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The vomeronasal organ in tree shrew and slow loris

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INTRODUCTION

The vomeronasal organs have been the subject of some light microscopic observations in amphibia, reptiles, non-primate mammals (Negus, 1958) and prosimian primates (Broom, 1915; Woollard, 1925; Le Gros Clark, 1926; Loo & Kanagasuntheram, 1971), while their ultrastructure has been studied in the slow worm (Bannister, 1968), turtle (Graziadei & Tucker, 1970) and sheep (Kratzing, 1970). Apart from a brief account of the fine structure of these organs in *Tupaia belangeri* (Kolnberger, 1971), there has not been any serious attempt at ultrastructural studies of these organs in prosimian primates. It was, therefore, thought worthwhile to compare the light and electron microscopic appearances of the vomeronasal organs in *Tupaia glis* and *Nycticebus coucang* and to ascertain whether such a study might provide some implications on the phylogenetic status of these two prosimian primates.

MATERIALS AND METHODS

Eight adult tree shrews (*Tupaia glis*) and six slow lorises (*Nycticebus coucang*) were available for study. Three tree shrews and two slow lorises were perfused with 10% formalin and their heads were decalcified in 5% formic acid. Paraffin sections were made of the nasal cavity at 10 μm and 20 μm thickness in the coronal plane, and every tenth section was stained with haematoxylin and eosin for morphological studies. Two tree shrews and two slow lorises were perfused with 10% formalin containing 2% calcium acetate (Spicer, 1960; 1965). Paraffin sections of the vomeronasal organs at 5 μm thickness were stained for mucosubstances by the PAS method (McManus & Mowry, 1960). Diastase digestion was carried out on alternate sections (Barka & Anderson, 1963). Control sections were treated with buffer solution containing no enzyme. Mayer's mucicarmine (Culling, 1963) and Alcian blue 8GX (Mowry, 1963) were used to detect the presence of acid mucins. For fine structural studies, three tree shrews and two slow lorises were perfused with 5% chilled glutaraldehyde. The vomeronasal organs were then dissected out, small blocks were cut and fixation continued in 5% chilled glutaraldehyde for 2 hours. Blocks were then washed in 0·2 M sucrose solution in 0·1 M phosphate buffer at pH 7·3 and post-fixed in Dalton's chrome-osmium fixative. Specimens were dehydrated in graded series of acetone and embedded in Araldite. Sections were cut on a Porter-Blum MT2 ultramicrotome and stained with uranyl acetate and Reynold's lead citrate. A Hitachi HS8 electron microscope was used for viewing the sections.

OBSERVATIONS

Tree shrew

The vomeronasal organs of tree shrew were two tubular structures situated at the base of the nasal septum and occupying almost the anterior one-third of the nasal cavity. Their average length in three animals was 9·8 mm against a nasal cavity length of 33·5 mm. Anteriorly, each organ communicated with the mouth cavity through the corresponding nasopalatine duct while posteriorly it terminated as a blind pouch. Coronal sections showed that it was crescent-shaped in its middle portion where it was surrounded by a plate of hyaline cartilage, deficient dorsolaterally (Fig. 1). 'Serous' glands, in this dorsolateral region drained into the organ along its dorsomedial angle. The collection of serous glands increased from before backwards so that an abundance of these glands was seen in its posterior portion. The glands stained intensely with PAS and the reaction was unaffected by diastase digestion, indicating the presence of neutral mucosubstances. Alcian blue and mucicarmine gave negative results, showing the absence of acid mucins.

Medially, the organ was lined by a thick sensory epithelium. This consisted of a superficial clear zone, followed by one or two layers of darkly stained oval nuclei, deep to which were three to four additional layers of rounded nuclei. Occasionally, a secretory crypt lined by columnar cells was also seen (Fig. 2). Large nerve bundles and vascular spaces were present in the lamina propria. Laterally, the lining consisted of pseudostratified columnar ciliated epithelium 2–3 cells thick, with vascular spaces in the lamina propria. Scattered lymphocytes were present in this epithelium while larger collections of lymphocytes were concentrated at the dorsal end of the organ anteriorly and at its ventral end posteriorly.

