

THE FINE STRUCTURE OF THE SENSORY EPITHELIUM OF THE VOMERONASAL ORGAN IN SUCKLING RATS

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Abstract

The sensory epithelium of the vomeronasal organ of the suckling rat consists of three cell types: receptor, supporting, and basal. Receptor cells are peripherally situated neurones and have dendritic and axonic processes extending from the perikaryon. The former expands near the epithelial surface to an enlarged area containing vacuoles, numerous centrioles, and electron-dense granules. The free surface carries numerous microvilli but no cilia. The perikaryon contains extensive rough endoplasmic reticulum and prominent Golgi apparatus. Microtubules occur in both processes. Supporting cells are enlarged near the surface to surround receptor dendrites. In the first 7 days after birth many supporting cells carry a single cilium of the "9+0" pattern.

I. INTRODUCTION

The structure of sensory cells in the vomeronasal organ has only recently come under investigation with the electron microscope and reports in the literature are limited in number and in the species investigated. Bannister (1968) and Altner and Muller (1968) published short reports on the nature of the receptor cells in reptiles, and recently descriptions of the organ in *Lacerta sicula* and *Natrix natrix* (Altner, Muller, and Brachner 1970) and in turtles (Graziadei and Tucker 1970) have appeared. At the time of writing no descriptions have yet appeared of the vomeronasal organ in mammals. Light microscope investigations describe the organ in mammals as having sensory epithelium on the medial aspect and ordinary non-sensory epithelium laterally (Allison 1953; Kadowaki 1959).

The present study was undertaken to investigate the structure in rats, in conjunction with a study of the olfactory epithelium, since the vomeronasal organ is usually regarded as a special division of the olfactory organ. Specimens were taken from rats in the first 3 weeks after birth as older animals maintained in colonies usually have a considerable bacterial population in the upper respiratory tract.

II. MATERIALS AND METHODS

Samples of the vomeronasal organ were taken from rats of both sexes from Wistar and Buffalo strains at 1, 2, 3, 4, 7, and 22 days after birth. Immediately after the animals were killed by decapitation the snout was sectioned in the region of the vomeronasal organ. The specimens were then immersed in ice-cold 4% glutaraldehyde in cacodylate buffer at pH 7.2. While under

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this fixative, small sections of the organ were dissected free of their fine bony investment with the aid of a dissecting microscope and transferred to fresh fixative. Specimens were post-fixed in osmium tetroxide, dehydrated in alcohol, and embedded in Araldite. Sections were stained with 5% uranyl acetate and Reynold's lead citrate. Thick sections for orientation were cut from the Araldite blocks and stained with methylene blue.

III. RESULTS

Since the study revealed no differences in structure attributable to sex or strain characteristics, the following description applies to all specimens except for certain developmental differences due to age.

The vomeronasal organ in the rat consists of a pair of blind tubes lying along the base of the nasal septum, opening into the nasal cavity close to the vestibule. The lumen is a modified C-shape since the sensory epithelium on the ventral aspect of the tube is very much thicker than that on the non-sensory dorsal aspect. The thinner epithelium bulges slightly into the lumen due to the presence of a fairly large artery in the underlying propria. For most of the length of the tubes, glands are absent but towards the caudal end they occur in the propria of the dorsal epithelium and extend up into the region of the nasal septum. The whole length of each tube is enclosed almost completely by fine bone, similar to the turbinates in structure and incomplete dorsally to allow egress for the fibres of the vomeronasal nerves. Figure 1 shows a diagrammatic representation of a section about midway along the organ in a young rat.

There are three types of cells in the sensory epithelium: basal, supporting, and sensory or receptor. Basal cells are not very numerous and are restricted to the lower part of the epithelium. Both other cell types extend the full height. Most of the ovoid nuclei of the supporting cells are found at the top of the nuclear stratum, but there is a columnar arrangement of these nuclei in some sections, particularly in the younger specimens, so that a clear division into zones of supporting and receptor nuclei cannot always be made. The latter are much more numerous, rounded, and mainly located in the deeper part of the nuclear stratum. The epithelium is a dense one with little intercellular space. Figure 2 is a diagrammatic representation of the three cell types and their relationship to one another. The total depth of the epithelium has been reduced for clarity, since there is a depth of 8-10 nuclei in the nuclear zone.

