J. Anat. (1986), **147**, pp. 191–199
With 13 *figures*
Printed in Great Britain

The ultrastructure of the paratympanic organ in the domestic fowl (*Gallus gallus domesticus*)

F. GIANNESSI AND L. PERA

Istituto di Anatomia Umana Normale, Facoltà di Medicina e Chirurgia, Università di Pisa, Via Roma, 55, 56100 Pisa, Italy

(*Accepted* 31 *October* 1985)

INTRODUCTION

The paratympanic organ, a characteristic formation in birds, was discovered by Vitali in 1911, in the middle ear of the house sparrow, *Passer domesticus* (*Passer italiae*); subsequently this author described the same organ in several other species of birds (Vitali, 1912, 1913, 1923). In addition to the fundamental work carried out by Vitali, contributions to knowledge of the structure of the paratympanic organ were made by Benjamins (1925, 1939) and Federici (1927); moreover a number of recent investigations have thrown light on its ultrastructural features (Maderson & Jaskoll, 1976; Petrash, Andres, Von During & Delius, 1983; Jørgensen, 1984; Giannessi & Pera, 1984*a*, *b*).

The paratympanic organ looks like an elongated vesicle, sometimes with diverticula, situated in the medial wall of the tympanic cavity, above the opening of the pharyngotympanic tube and near the mucosal surface. The organ comprises an epithelial-lined cavity containing a fluid whose nature is unknown. According to Vitali (1912), the paratympanic organ of the chicken is smooth walled, tapers at both ends and, in adult animals, measures nearly 1100 μm in length, while its maximum diameter is 320–400 μm. Most of the medial wall of the paratympanic organ is formed by a high sensory epithelium which rests on a basement membrane, beneath which numerous myelinated fibres are to be found. The remaining parts of the wall of the organ are formed by a simple epithelium which is usually squamous or cuboidal in shape.

MATERIAL AND METHODS

In the present study, 50 adult domestic fowl (*Gallus gallus domesticus*), Hubbard strain, weighing 1300–1500 g were killed by decapitation. The paratympanic organ was extirpated through the external acoustic meatus after the tympanic membrane had been broken and immersed for 8 hours in a fixative consisting of a mixture of paraformaldehyde (4%) and glutaraldehyde (2·5%) in 0·1 M phosphate buffer, pH 7·3. After rinsing in the buffer for 12 hours, the specimens were postfixed for 2 hours in 1·3% osmium tetroxide in the same buffer, dehydrated and embedded in Epon. Finally, the paratympanic organs were sectioned in a Porter–Blum ultramicrotome, stained with uranyl acetate (2·5% in water) and lead citrate (Reynolds, 1963) and observed through a Siemens Elmiskop electron microscope.

