OLFACTORY CILIA IN THE FROG

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ABSTRACT

Olfactory epithelium from the frog was examined in the living state by light microscopy and in the fixed state by electron microscopy. Particular attention was paid to the layer of cilia and mucus which covers the surface of the epithelium. The olfactory cilia differed from typical cilia in that they (*a*) arose from bipolar neurons and had centrioles near their basal bodies, (*b*) were up to 200 microns in length, of which the greater part was a distal segment containing an atypical array of ciliary fibers, (*c*) were often immotile, (*d*) had their distal segments arranged in parallel rows near the surface of the mucus, and (*e*) had many vesicles along their shafts and had splits in the array of fibers in their distal segments. These specializations make the olfactory cilia similar to cilia found on other sensory cells and support the theory that they are the locus where electrical excitation in the olfactory organ is initiated by contact with odorous substances.

INTRODUCTION

Schultze, one of the first histologists to study the olfactory epithelium of the frog, described fine "hairs" arising from the bipolar neurons and postulated that these hairs are important for sensing odors (29). Later, Hopkins (19) confirmed that these hairs are prevalent and well developed in the frog, reaching lengths of 200 μ, and Ottoson (22) found that the olfactory epithelium of the frog, like that of mammals, can respond to odors with specific electrical responses. Bloom examined the frog's olfactory hairs with the electron microscope and showed that, near their origins from the bipolar neurons, they are typical cilia (4). Since parts of other sensory cells, such as the outer segments of the retinal rods, are actually cilia with certain structural modifications at their ends (9), it was thought that the distal ends of the olfactory cilia might be modified also. The present study was undertaken in order to examine in some detail the layer of mucus and cilia covering the surface of the olfactory epithelium in the frog.

MATERIALS AND METHODS

Adult *Rana clamitans* or *R. catesbeiana*, obtained from neighboring ponds or from the Carolina Biological Supply Company, were used for this investigation. No differences were found between these species.

For examination with the phase microscope, olfactory epithelium was stripped from the nasal cavity, folded once in a drop of Ringer-Locke's solution in a small chamber on a slide, and sealed under a coverglass in such a way that one could look across the edge of the fold in the epithelium. In this manner the surface of the epithelium was examined within a few minutes of decapitation. Isolated cells were prepared by maceration of the olfactory epithelium in calcium-free Ringer's solution containing 0.01 per cent Versene. For examination by reflected light with a Reichert Universal Opaque Illuminator, the roof of the nasal cavity was removed immediately after decapitation and its undisturbed surface examined in a humid chamber, or, in some cases, the surface of the olfactory tubercle was examined *in situ* in a pithed but living frog.

For electron microscopy, the tissue was fixed by immersion in a solution of 1.0 per cent osmium tetroxide buffered with either sodium phosphate or Veronal-acetate to pH 7.6–7.8. Small amounts of sodium chloride and calcium chloride were added to the fixative so that its calculated osmolarity was equivalent to 0.65 per cent sodium chloride. The whole floor or roof of the nasal cavity was immersed in cold fixative for 30 minutes, the tissue being cut into small blocks for sectioning only after embedding was completed. This method avoided damage to the delicate mat of cilia and mucus covering the epithelium and allowed the orientation of the cilia to be observed by reflected light microscopy so that sections could be cut with a suitable orientation to the cilia. The material was dehydrated in acetone or alchohol and embedded in Araldite. Sections were cut with glass knives on a Porter-Blum microtome, picked up on Parlodion-coated grids, and stained with lead citrate (25), uranyl acetate, or both. Sections for light microscopy were cut at 0.5 μ and stained with toluidine blue (26). Serial sections for the electron microscope were cut at about 75 mμ, picked up with a Parlodion film on a loop, double stained, and then transferred to a slotted grid and stabilized with carbon. Six series of 10 to 40 sections each were examined in this manner, as many as 16 areas in each section being photographed. Observations were made with a RCA 3D microscope with a 25-μ aperture.

OBSERVATIONS

Light Microscopy

Examination of living olfactory epithelium *in vitro* with a phase microscope in a manner similar to that used by Hopkins (19) served to confirm his results. In some preparations, regardless of whether the roof of the nasal cavity or the olfactory tubercle was examined, two types of cilia were found, an immotile type 80 to 200 μ long and a motile type usually 25 to 50 μ long but occasionally as long as 75 μ. Although the most frequent finding was this intermingling of motile and non-motile cilia, in some areas in the same preparation only one type was present. The beat of the motile cilia was quite different from the synchronous beat of the cilia in the neighboring respiratory region, for each olfactory cilium moved in several different directions with dissociated pedular and uncinate movements which appeared to be independent of surrounding cilia. When preparations were made quickly with minimal mechanical disturbance, the cilia were found embedded in a dense coat of mucus 20 to 35 μ thick which appeared as a refractile mass in which few individual cilia could be distinguished and in which few motile cilia were seen. However, when the epithelium was soaked in Ringer's solution for some minutes before mounting, the mucus dissolved and the cilia which had been matted down parallel with the surface of the epithelium could then extend to lie perpendicular to the surface (Fig. 5). Isolated bipolar neurons were also examined in order to count the cilia originating from each neuron. The average number was 6 and the maximum number was 8. Supporting cells had no cilia.

As preparation for examination with the phase microscope involved tearing the axons of the bipolar neurons, as well as other damage to this material, the epithelium was examined *in situ* by means of reflected light microscopy. With this method, only those parts of cilia which reach the surface of the mucus can be seen, and these were the shafts of the cilia long enough to cross the 20 to 35 μ thick layer of mucus covering the epithelium. In these preparations all the cilia in the olfactory region appeared immotile, in contrast to the synchronously beating cilia in the respiratory regions (Fig. 4).

When carbon particles were put on the surface of the mucus, they moved with the mucus to the neighboring respiratory regions at a rate of less than one millimeter per second, and were then carried rapidly to the posterior nares. The lines of flow of the carbon particles in the various areas of the olfactory region were consistent from preparation to preparation, and the immotile olfactory cilia were passively arranged in bundles lying in parallel rows along these lines of flow. Probing the mucus with a fine needle indicated that a very thin layer of watery mucus covered a thicker viscid layer on which the cilia floated, and that it was the movement of this thin watery layer which moved the carbon particles. Continuous production of watery mucus in the olfactory region with its continuous removal by the motile cilia in the respiratory region appeared to be the cause of this flow.

Electron Microscopy

When the olfactory epithelium was prepared for electron microscopy without disturbing its layer of mucus, the mucus was condensed to less than one-half its former thickness and its surface typically appeared as a distinct dense line (Fig. 8).

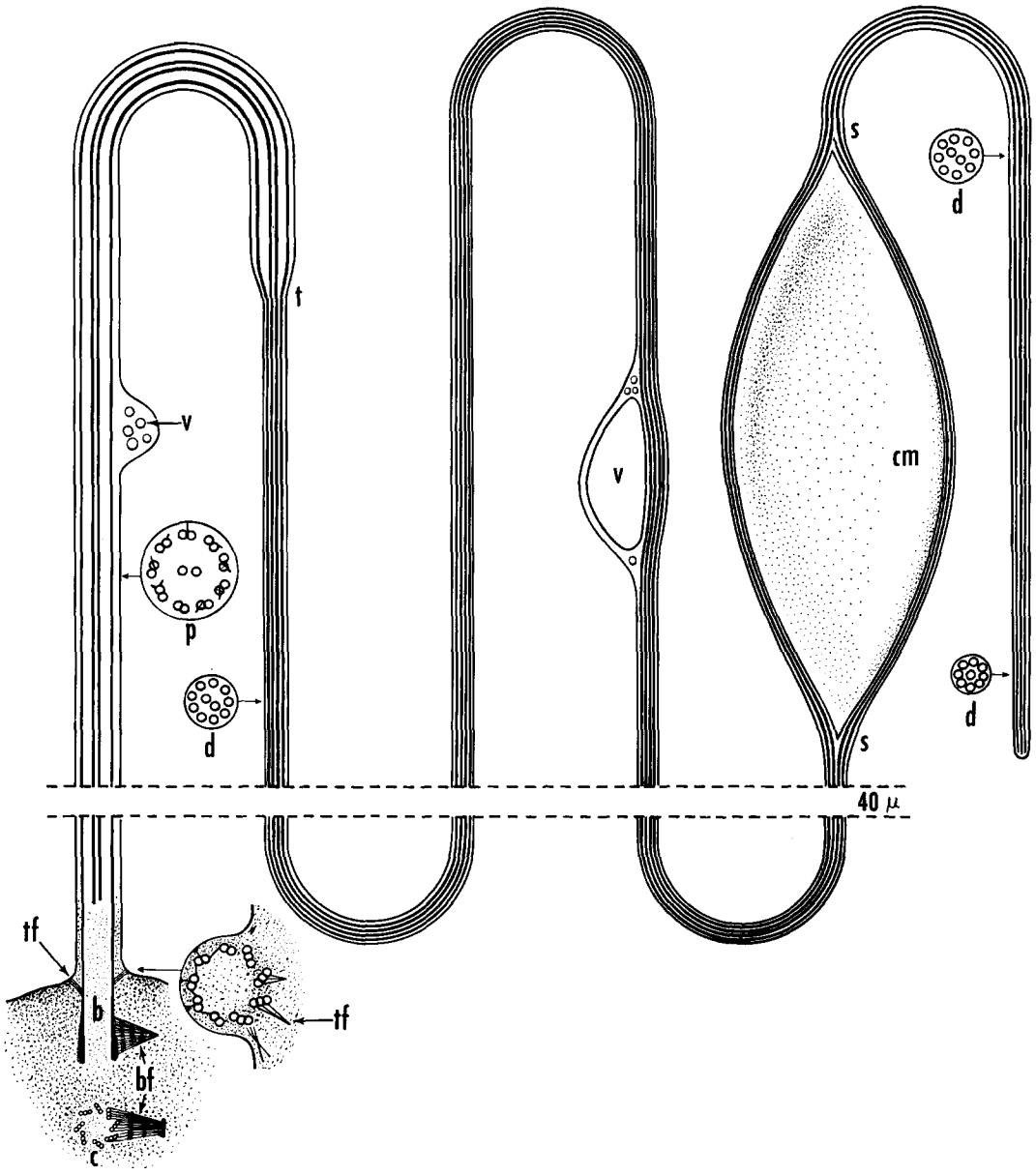


FIGURE 1 Diagrammatic reconstruction of an olfactory cilium originating from the dendrite of a bipolar cell. In the natural state the cilium would be fully extended, with its long distal segment lying near the outer surface of the mucus which covers the epithelium. As shown here, it is 200 μ long, which is the maximum length for olfactory cilia. Splits in the array of fibers (*s*) bridged by a double fold of ciliary membrane (*cm*) occur on approximately one-tenth of the olfactory cilia; vesicles (*v*) probably occur on all the olfactory cilia. *b*, basal body; *bf*, basal foot; *c*, centriole; *cm*, double fold of ciliary membrane; *d*, cross-section of distal segment; *p*, cross-section of proximal segment; *s*, split in the array of fibers; *t*, transition between proximal and distal segment; *tf*, transitional fibers; *v*, vesicle.