(a) *Receptor Cells*

The receptor cell of the vomeronasal organ, like its olfactory counterpart, is a peripherally located neurone. In form it is bipolar with a proximal axonic process, and a distal dendritic process extending from the perikaryon to, or just beyond, the epithelial surface (Fig. 3). The axons pass through the basal lamina to become fibres of the vomeronasal nerve (Fig. 4) and finally synapse in the glomerular layer of the accessory olfactory bulb.

Receptor nuclei are rounded, with fairly finely stippled chromatin and one or two dense clumps, one of which is in the region of the nucleolus. The cytoplasm in the perikarya contains a Golgi complex, and extensive endoplasmic reticulum, most of which is the granular type. The narrow cisternae between this rough endoplasmic

reticulum are often distended and may contain a fine grey amorphous material (Fig. 5). In specimens taken at 22 days the perikaryon in many cells contains electron-dense bodies bounded by a single membrane and showing an inner structure of darker lamellae (Fig. 6). Below the nucleus the cytoplasm narrows to a fine process in which longitudinally orientated microtubules and mitochondria are the only organelles.

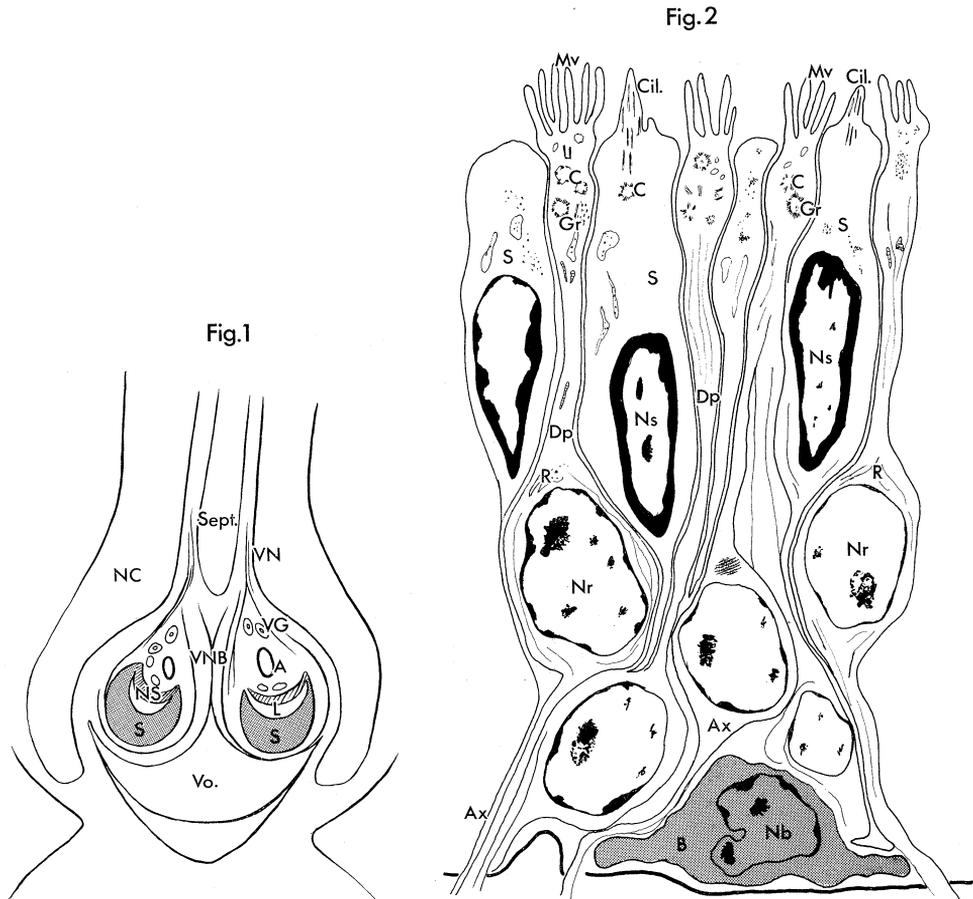


Fig. 1.—Diagrammatic cross section of the vomeronasal organ of the rat. *A*, artery; *L*, lumen; *NC*, nasal cavity; *NS*, non-sensory epithelium; *S*, sensory epithelium; *Sept.*, nasal septum; *VG*, vomeronasal glands; *VN*, vomeronasal nerve fibres; *VNB*, vomeronasal bone; *Vo.*, vomer.

