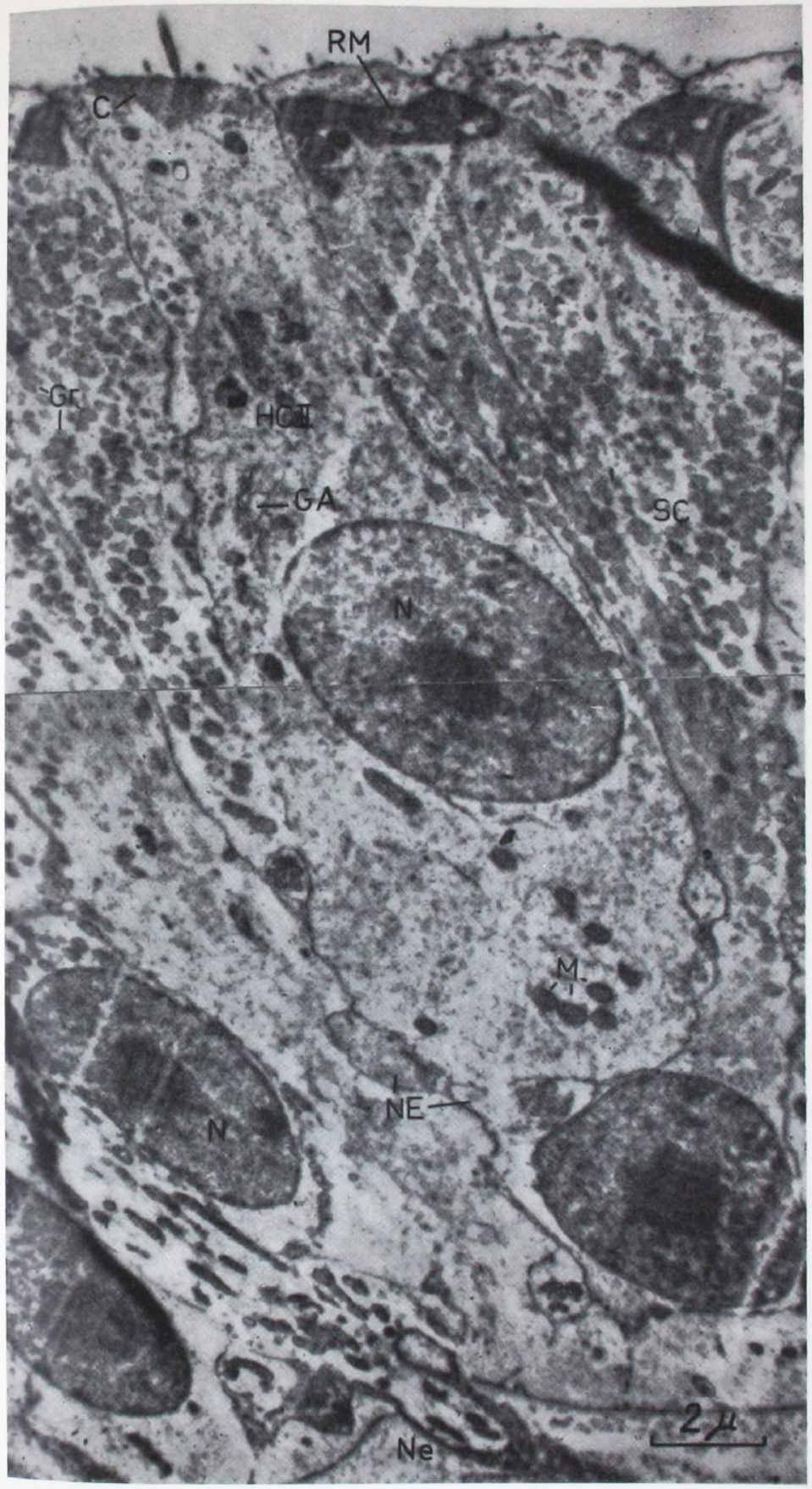

Fig. 17. General view of type II hair cell (HC II). GA, Golgiapparatus; N, nucleus; M, mitochodria; SC, supporting cell; RM, reticular membrane; C, cuticle; Gr, supporting cell granules; NE, nerve ending; Ne, nerve passing through the basement membrane. Electronmicrograph, 8500 ×.

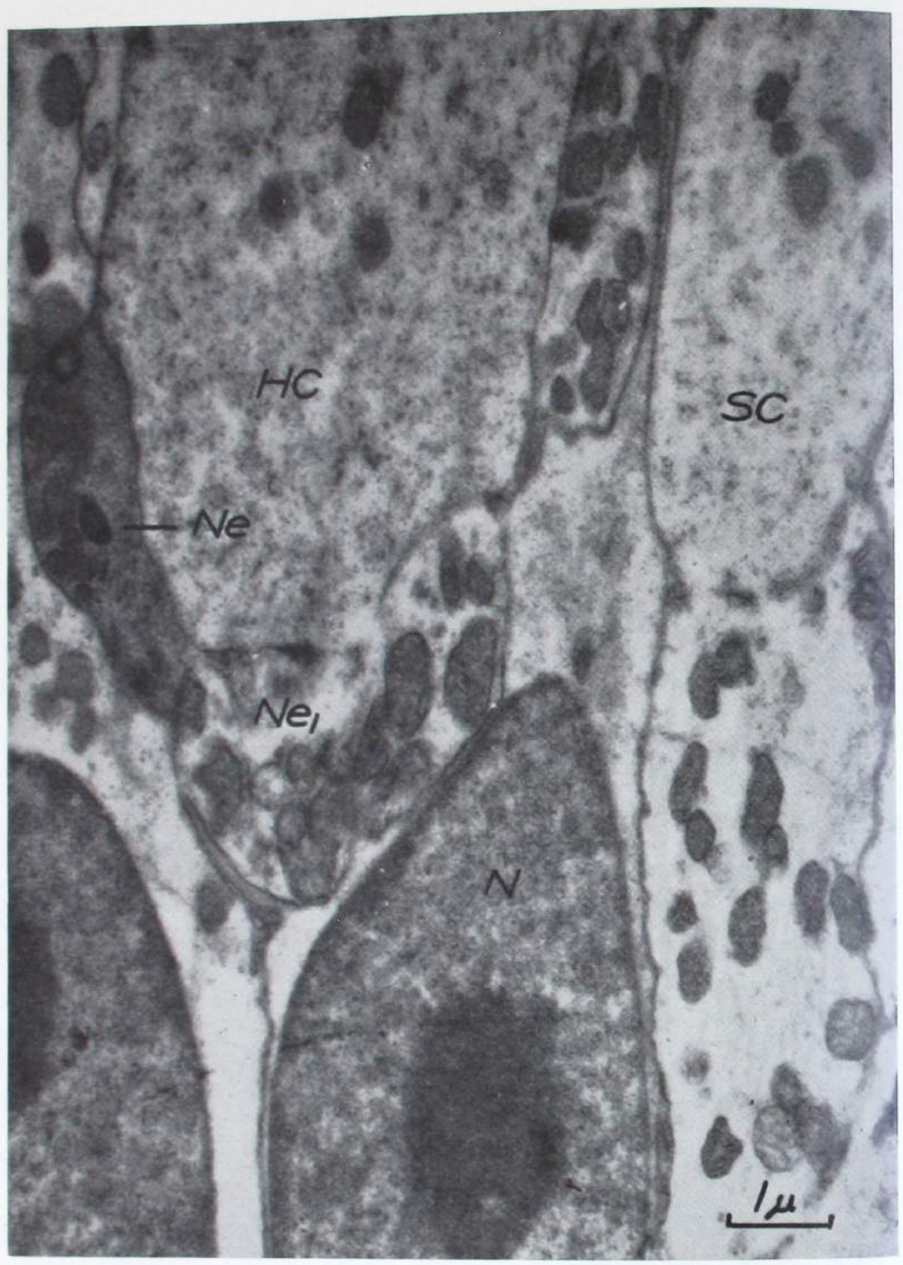

Fig. 18. Basal part of a type II hair cell (HC) with nerve endings (Ne) and (Ne1) and surrounding supporting cells (SC). N, nucleus. One of the nerve endings (Ne) contains a large number of small granules and a few strikingly slender mitochondria. Electronmicrograph, 13,000 ×