Electron microscopic studies showed that the sensory epithelium consisted of three cell types: receptor cells, supporting cells and basal cells. Receptor cells ended distally in expansions, which were roughly triangular in section and projected above the surface of the epithelium (Fig. 3). They bore numerous long slender unbranched microvilli on the surface, and these were more electron-dense than the microvilli of supporting cells. Glycogen granules, neurofilaments, vesicles of various sizes and centrioles numbering up to ten were present below the neck of the expansion. Centrioles were not seen in all random sections. The cytoplasm of the dendrites of the sensory cells was lighter in some but darker in others. Some large osmiophilic granules

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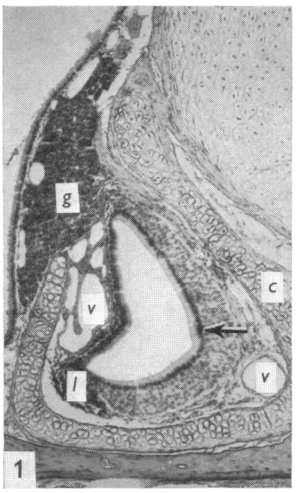
Fig. 1. Coronal section of *Tupaia* vomeronasal organ. Hyaline cartilage (*c*), glands (*g*) and vascular spaces (*v*) are seen. Arrow indicates dark band of supporting cell nuclei of the sensory epithelium. A collection of lymphocytes (*l*) is seen in the lower end of the organ. Haematoxylin and eosin. × 50.

Fig. 2. Coronal section of *Tupaia* vomeronasal organ. Secretory crypt in sensory epithelium is arrowed. Lymphocyte collection is labelled (*l*). Haematoxylin and eosin. × 50.

Fig. 3. Sensory epithelium of *Tupaia* vomeronasal organ showing receptor cells (*rc*) alternating with supporting cells (*su*). Microvilli (*mv*), vesicles (*v*), neurofilaments (*nf*) and large numbers of mitochondria are seen. Note centrioles in two of the receptor cells (arrows). × 8400.