However, the cilia remained intact and, since they all ran in the same direction in the mucus, it was possible to cut virtually all of them in cross-section (Figs. 3, 6, and 7). Although both short motile and long immotile cilia appeared to be present in some of the living preparations, all the cilia looked alike when examined with the electron microscope. The proximal segments of the ciliary shafts, whose cross-sections lay near the olfactory cells, contained the typical arrangement of fibers in which nine outer pairs of subfibers surround two central fibers. The distal segments of the shafts, whose cross-sections lay near the surface of the mucus, contained only single fibers (Figs. 3, 6 to 17). Transitions from the double to the single pattern, accompanied by a decrease in the diameter of the shaft from about 250 mμ to 150 mμ, were observed in series of cross-sections, where the complete taper occurred in less than 0.5 μ. Transitions were difficult to identify in longitudinal sections, as the cilia undulated in and out of the plane of section, but Fig. 22 probably represents a transitional zone. The cilia ended near the surface of the mucus, and in longitudinal sections tips were found only on the distal segments, never on proximal segments. At the tip, the ciliary membrane folded over the array of single fibers, with the fibers ending just short of the tip (Fig. 21). Since 80 per cent of the ciliary cross-sections contained only single fibers, it was estimated that the distal segment of the average cilium accounted for about 80 per cent of its length. This estimate could be made because almost all the cilia ran in the same direction in the mucus, thereby contributing only one cross-section to any field of cross-sections. These findings permitted the con-

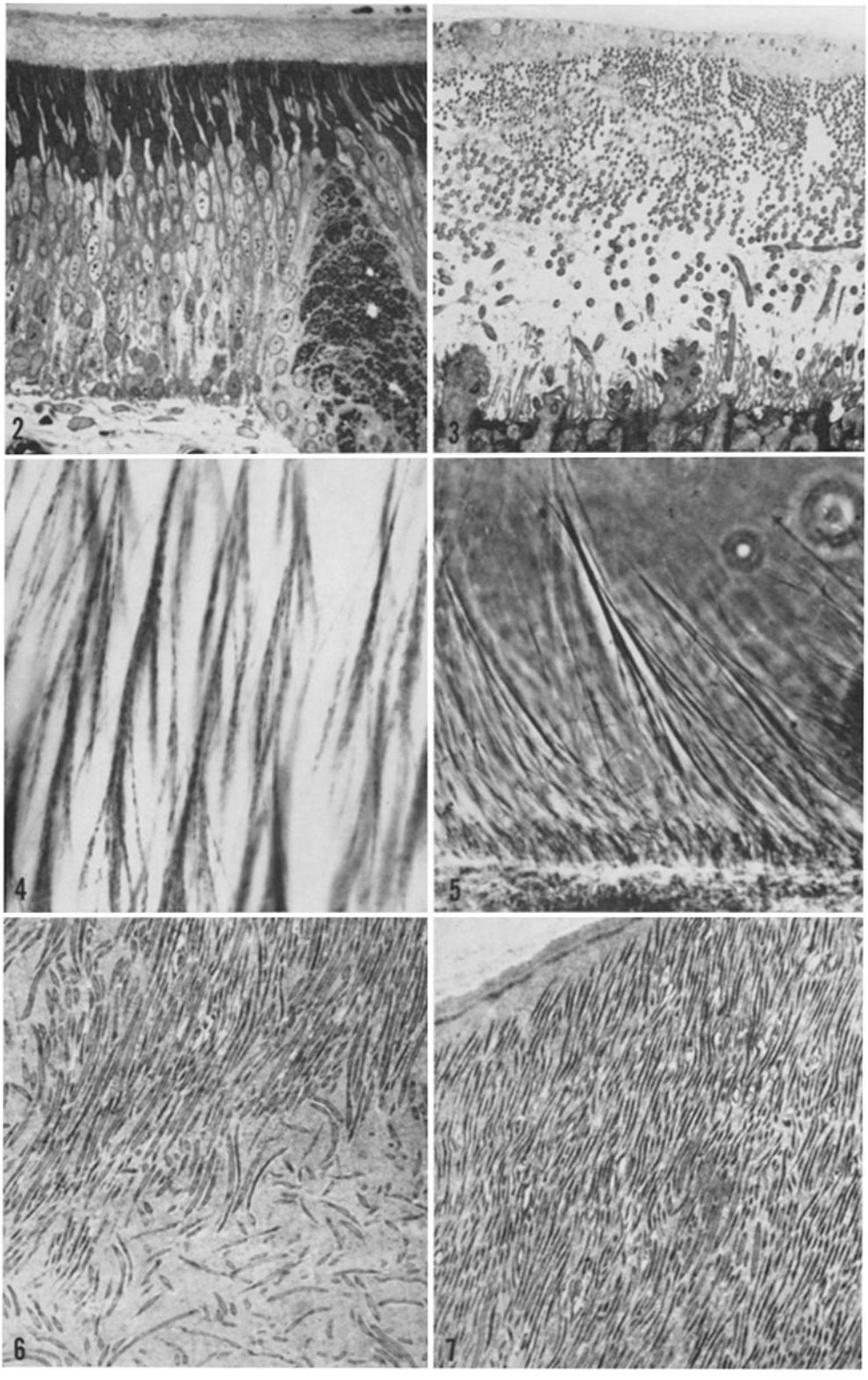
FIGURE 2 Light micrograph of olfactory epithelium from the roof of the nasal cavity. Dark-stained cells at the surface of the epithelium are supporting cells. Lighter-stained cells deeper in the epithelium are olfactory bipolar neurons. Their dendrites run between the supporting cells and end in small swellings, the olfactory "vesicles", at the surface of the epithelium. The mat of cilia and mucus covering the epithelium is preserved, as well as blood cells which floated on the mucus before fixation. × 310.

FIGURE 3 Electron micrograph of mat of cilia and mucus covering the epithelium. Olfactory vesicles extend up from the bottom of the picture. Practically all the cilia are cut in cross-section. Note how the larger cross-sections of the proximal segments of the cilia, lying near the olfactory vesicles, are outnumbered by the smaller cross-sections of the distal segments, lying near the surface of the mucus. Between the olfactory vesicles, microvilli from the supporting cells extend vertically for short distances. At the lower right is a space left by the discharge of a secretory droplet. Double stained. × 4900.

FIGURE 4 Negative of micrograph taken with light reflected from the surface of living, intact olfactory epithelium. Bundles of olfactory cilia lie in parallel rows near the surface of the mucus. At this magnification, individual cilia probably cannot be seen. It is not known why the bundles of olfactory cilia have a beaded appearance. Exposure, 30 seconds. × 310.

FIGURE 5 Phase micrograph looking across the surface of living olfactory epithelium mounted in Ringer's solution. Shorter cilia which move in an irregular manner arise from the surface of the epithelium, shown near the bottom of the picture. The longer immotile cilia are free to extend almost to the top of the picture because the Ringer's solution has dissolved the mucus which normally holds them parallel to the surface of the epithelium. × 470.

FIGURES 6 AND 7 Electron micrographs of sections cut nearly parallel to the surface of the epithelium. At the bottom of Fig. 6, deep in the mucus near their origins, proximal segments of cilia curve up to take their places in the parallel rows of cilia seen at the top of the picture. At the top of Fig. 7, the cilia end near the surface of the mucus covering the epithelium. At this level only distal segments of cilia are found. Lead citrate. × 3600.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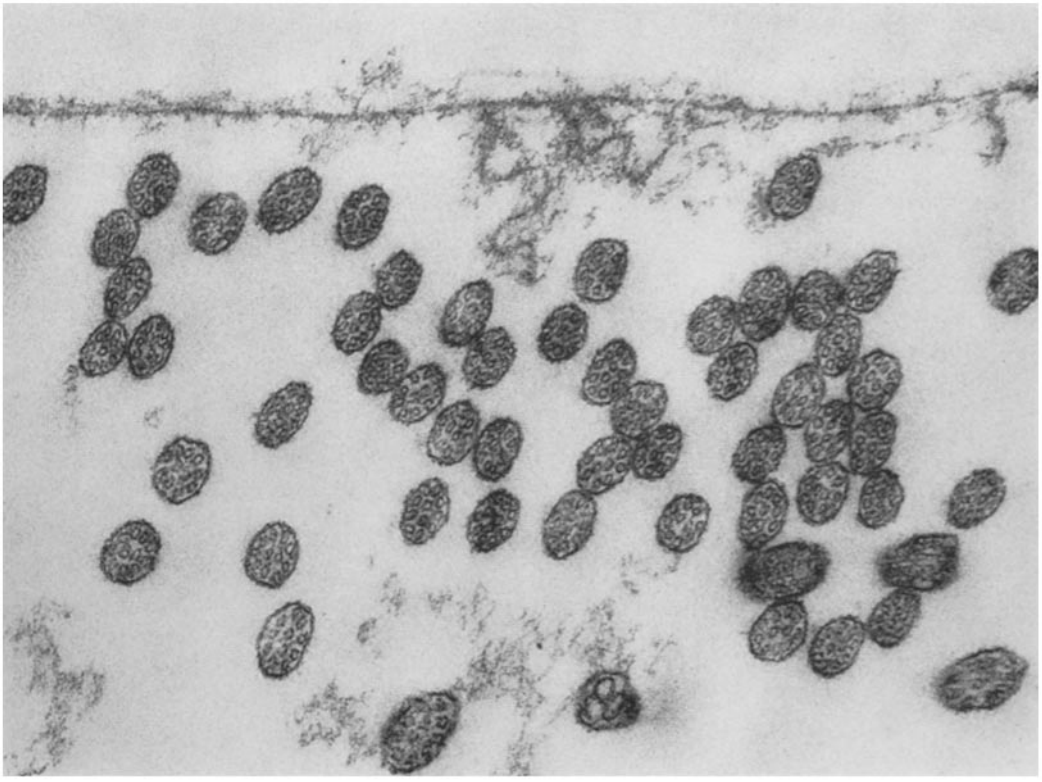


FIGURE 8 Section perpendicular to the surface of the epithelium. The surface of the mucus, typically appearing as a dense line, is at the top of the picture, and just below it the distal segments of many cilia are cut in cross-section. In places they are clumped together, the clumps perhaps corresponding to the bundles of cilia seen in the living preparations (Fig. 4). The group of vesicles at the bottom of the picture are probably an example of the vesicles which occasionally occur at the tips of the olfactory cilia. Double stained. × 76,000.

struction of a diagram of a typical olfactory cilium (Fig. 1).