Fig. 2.—Diagrammatic view of cells of the vomeronasal sensory epithelium. The height of the epithelium has been reduced. *Ax*, axonic process; *B*, basal cell; *C*, centriole; *Cil.*, cilium; *Dp*, dendritic process; *Gr*, granular material; *Mv*, microvilli; *Nb*, basal cell nucleus; *Nr*, receptor cell nucleus; *Ns*, supporting cell nucleus; *R*, receptor cell; *S*, supporting cell.

Bundles of these axonic processes may be seen between the basal cells at the bottom of the epithelium before they leave to join the bundles of nerve fibres just below the basal lamina (Fig. 4).

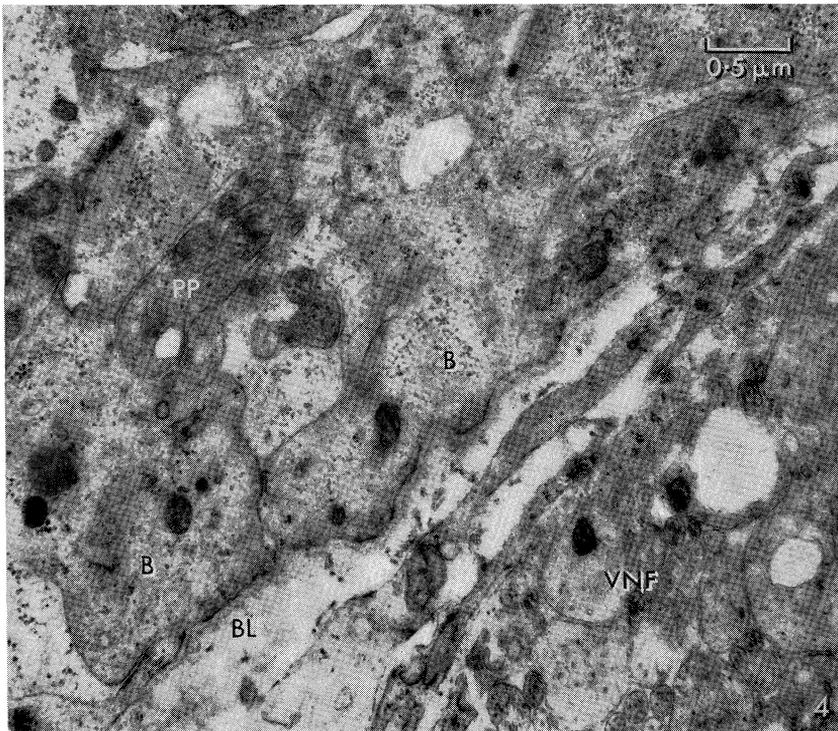
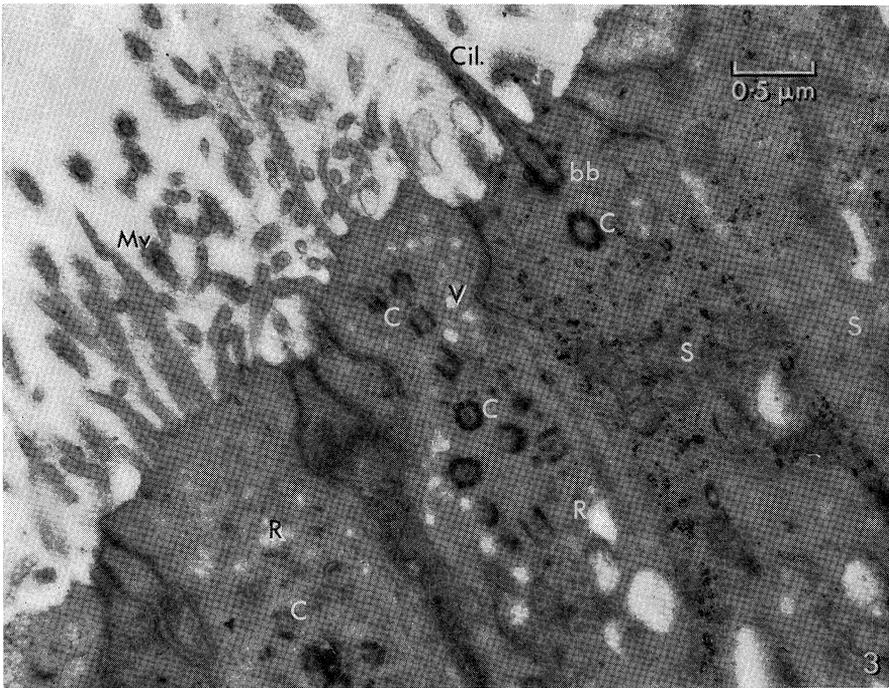