Fig. 4. *Tupaia* vomeronasal receptor cells (*rc*) showing nuclei and lamellated myeloid body (arrow). Rough endoplasmic reticulum (*rer*) and smooth endoplasmic reticulum (*ser*) are seen. Prominent Golgi apparatus in supranuclear region is labelled (*G*). × 8400.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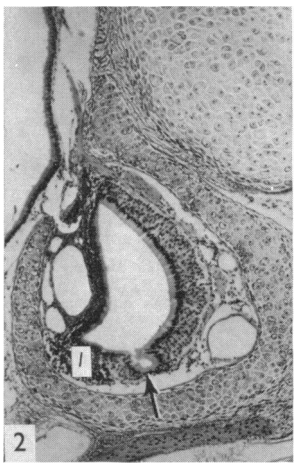
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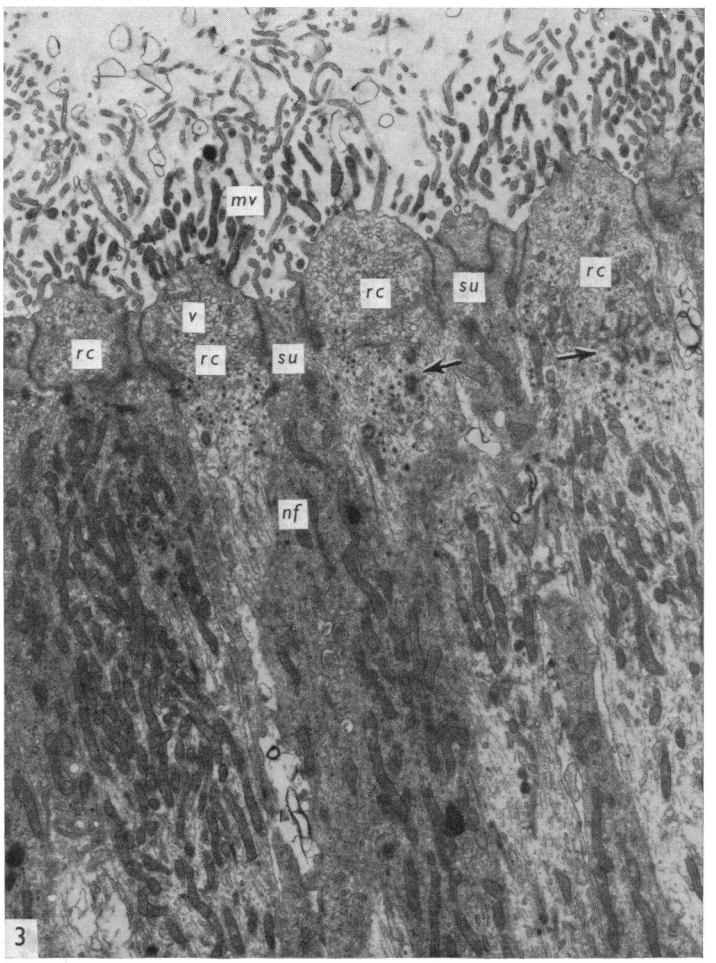
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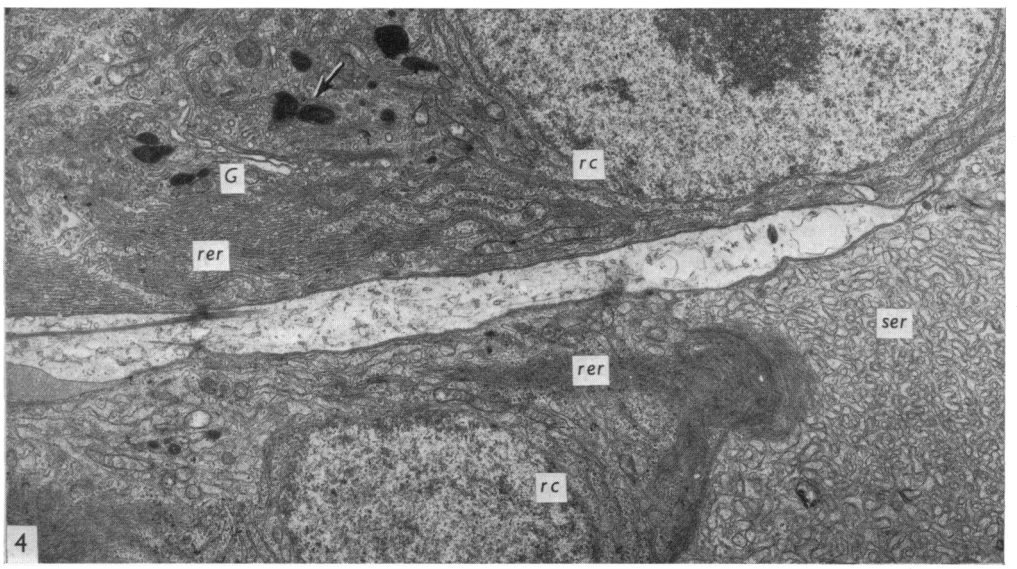
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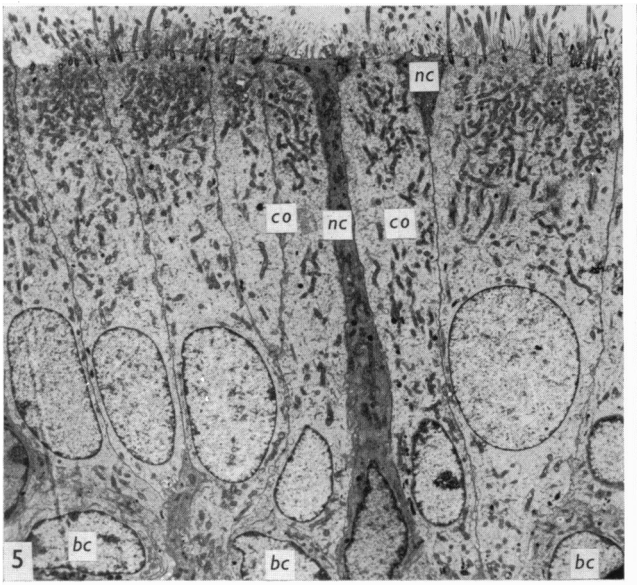
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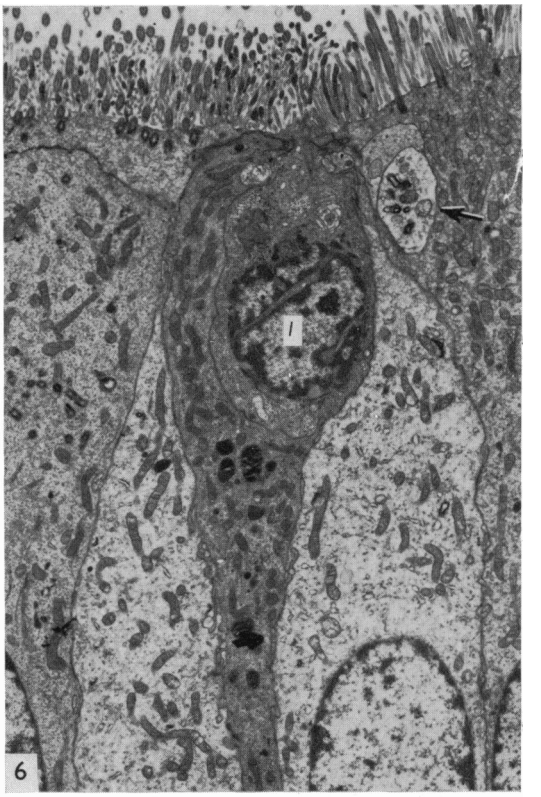
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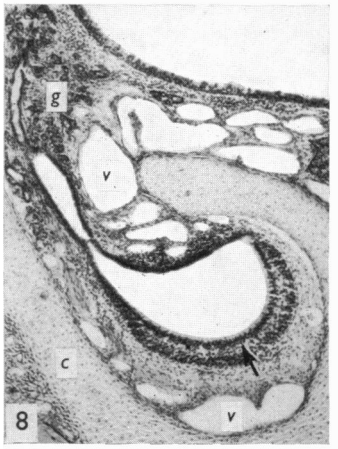