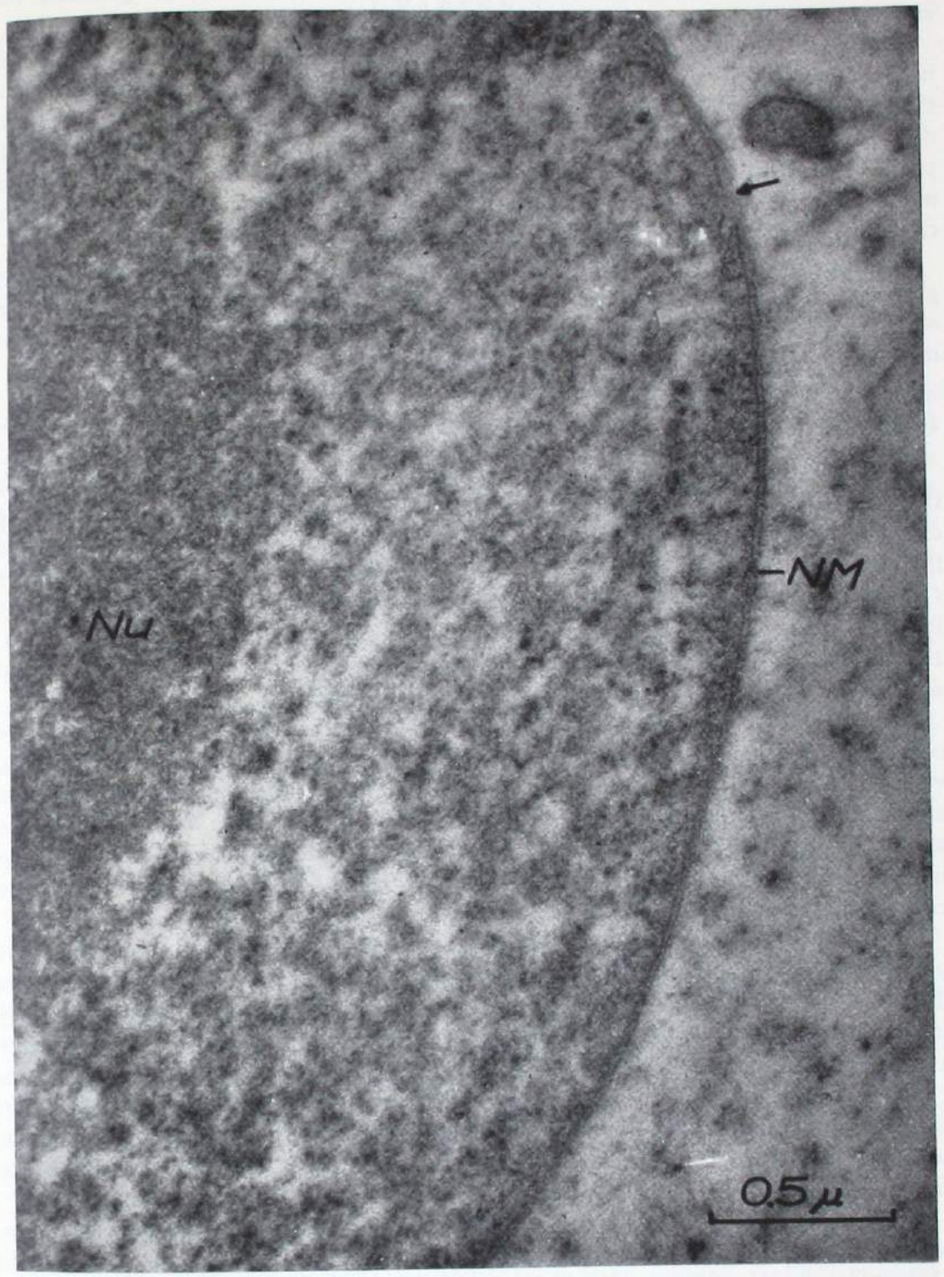

Fig. 19. Part of a supporting cell nucleus. Nu, nucleolus; NM, nuclear membrane. The arrow indicates a discontinuity of the nuclear membrane. Electronmicrograph, 47,000 ×.

amorphous intermediate substance within which can be seen darker granules. No enclosing membranes were observed.

The *Golgi apparatus* is localized to the middle of the cell. Usually it is assembled in a circumscribed zone, within which are membranes enclosing narrow spaces in the cytoplasm of the same type as those in the hair cells. However, it contains no demonstrable vacuoles of the magnitude observed in the hair cells. In the peripheral parts of Golgi's zone are numerous vesicular formations with a peripheral outer membrane and a lighter central part. These form, in some cells, somewhat dense clumps, often localized to the apicalmost part of the Golgi apparatus.

In the infranuclear portion of the cell are frequently observed one or more granules with diameters of up to 0.5 μ; they are very dense and apparently devoid of both inner structure and outer membrane. — No other types of intracellular membranes except those in Golgi's apparatus were observed in these cells.

Discussion

Earlier workers have attributed a supporting function alone to the prismatic cells that lie between sensory cells and nerve branches in the crista epithelium and extend from the basement membrane to the epithelial surface. They have compared the supporting cells in the organ of Corti with those of the cristae ampullares (Kolmer, Retzius, and others). Most workers examining the crista epithelium by light microscope have described fibrils in the supporting cells. Kolmer (1911, 1927) pointed out that these supporting fibrils stained with chromic acid and were mainly localized to the apicalmost part of the cells.

Wersäll (1954) described densely packed granules in the apical part of these cells, but was unable to observe any supporting fibrils. These observations were verified in the present investigation. It seems likely that the apical ring of dense substance in the supporting cells has a supporting function. The dense intracellular agglomeration of granules suggests, however, that the supporting cells may also have a secretory function—a function which earlier was chiefly attributed to the cells in the planum semilunatum.

The intraepithelial nerve branches in the sensory epithelium of the cristae ampullares consist of axons or branches devoid of neurilemma or myelin sheaths. These nerve fibers in the epithelium are surrounded by supporting cells, except in the region of contact between hair cell and nerve ending; here the nerve endings are in direct contact with hair cells and, in some cases, with adjacent nerve endings.

Thus the supporting cells have apparently assumed, in the epithelium, the role played by Schwann's cells in relation to the axon; hence they may possibly have some significance as supporting, isolating and nutrifying elements for the nerve fibers.

The relationship between supporting cells, nerve fibers and sensory cells in the sensory epithelium of the crista ampullaris has a counterpart in the organ of Corti (Engström & Wersäll, 1953), the retina (Sjöstrand, 1953), and the taste buds (Engström, 1956, Engström & Rytzner, 1956.)

CHAPTER V

FINE STRUCTURE OF NERVES AND NERVE ENDINGS

Introduction

Myelinated nerve fibers innervating the epithelium of the cristae ampullares were already described in the papers of Schultze (1858) and Odenius (1867). Schultze observed that the nerve fibers lost their myelin sheath and neurilemma on passing through the basement membrane, whereas the axon ramified in the epithelium. Kaiser and Niemack described granules in axons and nerve endings that were found to be composed of an otherwise homogeneous substance. These observations were criticized by Retzius, Kolmer and others, who, as mentioned above, pointed out that both axons and nerve endings had a fibrillar structure.

In recent years very extensive investigations have been conducted, with the use of polarization microscopy, X-ray diffraction technique and electron microscopy, into the structure of the nerve fibers in various nerves (Schmidt, 1936, 1937; Schmitt *et al.*, 1935, 1939, 1941; Sjöstrand, 1953 d). Here the structure of the myelin sheath and the axon has been meticulously studied, though there are still relatively few investigations dealing with the structure of the peripheral nerve end branches (Fernandez–Moran, 1952; Sjöstrand, 1953 f; Engström & Wersäll, 1953 a, b; Engström & Sjöstrand, 1954; De Robertis, 1955). A review of the literature in this field was given by Fernandez-Moran (1954).

A preliminary report on the structure of nerve endings in the sensory epithelium of the crista ampullaris was presented by Wersäll (1954). In the present investigation the fine structure was studied of the nerve fibers and nerve endings in the crista ampullaris.

Concurrently with these investigations the nerve fibers in the macula utriculi were studied, and were found to have an identical structure. Since the observed layer of nerve fibers in the macula utriculi permitted of excellent fixation, fibers therefrom were used for quantitative determination of the various layers in the myelin sheath.

The individual cristae in the vestibular apparatus are innervated by separate nerve-fiber bundles in which myelinated fibers with diameters of 1–9 μ preponderate. Between these fibers can be observed, however, a large number of unmyelinated ones with diameters between 0.3 and 1 μ. (Fig. 20.) While the myelinated nerve fibers could be visualized from the connective tissue into the sensory epithelium of the crista, it was not clear whether the unmyelinated ones innervated merely the numerous subepithelial capillaries or took part in the innervation of the sensory epithelium too.

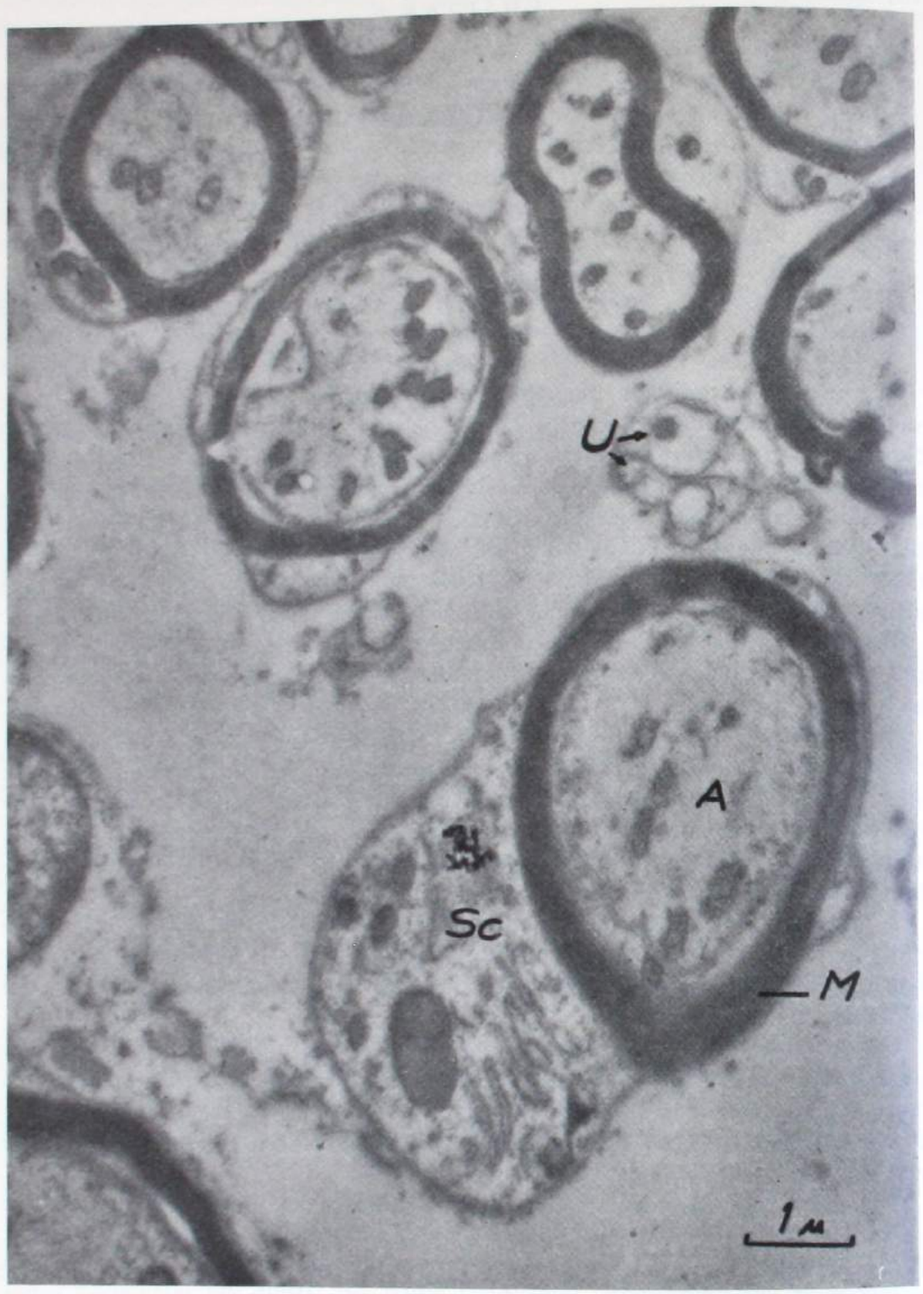

Fig. 20. General view of a transverse section through a part of the vestibular nerve. A, axon in myelinated nerve; Sc, Schwann cell; U, unmyelinated nerve fibers. A large number of mitochondria are present in each axon. Electronmicrograph, 14,000 ×.