Fig. 3.—Surface of sensory epithelium. Receptor cells (*R*) have surface microvilli (*Mv*), vesicles (*V*), and centrioles (*C*). Supporting cells (*S*) contain ribosome clusters and mitochondria. One supporting cell has a cilium (*Cil.*) with basal body (*bb*) and associated centriole.

Fig. 4.—Base of the sensory epithelium. Proximal processes (*pp*) of receptor cells can be seen between processes of basal cells (*B*). Below the basal lamina (*BL*) there is a bundle of fibres of the vomeronasal nerve (*VNF*).

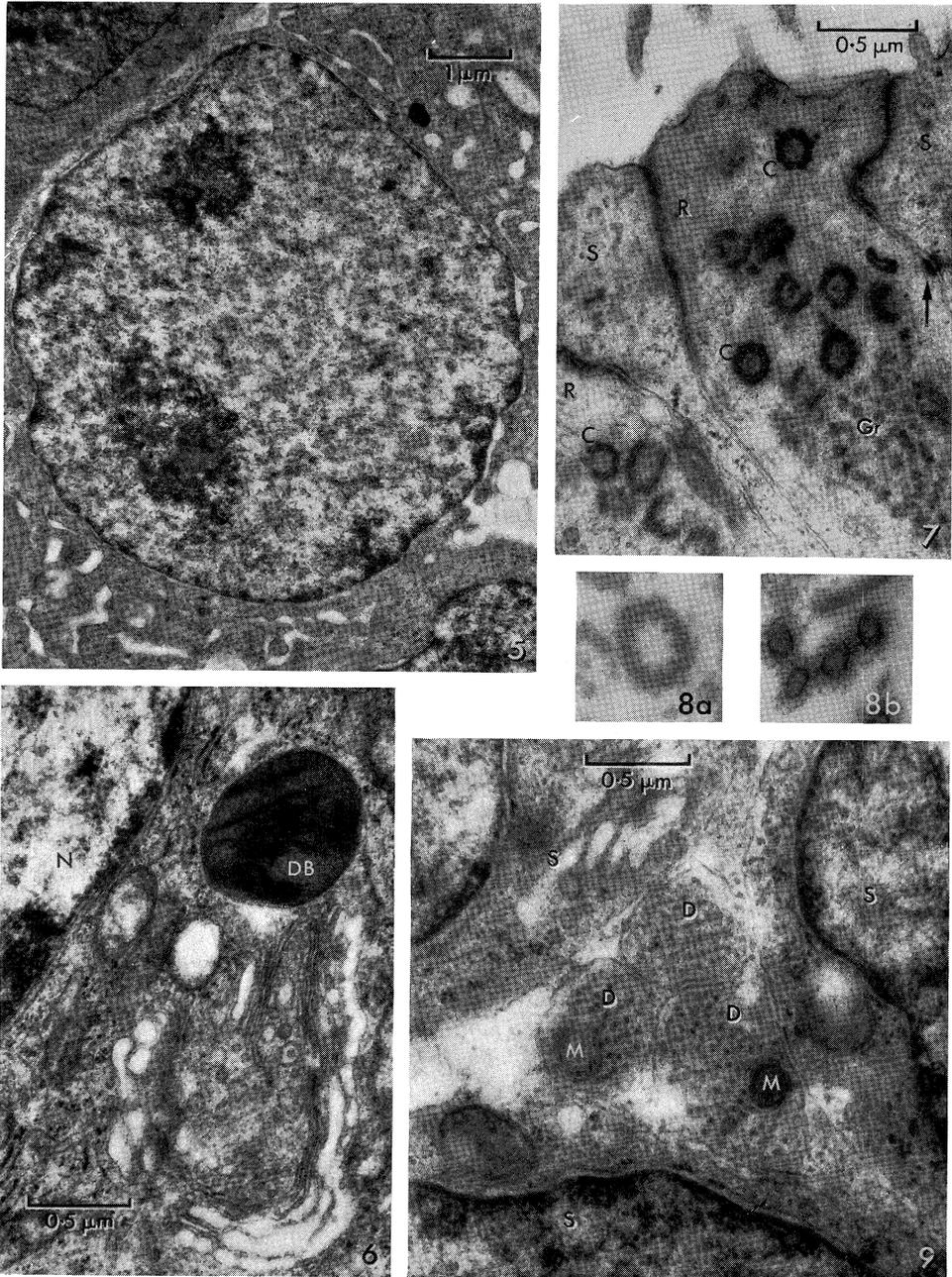


Fig. 5.—Receptor cell nucleus and perikaryon. Note the cisternae containing amorphous material between rows of rough endoplasmic reticulum.

Fig. 6.—Dark body (*DB*) with inner lamellar structure near the nucleus (*N*) of a receptor cell from a 22-day-old rat. The well-developed Golgi apparatus is characteristic of receptor cells.

Fig. 7.—Receptor cell surface showing a characteristic cluster of centrioles (*C*) and granular material (*Gr*). A desmosome indicated by an arrow forms the deepest part of the junctional complex between receptor (*R*) and supporting (*S*) cells.

Fig. 8.—Details of (*a*) a centriole and (*b*) surface microvilli from receptor cells. Note the fuzzy coat of microvilli.

Fig. 9.—Three dendritic processes of receptor cells (*D*) sectioned between the nuclear region of supporting cells (*S*). Mitochondria (*M*) and microtubules can be seen in the dendrites.