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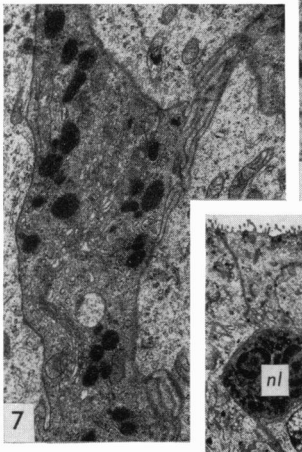
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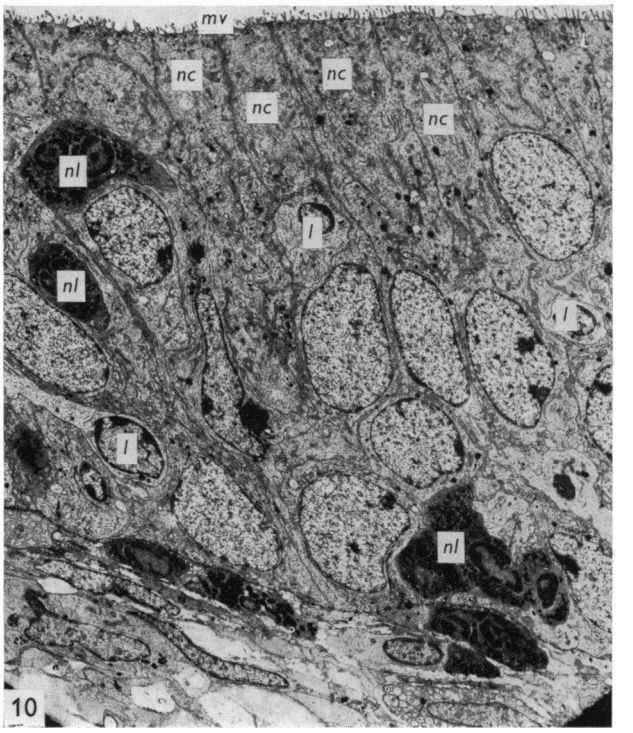
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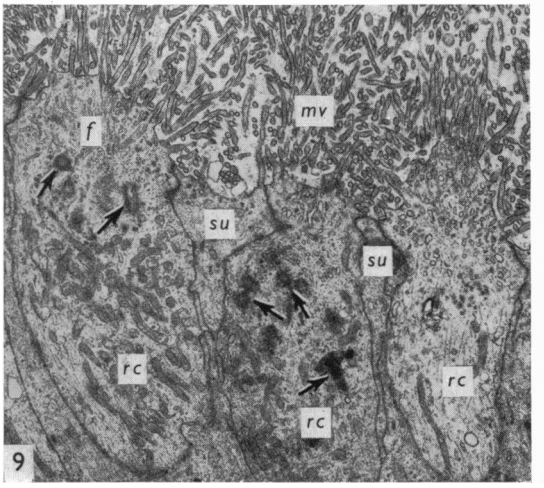
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and lamellated myeloid bodies were also seen (Fig. 4). Around the nucleus were parallel arrays of rough endoplasmic reticulum. However, as the layers of endoplasmic reticulum were followed outwards, ribosomes appeared to be reduced in number or even absent in some cases. Moreover, smooth endoplasmic reticulum was abundant in some cells (Fig. 4). A prominent Golgi apparatus was present in the supranuclear region of the cell. Nuclei of sensory cells were rounded and contained very little chromatin material and one or two nucleoli.