Myelinated Nerve Fibers

The myelinated nerve fibers pierce the connective tissue stroma of the crista up to the basement membrane, penetrate the latter to the epithelium, and there form their branches. Each nerve fiber consists of axon, myelin sheath and neurilemma. Immediately beneath the epithelium the myelin sheath tapers conically and disappears; the neurilemma ceases, and the axon penetrates into the epithelium. Here the axon divides into a number of branches, the course of which in the epithelium varies. The thick nerve fibers, as mentioned above, form a small number of branches, each of which terminates in a nerve calyx round a hair cell of type I. The fibers of medium caliber divide into several branches, some of which form nerve calyces, others nerve endings at hair cells of type II, while the fine fibers take part in the innervation of the type II cells. Each nerve ending constitutes a direct continuation of a branch of the axon that is in contact, though not united, with a sensory cell.

The Axon

The axon is composed of axoplasm bounded by a continuous membrane within which an opaque layer about 60 Å thick can be distinguished. The axoplasm in turn consists of an apparently amorphous ground substance containing a large number of fibrils having diameters of about 100 Å, scattered elongated mitochondria, and small granules with diameters of 200–400 Å.

The fibrils in the axoplasm are chiefly assembled in the long axis of the fiber. They have a definite length and appear to run quite independently of one another. (Fig. 21.) Due to the minute thickness of the sections it was not possible to determine the extension of the individual fibrils in the axon.

The mitochondria are bounded by an outer membrane within which can be distinguished two opaque layers separated by a lighter intermediate layer. Each mitochondrium contains a large number of similarly triple-layered inner membranes embedded in an intermediate substance. These inner membranes are disposed somewhat irregularly in the mitochondrium. Thus inner membranes are found running both transversely across the mitochondria, and more or less obliquely or longitudinally. Branched membranes appear to be commoner in the axonal mitochondria than in corresponding organelles in the hair cells.

The granules found in the axons are somewhat sparse within the axon itself, but increase in number towards the periphery. The number of mitochondria, too, is greater in the end branches than within the axon itself.

Nerve Calyces

Axons from nerve fibers of large and medium caliber form nerve calyces, each of which encloses a hair cell of type I. These calyces envelop like a shell the greater part of the hair cell, only a small apical portion of it being left free.

The nerve calyx is bounded throughout its extent by a plasma membrane representing a direct continuation of the axon membrane. In the region of contact between nerve ending and hair cell there is a light space about 150–200 Å thick that separates the opaque layers of the hair cell's plasma membrane from that of

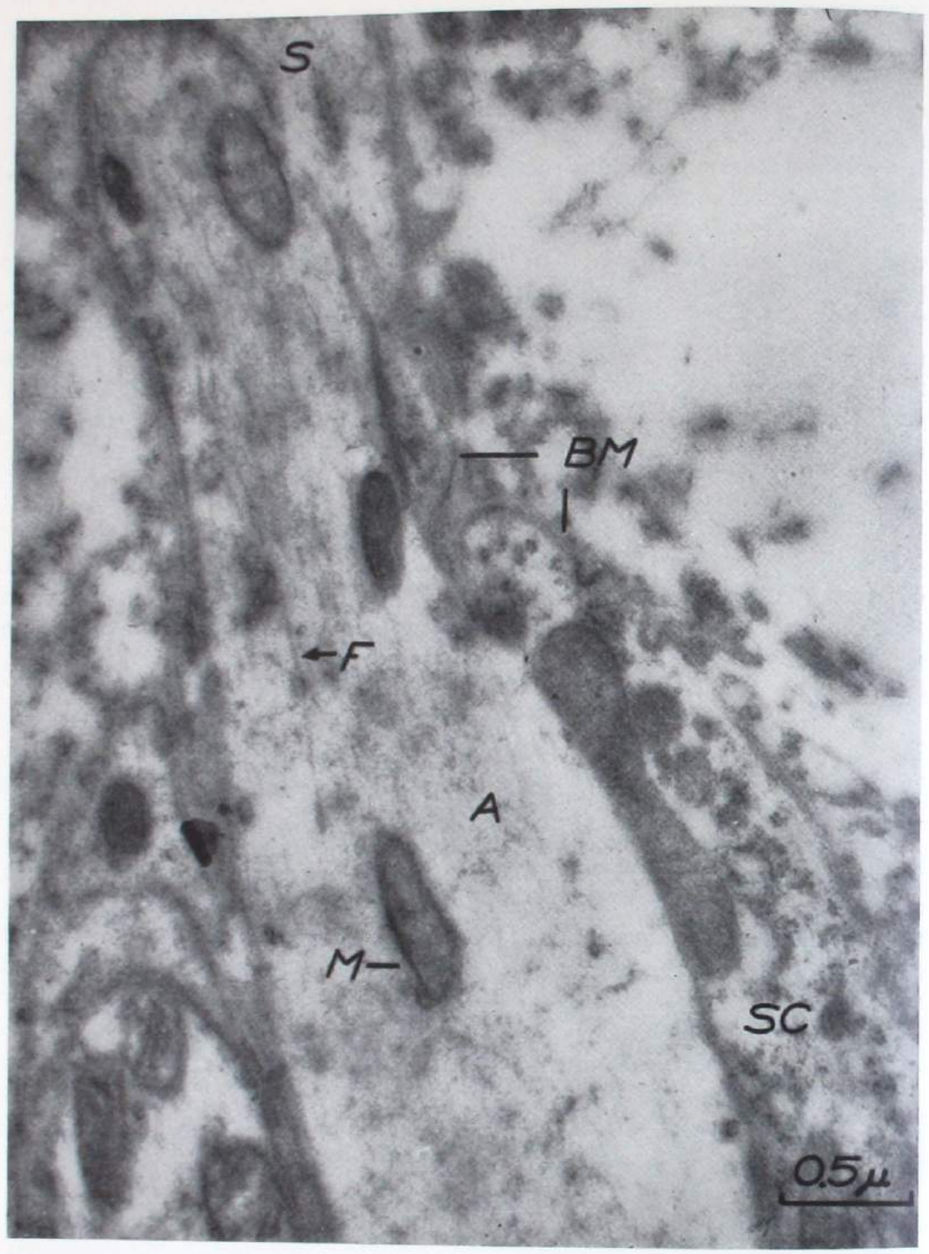

Fig. 21. Axon of myelinated nerve imediately after loss of the myelin sheath (A). In the lower part of the picture the axon is enclosed by a Schwann cell (SC); in the upper part the latter forms a boundary with a supporting cell in the sensory epithelium (S). The basement membrane (BM) runs from the supporting cell over to the Schwann cell. M, mitochondrium; F, neurofibril. Electronmicrograph, 35,000 ×.

the axon membrane. The nerve calyx has, in principle, the same structure as the axon, with an apparently amorphous ground substance within which are fibrils, elongated mitochondria, and granules. (Figs. 10, 11.)

Other Nerve Endings

Hair cells of type II are innervated by nerve end branches and bud-shaped nerve endings, lying against the basal parts of the hair cell. These nerve endings, like the nerve calyces, are surrounded by a plasma membrane with an opaque layer separated from that of the cell membrane by a lighter space 100–200 Å thick.

In silver-stained preparations these nerve endings in the light microscope have the appearance of thin loops round the hair cells, or small bud-shaped swellings on slender end branches. In preparations impregnated with osmium and embedded in plastic they are difficult to distinguish but may be observed in the form of small clumps and accumulations of granules at the base of type II hair cells.

The fine structure of these nerve endings varies, so that broadly speaking two different types may be distinguished. Some nerve endings thus show an assembly of mitochondria embedded in sparse axoplasm containing a few granules of the type observed in axons. Other nerve endings contain densely packed granules of the same type with diameters of 200–400 Å, embedded in a sparse intermediate substance, and occasional mitochondria or none at all. (Figs. 18, 22, 23.)

At this resolving power the granules found in axons and nerve endings have no detectable inner structure. It is nevertheless possible in each granule to distinguish a lighter inner zone enveloped by a thin opaque layer.

Myelin Sheath

In thin sections through myelinated nerve fibers in the different branches of the vestibular nerve are observed, within the myelin sheath, concentrically disposed opaque layers characterized by very high contrast on the electronmicrograph. These opaque layers alternate with less dense layers which, show an exceedingly thin, discontinuous line with fairly low contrast. These observations are fully consistent with Sjöstrand's description of the myelin sheath in the sciatic nerve of mouse (1953 d). Each opaque layer has a calculated thickness of 30 Å. The distance between the outer two opaque layer is 120 Å. (Table V, Fig. 26.)