Several arrangements of single fibers within distal segments of the ciliary shafts were found (Figs. 9 to 16), their frequencies allowing an estimate to be made of the proportion of the length of the distal segment which they occupied. In about 65 per cent of these cross-sections, nine single fibers surrounded two central ones (9 plus 2). The other frequencies were: (9 plus 1) 1.5 per cent, (8 plus 3) 5.5 per cent, (8 plus 2) 16 per cent, (8 plus 1) 5.5 per cent, (7 plus 1) 4 per cent, (6 plus 1) 0.5 per cent. Arrangements with less than eleven fibers had a smaller diameter and were usually found just at the surface of the mucus, probably representing a short portion of the distal segment lying near the tip of each cilium. In all cross-sections the thinner walls and slightly smaller diameters of the single fibers, in both the proximal and distal segments, distinguished them from the outer double fibers of the proximal segment (Figs. 9 to 17). In longitudinal sections of the proximal segments, the double fibers consistently appeared homogeneous and smooth in outline, whereas the pair of central fibers had a striated appearance and a scalloped outline with a period of about 125 A, which in other cilia has been interpreted as being due to an intrinsic periodicity in the fibers, as well as to a system of fine fibers surrounding them (16, 17; Figs. 18 and 19). The single fibers in the distal segments also had a striated appearance and a scalloped outline, both with a periodicity of about 160 A (Fig. 20). It was impossible to tell whether this was due to a system of surrounding fibers or was an intrinsic variation in their wall. The single fibers in the distal segments

appeared in serial sections to be continuous with the outer fibers of the proximal segments. However, no pictures were clear enough to demonstrate which of the two subfibers in the proximal segments gave rise to the single fibers in the distal segments.

Fine radial fibers passing between the double and single fibers in the proximal segments were seen in some cross-sections. In serial cross-sections, they passed from the region of the central fibers to different outer fibers in different sections. In longitudinal sections, these fine fibers appeared to be incomplete in most instances, a consistent finding even in preparations where the tonicity and type of buffer used with the fixative were varied. Fine fibers also passed between some of the single fibers in the outer segments (Figs. 9 to 16).

Consideration of all the findings with the light and electron microscopes raised the possibility that the short moving cilia could be remnants of the longer ones, broken during the extensive handling needed to prepare living specimens for phase microscopy. Since the olfactory cilia are made up of a thick proximal segment and a thinner distal one, it is apparent how random breakage of the shafts of these cilia could seem to produce a long and a short type of cilium. The thin distal segment would be more subject to breakage, leaving the thicker proximal segments 20 to 40 μ long, which is the length reported for the short type of cilium.

Observations on the distribution of the two types of cilia were consistent with this hypothesis. The proportions of the two types were highly variable from preparation to preparation although their density was fairly constant. In some areas only the long immotile cilia were found. In others, where only short motile cilia were found, the mucus was missing and bits of mucus containing ciliary fragments were sometimes found free in the mounting medium.

In order to accept this hypothesis, one has to suppose that the motion of these cilia could be initiated by breaking their shafts, a supposition supported by no direct evidence. However, the motions of the shorter cilia were unlike those of any other known cilia in that they were irregular and uncoordinated; this was certainly not a ciliary beat comparable to that in other cilia.

Finally, examination of the living epithelium *in situ* with reflected light microscopy, involving minimal handling of the tissue, failed to show a ciliary beat in the olfactory region, although a beat was easily seen in the respiratory regions.

Two variations in structure were observed in the shafts of the olfactory cilia. The first variation was found only in the distal portions of the shafts which contained single fibers (Figs. 26 to 31). The array of fibers divided and progressively diverged, leaving suspended a double fold of ciliary membrane. As few as three fibers might be found on one side. After approximately three to five microns, the divided array of fibers came together again. In a field containing 2000 cross-sections of cilia, this structure was found in 5, or 0.25 per cent, of the cross-sections. If each of these cross-sections is from a structure 3 to 5 μ long occurring on the shafts of cilia 150 μ long, it can be calculated that there are enough of these structures for one to be present in roughly 10 per cent of the cilia. A second variation consisted of groups of vesicles of different sizes between the ciliary membrane and the array of fibers in the shaft of a cilium (Figs. 23 to 25). In the proximal portion of the shafts, small vesicles or groups of small vesicles were found between the outer fibers and the ciliary membrane. These vesicles occurred in a high enough proportion of cross-sections for the assumption to be made that each cilium had some in the proximal portion of its shaft. Similar vesicles were found in the shafts of cilia from respiratory regions. Their presence in both locations did not seem to depend on the tonicity or the type of buffer used in the fixative. Vesicles were also found in the distal portions of the ciliary shafts (Figs. 23 and 24). Again there were enough of these vesicles to permit the assumption that one could occur in each cilium. Most of these vesicles were of greater diameter than those found in the proximal segments of olfactory cilia or in the respiratory cilia. The largest were 0.5 μ in diameter, and these were surrounded by a few smaller vesicles. The wall of these vesicles was similar in thickness to the ciliary membrane.

All the cilia in the olfactory region arose from basal bodies in the bulbous apices of the dendrites of the bipolar cells. These distal enlargements of the bipolar cells corresponded to the olfactory "vesicles" which extend above the surface of the epithelium (Figs. 2, 3, 58, and 59). They were like vesicles in that a mantle of relatively dense fibrillar cytoplasm, resembling a terminal web, surrounded a less dense core containing microtubules, microvesicles, and mitochondria. The

basal bodies that gave rise to the olfactory cilia were embedded in this dense mantle; centrioles unattached to cilia were dispersed through the entire tip of the dendrite. Microtubules 180 to 200 A in diameter lay in parallel rows in the shafts of the dendrites, and in the apices of the dendrites ran in different directions around the centrioles and basal bodies (32; Figs. 58, 59). Few mitochondria were found in the apices of the dendrites, but many lay among the parallel rows of tubules in the dendritic shafts and, like mitochondria in other nerve cells, contained no granules (6; Fig. 57). Vesicles of various diameters, some with "coated" rims (27), were found in the apices of the dendrites in numbers which varied greatly from one dendrite to the next. Some connected with the surface of the dendrite, as in pinocytosis (Fig. 56).

The centrioles in the tips of the dendrites usually lay deep in the cytoplasm but occasionally were found in close proximity to the cell membrane (Fig. 47). Since the apex of a dendrite might contain five or six centrioles and five or six basal bodies, it was difficult, on the basis of a single section, to determine the relationship of a centriole to other centrioles, to basal bodies, or even to the cell membrane. These relationships were seen in serial sections through the apices of the dendrites. In one series, one centriole lay free in the cytoplasm proximal to a basal body, while another centriole, in a similar position, was found in deeper sections to reach the cell membrane (Figs. 32 to 37). Examination of other series of sections through the apices of the dendrites showed many different degrees of contiguity between centrioles and basal bodies, and it was impossible to find any evidence of pairing of centrioles, or of centrioles and basal bodies. The numbers of basal bodies, centrioles in close proximity to the cell membrane, and free centrioles appeared to vary independently.

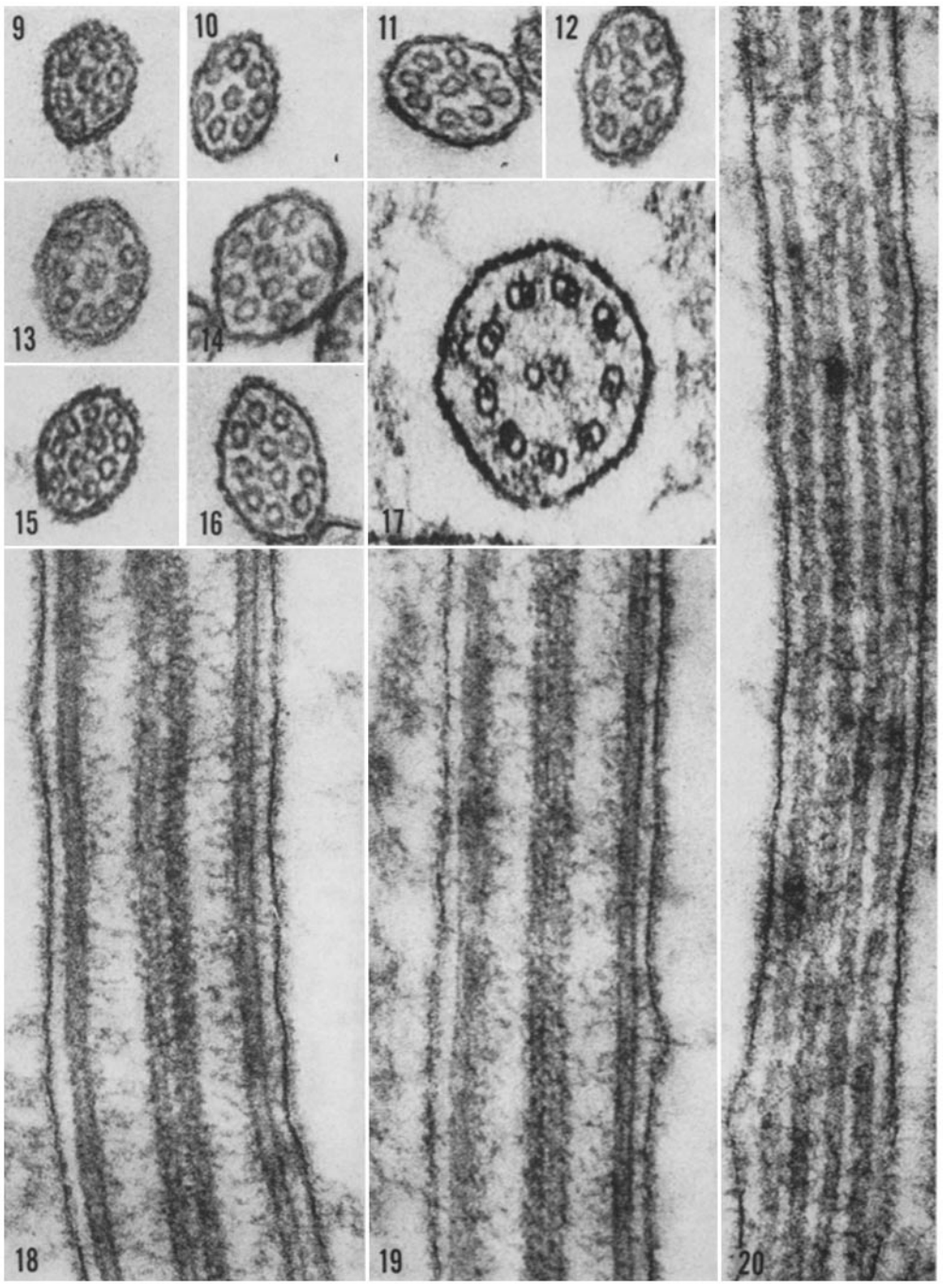
All the centrioles and basal bodies in the tips of the dendrites had the same basic structure, consisting of nine sets of three tubules or subfibers forming a cylinder 300 to 400 mμ long (Figs. 49 to 53). The subfibers in the shaft of a cilium were continuous with two of the subfibers in each triplet in a basal body. As in other cilia, the pairs of subfibers in an olfactory cilium, near a basal body, increased their inclination away from the tangent to about 20 degrees, while the sets of subfibers at the distal end of a basal body inclined about 30 degrees and at the proximal end the inclination had increased to more than 60 degrees (17). About a micron from the basal body, the central fibers of the proximal segments ended and arms were no longer present on the outer fibers. Bridges connected each pair of subfibers to its neighbor and to the ciliary membrane, where the radial arm of the bridge ended in a dense mass lying against the ciliary membrane (Fig. 48). These bridges were not identified in longitudinal sections but they were present down to the level of the surface of the dendrite. Somewhat below the beginning of the bridges, a variable amount of dense amorphous material appeared around

