

Freeze-etching studies on the ciliary necklace in the rat and chick

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INTRODUCTION

The 'Ringmanschette', a highly specialized circular structure in the membrane of the proximal part of olfactory cilia, was first described electron microscopically by Andres (1969). Its existence has since been confirmed by many authors in a variety of cilia with freeze-fracturing techniques and later it was named 'ciliary necklace' (Satir & Gilula, 1970; Dirksen *et al.* 1971; Gilula & Satir, 1972; Bergström & Henley, 1973; Warner, 1974). Its functional meaning has been variously interpreted (Gilula & Satir, 1972; Matsusaka, 1974; Tani, Ikeda, Nishiura & Higashi, 1974; Röhlich, 1975; Boisvieux-Ulrich, Sandoz & Chailley, 1977) and remains obscure.

The main purpose of this paper is to compare the ciliary necklace in typical kinocilia, atypical cilia and mature and immature olfactory cilia, with special attention to the question whether olfactory sensory hairs are motile or not.

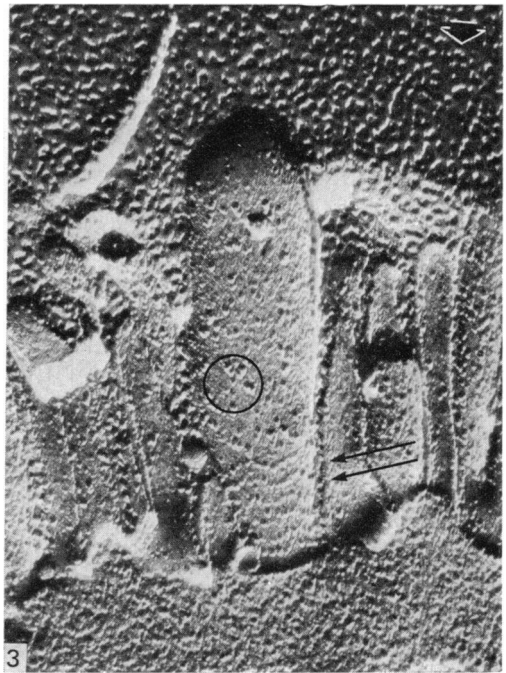
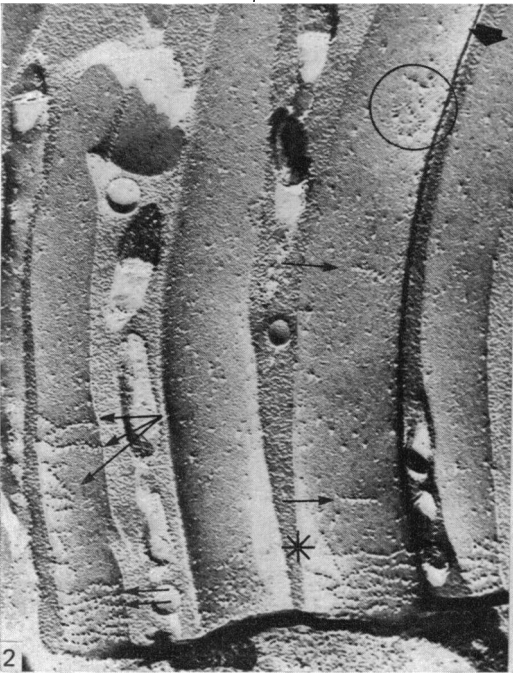
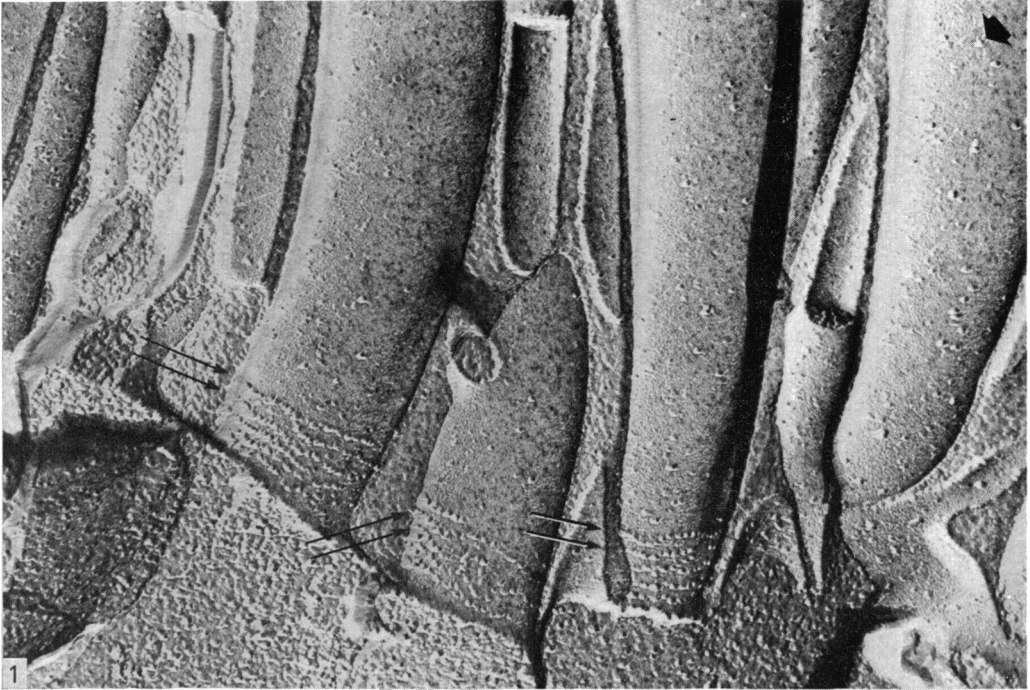
MATERIAL AND METHODS