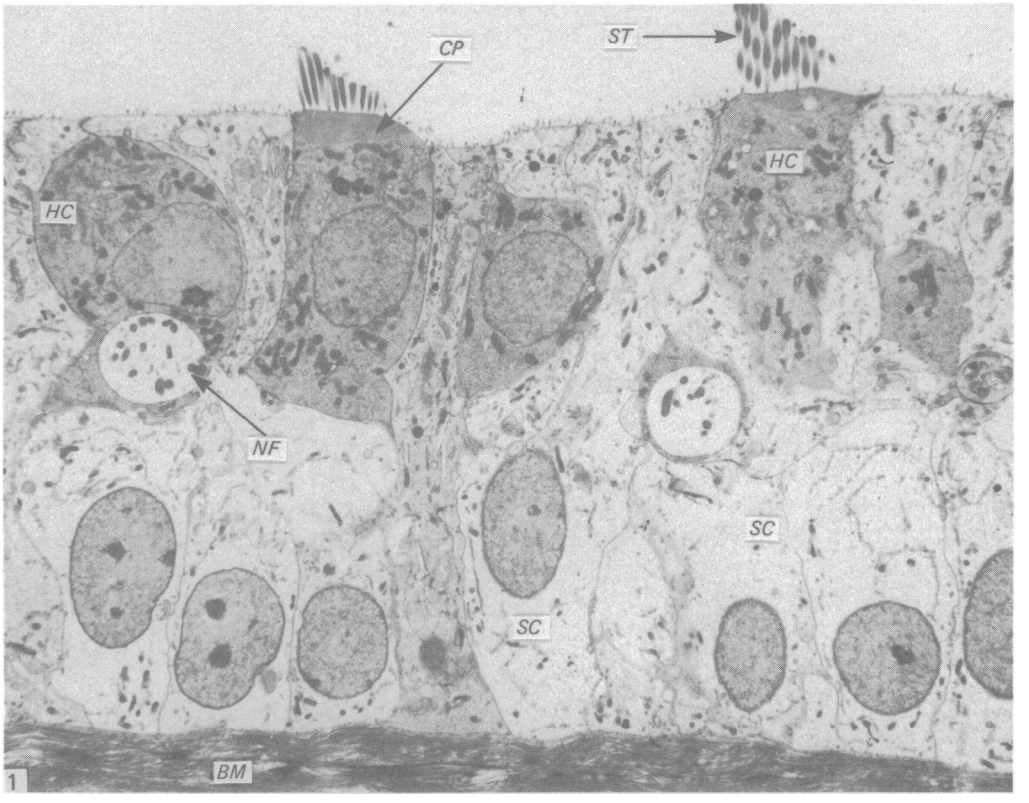
RESULTS

Sensory epithelium

The sensory epithelium of the paratympanic organ consisted of hair cells and supporting cells. The supporting cells (Fig. 1) were slender and electron-lucent and rested on a basement membrane, while their distal part was situated between the hair cells and extended up to the surface of the epithelium. The nucleus was ellipsoid and was located basally; it contained very dense chromatin and distinct nucleoli. A number of short microvilli were found on the luminal surface (Fig. 1). The apical ends of the supporting cells were connected by tight junctions to both one another and the hair cells (Figs 1, 3). The hair cells were cylindrical or pear shaped and occupied the terminal half of the epithelium. They were separated from the basement membrane (Fig. 1) and, partly, from one another (Figs. 1, 2) by supporting cells. The nucleus, which was spherical or slightly oval, was situated near the basal end of the cell or in the middle of it. The cytoplasm, which was denser than that of the supporting cells, contained scattered mitochondria and numerous vesicles, especially in the basal part of the cell (Fig. 6), whereas a Golgi apparatus, lysosomes and multivesicular bodies were found in the supranuclear cytoplasm (Fig. 3). At the apex of each hair cell there was a bundle of sensory hairs consisting of 40–70 stereocilia and an eccentrically placed kinocilium (Figs. 4, 5). The stereocilia increased regularly in height from one side of the cell surface to the other. Their bases were thinner than the rest of the filament and were embedded in a cuticular plate, finely granular in appearance, which occupied a large part of the luminal part of the cell cytoplasm (Fig. 4). The stereocilia were arranged in such a way that regular hexagonal figures were formed, with six stereocilia surrounding a central one (Fig. 5). The kinocilium, 0·2–0·3 μm in diameter, was adjacent to the tallest stereocilia and terminated in a basal body in a cuticular plate-free portion of the apical cytoplasm (Fig. 4). It contained nine peripheral double tubules which surrounded a pair of central tubules; this central one was not always clearly visible (Fig. 5).

Innervation

Afferent nerve fibres, which originate from the facial ganglion (Vitali, 1911), penetrated the basement membrane and lost their myelin sheaths. They passed between neighbouring supporting cells and made synaptic contact with the basal region of the hair cells where they frequently made deep invaginations (Fig. 1). A synaptic cleft, about 18 nm in width, separated the plasma membrane of the nerve ending from that of the hair cell (Fig. 6). In the synaptic region, the cytoplasm of the hair cells contained ribosomes and mitochondria as well as smooth and coated vesicles; infoldings of the plasma membrane might be observed also (Fig. 6). Inside the afferent terminals, there were mitochondria and several vesicles of various sizes (Fig. 6). Synaptic bodies were always present in the hair cells adjacent to the nerve fibres (Fig. 6); they were surrounded by a ring of small round vesicles and, near them, both the plasma membrane of the hair cell and that of the nerve ending were thicker. The synaptic bodies appeared irregularly spherical in shape, sometimes with a stalk which extended as far as the presynaptic membrane and touched it. When the stalk was not visible they did not make contact with the plasma membrane.