Table V. *Distance between the opaque layers in the myelin sheath of nerve fibres from one vestibular nerve. Each figure represents the mean value of between fifty and sixty measured spacings. Total number of measured spacings: 547.*

Fibre No.	Distance between the centers of two opaque layers in Å	Fibre No.	Distance between the centers of two opaque layers in Å
1	116	6	126
2	110	7	128
3	123	8	119
4	124	9	117
5	111	10	107
		Mean	118

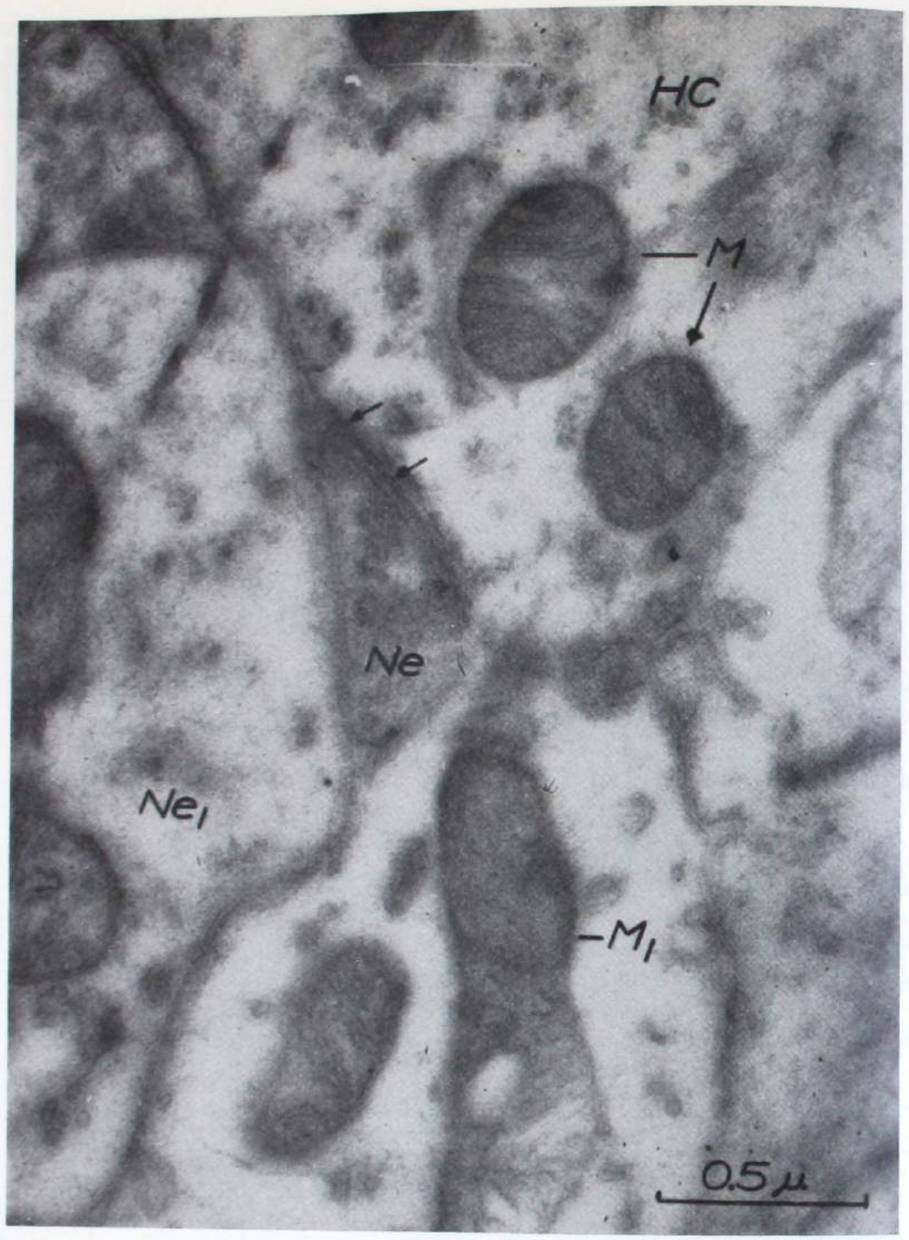

Fig. 22. Nerve endings surrounding the basal portion of a type II hair cell. "Ne" indicates a nerve ending having a large number of granules. In part of the region of contact between nerve ending and hair cell, the cell membrane shows a double layer indicated by arrows. M, mitochondria with a distinct membrane structure in the hair cell; M_1, mitochondrium in a nerve ending. Electronmicrograph, 55,000 ×.

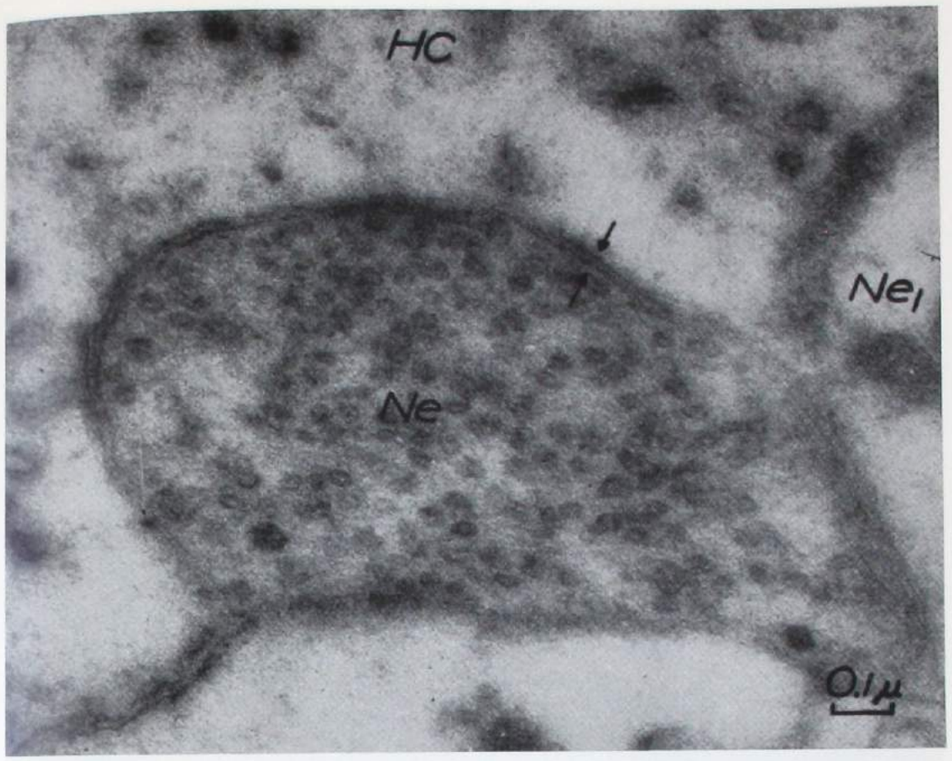

Fig. 23. Nerve ending below a type II hair cell. The nerve ending contains densely packed granules having diameters of 200–400 Å. The arrows indicate plasma membranes separating nerve ending and hair cell. Electronmicrograph, 74,000 ×.

Schwann's Cells and Their Relation to the Myelin Sheath

The cells of Schwann are disposed in a single continuous layer round the myelin sheath. Each cell has a thicker central zone containing the nucleus, the Golgi apparatus and a number of elongated or spherical mitochondria, and is thinner towards the periphery, forming a thin neurilemmal layer round the myelin sheath.

The plasma membrane of Schwann's cell communicates, via a connecting membrane, with the outermost layer of the myelin sheath. This connecting membrane is composed of two opaque layers separated by a less dense interspace, and communicates with the outermost layers of the myelin sheath. This finding lends weight to the view expressed by Geren (1954) and Robertson (1955) that the myelin sheath is composed of a closely packed, helically wounded double membrane derived from the Schwann cell plasma membrane. Similarly to these observations, it was found in the present investigation that the two opaque layers of the myelin sheath nearest the axon form an inner connecting membrane that deviates from the myelin sheath towards the axon. The two opaque layers in this inner connecting membrane branch, each layer running in its own direction round the axon membrane, so that the axon plasma membrane is surrounded by a single outer layer derived from the Schwann cell plasma membrane. The Schwann cell is

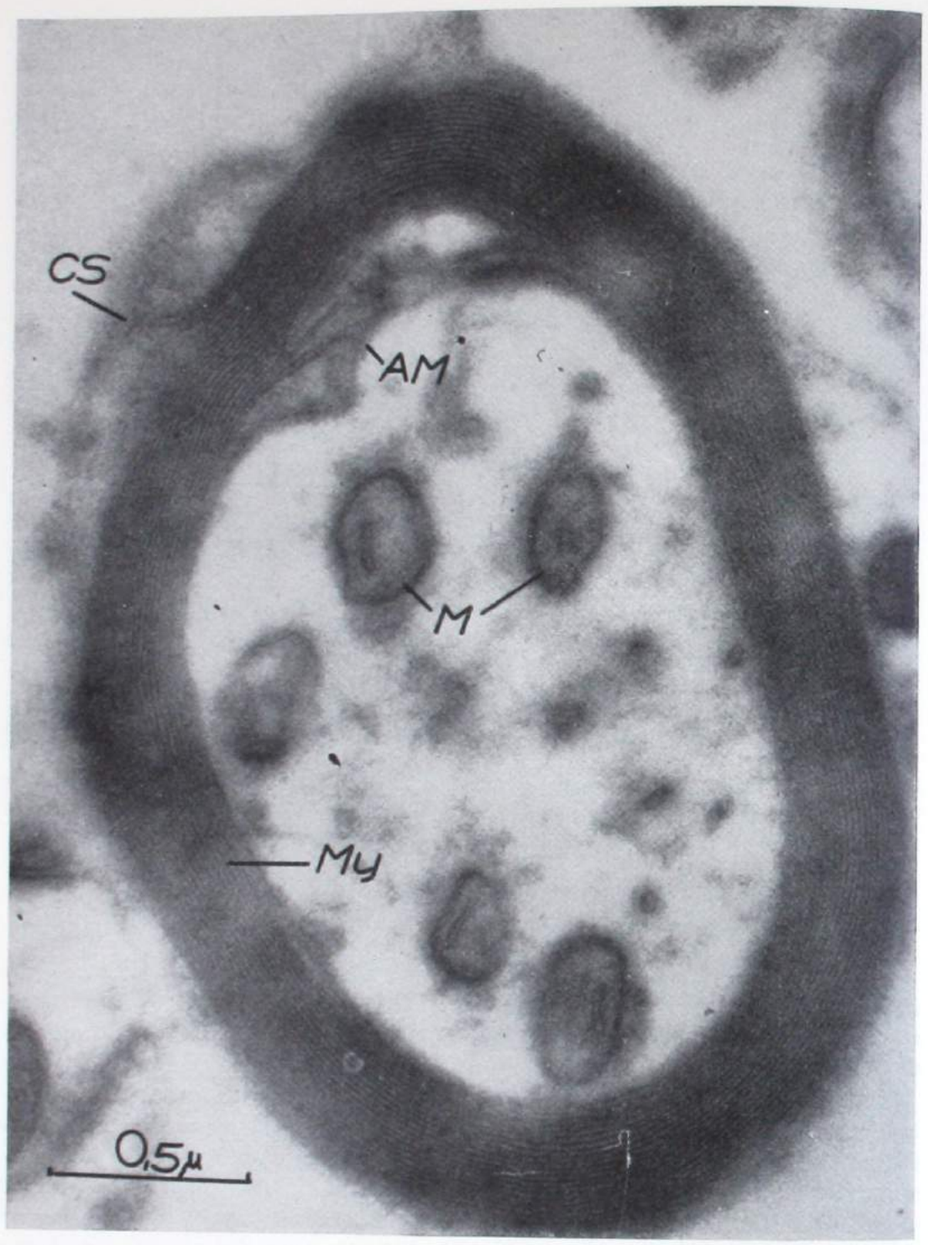

Fig. 24. Transverse section of myelinated nerve fiber from the vestibular nerve. The axon contains a large number of mitochondria (M). The myelin sheath communicates, via a double outer membrane, with the plasma membrane of the Schwann cell (CS) and, via a double inner membrane, with a single layer surrounding the axon membrane (AM). MY, myelin sheath. Electronmicrograph, 50,000 ×.