FIGURES 9 TO 16 Cross-sections of distal segments of olfactory cilia. Nine single fibers surrounding two central ones is the arrangement most frequently seen. The patterns with less than eleven fibers probably occur in a short portion of the tips of the distal segments. The cilia are oriented so that the surface of the mucus is upward. Where two central fibers are present, they are usually oriented vertically. Double stained. × 170,000.

FIGURE 17 Cross-section of the proximal segment of an olfactory cilium. This is a typical ciliary cross-section. In this section radial links between outer and central fibers are not seen, but occasionally they are seen in other cross-sections. Double stained. × 170,000.

FIGURES 18 AND 19 Longitudinal sections of proximal segments of olfactory cilia. The slightly narrower central fibers have a striated appearance which clearly distinguishes them from the outer fibers. Uranyl acetate. × 135,000.

FIGURE 20 Longitudinal section of the distal segment of an olfactory cilium. All fibers look alike. They are narrow and, like the central fibers of the proximal segment, have a striated appearance. The period, however, is slightly greater than that of the central fibers in the proximal segments. Uranyl acetate. × 135,000.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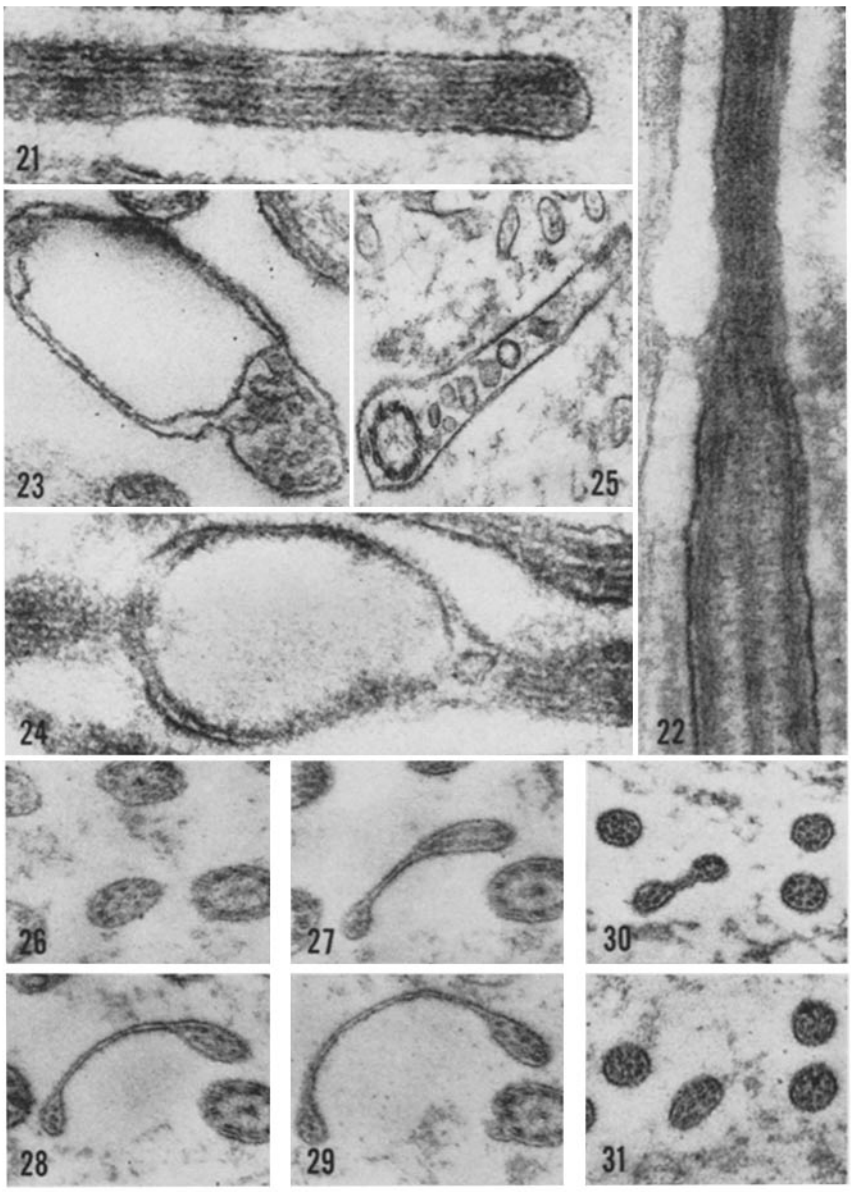

FIGURE 21 Tip of an olfactory cilium. The distal segments end near the surface of the mucus covering the epithelium. Vesicles are occasionally found under the ciliary membrane at the tip. Lead citrate. × 96,000.

FIGURE 22 Longitudinal section of an olfactory cilium showing a transition between a proximal and a distal segment. A sudden decrease in the diameter of the cilium is accompanied by a change from double to single outer fibers. These changes have been followed in serial cross-sections. Lead citrate. × 72,000.

FIGURE 23 Cross-section of a large vesicle in the distal segment of an olfactory cilium. Double stained. × 96,000.

the ciliary fibers, and in some cilia a structure resembling a more or less complete basal plate was present at this level, but it differed from the basal plates of other cilia in that the surrounding ciliary membrane was not adherent to the ciliary fibers (compare Figs. 54, 55, and 57). The transition from the doublet to the triplet pattern occurred farther down the shaft at the surface of the dendrite (Fig. 49). Just below this transition, three "transitional fibers" (17) left each triplet and ran obliquely to the cell membrane of the dendrite (Fig. 49). Transitional fibers were also found on centrioles whether or not they were in close proximity to the cell membrane, but when a centriole was not in close proximity to the cell membrane the transitional fibers were without attachment to another structure (Fig. 38). Just below the origins of the transitional fibers, there were no appendages on the centrioles or basal bodies but their sets of triple fibers were surrounded by a dense, amorphous material which became more prevalent in proportion to the nearness of the proximal end. Most centrioles and basal bodies gave rise proximally to a basal foot formed of many fine fibers originating from three of the sets of triplets and converging on a dense "basal bar" (Figs. 36, 40, 45, 51, 52, and 56). Two or three double bands of greater density crossed each basal foot, spaced 35 to 40 mμ apart (Fig. 56). In a few instances, serial sections through centrioles or basal bodies failed to reveal a basal foot, but, when present, the basal foot was never directed centrifugally or proximally in the tip of the dendrite (16; Fig. 46). Occasionally a basal foot appeared to bifurcate, or two basal feet arose from one centriole (Fig. 51). Many microtubules in the apices of the dendrites converged on basal bars (16; Fig. 56). On the proximal ends of some of the centrioles and basal bodies there was a very fine non-striated root about 250 mμ long. A few centrioles and basal bodies had vesicles or granules in their centers (Figs. 50, 51, and 59).

Rootlets, granules, vesicles, or basal feet were present in some centrioles and basal bodies and were absent in others. However, on the basis of serial sections through centrioles and basal bodies from different regions of the dendrites, it was found that, except for their continuity with a ciliary shaft, there were no structural criteria for distinguishing basal bodies from centrioles (Figs. 32 to 45). The basal bodies in the dendrites of the bipolar cells were, however, easily distinguishable from those at the proximal ends of cilia found in the respiratory region, which had prominent striated roots and a dense, double basal plate with a closely adherent ciliary membrane (Fig. 57).

The apical ends of supporting cells were also found at the surface of the olfactory epithelium, where several dendrites surrounded each supporting cell. Supporting cells were joined to each other and to dendrites of bipolar cells by terminal bars (Figs. 59 to 61), but, at this level, contacts be-

FIGURE 24 Longitudinal section of a large vesicle in the distal segment of an olfactory cilium. The vesicle deflects the fibers of the cilium so that they pass under the plane of section. A smaller vesicle accompanies the larger vesicle. Large vesicles probably occur in the distal segments of most of the olfactory cilia. Lead citrate. × 96,000.

FIGURE 25 Cross-section of a group of small vesicles in the proximal segment of an olfactory cilium. This cilium is cut very near its origin, so that central fibers are absent. Groups of small vesicles probably occur in the proximal segments of most of the olfactory cilia. Double stained. ×41,000.

FIGURES 26 TO 29 Sections 1, 7, 14, and 20 from a series which is approaching the tip of the distal segment of an olfactory cilium. The array of fibers divides and progressively diverges, leaving suspended a double fold of ciliary membrane. The surface of the mucus is upward. Double stained. × 45,000.