The receptor-free epithelium of the vomeronasal organ and olfactory epithelium from septum nasi of adult male Wistar rats (300–350 g) was investigated. The olfactory epithelium from the superior nasal concha of chick embryos of 11 and 15 days, and respiratory epithelium from the septum nasi of 7 days old chickens were also investigated. The rats and chickens were anaesthetized with Nembutal and perfused intravascularly with 3.2% glutaraldehyde and 2.6% paraformaldehyde in cacodylate buffer (0.09 M, pH 7.35). The chick embryos were fixed by immersion in the same fixative. The tissue samples were dissected free while immersed in the fixative.

After fixation the tissues were washed in Hank's solution, and then immersed in 10 and 20% glycerine in Tyrode solution for 30 minutes. The samples were transferred to 30% glycerine in Tyrode solution for several hours, frozen in Freon 22 and stored in liquid nitrogen. Fracturing of the samples was accomplished in a Balzers 360 M Unit at -110°C at a pressure of 2×10^{-6} Torr. The tissues were then etched for 30 seconds, shadowed with carbon-platinum and replicated with carbon. The replicas were immersed in 40% chromic acid for cleaning and then washed several times in distilled water.

The samples for transmission electron microscopy were prepared as previously described (Breipohl, Bhatnagar & Mendoza, 1979). Both replicas and ultrathin sections were picked up on Pioloform F-coated 200 mesh copper grids and examined in a Philips 200 or 400 electron microscope at 60–80 kV.

Dedicated to Prof. Dr K. H. Andres on the occasion of his 50th birthday.



RESULTS

The ciliary necklace of kinocilia in the respiratory epithelium of the chicken consisted mainly of five to six nearly parallel rows of intramembranous particles (IMP) approximately 8.5 nm in diameter. The distance between the rows was about 19–21 nm (Figs. 1, 2). In other samples of chicken material less regular patterns in the orientation of the ciliary necklace rows were seen and the rows often showed cross linking (Fig. 2). Besides the mainly horizontal, diagonal and longitudinal orientations of the IMP rows also occurred. The latter two were either in contact with the basally located ciliary necklace or joined more distally located rows of IMP. The distally situated rows also occurred isolated. Often they only partly encircled the ciliary membrane. Further distally, irregular aggregations of IMP were noted, but regardless of their variable pattern, the size of the IMP always resembled that seen in a typical ciliary necklace (Fig. 1). Small irregular aggregations of IMP distal to the ciliary necklace were also seen on the atypical cilia of the receptor-free epithelium of the rat vomeronasal organ (Fig. 3), and consisted of three to four unevenly distributed IMP (Fig. 3). Additional rows of irregularly arranged IMP interconnected with the ciliary necklace have not been seen in the receptor-free epithelium of the vomeronasal organ.