The supporting cells, which usually alternated with sensory cells, were narrow at their distal ends and had a few short microvilli on the surface. Occasionally one or two centrioles were seen inside the supporting cells. Mitochondria and some electron-dense granules were present throughout the cytoplasm. Cytosegregosomes were sometimes observed. Nuclei were oval, and clumps of chromatin material were deposited at their periphery. Basal cells were seen only occasionally and were polyhedral in shape with large nuclei. Cytoplasm was scanty. Cytoplasmic processes, a few mitochondria, some vacuoles and ribosomes were also observed. Mitosis of a basal cell was observed in the dorsolateral region of the organ close to the junction between the sensory and non-sensory epithelia.

In the non-sensory part of the vomeronasal organ four cell types were seen: ciliated cells, non-ciliated cells with microvilli, basal cells (Fig. 5) and lymphocytes which had invaded the epithelium. Ciliated cells bore both cilia and microvilli at their distal borders. Centrioles were present at the bases of the cilia. Cytoplasm was paler than in other cell types and contained mitochondria, free ribosomes, microvesicles and dense bodies. Moreover, multivesicular bodies and cytosegregosomes were sometimes seen (Fig. 6).

Non-ciliated cells were dark and narrow, and were randomly distributed between ciliated cells. Short microvilli were seen at their distal borders. The cytoplasm contained some mitochondria and electron-dense osmiophilic granules (Fig. 7). Centrioles were occasionally seen and sometimes cytosegregosomes were present near the distal border of the cell. Nuclei were polyhedral in shape and situated near the base of the cell. They contained clumps of chromatin material deposited at the periphery.

Fig. 5. *Tupaia* vomeronasal organ showing non-sensory epithelium. Ciliated cells (*co*), non-ciliated cells (*nc*) with microvilli and basal cells (*bc*) are seen. ×2000.