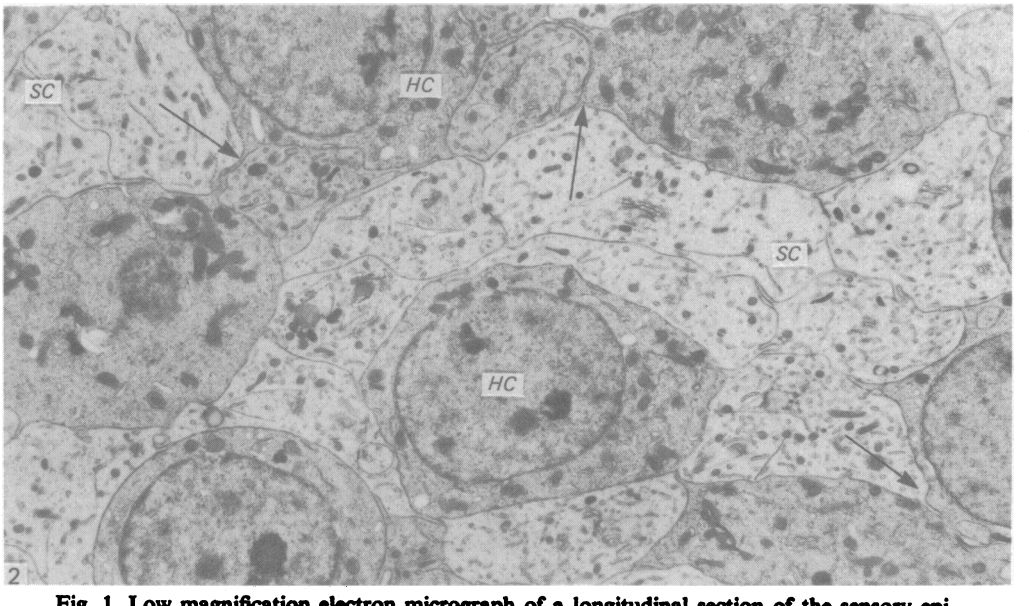
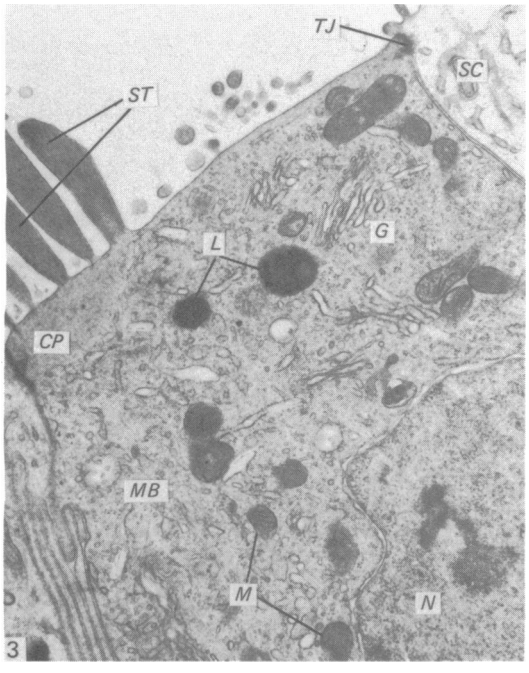
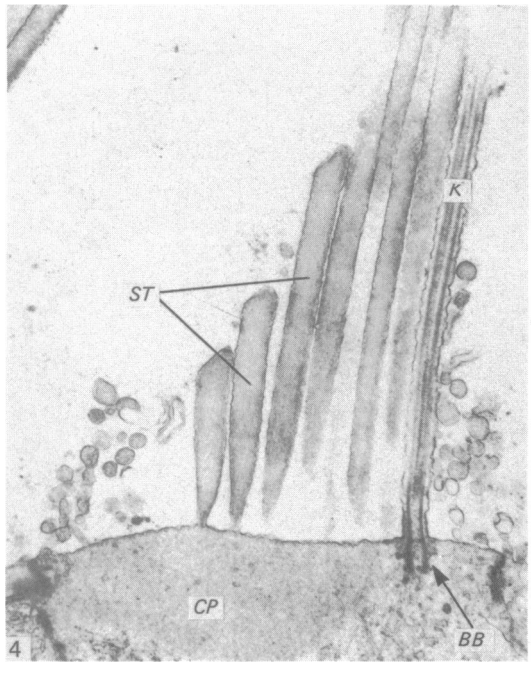