In the rat olfactory cilia a typical ciliary necklace was also observed (Fig. 4). By transmission electron microscopy at the level of the ciliary neck, the cilium displayed the typical arrangement of nine axonemal doublets. These were always connected with each other by a short filament (Fig. 5), and laterally the axonemal doublets were linked with the ciliary membrane. Further distally, however, the lateral linking disappeared and the tubuli were connected by radially oriented electron-dense material with the centre of the cilium. Most of them displayed a pair of dynein arms in the A tubule with the internal arm hardly developed (Figs. 6, 7).

The ciliary necklace was not restricted to fully developed olfactory cilia since in maturing olfactory knobs of 11 days old chick embryos, a circular arrangement of IMP was seen prior to the outgrowth of cilia (Fig. 8); it was missing, however, in the regions where microvilli were outgrowing (Fig. 9). In 15 days old chick embryos the more mature olfactory cilia always exhibited a typical, well developed ciliary necklace (Fig. 9).

DISCUSSION

Since the first descriptions of the ciliary necklace by freeze-etching (Satir & Gilula, 1970) on motile and on non-motile cilia, this structure has been described in flagella and in a variety of other cilia in different species (Bergström & Henley,

Fig. 1. Kinocilia of respiratory epithelium in chicken showing typical ciliary necklace (double arrows). In addition to definite parallel rows of IMP a slight deviation in the arrangement of IMP is also seen. The arrowhead in this and subsequent figures indicates the direction of shadowing. $\times 114\,500$.

Fig. 2. Cilia from respiratory epithelium in chicken. Note typical ciliary necklace (double arrows) and irregularly patterned necklace (asterisk), irregular oriented and interconnecting rows of IMP (multiple arrow), single rows of IMP (single arrows) and irregular aggregation of IMP (circle). $\times 51\,800$.

Fig. 3. Cilium of the receptor-free epithelium of the rat vomeronasal organ. Typical necklace (double arrow) and irregular aggregation of IMP (encircled) are indicated. $\times 82\,000$.

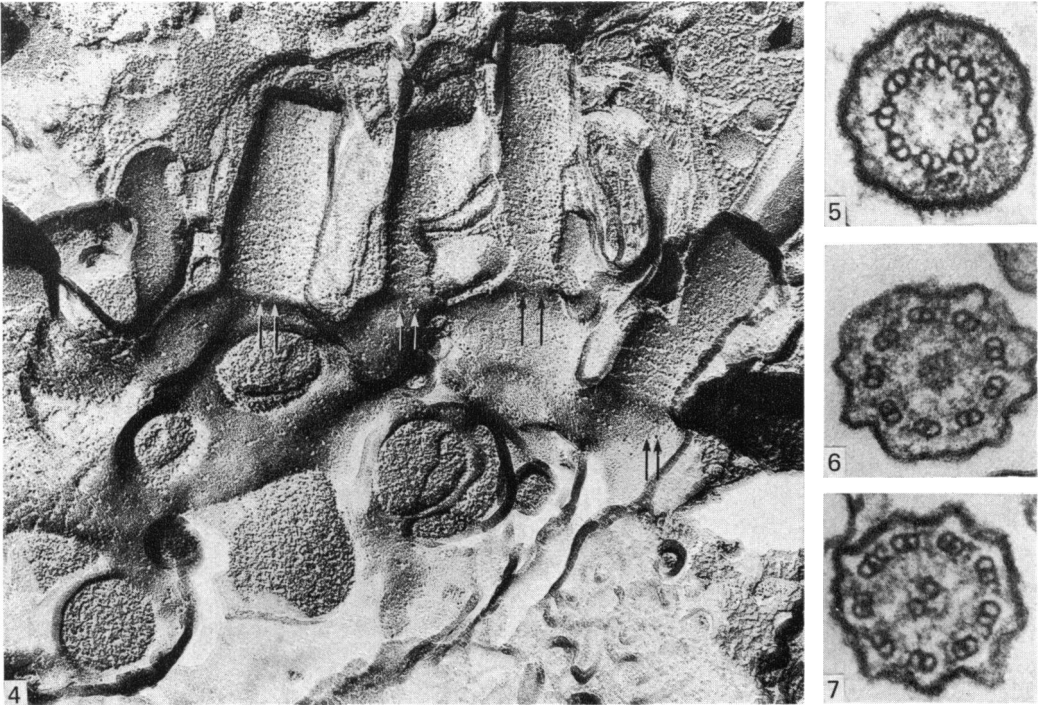


Fig. 4. Ciliary necklace (double arrows) on olfactory cilia from the septum nasi of the rat. $\times 68700$.