Fig. 6. Non-sensory epithelium of *Tupaia* vomeronasal organ. Lymphocyte (*l*) is seen invading the epithelium. Cytosegregosome in ciliated cell is arrowed. ×8400.

Fig. 7. Part of ciliated cell in non-sensory epithelium of *Tupaia* vomeronasal organ. Electron-dense osmiophilic granules are seen. ×8400.

Fig. 8. Vomeronasal organ of *Nycticebus* in coronal section. Hyaline cartilage (*c*), glands (*g*) and vascular spaces (*v*) are seen. Arrow indicates dark band of supporting cell nuclei below which is a clear zone. Note that sensory epithelium is much thicker than non-sensory epithelium. Haematoxylin and eosin. ×50.

Fig. 9. Vomeronasal receptors (*rc*) of *Nycticebus* showing numerous microvilli (*mv*) with fibrils (*f*) extending into apical cytoplasm of receptor expansion. Centrioles (arrows) and large numbers of mitochondria are seen. Supporting cells are labelled (*su*). ×8400.

Fig. 10. Non-sensory portion of vomeronasal organ of *Nycticebus* showing columnar cells (*nc*) with short microvilli (*mv*). Lymphocytes (*l*) and neutrophil leucocytes (*nl*) are seen invading the epithelium. ×2000.

Basal cells did not differ markedly from those of the sensory epithelium. Lymphocytes and plasma cells were present at the base of the epithelium and some lymphocytes could be seen invading the epithelium at all levels. Myelinated and unmyelinated nerve bundles were present in the lamina propria.

Slow loris

In slow loris, the organ was situated at the base of the nasal septum, but was relatively shorter as it occupied less than the anterior one-fourth of the nasal cavity. The average length in two animals was 7 mm against a nasal cavity length of 32 mm. In coronal sections, the organ was somewhat D-shaped, with the vertical limb of the D lying dorsally (Fig. 8). Hyaline cartilage surrounding the organ was J-shaped. 'Serous' glands in the dorso-lateral region were fewer and less closely packed. They did not increase in number as the organ was traced from before backwards (Fig. 8) but they gave the same reactions to the mucin stains as noted in tree shrew.

Both sensory and non-sensory types of epithelia were present, but the sensory epithelium occupied only the lower and medial portions of the organ. A relatively clear zone was seen just deep to the supporting cell nuclei which were placed more superficially beside the lumen. The non-sensory epithelium consisted of one to two layers of non-ciliated pseudostratified columnar epithelium. This epithelium was infiltrated with lymphocytes from the underlying lamina propria, but the infiltration was not as heavy as that seen in the tree shrew nor were there any large collections of lymphocytes.

Electron microscopic studies showed that the sensory epithelium was composed of the same three cell types as in tree shrew. The expanded ends of the receptors, however, differed in that they were more rounded and bore microvilli which were more numerous and much longer than those in tree shrew (Fig. 9). Moreover, some of these microvilli were branched and covered by glycocalyx. Fibrils from the cores of microvilli passed into the cytoplasm of the receptors for some distance. Receptors with light and dark cytoplasm were also seen. Otherwise, the receptor, supporting and basal cells resembled those of tree shrew.

The non-sensory epithelium was composed of two layers of pseudostratified columnar epithelium (Fig. 10). A few short, unbranched microvilli, coated with glycocalyx, were seen on their distal surfaces. The cytoplasm was rich in mitochondria and ribosomes. Centrioles were present in some sections. The cytoplasm also contained a few osmiophilic bodies and some rough endoplasmic reticulum in the supranuclear region. Nuclei were oval, with chromatin material deposited in small clumps in the periphery. Mitoses of columnar cells were quite frequently seen. Moreover, the epithelium was invaded by numerous lymphocytes and in one animal by neutrophil leucocytes as well.