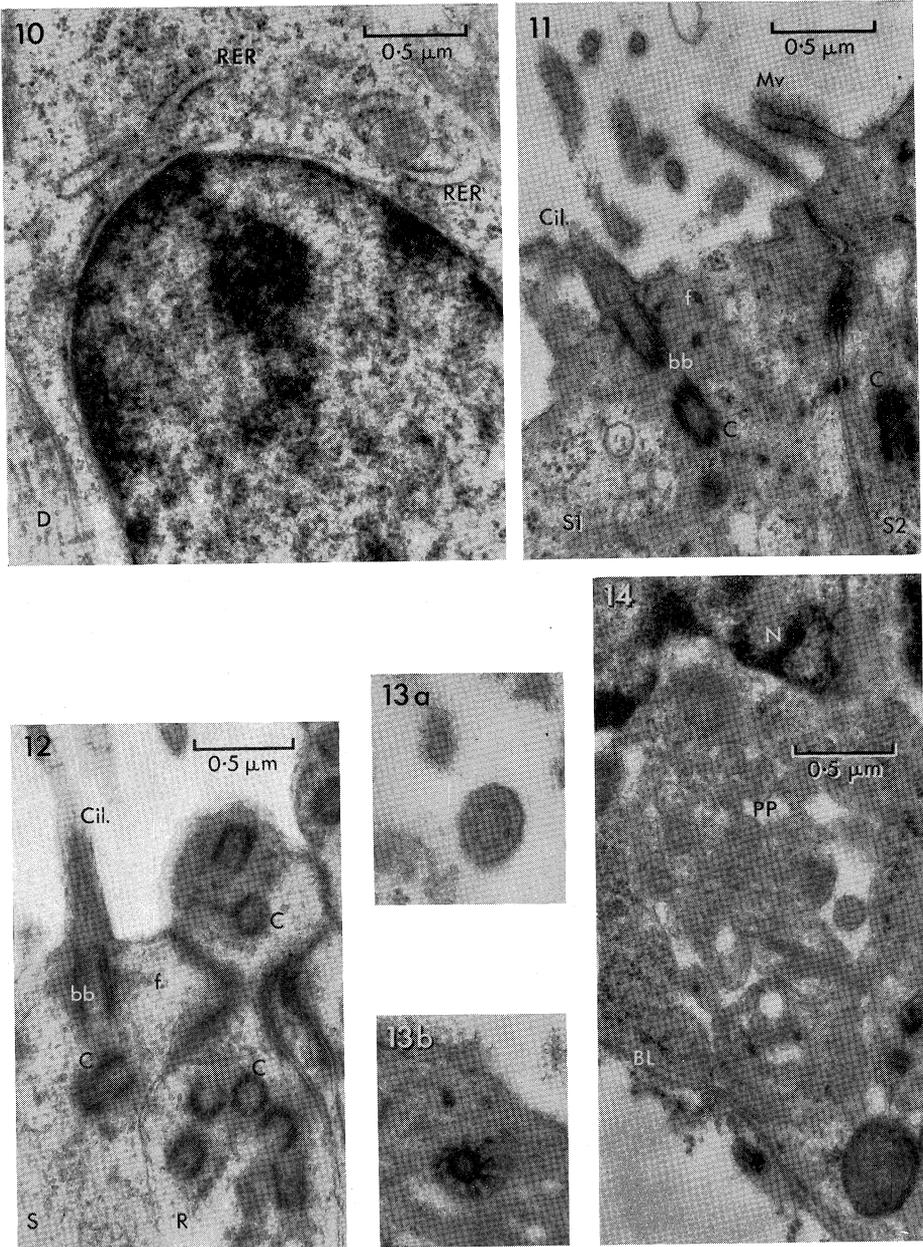


Fig. 10.—Supporting cell nucleus and perinuclear cytoplasm containing ribosome clusters and a few parallel rows of rough endoplasmic reticulum (*RER*). Part of a sensory cell dendritic process (*D*) can be seen.

Fig. 11.—Surface of adjacent supporting cells. Cell *S1* has a cilium (*Cil.*) with a basal body (*bb*) and striated lateral foot (*f*). Orientated nearly at right angles to it is a centriole (*C*). Cell *S2* has a similar centriole. Microvilli (*Mv*) on the cells resemble those on receptors.

Fig. 12.—Surface area of a receptor (*R*) and a supporting (*S*) cell. The receptor cell has a well-defined neck separating the two terminal expansions, in each of which centrioles (*C*) appear. The supporting cell has a single cilium with basal body (*bb*) and associated centriole. The basal body has a striated lateral foot (*f*).

Above the nucleus the cytoplasm narrows again to a lesser degree to form the dendritic process. At first the only prominent organelles are longitudinally orientated microtubules and mitochondria (Fig. 9), but close to the surface the process increases in diameter, the number of mitochondria increases and there is a group of centrioles with axes in several directions, usually accompanied by a cluster of dark granules (Fig. 7). The centrioles have the typical structure of nine groups of three partially fused tubules arranged around a clear central area (Fig. 8*a*). In most cases this expanded section of the dendrite is separated by a constricted neck from a terminal expansion which finishes at, or just beyond, the general level of the epithelium. In some cells centrioles may also be found in this second enlargement but often they are confined to the subterminal area (Fig. 3). Vesicles are common in both the terminal swellings of the dendrite. The surface of the receptor cell carries a fringe of microvilli, about $1.3 \mu\text{m}$ in length and usually unbranched. They show very little internal organization, though a central core of fine filaments can be seen in some longitudinal views. In almost all cases they are surrounded by a fuzzy coat which shows well in transverse sections (Fig. 8*b*). There are no cilia on the receptor cells.

Receptor cells are anchored to supporting cells near the surface of the epithelium by junctional complexes, the deepest component of which is a desmosome situated below the neck. Each receptor dendrite seems to be surrounded by supporting cells, and no junctions between neighbouring sensory cells were seen. At the nuclear level, processes of supporting cells usually extend between receptor perikarya but frequently two receptor cells are in contact though there are no junctional structures. At the epithelial base, bundles of receptor axons occur with no supporting or basal cell processes to separate individual axons.