Fig. 5. Transverse section through ciliary necklace region of an olfactory cilium in chicken. Central tubular pair is absent. Note the connection of the outer doublets with each other and their lateral projections. $\times 112000$.

Fig. 6. Transverse section through olfactory cilium in chicken where the central pair of tubuli originates. Note dynein arms in the outer doublets and dense matrix between the axonemal structures. $\times 91000$.

Fig. 7. Transverse section through olfactory cilium in chicken with central pair of tubuli. Note dynein arms and spoke linking of outer doublets. $\times 101750$.

1973; Tani *et al.* 1974; Boisvieux-Ulrich *et al.* 1977; Inoue & Hogg, 1977). A ciliary necklace was also shown in the atypical short cilia of the vomeronasal receptor-free epithelium that lack rootlet fibres (Miragall, Breipohl & Bhatnagar, 1979). Even in non-motile sensory cilia (Matsusaka, 1974; Röhlich, 1975) and in sensory cilia of doubtful motility (Kerjaschki & Hörandner, 1976; Menco, Dodd, Davey & Bannister, 1976) a ciliary necklace has been reported in many species. The localization of the ciliary necklace is considered to be restricted to the transition region between the doublets and triplets of a cilium or to the region where the two central microtubules are lacking (Sattler & Staehelin, 1974; Boisvieux-Ulrich *et al.* 1977).

The ciliary necklace has been said to have no 'significant role in the actual mechanism of motility or beating', although it has been thought to be involved in energy transduction and so, perhaps, in timing the ciliary beat (Gilula & Satir, 1972). Motility or immotility of cilia has also been linked with the presence or absence of dynein arms respectively (Gibbons, 1965; Stephens & Levine, 1970; Afzelius, Eliasson, Johnson & Lindholmer, 1974), but while dynein arms could be found in the olfactory cilia of some species (Thornhill, 1967) their absence has been stressed in others (Bannister, 1965; Theisen, 1973; Kratzing, 1975). Millecchia &

Rudzinska (1970) interpreted the absence of the dynein arms in kinocilia as the result of developmental immaturity. Menco (1977) has denied the existence of dynein arms in mature bovine olfactory cilia and although he showed that they possessed a ciliary necklace, believed them to be immotile. On the other hand, olfactory cilia have been reported to be motile in some species (Graziadei, 1971; Menco, 1977). Our results clearly demonstrate the existence of both dynein arms and the typical ciliary necklace in the olfactory cilia of the chick: the asymmetry of the dynein arms in the olfactory cilia seems to be typical for kinocilia (Stephens & Levine, 1970) and we, therefore, believe the olfactory cilia to be motile. However, the relationship between motility and the existence of dynein arms and/or a ciliary necklace needs further investigations.

Our results also provide some evidence that the ciliary necklace, in addition to involvement in ciliary motility and in control of localized membrane permeability (Tani *et al.* 1974), may be involved with ciliogenesis or renewal of ciliary membranes. At the end of ontogenesis, ciliogenesis is frequently seen in the rat olfactory epithelium. However, not only perpendicularly arranged precursor bodies of ciliary necklaces can be seen here, as described by Kerjaschki, Sleytr & Stockinger (1972), but also circular arrangements of up to three IMP rows. Those cilia which have started outgrowth already show a typical ciliary necklace of up to six rows of IMP.