Fig. 1. Low magnification electron micrograph of a longitudinal section of the sensory epithelium. The sensory epithelium is formed by hair cells (*HC*) which are surrounded by supporting cells (*SC*); a nerve fibre (*NF*) appears to be deeply invaginated into a hair cell. *BM*, basement membrane; *CP*, cuticular plate; *ST*, stereocilia. ×4500.

Fig. 2. Tangential section of the sensory epithelium. The micrograph shows that the supporting cells (*SC*) do not entirely separate the hair cells (*HC*) from one another and these often come into contact with each other (arrows). ×9800.





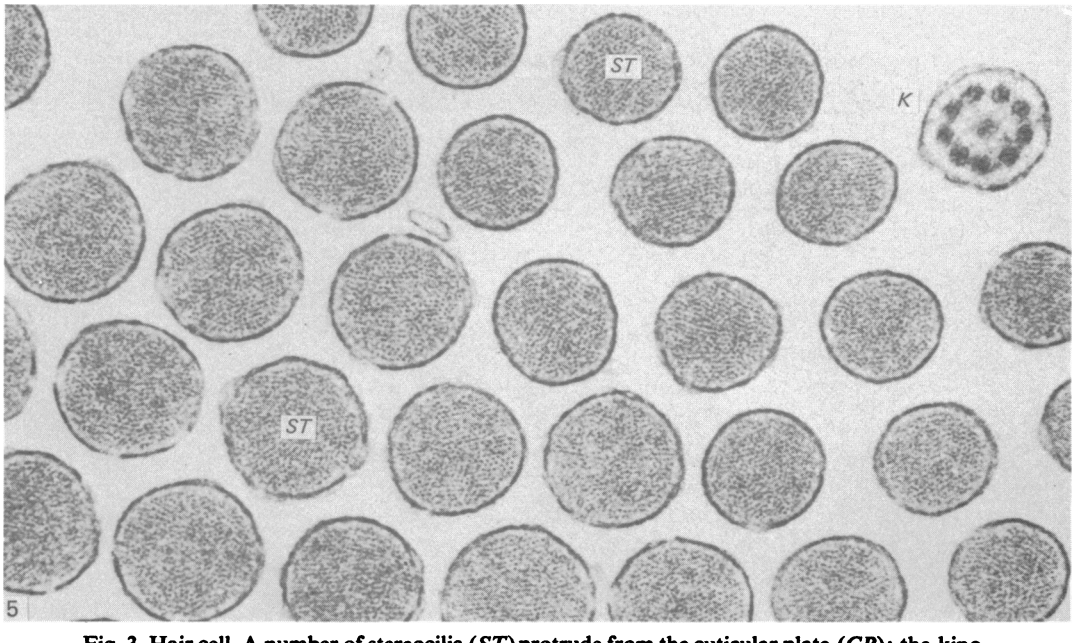


Fig. 3. Hair cell. A number of stereocilia (*ST*) protrude from the cuticular plate (*CP*); the kinocilium is not visible in the picture. *G*, Golgi apparatus; *L*, lysosomes; *M*, mitochondria; *MB*, multivesicular body; *N*, nucleus; *SC*, supporting cell; *TJ*, tight junction. × 17800.

Fig. 4. Longitudinal section of a hair bundle of a sensory cell. The kinocilium (*K*) emerges from a basal body (*BB*) located in an area of the cytoplasm devoid of cuticular plate (*CP*). The stereocilia (*ST*) gradually decrease in height as the distance from the kinocilium increases. × 15200.