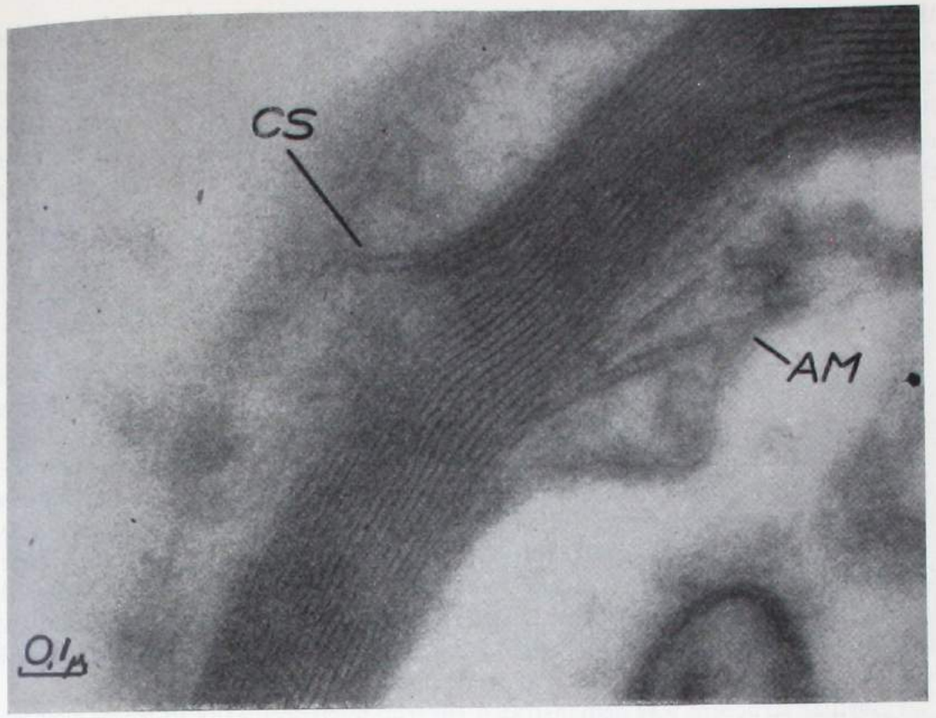

Fig. 25. Part of the same nerve as that in Fig. 24. Electronmicrograph, 110,000 ×.

covered by a basement membrane having the same appearance as that separating the epithelium from the connective tissue. (Figs. 20, 24, 25.)

Immediately before the axon of the myelinated nerve fiber passes from the connective tissue into the epithelium, the myelin sheath tapers off and ceases. The neurilemma forms a thin layer round the axon, and ceases only when the latter penetrates between the supporting cells in the epithelium. In this way the axon is always surrounded by a cell layer, formed, in the connective tissue, by the Schwann cells and, in the epithelium, by the supporting cells. (Fig. 21.)

Unmyelinated Nerve Fibers

The unmyelinated fibers in the peripheral parts of various branches of the vestibular nerve closely resemble the *C* fibers described by Gasser (1952) and the nerves of the chick embryo in an early stage of development, described by Geren (1954).

The axon is composed of a simple tube with an axon plasma membrane surrounding an apparently amorphous axoplasm ground substance in which are found an abundance of thin neurofibrils of the same type as those described in the axon of the myelinated nerve. In the axoplasm are also embedded a few typical mitochondria and granules with diameters of 200–400 Å. (Fig. 27.)

The bulk of the unmyelinated nerve fibers are completely enclosed by a Schwann cell. Usually two or more fibers are in association with each Schwann cell. Here

the axon membrane is surrounded by a thin membrane communicating by a double membrane with the Schwann cell plasma membrane. Some of the fibrils, however, are not completely surrounded by the Schwann cell; they merely run in a groove in the cell wall formed by impression of the Schwann cell plasma membrane. These nerve fibers are thus bounded on one side by the Schwann cell, but on the other they are separated from the connective tissue only by a basement membrane representing a continuation of that enclosing the Schwann cell. (Fig. 20.)

Discussion

The axon.—Very great interest has been shown in the fine structure of the axon, due to its ability to conduct electrical impulses. The studies thereof have chiefly been concerned with the neurofibrils, their structure and occurrence.

Under the light microscope, intra-axonal fibrillar structures can be observed in silver-stained nerve fibers (Cajal, 1909, and others). However, the appearance of these structures greatly varies in different preparations, and hence the silver-staining methods cannot yield a complete picture of the axon's structure. Bear, Schmitt and Young (1937) showed, in polarization microscopic studies of fresh squid giant fibers, a slightly positive uniaxial birefringence in the axoplasm, and therefore assumed that the latter contained a relatively small fraction of well oriented particles having widths that were small with respect to the light.

A fibrillar structure was demonstrated electron microscopically in teased preparations of squid giant fiber axoplasm by Richard *et al.* (1943).

Schmitt and Geren (1950) observed, in thin sections from nerve fibers fixed in osmium, fibrils having diameters of 100–300 Å. Similar fibrils were also found by Fernandez-Moran (1950) in frozen sections of unfixed nerves.

Hess & Lansing (1953) observed slender fibrils of the order of 70 Å in sectioned nerves fixed in osmium tetroxide. However, they considered these fibrils to be "artefacts caused by longitudinal tension forces in the elongated axis cylinder exaggerated by the treatments involved in fixation and embedding".

Earlier investigations, both by polarization microscope and electron microscope, point to the occurrence of fibrils with diameters of 100–200 Å in the axon. In the present investigation such fibrils were observed both in the axons themselves and in the intraepithelial nerve branches. These fibrils were of a definite length, had a diameter of around 100 Å, and ran more or less independently of each other, longitudinally in the axon.—Since no fibrils of similar type have been observed in the Schwann cells or the cells of the sensory epithelium, it seems improbable that they are due to tension forces, as suggested by Hess & Lansing, or are fixation artefacts. More likely is that fibrils with diameters of around 100 Å constitute an important component of the axon and nerve ending, even in fresh condition.

Structures containing the characteristic internal organization of mitochondria were first described in the axoplasm by Hess & Lansing (1953).

The mitochondria that were observed in the fibers of the vestibular nerve showed a very irregular membrane system and were remarkably elongated, but in other respects did not differ from the mitochondria that have been described in the hair cells.

Nerve endings.—Two different types of nerve endings, as described above, are

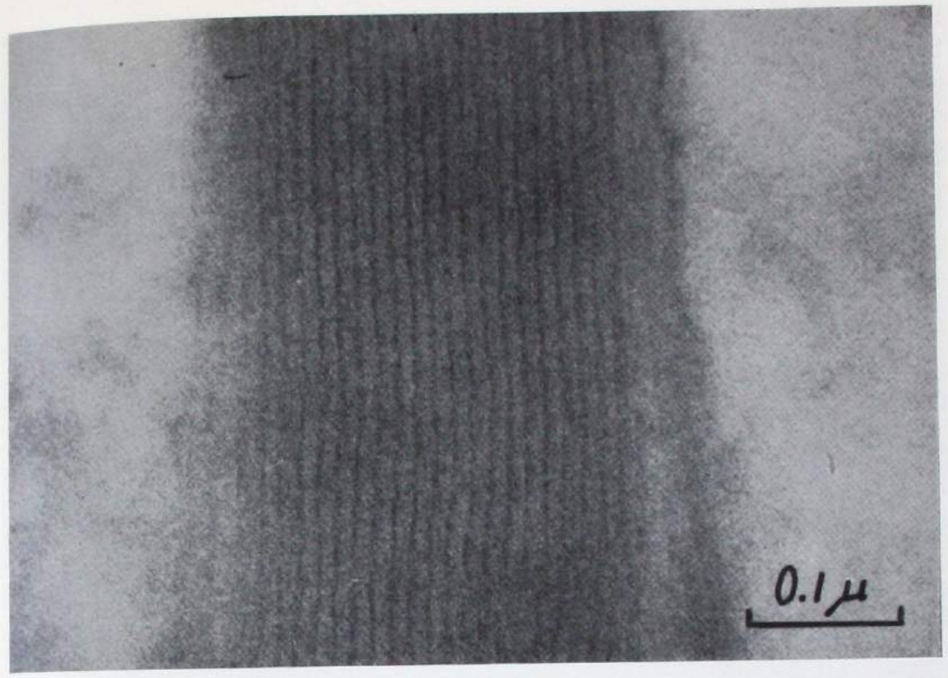

Fig. 26. Part of transverse section through myelin sheats, showing the layered structure of the myelin. Between two continuous opaque layers can be distinguished a discontinuous line dividing the lighter space between two opaque layers into two almost equal parts. Electron-micrograph, 200,000 ×.

found in the sensory epithelium; namely, nerve calyces and slender nerve end branches. The nerve calyces envelop sensory cells of type I, while the nerve end branches form loops round the basal part of type II hair cells or terminate in the form of bud-shaped nerve endings.