(b) Supporting Cells

Most of the cytoplasm of supporting cells is found in the surface zone of the epithelium, and their nuclei form the most superficial nuclear stratum in rats older than 3 days. In young rats of 1–3 days, this nuclear arrangement is not always very regular, and there is a tendency to form columns of supporting cell nuclei extending right down to the basal lamina. The nuclei are ovoid in shape, with a heavier peripheral arrangement of chromatin than receptor cell nuclei. Perinuclear cytoplasm contains clusters of ribosomes, mitochondria, and a few parallel rows of rough endoplasmic reticulum (Fig. 10). The main cytoplasmic area towards the surface contains numerous clusters of ribosomes, some smooth endoplasmic reticulum, and mitochondria. Many sections of supporting cells from rats up to 7 days of age show a single cilium projecting from the surface, whose basal body usually has an accompanying centriole orientated approximately at right angles to it (Figs. 3, 11, 12). The basal bodies of these cilia are accompanied by radiating dark areas which appear as lateral "feet" in longitudinal sections in Figures 11 and 12 or as a catherine-wheel

Fig. 13.—Details of the cilium (*a*) and basal body (*b*) of a supporting cell. The cilium lacks the central pair of tubules in typical ciliary structure. The basal body is sectioned at the level of the lateral feet (*f*) in the previous figure.

Fig. 14.—Base of the sensory epithelium. Above the basal lamina (*BL*) processes of basal and supporting cells surrounded the proximal process (*pp*) of receptor cells. Basal cell nuclei (*N*) are often deeply indented.

formation if sectioned transversely (Fig. 13*b*). The cilia on the supporting cells appear to lack a central pair of tubules (Fig. 13*a*). However, in specimens taken from 22-day-old rats no cilia were seen projecting from the cell, though paired centrioles close to the surface were seen in many sections. The supporting cell surface produces a few microvilli which resemble those on the receptor cells, and are covered by the same fuzzy coat.

Below the nucleus, supporting cells narrow to very thin processes which can be seen between the perikarya of receptor cells. These processes expand at the base of the epithelium, but are difficult to distinguish from the basal cells, though generally this area of the supporting cell has pale cytoplasm and fewer ribosome clusters.

(c) *Basal Cells*

The cytoplasm of basal cells resembles that of the supporting cells. Nuclei are situated close to the basal lamina and are often quite deeply indented (Fig. 14). Processes of these cells abut on to the basal lamina and loosely invest the proximal (axonic) processes of the receptor cells. In the young material used in this study, junctional specializations between neighbouring basal cells were very rarely seen, and seemed to consist of not very marked thickening of the cell membrane in the junctional region. No hemidesmosomes were seen where the cell is in contact with the basal lamina.

IV. DISCUSSION

Ultrastructural studies of the vomeronasal organ in Reptilia (Bannister 1968; Altner and Muller 1968; Altner, Muller, and Brachner 1970; Graziadei and Tucker 1970) have revealed that the surface of the receptor cell in this organ is devoid of cilia. Non-ciliated receptor cells in mammals have been described by Altner, Muller, and Brachner (1970) for insectivores and Kratzing (1971) for sheep. It is this feature which differentiates the vomeronasal receptors from those of the olfactory organ. Furthermore, since the cilia have been considered a probable site for initiating olfactory response, their absence in the vomeronasal receptors raises the question of the probable site of response in these cells, and perhaps in the olfactory cell as well. So far as the vomeronasal organ is concerned, all specimens so far described with the aid of the electron microscope show that the surface of the sensitive cell carries numerous microvilli, which provide an extensive area of plasma membrane to come into contact with odorous material.

In mammals, a large number of centrioles located towards the top of the dendrite are a striking feature of the receptor cell. It is tempting to ascribe to them a probable function in the reception of stimuli. However, Altner, Muller, and Brachner (1970) have reported that centrioles are absent in the receptor cells of *Lacerta sicula*, and are only few in number in *Natrix natrix*. If they are not necessary for the reception of stimuli in these reptilian species, where the vomeronasal organ reaches a high level of development, then it is improbable that they play a major role in this function in mammals.