FIGURES 30 AND 31 Sections 1 and 5 from a series which is also approaching the tip of the distal segment. The subfibers here converge and re-form into a single array. Similar splits in the array of fibers probably occur in about 10 per cent of the olfactory cilia. Double stained. × 40,000.

tween dendrites were not found. Near the surface of the mucus, at the distal ends of the terminal bars, were typical tight junctions or zonulae occludentes (13). Here, the intercellular space was approximately 80 A and cell membranes lay strictly parallel to each other. In places a fine line bisected the extracellular space, probably representing a fusion of the outer leaflets of the cell membranes. In sections parallel to the surface of the epithelium, the tight junctions were seen continuing for long distances around the dendrites. They were continuous with similar junctions around the supporting cells, so it is likely that tight junctions completely surround the distal ends of the olfactory and supporting cells (Fig. 62). Proximal to the tight junctions were typical intermediate junctions or zonulae adhaerentes (13). Here, the intercellular space widened to approximately 180 A and a dense material was found between and around the cell membranes, in places forming a dense layer or plate just inside the cell membrane. The intermediate junctions also appeared to form continuous belts around the distal ends of the supporting and olfactory bipolar cells (Fig. 62). Deeper still and occurring at several levels, junctions were found between supporting cells and between supporting cells and dendrites, which resembled desmosomes or maculae adhaerentes in that they were macular, had distinct dense plates on the inner surfaces of the cell membranes, had a wide intercellular space (approximately 220 A), and had distinct, coarse fibers running from these plates into the cytoplasm (13; Figs. 59 to 63). They differed from typical desmosomes, however, in that coarse fibers ran completely across the extracellular space (Fig. 60).

The distal processes of the supporting cells were packed with large secretory droplets resembling those in goblet cells (23; Figs. 61 to 63). They were smaller and stained much less intensely with toluidine blue than the secretion droplets in the Bowman's glands. A smooth vesicular endoplasmic reticulum surrounded the droplets and in places appeared continuous with the membranes surrounding them. Multivesicular bodies and dense granules 90 to 100 A in diameter, probably ribonucleoprotein, were present among the secretion droplets, but ergastoplasm was not present at this level (Figs. 61 and 63). In places a terminal web occurred between the secretion droplets and the distal ends of the supporting cells, but elsewhere droplets were seen which reached the cell membrane, or were penetrating it, or were being discharged into the mucus covering the epithelium (Fig. 61). Unlike the droplets in goblet cells, however, these secretion droplets appeared to break through the cell membrane only one or a few at a time, and depleted or empty supporting cells were not found. The distal ends of the supporting cells were covered by a dense mat of microvilli of fairly uniform diameter but varying in length up to 3 μ (Figs. 3, 58, and 59).

DISCUSSION

Investigations of the electrical responses of olfactory epithelium to odors have shown that the olfactory cilia may be points at which electrical stimulation is initiated by contact with odorous

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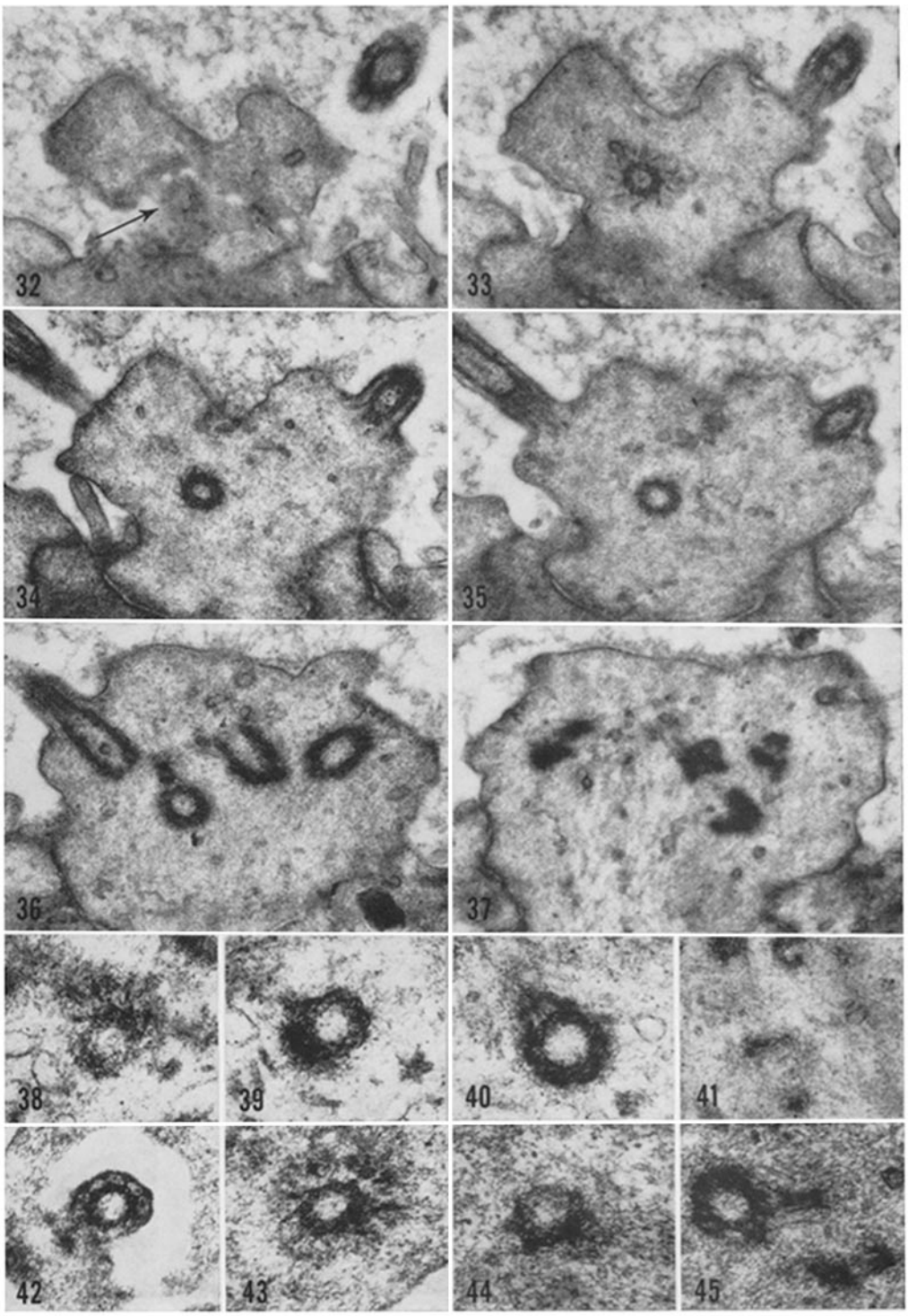
FIGURES 32 TO 37 Sections 1, 2, 3, 4, 6, and 8 from a series cut perpendicular to the surface of the epithelium. Two basal bodies, two centrioles, and part of a third centriole are present in the part of an olfactory vesicle shown here. One centriole reaches the cell membrane (arrow, Fig. 32) while the other centriole does not. The centriole which reaches the cell membrane has transitional fibers at its distal end (Figs. 33 and 34) and a basal foot at its proximal end (Fig. 36). Double stained. × 32,000.

FIGURES 38 TO 41 Sections 1, 3, 4, and 7 from a series passing through a centriole which does not reach the cell membrane. Transitional fibers are found at one end (Fig. 38) and a basal foot at the other (Fig. 40). Fig. 41 shows the proximal end of a basal body lying just above the end of the centriole. Double stained. × 55,000.

FIGURES 42 TO 45 Sections 1, 3, 5, and 11 from a series passing through a basal body. Like the centriole shown above, it has transitional fibers at its distal end (Fig. 43) and a basal foot at its proximal end (Fig. 45). Double stained. × 55,000.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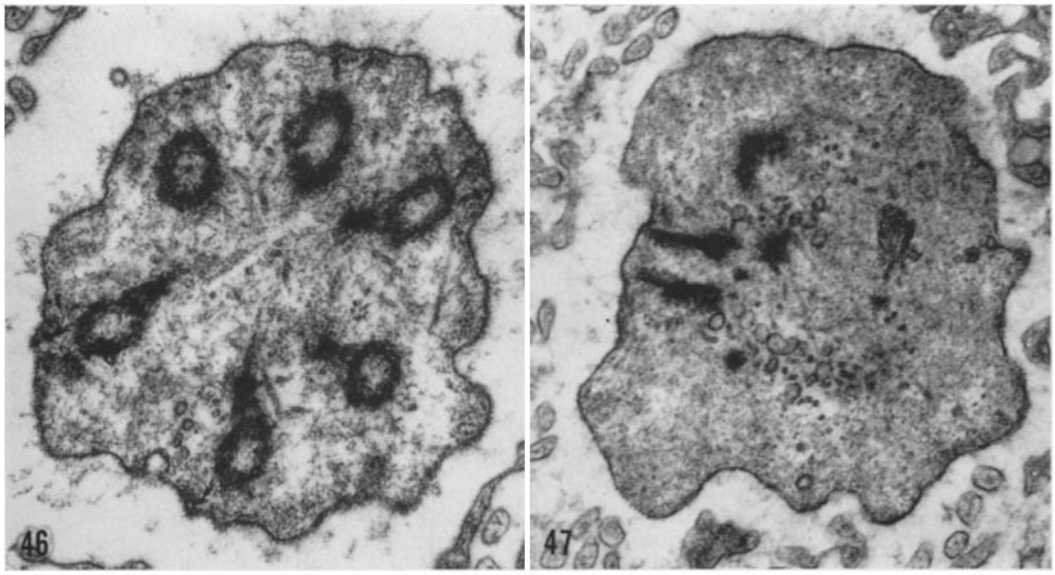


FIGURE 46 Section parallel to the surface of the epithelium which passes through an olfactory vesicle. Basal feet, as seen here, typically point in a centripetal direction, and in vertical sections point toward the surface of the mucus. Double stained. × 35,000.

FIGURE 47 Section parallel to the surface of the epithelium passing through an olfactory vesicle, to show a centriole in close proximity to the cell membrane. Double stained. × 34,000.

FIGURE 48 Cross-section of a cilium just above its basal body, showing bridges between pairs of subfibers and between the subfibers and the ciliary membrane. An amorphous material is present around the fibers at this level. The circular cross-section in the center is probably the end of one of the central fibers. Double stained. × 110,000.

FIGURE 49 Transition between a cilium and its basal body, just at the level of the cell membrane. The open space at the top of the picture is extracellular, and the fibers just below it are double while the fibers at the bottom of the picture are triple. Transitional fibers originate at this level. Uranyl acetate. × 110,000.