The inner structure of the nerve ending differs from that of the axon notably with regard to the disposition of mitochondria and granular formations. In the nerve calyces and most endings round hair cells of type II there is an accumulation of both mitochondria and granules having diameters of 200–400 Å. The granular mass is especially conspicuous in some nerve endings round type II hair cells, where only occasional mitochondria or none at all can be observed. Whether this variation in the structure of the nerve endings has any functional significance or not is obscure.

The nerve endings round type II hair cells show major structural similarities with those in the organ of Corti. Engström & Wersäll (1953 a, b) thus showed that the latter contained a large number of mitochondria embedded in fine, granulated cytoplasm. Further investigations into the fine structure of these endings demonstrated that the cytoplasm therein contained densely packed granules or rods with diameters around 400 Å (Engström & Sjöstrand, 1954).

The granules observed in the nerve endings in the crista ampullaris and organ of Corti are consistent in their appearance with the rods or granules described by Sjöstrand (1953 f) in the retinal synapses of the guinea-pig eye and the synaptic

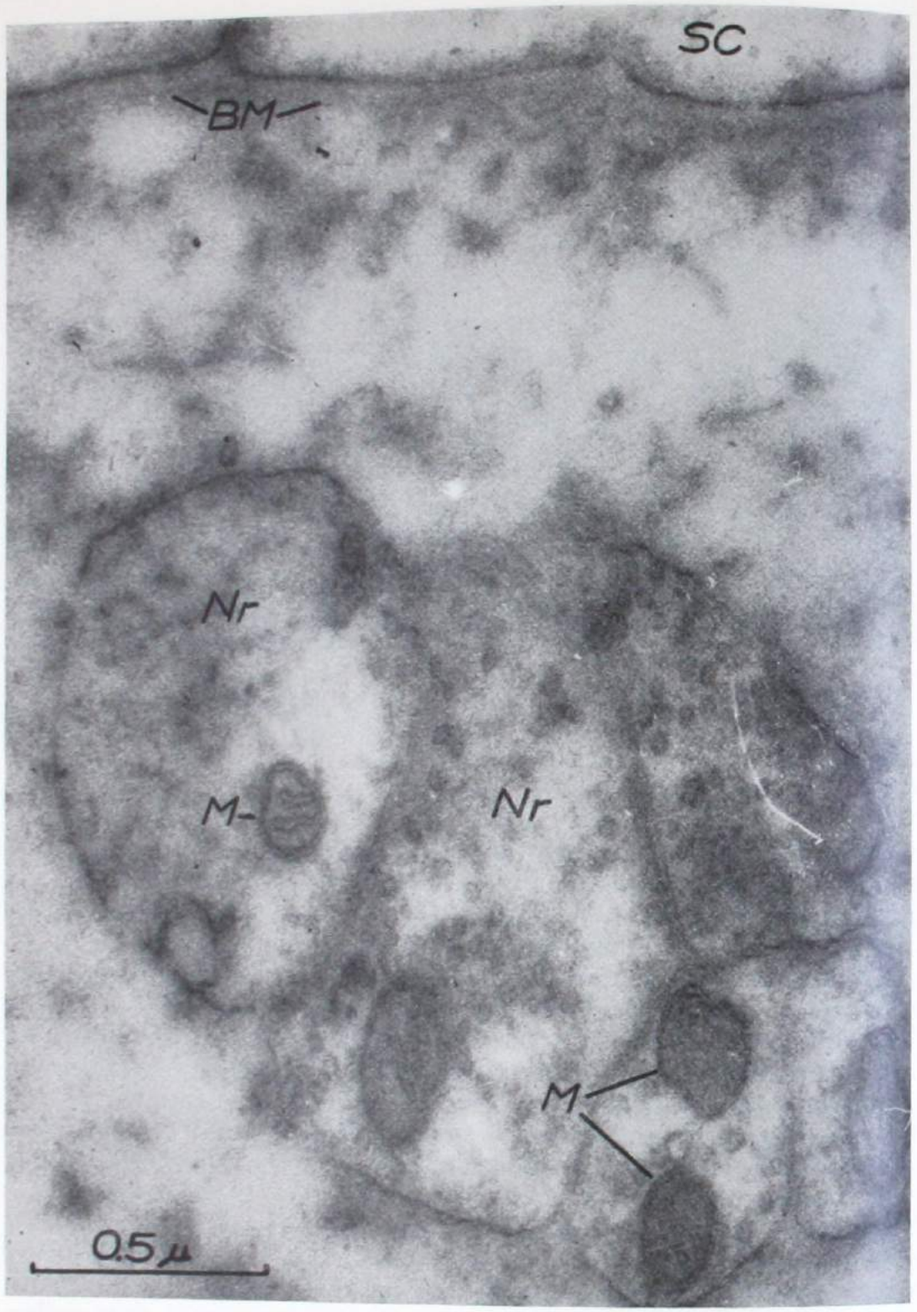

Fig. 27. Slender unmyelinated nerve fibers (Nr) beneath the basement membrane (BM). Each nerve fiber contains a large number of granules 200–400 Å in diameter and mitochondria (M) having typical membrane structure. The basement membrane has a somewhat diffuse boundary with the connective tissue. SC, supporting cell. Electronmicrograph, 65,000 ×.

vesicles observed on the presynaptic side of synapses by Palade (1954). De Robertis & Bennet (1954, 1955) and De Robertis (1955).

Since granules or vesicles having diameters of 200–400 Å have been observed in nerve terminals of synapses of varying localization, and in nerve endings in Corti's organ and the sensory epithelium of the vestibular apparatus, they may well be characteristic components of the synapse cytoplasm, of fundamental importance to the conduction of impulses in different types of nerve endings.

Myelin sheath.—Our present knowledge of the structure of the myelin sheath in myelinated nerves is based on a large number of investigations with the use of varying techniques. In a series of polarization microscopic studies reported by Göthlin (1913), Schmidt (1936), and Schmitt *et al.* (1937), a marked birefringence was observed in the myelin sheath which was positive referred to the radial direction. Schmidt correlated the data from those studies with results obtained from chemical analysis and polarization optic studies of extruded myelin and deduced that the myelin sheath is essentially built up of concentrically arranged protein lamellae alternating with lipid layers.

This structure was confirmed by X-ray diffraction studies of the myelin sheath made by Schmitt and coworkers (1935, 1941). Characteristic long spacing equatorial diffractions were observed which appeared to be the first four orders of a fundamental spacing of 184 Å in fresh mammalian nerve and 159 Å in dry nerve.

On the basis of these and earlier investigations, Schmitt, Bear and Palmer (1941) concluded that the myelin sheath in dry nerve was composed mainly of lipid-protein layers about 160 Å thick, wrapped concentrically about the axon. These were considered to contain layers of mixed lipids about 134 Å thick, and 25 Å of protein. Finian (1954) studied myelin sheaths fixed in osmium tetroxide and found a fundamental repeating unit of 162 Å.

The above findings were confirmed by Sjöstrand (1949), who found lamellar structures in fragmented nerves presumably derived from the myelin sheath. Fernandez-Moran (1950, 1952) later demonstrated a lamellar structure in thin frozen sections of nerves from cat and frog. Conclusive evidence of the lamellar structure of the myelin sheath was obtained when Sjöstrand (1953 c) demonstrated, in ultra-thin sections from mouse sciatic nerve, a system of concentric lines completely encircling the myelin sheath, having a thickness of around 25 Å and fairly constant spacing of 120 Å between the lines.

The lamellar structure of the myelin sheath was clearly demonstrated in the present investigation too, in myelinated nerve fibers of the vestibular nerve. The thicknesses of the various layers that were observed here closely coincided with those reported by Sjöstrand. The pictures obtained of the relationship between neurilemma and myelin sheath fully support the observations made by Geren (1954) and Robertson (1955), which showed that the myelin sheath consists of a double membrane encircling the axon like a spiral and arising from the plasma membrane of the Schwann cell.

CHAPTER VI

STRUCTURE OF THE CUPULA

Introduction

The structure and function of the cupula have been extensively discussed ever since Lang, in 1863, reported his investigations into "*das Gehörorgan der Cyprinoiden*". He observed, between the apical surface of the crista and the ampullar wall, an extremely delicate, finely streaked cupula (the "cupula terminalis"), composed of fine retracting fibrils. Unlike Schultze (1858), he did not find any trace of sensory hairs emerging from the upper surface of the crista, and therefore assumed that the sensory hairs described by Schultze were residue of the shrunken cupula. The relationship between cupula and sensory hairs was studied by Haase, who, like Lang, observed a cupular structure between the crista surface and the ampullar wall in cyprinoids. In his first paper (1870) he assumed that this formation was peculiar to cyprinoids, but later (1871 a, b) described a similar structure in birds and various fishes too. He described this cupula as being composed of homogeneous substance containing a large number of canals. In these canals sensory hairs extended from the crista surface into the cupula.

Hensen (1878) considered that the cupula did not exist in fresh condition but that its fibers were made up of the greatly swollen auditory hairs. In his opinion the hairs normally projecting into the lumen became enlarged under the influence of the reagents used as fixatives, and were held together through coagulation of the intermediate substance. Bowen (1931) held a similar view. He was unable to demonstrate any cupula in fresh ampullae from catfish, but claimed to have studied the development of a cupula under the influence of various fixatives upon some fluid or semifluid substance within the ampullae. Van der Stricht (1921) considered that the cupula, like the tectorial membrane, was composed of a system of weakly staining prisms separated by clear interplasmatic spaces in which sensory hairs were localized. Kolmer (1911, 1927), in studies of preparations from various animals and man, reported findings well supporting his opinion that the cupula was a gelatinous substance which covered the crista like a cap, separated from the epithelial cells by a fine subcupular space. He found canals passing from the subcupular space into the cupula over each hair cell. The lower part of the canals contained the upper ends of the hairs protruding from the sensory cells. Kolmer also showed that the cupula was easily dislodged from the hairs and that its structure and form were retained after such removal. Donadei (1925) demonstrated the existence of the cupula in fresh sections from frozen specimens (unfixed preparations) from frog.