In a great many of the receptor cells studied, clusters of dark granular material could be seen in the region of the centrioles. Each such area consisted of an aggregate of granules of about 600–700 Å in diameter, with a substructure of four or five denser

units in cross or star formation. The size and position of these particles strongly resemble the "axonemal precursor bodies" described by Steinman (1968, 1970) in studies of ciliogenesis in *Xenopus laevis*. This author describes their occurrence during ciliogenesis, after the appearance of numerous centrioles in the cells but before the development of axonemal filaments in the growing cilia. The precursor bodies follow the centrioles in their migration to the surface of the cell, and are no longer seen in cells with mature cilia. The resemblances between these structures in two cells of very different origin cannot, of course, assert their similarity, but they do suggest the possibility that the normal process of ciliogenesis has not been carried through to completion in the vomeronasal receptor cells. The material described in this study was all young but vomeronasal receptor cells were not ciliated in sections from adult rats. In addition, similar dark granular bodies occur near the centrioles in sheep vomeronasal cells from mature animals. Altner, Muller, and Brachner (1970) consider the possibility that deviations from typical ciliary structure seen in olfactory and vomeronasal receptors should be considered as reductions of the normal pattern rather than as specializations for receptor function; an interpretation of rat vomeronasal receptors as cells in which the later steps of ciliogenesis remain incomplete is in agreement with this point of view.

Structures which are strikingly similar in olfactory and vomeronasal cell dendrites are microtubules and vesicles of varying sizes bounded by smooth membranes. The microtubules are orientated along the long axis of the cell, and also make up the distinctive organelle of the axonic process. They terminate in the apical enlargements of the dendrite but no definite association could be established with centrioles or vesicles. The latter are only numerous in the apical expansions of the dendrite, and do not appear closer to the nucleus. Formation of vesicles by pinocytosis at the base of the microvilli was sometimes apparent in sections but these sites were not common.

Resemblance to the olfactory cell is most marked in the axonic part of the vomeronasal receptor, especially when these axons leave the epithelium to form bundles of the vomeronasal nerve. Microtubules are the only prominent organelle, except for mitochondria. In rats of similar age, bundles of the vomeronasal nerve show a greater variability of axon diameters than olfactory nerve bundles, but the number of microtubules is an average of 3-4 in both cases. Because it is a much wider epithelium than its olfactory counterpart, bigger bundles of axons gather as conspicuous features of the base of the epithelium and leave as larger groups to join the bundles in the lamina propria than they do in the olfactory area. As it leaves the epithelium each group is surrounded by cytoplasm of basal cells.

An unexpected result in the present study is the presence of a cilium on the supporting cells in the younger material. Only one has been seen per cell, but these are large cells, embracing more than one dendritic process, and serial sections would be necessary to exclude the possibility of others. The supporting cell cilium contains the typical nine peripheral double filaments but the two central filaments are absent. Its basal body has a 9 triplet fibre structure as has the centriole at right angles to it in the typical alignment of a centrosomal pair. Thus the structure fits the category of "9+0" cilia described by Barnes (1961) in the mouse hypophysis and Currie and Wheatley (1966) in a number of other tissues. Older supporting cells do not appear to have cilia projecting into the lumen, though a short ciliary shaft enclosed in an

invagination of the cell membrane is occasionally seen close to the surface, and a pair of centrioles frequently occurred in this position. Previous descriptions of supporting cells do not mention cilia. In some species, the cells end in microvilli which resemble in all respects those on the receptors, in others, microvilli only occur occasionally. In the suckling rats, supporting cell microvilli are much fewer than those on receptor cells, but resemble them otherwise in size and structure.

In the period covered by the study (birth to 22 days) comparatively few structural changes were evident in the epithelium. Most noticeable were the reduction in the occurrence of cilia on supporting cells, and the appearance of electron dense lamellated bodies in the perikarya of some receptor cells. Structures of very similar appearance were seen in the same position in receptor cells of adult sheep (Kratzing 1971), suggesting that they may be a product of continued activity in the cell.

V. ACKNOWLEDGMENTS

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