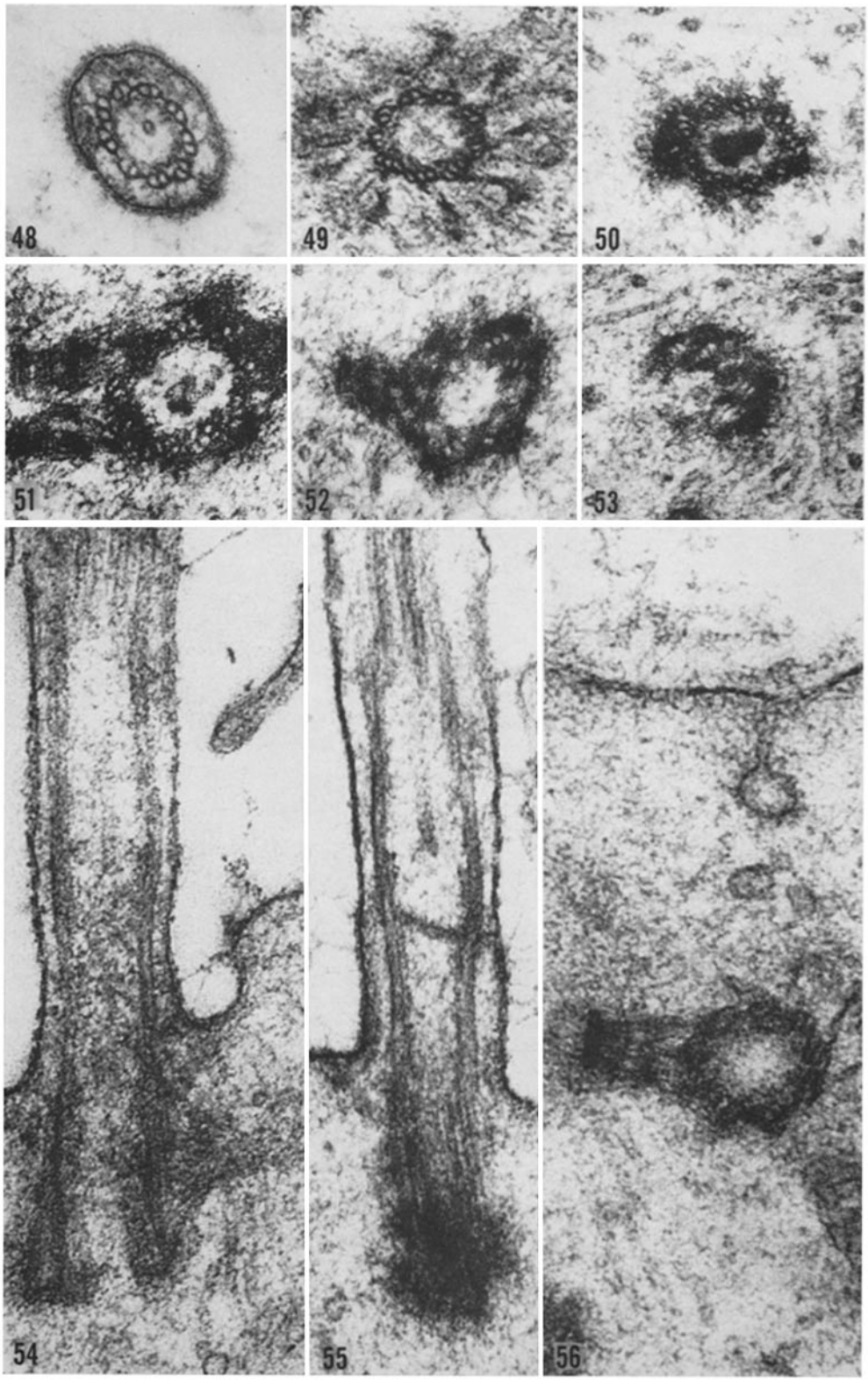
FIGURE 50 A region proximal to the origin of the transitional fibers where an amorphous material surrounds the fibers of a basal body or centriole. Dark granules are occasionally found in basal bodies and centrioles. Double stained. × 110,000.

FIGURES 51 AND 52 Cross-sections of basal bodies or centrioles showing double and single basal feet. At this level, near the proximal end of a basal body, an amorphous dense material surrounds its fibers. Uranyl acetate. × 110,000.

FIGURE 53 Proximal end of a basal body or centriole. The dense material around the fibers is even more apparent at this level. Uranyl acetate. × 110,000.

FIGURES 54 AND 55 Longitudinal sections of cilia and their basal bodies, showing cilia with and without basal plates. Fibers in the cilium become triple only at the level of the cell membrane, as seen in Fig. 49. Transitional fibers are not seen well in longitudinal sections. Double stained. × 110,000.

FIGURE 56 Part of an olfactory vesicle, showing a basal foot which ends in a prominent "basal bar." Longitudinal and cross-striations are apparent in the basal foot, and microtubules approach it from the bottom of the picture. A vesicle surrounded by an amorphous material appears to connect with the cell membrane. Double stained. × 110,000.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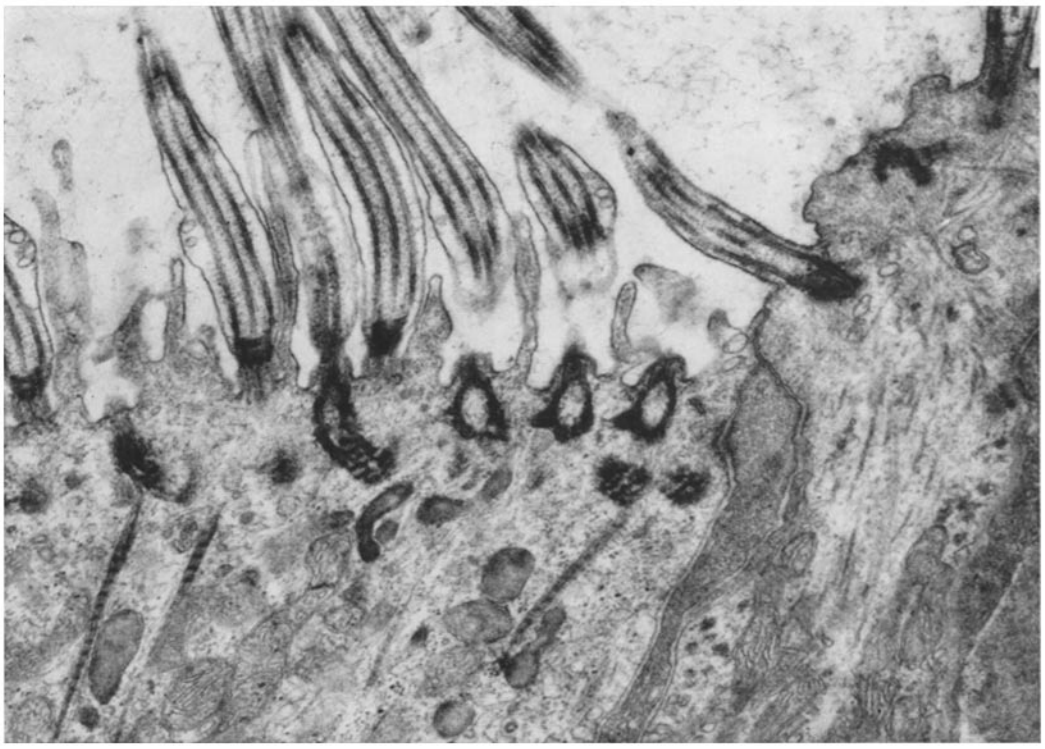
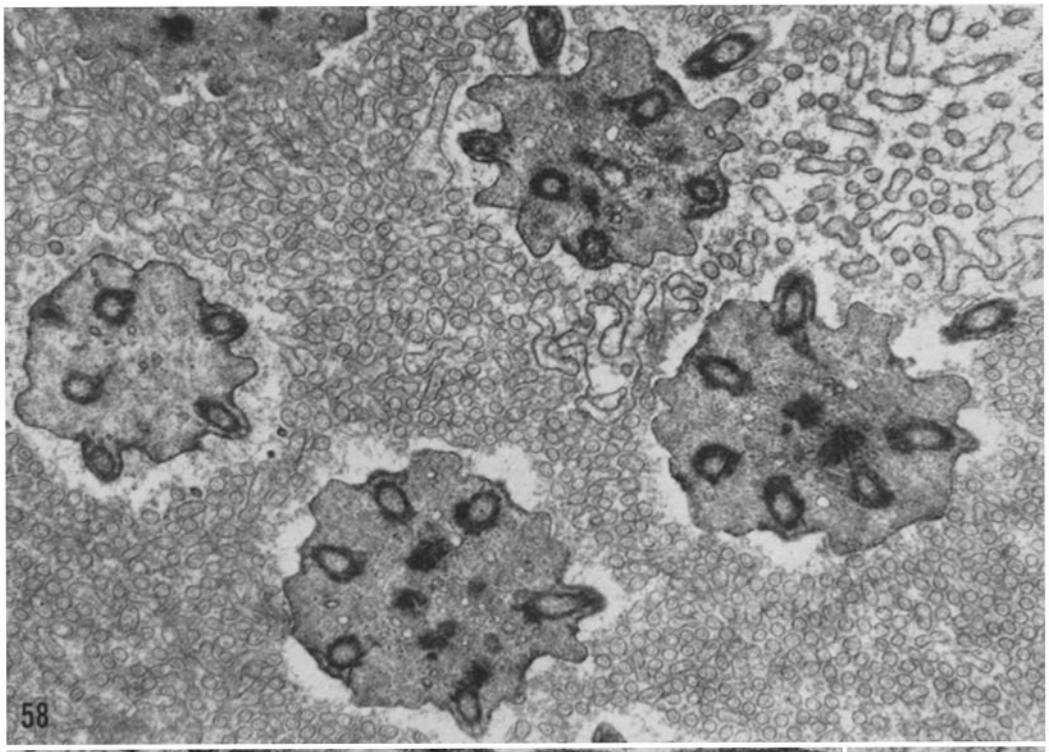


FIGURE 57 Point of transition from the olfactory to the respiratory region. On the right is an olfactory bipolar neuron and on the left a ciliated cell from the respiratory region. The basal bodies of the respiratory cilia are distinctly different from those of the olfactory cilia, but small vesicles similar to those found in the proximal segments of the olfactory cilia are present in the respiratory cilia. Double stained. × 25,000.