Wittmaack (1935) described the cupula as a relatively voluminous formation of constant shape, made up of a colloidal substance. In his opinion, it arose from an ectoplasmatic layer in the sensory hairs and consisted of a network of fine fibers, showing a conspicuously regular structure, the interspaces of which were filled

with fluid. Vilstrup (1950) found the cupula, in shark and cod, to be composed of two parts: one immediately above the crista surface and having a cytoplasmatic character; the other an outer fibrillar portion. In the inner part he found cell-like structures and fibrillar elements, by which, he assumed, these cells were attached to the crista.

Personal Observations

In osmium-fixed, well preserved preparations from guinea-pig the cupula is observed as a long hood covering the sensory epithelium of the crista ampullaris and extending into the endolymph between the upper surface of the crista and the ampullar summit. In fixed preparations it usually occupies two-thirds or more of the distance between the crista and the ampullar summit. In direct studies of the fixed ampulla the cupula is generally visualized as a formation of definite shape. Although it appears to be only slightly osmiophilic it can be distinctly seen, in most fixed preparations, through the ampullar wall. If a slender pipet be inserted into that part of the semicircular duct that commonly accompanies the preparation, the cupula will be seen to be displaced by the action of pressure or suction therefrom (*cf.* Steinhausen, 1933 and Dohlman, 1941). Indeed it may easily be dislodged altogether, but will retain its shape completely. In ampullae that have been dissected out and embedded in plastic, the cupula is often either absent or dislodged from the crista ampullaris and lying alongside the latter. (Fig. 4.) Worthy of note is that in these specimens the cupula has the same form as that in its normal position.

In transverse sections through the crista and cupula the under surface of the latter is concave and coincides with the upper surface of the crista. The cupula covers the whole of the specific sensory epithelium. Between it and the epithelium there is usually found, however, a subcupular space with a depth of about 2–5 μ. The entire cupula is permeated by canals extending from its lower to its upper surface. In each canal there are sensory hairs from a sensory cell that enter and partially fill the basal part. This latter part has a diameter approximating that of the hair cells—i.e. 3–5 μ—but apically the canals taper to a width of 1 μ or less. The peripheral canals in the cupula converge somewhat towards the center, so that all canals open on the upper surface. The sensory hairs of the hair cells in the summit of the crista emerge at right angles to the cuticle and pass directly into the cupular canals. In the periphery too, they emerge at right angles to the cuticle, but then turn markedly towards the center of the cupula.

Electron microscopic studies of the cupula in specimens fixed in osmium tetroxide reveal an exceedingly loose structure composed of innumerable fibrils having a reticular disposition and diameters of about 100 Å. No separate outer membrane can be observed. There is, however, a dense outer layer forming a boundary between cupula and endolymph. No boundary membrane is detectable round the canals within the cupula.

In this investigation a large number of preparations showed, in both the light microscope and the electron microscope, discrete structures between the sensory hairs, sometimes isolated from the epithelium in the subcupular space, and sometimes forming spherical protrusions from the epithelium. These formations were usually rounded and had diameters of 1–5 μ. They were bounded by a membrane

and mostly had a very sparse inner structure. In most cases they appeared to rise from the sensory cells and not, as reported by Wersäll (1954), from the supporting cells.

Discussion

Although fixation in osmium tetroxide solution followed by embedding in plastic has proved to be the best suited of all known preparative methods for ultrastructural studies, some shrinking of the cupula appears to occur during the preparative treatment of the crista ampullaris. Hence the extension of the cupula reported here may not accord with that existing in the fresh preparation. Steinhausen (1933) and Dohlman (1941) thus showed that in the living animal the cupula extends from the crista ampullaris surface as far as the ampullar summit, and terminates in its immediate proximity.

The fine structure of the cupula appears to coincide largely with that of the tectorial membrane; for ultrastructural fibrils have also been demonstrated in the latter, though disposed more regularly than in the cupula (Smith, 1955; Engström, Sjöstrand & Wersäll, 1956). Both of these structures are probably of gelatinous nature in fresh condition.

The chemical structure of the cupula is not yet fully elucidated. A series of histochemical investigations conducted in recent years suggest, however, that sulphomucopolysaccharides are an important chemical constituent of its structure, and that chemically it is closely consistent with the tectorial membrane. Belanger (1953) and Friberg & Ringertz (1955) thus demonstrated an uptake of S_{35} in the otolithic membranes, the cupulae and the tectorial membrane of the inner ear. Wislocki & Ladman (1954, 1955) showed that these structures in rat were characterized by their selective staining with alum hematoxylin and aldehyde fuchsin (Gomori's methods), by a strong periodic acid Shiff reaction, and by the presence of the sulphide groups associated with cystin. Vilstrup (1950) demonstrated metachromasia in the cupula in studies of preparations treated by the freezing and drying method, and Plotts & Pearlman (1955) observed it in the tectorial membrane; but it was not found in chemically fixed preparations studied by Wislocki & Ladman (1954, 1955) and Friberg & Ringertz (1956).

Kaiser (1891) and Kolmer (1911) observed, between the sensory hairs in the subcupular space, spherical formations that could even be visualized in apparently well fixed preparations. However, both of them regarded these formations as artefacts due to postmortal changes. Vilstrup (1950) found in the subcupular space, in frozen-dried preparations, cell-like structures which he assumed to be residue of cells that had migrated from the crista epithelium. Wersäll, Engström & Hjort (1953) described vesicular protrusions between the hair tufts of the sensory cells in the macula utriculi, which were thought to be derived from the sustentacular cells. Similar formations were reported by Wersäll (1954) in the epithelium of the crista ampullaris in guinea-pigs.

Although large numbers of preparations were studied in the present investigation, it could not be definitely established whether these formations consisted of postmortal artefacts or secretions from the cells in the crista epithelium. Having regard to the extent of this series, which included well-fixed preparations, they

are not likely to have been artefacts. Their function in the crista is, however, obscure.

Vilstrup (1950) considered it impossible with the light microscope alone to establish whence those fibrils arose that were observed in the subcupular space. He wrote: "When 'hair cells' are reckoned as identical with 'sensory cells', this is probably in some degree a result of synthetic speculation, as it is almost impracticable in these sections to determine the character of the cell from which such hairs arise." Also: "It is no hazardous conclusion to assume that the elements mentioned in the literature as 'cilia' or 'sensory hairs' are identical with those here described as cytoplasmic extensions from the cristal cells."

In the present investigation the greater potentialities of the electron microscope, in studies of the fine structure of the cupula and sensory hairs, enabled Kolmer's observations on their relationships to be verified. At the same time, communications between cupula and sensory hairs of the type described by Wittmaack and others were ruled out. The sensory hairs, accordingly, are not cytoplasmic filaments connecting migrated cells in the subcupular space with the sensory epithelium, as suggested by Vilstrup, but are specially differentiated processes arising from sensory cells having a characteristic structure and a significant bearing on the function of the sensory epithelium.

GENERAL DISCUSSION

Since each chapter in this paper concludes with a brief discussion, it will suffice here to present some further considerations regarding the structure and function of the crista epithelium.

The sensory epithelium in the superior and posterior semicircular ducts shows, as mentioned before, an asymmetrical distribution over the surfaces of the respective cristae ampullares, a greater number of sensory cells being found on the utricular than on the canalicular side. Corresponding anatomical conditions were demonstrated in *Acanthias vulgaris* by Vilstrup (1950), who thought that "the asymmetrical distribution of the epithelium might be the anatomical manifestation of the physiological condition expressed in Ewald's law".

Ewald (1892) found that the sensory epithelium of the vertical semicircular ducts was chiefly stimulated by ampullofugal (i.e., passing from the ampulla to the duct) "currents", while the horizontal semicircular duct was stimulated only by ampullopetal "currents". In guinea-pigs, like *Acanthias vulgaris,* the sensory epithelium of the vertical semicircular ducts is thus most highly developed on that side of the crista which, in these ampullae, encounters the "most active current". This lends weight to Vilstrup's assumption that the asymmetrical distribution of the sensory epithelium has a bearing on the crista function. Vilstrup also found, in *Acanthias vulgaris,* a less conspicuous asymmetry in the crista ampullaris lateralis, with a preponderance of sensory cells on the canalicular side. In the guinea-pig, however, the sensory epithelium in the crista of the lateral ampulla is equally distributed on both sides, so that Ewald's phenomenon may exist in that duct without an asymmetrical distribution. This may possibly be connected with different conditions with respect to "currents" in the two vertical semicircular ducts, on the one hand, and the horizontal duct on the other, so that a relationship between the sensory epithelium distribution and Ewald's phenomenon cannot be ruled out.

The two types of sensory cells that were found in the crista epithelium in this investigation, and which have been designated as hair cells of type I and type II respectively, differ both in ultrastructural characteristics and innervation. Ultrastructurally the difference lies chiefly in the disposition of the intracellular membrane systems of the respective types. In type I hair cells there is a special system of intracellular membranes enclosing narrow spaces in the infranuclear zone. In hair cells of type II these membranes are either absent or occur in the form of short fragments within the cell.

Oberling *et al.* (1953) and Weiss (1953), among others, considered that membrane systems of similar type, demonstrated in other kinds of cells, had a bearing on their protein metabolism and chiefly occurred in cells with a particularly high protein synthesis. But since membrane systems of characteristic appearance (alpha-cytomembranes—Sjöstrand, 1956) have been demonstrated in a number of completely different types of cells, it seems more likely that these systems, though morphologically similar, have a function characteristic for each particular type

of cell. Bernhard *et al.* (1952) and Porter (1954), among others, considered that there is a correspondence between the quantity of these membranes present in the cell and the basophilic substance "ergastoplasm" of the cell. This does not however seem to be quite proved yet.

Worthy of note is that the inner hair cells in the organ of Corti contain a membrane system similar to that described here in type I hair cells in the sensory epithelium of the crista ampullaris, but that the outer hair cells do not.