Fig. 5. Transverse section of a hair bundle of a sensory cell. The kinocilium (*K*) is located on one side of a group of stereocilia (*ST*) and its arrangement of 9+2 microtubules is typical of motile cilia. The stereocilia have a protoplasmatic core with several fine fibrils and are arranged hexagonally. × 65000.

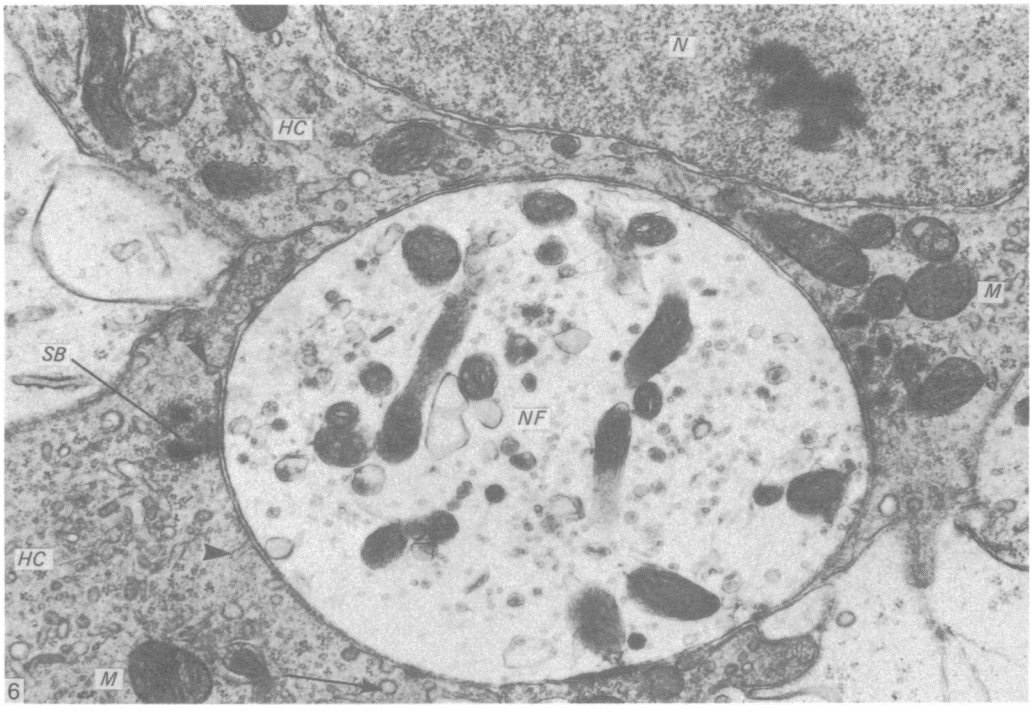


Fig. 6. A nerve fibre (*NF*) makes synaptic contact with two hair cells (*HC*) seen in the upper part and in the lower left part of the picture. Arrow, coated vesicle; arrowheads, infoldings of the presynaptic membrane; *M*, mitochondria; *N*, nucleus; *SB*, synaptic bodies. × 28 500.