The number of rows of the ciliary necklace described ranges from one to thirteen (cf. Tani *et al.* 1974). Except to suggest that this feature represents species specific differences (Dirksen *et al.* 1971; Gilula & Satir, 1972; Sattler & Staehelin, 1974; Boisvieux-Ulrich *et al.* 1977) no other functional implications have been suggested. Differences in the direction or regularity of IMP rows in the region of the ciliary necklace have been described. The appearance of a scalloped ciliary necklace has been said to be typical of molluscs (Gilula & Satir, 1972; Bergström, Henley & Costello, 1973). Minor deviations of the typical straight and parallel or only slightly wavy orientation of the necklace bands were also observed in the quail oviduct by Boisvieux-Ulrich *et al.* (1977) and in the present material, on olfactory cells and especially on ciliated cells of respiratory epithelium of chick, and were most marked distally. This observation suggests that the ciliary necklace may be related to the process of membrane renewal, comparable to that occurring in the connecting cilium of photoreceptors (see Discussion in Matsusaka, 1974). Boisvieux-Ulrich *et al.* (1977), however, suggested that the ciliary necklace, stabilized by its linkage to the axonemal microtubules, is implicated in barrier phenomena of membrane fluidity. According to their interpretation, "the ciliary necklace could stop the migration of large proteins and other oligomeric intramembranous complexes toward the ciliary shaft." Another possible explanation for the great diversity in the arrangement of the rows of the ciliary necklace has been suggested: that it may represent the result of different membrane-microtubular interactions during the phases of ciliary beating (Sattler & Staehelin, 1974).

SUMMARY

The existence of a ciliary necklace in kinocilia, atypical cilia and mature and differentiating sensory cilia is described in rat and chicken. In addition to parallel horizontally oriented rows of the ciliary necklace, irregular forms of intramembranous particle (IMP) aggregations are also described.

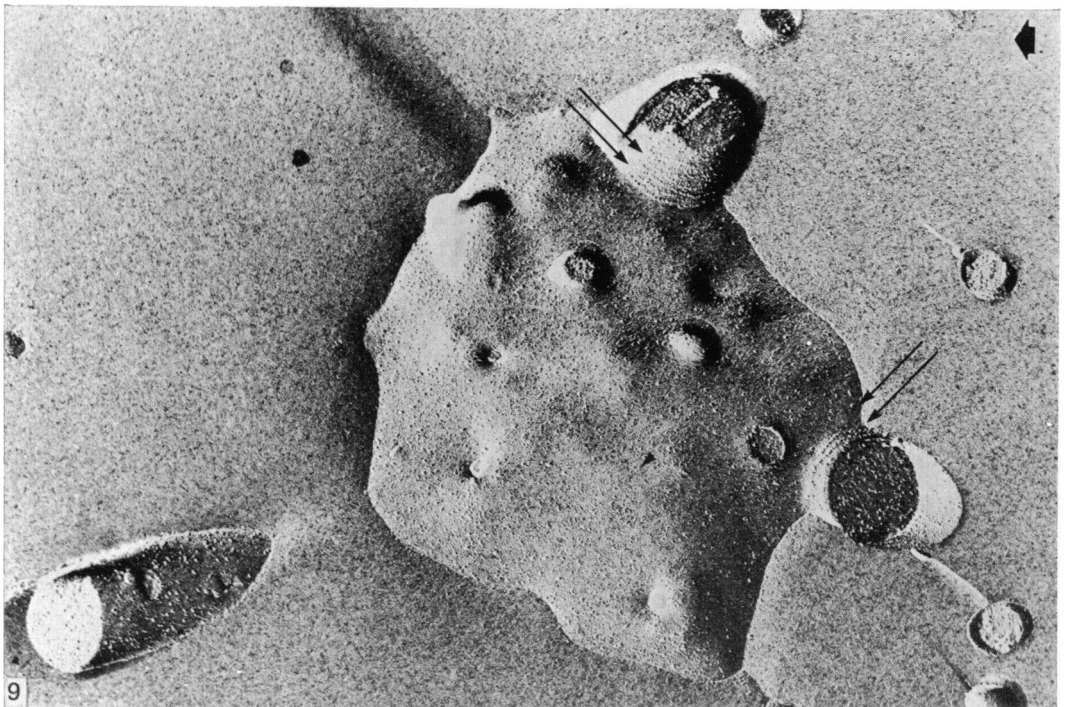


Fig. 8. Olfactory knob of 11 days old chick embryo. Prior to the ciliary outgrowth a typical circular arrangement of IMP can be observed (arrows). $\times 91100$.

Fig. 9. Olfactory knob of 15 days old chick embryo. The newly emerged cilia depict typical necklace structures (double arrows). $\times 67000$.

The function of the ciliary necklace is discussed, special attention being given to the appearance of circular rows of IMP prior to the outgrowth of olfactory cilia and to the existence of dynein arms in these.

Evidence is provided for the motility of olfactory cilia in the chick.

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