As pointed out earlier, the ultrastructural difference between type I and type II hair cells, and the conspicuous difference in their innervation, probably means that they have different functions in the sensory epithelium. However, full knowledge of their respective functions can scarcely be acquired other than by more detailed electrophysiologic studies of the epithelium, with the use of microelectrodes and direct conduction from the individual cells. Bearing in mind the advances made in this field by Békésy (1954) and Katsuki *et al.* (1954), among others, such leads should be practicable. Further teamwork by morphologists and physiologists might well, therefore, enhance our knowledge of the structure and function of the vestibular apparatus, and perhaps resolve the problems relating to the physiologic background of Ewald's law.

SUMMARY

The investigation reported here is presented in six chapters. The first chapter deals with the fixation and embedding technique employed in the work. Chapter II contains a description of the anatomy of the guinea-pig ear with special reference to problems associated with the preparative technique. Chapter III is concerned with the general structure of the sensory epithelia in the cristae ampullares of the guinea-pig and their innervation. In Chapters IV and V a description is given of the fine structure of cells, nerves and nerve endings in the sensory epithelium; and Chapter VI, lastly, is concerned with the structure of the cupula.

Chapter I. Material and Methods

The studies were performed on specimens from guinea-pig ears. Some preparations were studied in the light microscope after fixation and embedding by classical histologic methods. In other specimens the ampullae of the membranous labyrinth were dissected free from the surrounding bone after fixation in osmium tetroxide solution buffered to pH 7.2–7.4 and isotonic with blood. These ampullae were embedded in a butylmetacrylate metamethylacrylate mixture and sectioned either for phase contrast microscopy or electron microscopy. The electron microscope used was a RCA EMU 2 c model.

Chapter II. Gross Anatomy of the Guinea-Pig Ear

The guinea-pig ear lends itself admirably both to physiologic and histologic studies. A major portion of the petrous bone is occupied by an air-filled cavity, the bulla tympanica, forming a dilation of the middle ear. In this cavity the greater part of the cochlea and some portions of the semicircular canals bulge, surrounded only by very thin bony walls. It is relatively simple, therefore, to mobilize large parts of the membranous labyrinth, and to fix and embed them for examination by light and electron microscope, without the use of decalcification methods that damage the tissues. An outline is presented of the anatomy of the middle and inner ear in the guinea-pig.

Chapter III. General Structure and Innervation of the Crista Epithelium

Studies of the crista epithelium by light microscope and electron microscope show that it is composed of three types of cells: two different types of hair cells—in this paper, termed type I and type II—and supporting cells. Type I hair cells are bottle-shaped, and each of them has a nerve calyx enclosing, like a shell, the greater part of the cell. Type II hair cells are more cylindrical, with a rounded base. Each of them is innervated by a number of peripheral nerve end branches,

lying against the basal part of the cell in the form of loops or budshaped nerve endings. Both type I and type II hair cells are distributed throughout the crista epithelium. However, the former are mainly localized to the summit, and the latter to the periphery of the cristae ampullares. The supporting cells show a regular distribution in the sensory epithelium, and vary only very slightly in appearance in different parts of it.

Quantitative caliber analysis of the myelinated fibers in six posterior ampullar nerves from five animals yielded the following results: 66.1 per cent had diameters of 3–5 μ, 24.8 per cent diameters of 1–2 μ, and 9.1 per cent diameters of 6–9 μ. No nerve fibers exceeding 9 μ in diameter were observed.

Studies of specimens treated either by silver impregnation *ad modum* Palmgren (1948) or by osmium tetroxide impregnation, showed a characteristic distribution of nerve fibers of varying caliber in the crista. The thickest fibers pass to the summit of the crista and there give off a small number of branches, forming nerve calyces around type I hair cells. The fibers of medium caliber are distributed throughout the crista. Some of them form nerve calyces round type I hair cells, several calyces being innervated by one and the same fiber. Others give off a large number of fine branches which, together with the finest nerve fibers, form an intraepithelial nerve plexus. From this latter emerge branches that innervate hair cells of type II. Each of the last-named cells is innervated by several different nerve fibers, and each nerve fiber innervates several hair cells.

Chapter IV. Fine Structure of the Cells of the Sensory Epithelium in the Cristae Ampullares

Hair cells of type I are separated from the nerve calyx and surrounding supporting cells by a continuous plasma membrane. The cytoplasm is composed of a homogeneous ground substance and granules having diameters of about 100 Å, assembled in clumps around 500 Å in diameter. Further, larger granules of somewhat varying appearance may be observed.

In the infranuclear portion of the cell is found a membrane system composed of thin spaces in the cytoplasm, usually parallel with the nuclear surface surrounded by membranes about 50 Å thick. The side of the membrane turned away from the space is lined by granules about 100 Å in size.

In the Golgi zone, i.e., the supranuclear portion, are observed membranes and vacuoles of typical appearance. The membranes enclose lighter spaces in the cytoplasm that are sometimes dilated to form vacuoles of varying size. The Golgi apparatus is divided into a number of smaller zones having diameters of about 2 μ, where the cytoplasm is denser than in other parts of the cell. In the periphery of each such zone can be observed a number of small vesicular formations with diameters of 200–300 Å.

The mitochondria in the hair cells are spherical or oval in shape. They are bounded by an outer membrane and contain a large number of inner membranes, mostly parallel. Both outer and inner membranes are composed of two opaque layers separated by a less dense interspace.

The nucleus has an envelope composed of two opaque layers about 50 Å thick, separated by a lighter interspace varying between 100 and 200 Å in thickness.

The nuclear substance consists of filaments, some of them interconnected, and masses of an almost amorphous opaque substance containing a large number of small granules. Usually the nucleus has a central nucleolus with the same structure as the rest of the nuclear substance.

Immediately beneath the apical surface of the hair cell is a cuticle 0.2–0.5 μ thick. From each hair cell emerge about fifty sensory hairs. One of them has a kinocilium-like structure and the others have stereocilial structures.

The stereocilia are composed of finely granulated protoplasm bounded by a plasma membrane, and an axial fibril. This latter has an intracellular portion extending into the cuticle and surrounded by a lighter zone. Its extracellular part runs along the cilium, though in this investigation it could not be visualized for a greater distance than about 4 μ of the approximately 40 μ long cilium.

The kinocilia, like the stereocilia, have an extracellular and an intracellular portion. The former is composed of a fibrillar bundle with two single central fibrils surrounded by nine double fibrils embedded in a finely granulated protoplasm bounded by a plasma membrane. This latter merges basally with the plasma membrane of the cell, while the fibrillar bundle forms a budshaped basal corpuscule in the cuticle.

The hair cells of type II do not differ, with respect to structure of the cytoplasm, nucleus and nuclear envelope, from those of type I. They have, like the latter, a system of Golgi membranes in the supranuclear portion of the cell which is usually divided into a greater number of zones than those in type II hair cells.

Granulated membrane systems of the type described in type I hair cells are not found here. However, short fragments of similar membranes were observed in the infranuclear portion of the cells. Cuticles, stereocilia and kinocilia have the same structure as their counterparts in type I hair cells.

In those regions of the cell where nerve endings and end branches lie in contact with the cell, the plasma membrane shows a depression or shallow groove in which the nerve ending rests. In these zones the plasma membrane often has a double structure and the cytoplasm is somewhat denser near the membrane than in the rest of the cell.

In the apicalmost part of the *supporting cells* is a ring of dense substance having a depth of about 0.5–1 μ and constituting a part of the reticular membrane. Otherwise this uppermost part is filled by densely packed granules with diameters of 0.2–0.3 μ. The Golgi apparatus consists of a number of thin membranes enclosing spaces in the cytoplasm, located centrally and usually coinciding with the long axis of the cell, as well as narrow vesicular formations.

The nucleus is oval and enveloped by a double membrane. Its substance is denser than that of the hair-cell nuclei, and each nucleus contains one or two opaque nucleoli.

Chapter V. Fine Structure of Nerves and Nerve Endings

The various branches of the vestibular nerve are dominated by myelinated nerve fibers that innervate the sensory cells in the different sensory epithelium zones. Between these are observed, however, a large number of unmyelinated nerve fibers the function of which is still obscure.

In the myelinated fibers the axons are composed of a ground axoplasm that is amorphous at this resolving power and is bounded by a continuous plasma membrane. Embedded in this axoplasm are a large number of neurofibrils with diameters of about 100 Å, rod-shaped mitochondria, and granules around 200–400 Å in diameter. Mitochondria and granules increase in number towards the nerve periphery.

In the sensory epithelium of the crista ampullaris the axons divide and form either calyces round hair cells of type I or slender end branches in contact with type II hair cells. Some of these end branches form bud-shaped nerve endings in the region of contact between nerve and hair cell. All nerve endings are direct continuations of the peripheral branches of the axons, and have structures characteristic of the axon. Most of them thus contain neurofibrils, small granules and mitochondria. However, some of them that join hair cells of type II are virtually filled with granules 200–400 Å in diameter. Each nerve ending is bounded by a continuous plasma membrane connected with that of the axon, so that there is no directly demonstrable communication between the axoplasm and the hair cell cytoplasm.

The myelin sheath is composed of opaque layers with a calculated thickness of 30 Å, alternating with less dense layers around 90 Å thick. The spacing between the opaque layers is found to be 120 Å. The two peripheralmost layers of the sheath are connected with the plasma membrane of the surrounding cell of Schwann. These findings support the theory propounded by Geren (1954) that the myelin sheath is composed of a double membrane derived from the plasma membrane of the Schwann cell, closely wound about the axon like a helix.

The unmyelinated nerve fibers have the same structure, in principle, as the myelinated nerve axon. Each unmyelinated fiber is partly or completely enclosed by a single layer of the plasma membrane of a Schwann cell. Generally, two or more fibers are enclosed by each Schwann cell.

Chapter VI. Structure of the Cupula

In osmium-fixed preparations the cupula resembles a hood of more or less constant form, which covers the sensory epithelium and from there extends into the endolymph between the epithelium and the ampullar summit.

The entire cupula is permeated by narrow canals which pass from the concave surface facing the sensory epithelium to the convex upper surface. These canals are partially filled by the cilia arising from the sensory cells.

In fixed preparations the cupula is composed of slender fibrils forming a three-dimensional network, probably arising during fixation of a structure gelatinous in the unfixed state. The cupula is bounded by a somewhat denser outer layer, though this apparently does not form a separate membrane.

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