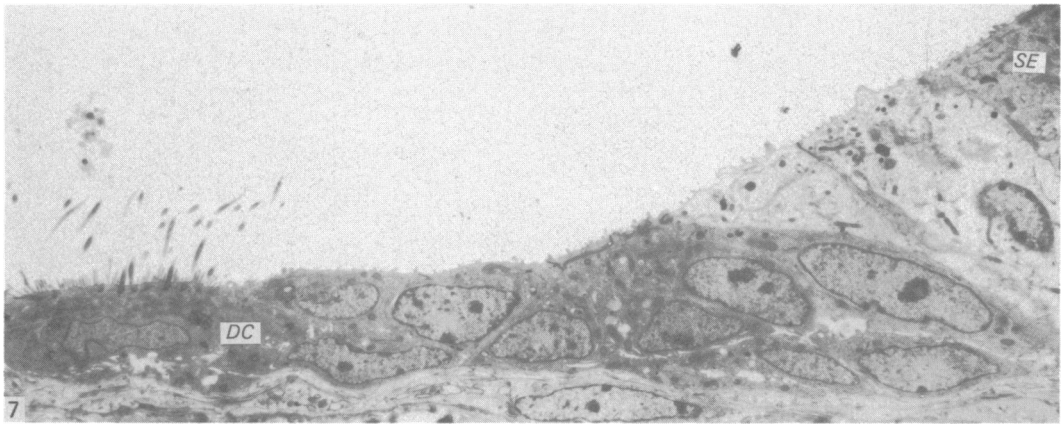
Non-sensory epithelium

Most of the non-sensory epithelium was formed by dark and light cells. The former (Figs. 7, 9, 10) were squamous, irregularly overlapping and sometimes separated by narrow intercellular spaces. Their cytoplasm, which was very dense, contained mitochondria and a small Golgi apparatus; a few ciliated dark cells were also observed.

The light cells, which were more numerous than the dark cells, were cuboidal or slightly flattened in shape and were arranged in a single layer (Fig. 8). They contained mitochondria, cisternae of the granular endoplasmic reticulum (Fig. 13) and a well developed Golgi apparatus (Fig. 11). The apical cell portion often contained an accumulation of electron-lucent material which was homogeneous, contained no organelles and might cause irregular protrusions (Fig. 8). Moreover, inside the cytoplasm of the light cells there were often vesicles containing a moderately dense substance, which was mostly localised in the basal part of the cells. These were sometimes homogeneous in appearance (Figs. 8, 12), while at other times they were characterised by a clearer peripheral region (Fig. 11). Near the luminal surface, on which there were few irregular microvilli, globular formations variable in volume were present (Figs. 8, 11, 13).

DISCUSSION

As far as the presence of a well defined sensory organ in the medial wall of the tympanic cavity in birds is concerned, this investigation confirms the data concerning the chicken obtained by Vitali (1912). The ultrastructure of the sensory



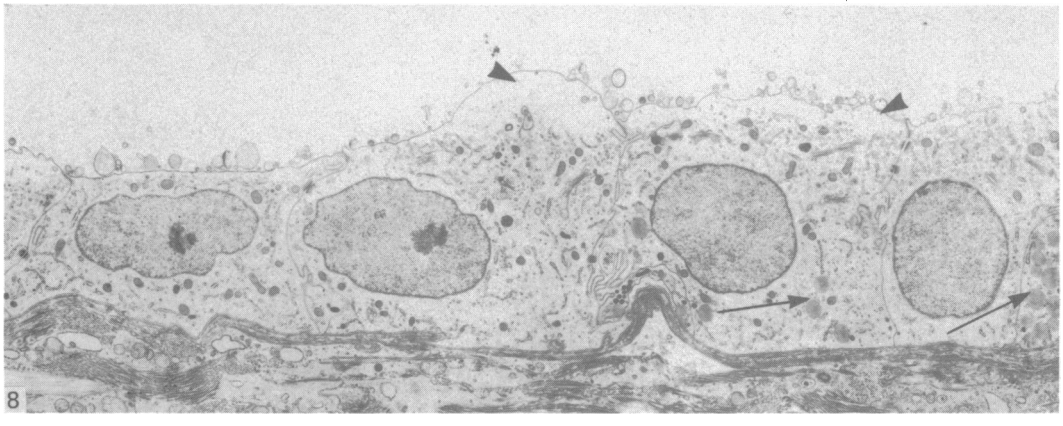


Fig. 7. Transition zone between the sensory epithelium and the non-sensory epithelium. The sensory epithelium (*SE*) is progressively reduced in height and is replaced by dark cells. A ciliated dark cell (*DC*) is present on the left of the picture. ×4100.