FIGURE 58 Section parallel to the surface of the epithelium, showing the apices of the dendrites or olfactory vesicles of four bipolar cells. Cross-sections of microvilli from the supporting cells surround the olfactory vesicles. Note the variability in the locations of basal bodies and centrioles in the dendrites. Double stained. × 23,000.

FIGURE 59 Section cut perpendicular to the surface of the epithelium. The dendrite of a bipolar cell comes up from the bottom of the picture and ends in a swelling, the olfactory vesicle. The basal bodies of two cilia and perhaps a third are embedded in the amorphous material around the outside of the vesicle, and a centriole or perhaps another basal body lies near the basal body of one of the cilia. Microtubules and small vesicles are found in its core. Microvilli originate from the distal ends of the supporting cell. Double stained. × 23,500.

FIGURE 60 Terminal bar between the dendrite of a bipolar cell (left) and a supporting cell (right). The terminal bar has three distinct zones. At the surface of the epithelium (top) there is a tight junction, deeper in the epithelium is an intermediate junction, and deeper still a desmosome. Double stained. × 100,000.



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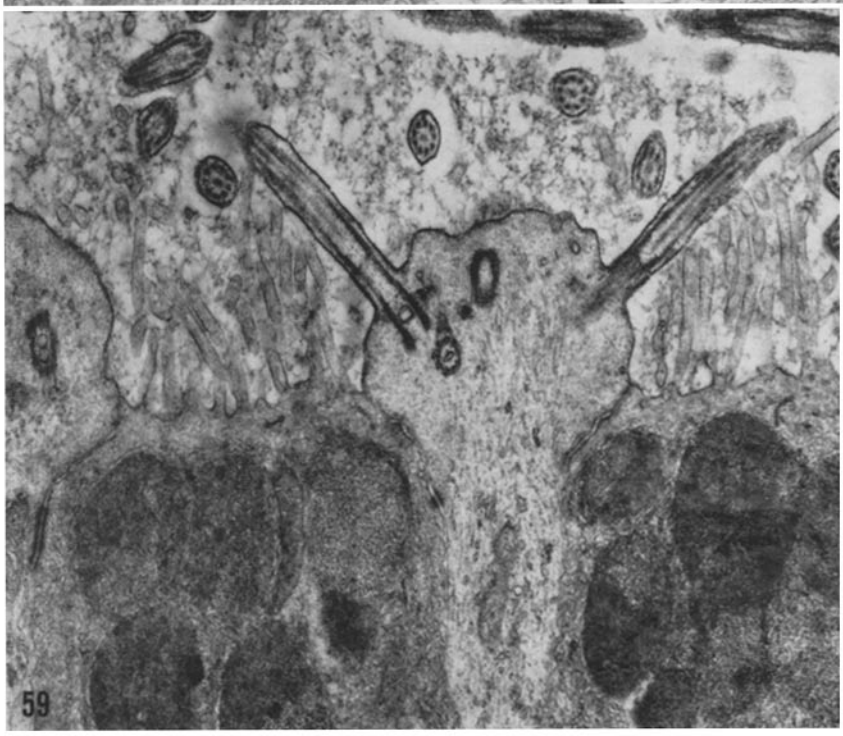

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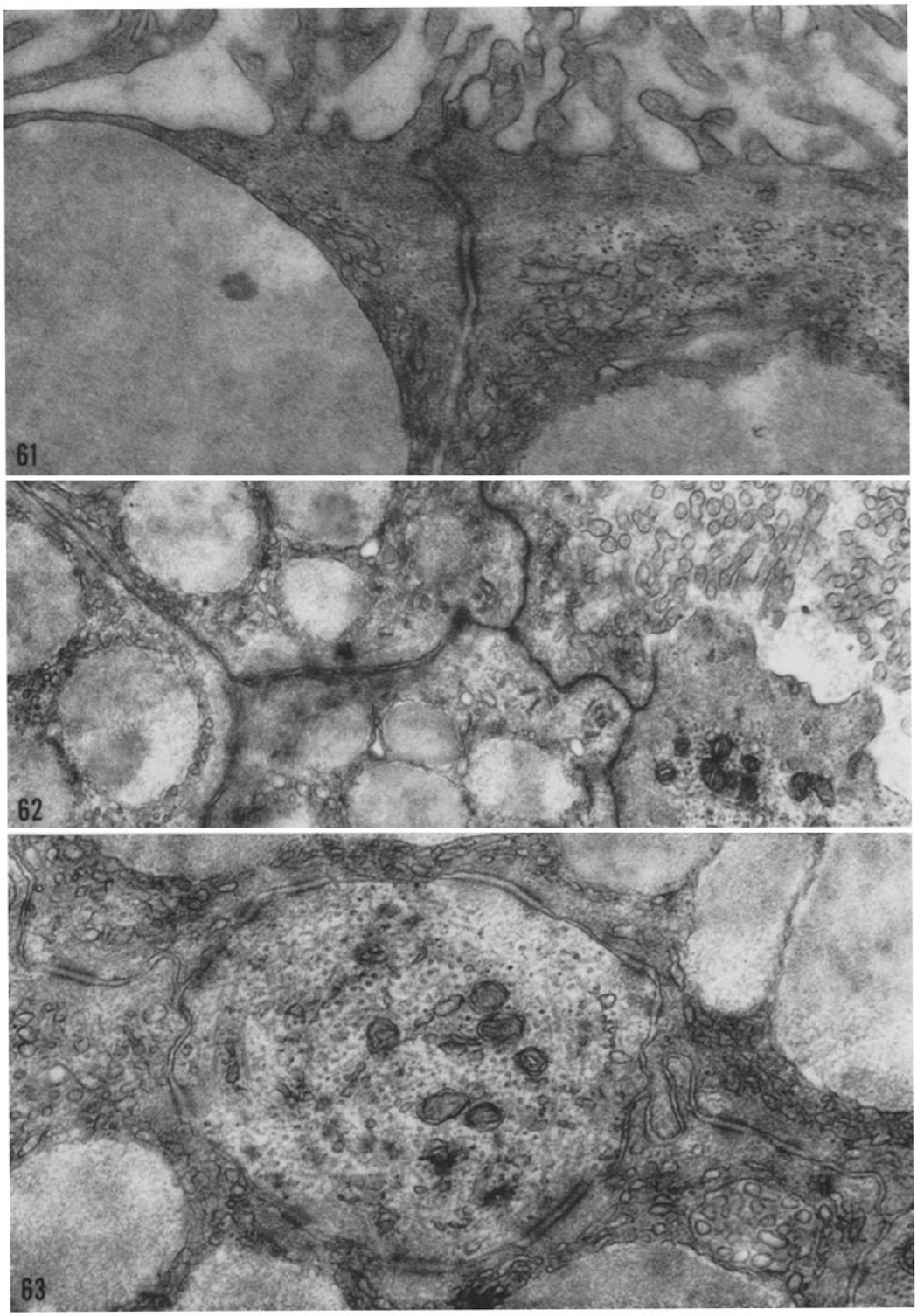
substances (22). This is to be expected because the olfactory cilia are the only structures in the epithelium likely to have close access to odorous molecules in the ambient air. Also, cilia are found on sensory cells in sense organs mediating various kinds of stimuli in both vertebrates and invertebrates (9–11, 18, 20, 33–35, 38, 39). The cilia on these sensory cells, like those on the frog's olfactory bipolar cells, have modifications or are in positions which suggest that they are important in the initiation of electrical excitation. In spite of the diverse locations and sensitivities of these cells, certain similarities are found in their structure. They are, typically, bipolar cells whose dendrite or distal process contains a centriole and a basal body from which a modified cilium originates. Direct observation of living preparations is usually impracticable, but the cilia appear to be in positions where motility, if present, would be extremely limited. When the basal bodies of these sensory cilia are seen in favorable sections with sufficient detail, they have the typical pattern of nine sets of triple subfibers arranged as a cylinder, as well as appendages, such as basal feet and transitional fibers, which are similar to those found in olfactory cilia and a variety of non-sensory cilia (16, 17, 20, 33, 38). Nothing in the structure of their basal bodies distinguishes this group of sensory cilia from olfactory cilia or from non-sensory cilia. It has been pointed out, however, that sensory cilia, in addition to having a basal body or "distal centriole," commonly have a "proximal centriole" lying near their basal body (3). Indeed, the structure of basal bodies has been shown to be similar to that of centrioles (7), and, except in the locust ear (18), the basal bodies of sensory cilia are not an exception to this rule. The olfactory bipolar cells differ from other bipolar sensory cells, however, in that several basal bodies and several centrioles are present in each dendrite, and centrioles do not appear to be paired with basal bodies. Moreover, centrioles are occasionally found in close proximity to the cell membrane, an arrangement that has been described in cells which are developing cilia (28, 36). It is possible, therefore, that the centrioles in the olfactory cells are steps in the involution or evolution of cilia. One can conclude that the olfactory bipolar cells resemble other ciliated sensory cells in that centrioles are present in their dendrites, but more must be known about the development of cilia in sensory cells before one can say whether the centrioles in the olfactory cells are comparable with those in other sensory cells. The function of centrioles in mature sensory cells is unknown, but the finding that centrioles in apposition to the cell membrane of cochlear hair cells have a certain fixed relationship with the direction of movement required to excite this cell suggests the possibility of their having a role in the responses of sensory cells (12, 14).

The shafts of other sensory cilia typically contain nine outer pairs of fibers but, unlike the olfactory cilia, lack a central pair of fibers, a finding said to be characteristic of immotile cilia (3). Since all the cilia from the olfactory region contain a central pair of fibers, and since at least some of these cilia are immotile, they appear to be an exception to the generalization that immotile cilia lack a central pair of fibers. The possibility remains, however, that the olfactory cilia which are immotile *in situ* are capable of motion

FIGURE 61 Section perpendicular to the surface of the epithelium, showing the distal ends of two supporting cells. Release of the secretion droplet at the left appears to have been imminent. The secretion droplets are surrounded by smooth endoplasmic reticulum and small granules which are probably ribonucleoprotein. Lead citrate. × 42,000.

FIGURE 62 Section almost parallel to the surface of the epithelium. What appears, at this magnification, to be a tight junction surrounds the dendrite at the lower right and continues between the supporting cells. Deeper in the epithelium, between the supporting cells, is a continuous intermediate junction, and deeper still, at the upper left, are some desmosomes. Lead citrate. × 26,000.