Fig. 8. Non-sensory epithelium. The pale cells visible in the micrograph form a monolayered epithelium and are cuboidal or slightly flattened. The cytoplasm sometimes contains vesicles (arrows) and there may be clear material just beneath the free surface of the cells (arrowheads). ×4400.

epithelium of the paratympanic organ appears to be similar to that observed in the neuroepithelia of the vestibule and the lateral line organs. The phyletic affinity of the paratympanic organ with the lateral line organs was maintained by Vitali (1925) who demonstrated that the paratympanic organ originates from a thickening of the dorsal edge of the first branchial furrow (epibranchial placode). He considered this thickening to belong to the differentiated ectodermal strip from which the lateral line system develops.

As is well known, two kinds of sensory cell exist in the vestibule of sauropsids and mammals (Wersäll, 1956, 1967; Engström & Wersäll, 1958; Smith, 1967). One kind

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Fig. 12. Light cells. Detail of basal cytoplasm containing a number of dense bodies (arrows) and vesicles (*V*) that are homogeneous in content and of the same type as in Figure 8. ×9600.

Fig. 13. Light cells. Adjacent cells are connected by desmosomes (*D*). *RER*, granular endoplasmic reticulum. ×11100.

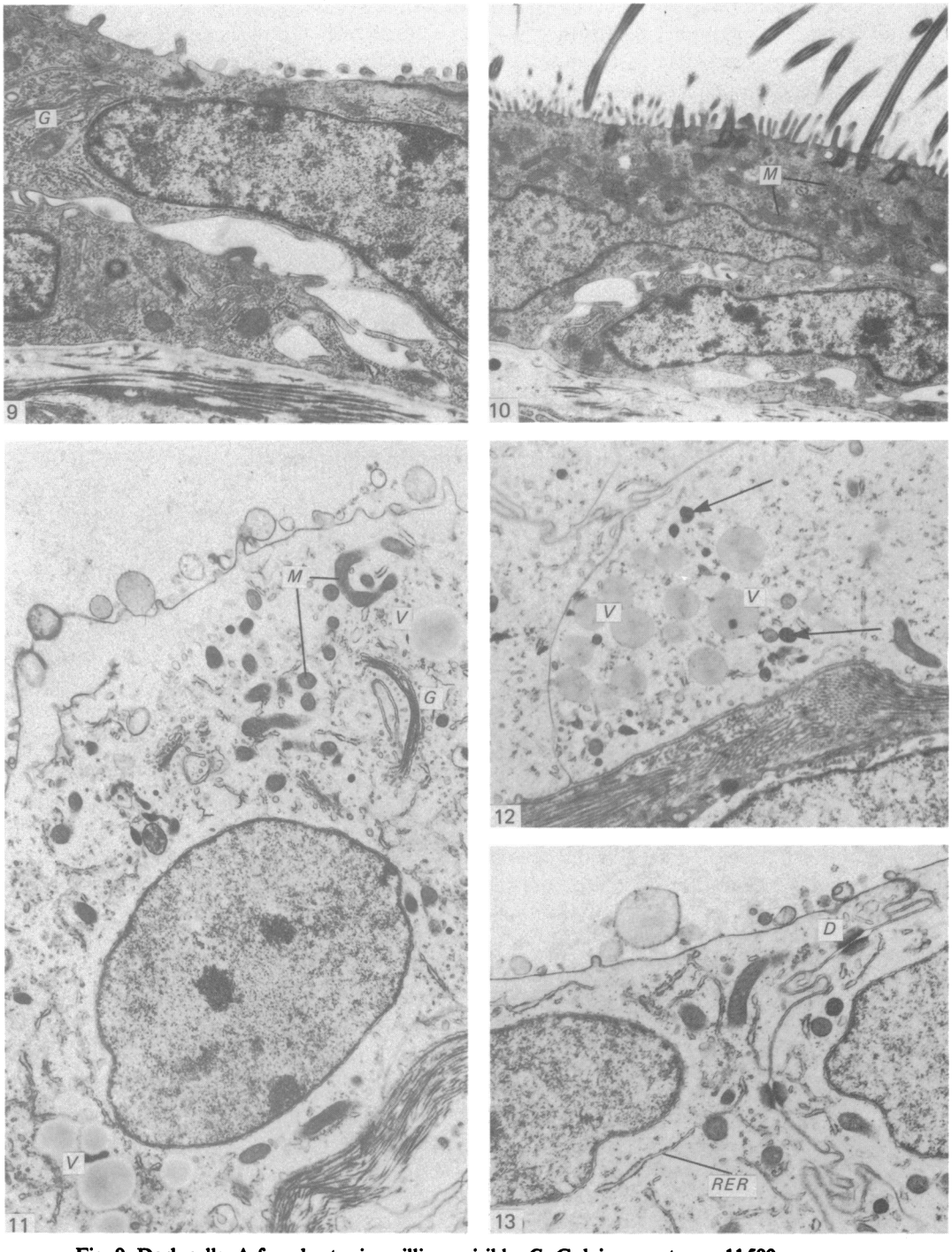

Fig. 9. Dark cells. A few short microvilli are visible. *G*: Golgi apparatus. ×11500.

Fig. 10. Ciliated dark cell. Numerous long microvilli are observed. *M*, mitochondria. ×8500.

Fig. 11. Light cell. The vesicles (*V*) present in the picture are characterised by a clearer peripheral region. There are globular formations present near the upper surface of the cell. *G*, Golgi apparatus; *M*, mitochondria. ×9900.

is pear shaped and is surrounded by a nerve chalice (type I sensory cell). The other cell (type II) is cylindrical and receives nerve fibres which end as small boutons. It has been observed that in the paratympanic organ of the chicken the sensory epithelium has type II receptors only; they are the sole type present in the lateral line organs and in the vestibule of the lowest vertebrates (Flock, 1965; Vinnikow, 1969; Wersäll & Flock, 1965). This supports Vitali's idea that the paratympanic organ in birds and the lateral line organs are homologous.

Vitali (1921), Federici (1927), Maderson & Jaskoll (1976) and Jørgensen (1984) have supposed that the paratympanic organ may be involved in the reception of variations of air pressure in the middle ear. These variations might result in movements of the liquid contained in the organ and consequently might cause stimulation or inhibition of the receptor cells. This hypothesis appears to be supported by the present ultrastructural findings which show that the hair cells of the paratympanic organ are similar to the mechanoceptors in the vestibule and lateral line organs. Moreover, the afferent fibres innervating the paratympanic organ come from the geniculate ganglion (Vitali, 1911) and therefore in birds, too, the facial nerve possesses that proprioceptor component which in fish innervates part of the lateral line organs.

The afferent synapses described in this paper do not differ significantly from the synapses of sensory cells similar to those in the paratympanic organ. The cytoplasmatic infoldings present in the synaptic region of the hair cells are similar to those in neuromuscular junctions (Heuser & Reese, 1973), in retinal receptors (Shacher, Holtzman & Hood, 1976) and in the inner ear (Hama & Saito, 1977). The consensus of opinion is that these infoldings are involved in pinocytosis, which permits reabsorption of an excess of plasma membrane caused by exocytotic processes involved in release of the chemical mediator.

The role of the paratympanic organ is still not known. The experiments carried out by Vitali (1921) would seem to indicate that this structure is connected in some way with flight. On the other hand, the fact that the paratympanic organ is well organised, as shown in the present investigation, in animals such as the chicken which has adapted to a basically terrestrial way of life suggests that the functional contribution of this organ to the life of birds has yet to be properly defined.

SUMMARY

The structure of the paratympanic organ in chickens was investigated by means of the transmission electron microscope. The epithelium lining the lumen of the paratympanic organ consists of sensory and non-sensory components. The sensory epithelium is composed of supporting and hair cells. The hair cells are similar to the type II receptor cells present in the neuroepithelia of the vestibule and of the lateral line organs. The afferent synapses at the bases of the hair cells are also described.

The non-sensory epithelium is made up of cells with a clear cytoplasm and arranged in a single layer. It also contains dark, flattened cells which sometimes possess motile cilia.

Special emphasis is given to the fact that the results agree with Vitali's theory that the paratympanic organ and the lateral line organs are homologous. It is concluded, therefore, that present knowledge about this structure is not yet sufficient to allow a definitive functional interpretation.

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