FIGURE 63 Section parallel to and about 0.5 μ below the surface of the epithelium. The dendrite of a bipolar cell is surrounded by desmosomes. At the lower right is a multivesicular body. Lead citrate. × 43,000.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when their distal segments are broken. Photoreceptor cilia in the hydromedusan eye (11) and, possibly, the olfactory cilia from insects (33, 34) appear to be other examples of immotile cilia with a central pair of fibers. A motile flagellum lacking central fibers has also been described (1), so that the relation between central fibers and motility is not clear. Cilia lacking central fibers usually have very short proximal segments, less than three to four microns in length (3, 9, 11, 18, 35, 39). Therefore, by the same reasoning used to connect central fibers with motility, it would be possible to correlate their presence with the ability of cilia to elongate.

All the sensory cilia have distal segments in a position or with structural modifications which would enable them to react to appropriate stimuli. In receptors mediating visual stimuli, these usually take the form of vesicles or lamellae of various shapes, which are thought to contain visual pigments (21). In mechanical receptors, dilatations containing short fibrils may occur near the tips of the cilia (18), or the distal segment of the cilium may lack the central fibers found in the proximal segment (39). For chemoreceptors, the distal segments of some olfactory cilia from insects have been described (33–35), and the resemblance of these cilia to the olfactory cilia in the frog is striking. In the olfactory receptors of these insects the olfactory cilia have proximal and distal segments. The proximal segments lie deep inside the antennae in dense sheaths, while the distal segments emerging from the sheaths contain only fine tubules about 200 A in diameter. The distal segments branch and travel up to 80 μ within olfactory hairs on the surface of the antennae, where parts of the ciliary membranes come in contact with fine pores in the surface of the olfactory hair, far removed from the origins of the olfactory cilia.

The olfactory cilia in the frog also have highly specialized distal segments. First, they are immotile and extremely long. They contain only single fibers, a pattern similar to that occurring for only a fraction of a micron at the tips of other cilia (16, 17). Vesicles are frequently found along the shafts of these cilia. Similar vesicles in some sensory cilia are thought to contain visual pigments (21), but vesicles have been found in cilia which do not appear to be sensory cilia, such as those on the ependymal cells (5) and the saccus vasculosus (2, 37), and in cilia from the respiratory region examined in the present study (Fig. 57). The significance of vesicles in the olfactory cilia, therefore, remains to be demonstrated. About one-tenth of the olfactory cilia have a completely unique structure in their distal segments, a double fold of ciliary membrane bridging a split in the array of subfibers. No analogous structures have been found in other sensory cilia, so that their significance here is unclear, but they are present in a high enough frequency so that they might be supposed to have a role in the response of the olfactory cilia to odors.

If the olfactory cilia initiate electrical excitation, they must conduct it to the dendrites of the bipolar cells. The same must be true for cilia on other sensory cells, but the extreme length and small diameter of the distal segments of the olfactory cilia pose a special problem. For a passive spread of excitation in the cilia to be effective in initiating impulses in the bipolar cells, the ratio of the resistance of the ciliary membrane to that of the internal and external media would have to be higher than that usually found in neurons (30). If excitation is propagated as an impulse in the cilium, the active properties of the membrane might be similar to those of the olfactory nerve fibers, which are of similar diameter (24). Such special electrical properties are apparently present in the olfactory cilia of insects, where the ends of the olfactory cilia are separated from their bipolar cells by distances comparable to those found in the frog (33–35).

The arrangement of the distal segments of the frog's olfactory cilia into bundles lying in parallel rows may represent a specialization of some significance, for it implies that potentials arising from individual cilia could interact with potentials in neighboring cilia. A possible explanation for some puzzling changes in the electrical activity of the epithelium reported to occur after manipulations of the surface of the mucus (31) is that these manipulations disturb the arrangement of the cilia.

The olfactory cilia in the frog are, like other sensory cilia, highly specialized components of a highly specialized sense organ. On the basis of the anatomical and physiological evidence now available, they appear to be the component of the sense organ excited by contact with odorous sub-

stances. To what extent their structure and organization is representative of other classes of vertebrates remains to be seen, for previous studies of vertebrate olfactory receptors have not dealt specifically with the layer of cilia and mucus which covers the epithelium. Studies in the mammal, however, have indicated that cilia are present on the olfactory bipolar cells and that these cilia may have processes which lie near the surface of the mucus (8, 15). It will be interesting to compare the structure and organization of these processes with those in the frog.

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REFERENCES

1. AFZELIUS, B. A., The contractile apparatus in some invertebrate muscles and spermatozoa, *Proc. 5th Internat. Conf. Electron Micr.*, 1962, **2**, M1.
2. BARGMANN, W., and KNOOP, A., Electronenmikroskopische Untersuchung der Krönchenzellen des Saccus vasculosus, *Z. Zellforsch. u. mikr. Anat.*, 1955, **43**, 184.
3. BARNES, B. G., Ciliated secretory cells in the pars distalis of the mouse hypophysis, *J. Ultrastruct. Research*, 1961, **5**, 453.
4. BLOOM, G., Studies on the olfactory epithelium of the frog and toad with the aid of light and electron microscopy, *Z. Zellforsch. u. mikr. Anat.*, 1954, **41**, 89.
5. BRIGHTMAN, M. W., personal communication.
6. BRIGHTMAN, M. W., and PALAY, S. L., The fine structure of ependyma in the brain of the rat, *J. Cell Biol.*, 1963, **19**, 415.
7. BURGOS, M. H., and FAWCETT, D. W., Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (*Felis domestica*), *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 287.
8. DE LORENZO, A. J., Electron microscopic observations of the olfactory mucosa and olfactory nerve, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 839.
9. DE ROBERTIS, E., Electron microscope observations on the submicroscopic organization of the retinal rods, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 319.
10. EAKIN, R. M., and WESTFALL, J. A., Further observations on the fine structure of the parietal eye of lizards, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 483.
11. EAKIN, R. M., and WESTFALL, J. A., Fine structure of photoreceptors in the hydromedusan, *Polyorchis penicillatus*, *Proc. Nat. Acad. Sc.*, 1962, **48**, 826.
12. ENGSTRÖM, H., ADES, H. W., and HAWKINS, S. E., Structure and functions of sensory hairs of the inner ear, *J. Acoust. Soc. Am.*, 1962, **34**, 1356.
13. FARQUHAR, M. G., and PALADE, G. E., Junctional complexes in various epithelia, *J. Cell Biol.*, 1963, **17**, 375.
14. FLOCK, Å., KIMURA, R., LUNDQUIST, P.-G., and WERSÄLL, J., Morphological basis of directional sensitivity of the outer hair cells of the organ of Corti, *J. Acoust. Soc. Am.*, 1962, **34**, 1351.
15. GASSER, H. S., Olfactory nerve fibers, *J. Gen. Physiol.*, 1956, **39**, 473.
16. GIBBONS, I. R., The relationship between the fine structure and direction of beat in gill cilia of a lamellibranch mollusc, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 179.
17. GIBBONS, I. R., and GRIMSTONE, A. V., On flagellar structure in certain flagellates, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 697.
18. GRAY, E. G., The fine structure of the insect ear, *Phil. Trans. Roy. Soc. London, Series B*, 1960, **243**, 75.
19. HOPKINS, A. E., The olfactory receptors in vertebrates, *J. Comp. Neurol.*, 1926, **41**, 253.
20. LOWENSTEIN, O., OSBORN, M. P., and WERSÄLL, J., Structure and innervation of the sensory epithelia of the labyrinth in the thornback ray, (*Raja clavata*), *Proc. Roy. Soc. London, Series B*, 1964, **160**, 1.
21. MOODY, M. F., Photoreceptor organelles in animals, *Biol. Rev.*, 1964, **39**, 43.
22. OTTOSON, D., Some aspects of the function of the olfactory system, *Pharmacol. Rev.*, 1963, **15**, 1.
23. PALAY, S. L., Morphology of secretion, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Conn., Yale University Press, 1958, p. 305.
24. REESE, T. S., unpublished data.
25. REYNOLDS, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.
26. RICHARDSON, K. C., JARRETT, L., and FINKE, E. H., Embedding in epoxy resins for ultrathin

sectioning in electron microscopy, *Stain Technol.*, 1960, **35**, 313.

27. ROSENBLUTH, J., and WISSIG, S. L., The uptake of ferritin by toad spinal ganglion cells, *J. Cell Biol.*, 1963, **19**, 91A (abstract).
28. ROTH, L. E., and SHIGENAKA, Y., The structure and formation of cilia and filaments in rumen protozoa, *J. Cell Biol.*, 1964, **20**, 249.
29. SCHULTZE, M., Untersuchungen über den bau der Nasenschleimhaut, namentlich die Strukture und Endigungsweise der Geruchsnerven, *Abhandl. Nat. Ges. Halle*, 1862, **7**, 1.
30. SHEPHERD, G. M., personal communication.
31. SHIBUYA, T., Dissociation of the olfactory neural response and mucosal potential, *Science*, 1964, **143**, 1338.
32. SLAUTTERBACK, D. B., Cytoplasmic microtubules. I. Hydra, *J. Cell Biol.*, 1963, **18**, 367.
33. SLIFER, E. H., and SEKHON, S. S., Sense organs on the antennal flagellum of the milkweed bug, *Lygaeus kalmii*, Stal. (Hemiptera, Lygaeidae), *J. Morphol.*, 1963, **112**, 165.
34. SLIFER, E. H., and SEKHON, S. S., Fine structure of the sense organs on the antennal flagellum of a flesh fly, *Sarcophagia argyrostoma* R.-D. (Diptera, Sarcophagidae), *J. Morphol.*, 1964, **114**, 185.
35. SLIFER, E. H., SEKHON, S. S., and LEES, A. D., The sense organs on the antennal flagellum of aphids (Homoptera) with special reference to the plate organs, *Quart. J. Micr. Sc.*, 1964, **105**, 21.
36. SOTELLO, J. R., and TRUJILLO-CENÓZ, O., Electronmicroscopic study of the kinetic apparatus in animal sperm tails, *Z. Zellforsch. u. mikr. Anat.*, 1958, **48**, 565.
37. SUNDARARAJ, B. I., and PRASAD, M. R. N., The histochemistry of the saccus vasculosus of *Notopterus chitals* (Teleostei), *Quart. J. Mikr. Sc.*, 1964, **105**, 91.
38. TOKUYASU, K., and YAMADA, E., The fine structure of the retina studied with the electron microscope. IV. Morphogenesis of outer segments of retinal rods, *J. Biophysic and Biochem. Cytol.*, 1959, **6**, 225.
39. WHITEAR, M., Chordotonal organs in Crustacea, *Nature*, 1960, **187**, 522.