DISCUSSION

The vomeronasal receptors of tree shrew and slow loris resemble those previously described in amphibia, reptiles and mammals in that they do not bear cilia but only microvilli. In sheep (Kratzing, 1970), tree shrew and slow loris the ends of the receptors are expanded and project above the surface of the epithelium. Owing to this expansion the distal ends of the supporting cells are squeezed and narrowed. How-

ever, in frog (Kolnberger, 1971), *Xenopus* and *Varanus* (Andres, 1970), slow worm (Bannister, 1968), and turtle (Graziadei & Tucker, 1970), the receptors do not end in such pronounced expansions, so that they do not always project above the surface of the epithelium. Thus, phylogenetically, microvilli increase in number from amphibia to the prosimian primates so that in slow loris they are most numerous and longest. This may perhaps be a device to increase the discriminatory sensitivity of the receptors.

Centrioles are seen only in a few instances in turtle (Graziadei & Tucker, 1970) and are not particularly numerous in slow worm (Bannister, 1968), *Xenopus* and *Varanus* (Andres, 1970). However, centrioles numbering two to eight are frequently seen in the distal expansion of the receptors in rat (Andres, 1970) and sheep (Kratzing, 1970), and were observed by us in tree shrew and slow loris. Nevertheless, the precise functional significance of these centrioles remains uncertain. It may be that they represent an arrested phase in the development of ciliated receptors, as found, for example, in the olfactory epithelium.

Mitochondria are evenly distributed all over the dendrite in turtle (Graziadei & Tucker, 1970), whereas there is a heavy concentration of these structures below the centriolar region in both tree shrew and slow loris. The presence of large numbers of mitochondria near the distal end of the dendrite probably implies an increased metabolic activity in this area. This concentration of mitochondria and the presence of centrioles resembles the condition seen in olfactory receptors. Thus, there seems to be an increasing similarity between olfactory and vomeronasal receptors as the phylogenetic scale is ascended. The main difference, however, is the absence of cilia in the sensory epithelium of the vomeronasal organ.

Supporting cells in the vomeronasal organ of amphibia are ciliated and are said to contain secretion granules (Andres, 1970; Kolnberger, 1971). In turtle, although secretion granules are present, the supporting cells are non-ciliated (Graziadei & Tucker, 1970) whereas the supporting cells of the vomeronasal receptors of tree shrew and slow loris are non-ciliated and do not contain any secretion droplets, thereby resembling those in rat (Andres, 1970) and sheep (Kratzing, 1970). Thus cilia and secretory granules in the supporting cells appear to undergo regressive changes as one ascends the phylogenetic scale. A similar regressive change appears to take place also in the non-sensory portion of the vomeronasal organ in slow loris, where no ciliated cells are present, whereas in tree shrew and other mammals, ciliated cells are constantly observed.

In conclusion, the vomeronasal receptors increase in complexity during phylogenetic development, with slow loris exhibiting the highest degree of elaboration of the receptor cells. It may, therefore, be argued that, with an overall phylogenetic reduction in size of the vomeronasal organs, there appears to be a compensatory elaboration of the vomeronasal receptor cells. Whether such a mechanism confers a greater discriminatory capacity upon these peripheral receptor cells remains hypothetical. Finally, the structural differences in the vomeronasal organs of the tree shrew and slow loris do not provide any definite answers on the phylogenetic status of the two primates, although it is tempting to speculate that there is a tendency for the vomeronasal receptors to evolve some form of olfactory function in mammals from their possible role as taste organs in non-mammalian vertebrates. Such a con-

cept seems to fit in well with the elaboration of microvilli, presence of centrioles and the reduction of the nasopalatine canals in an ascending scale of phylogeny.

SUMMARY

The vomeronasal (Jacobson's) organs of two prosimian primates, *Tupaia glis* and *Nycticebus coucang* were studied by light and electron microscopy. Vomeronasal receptors are non-ciliated in both prosimians, as in other vertebrates. However, even though the vomeronasal organ is reduced in the ascending scale of phylogeny, the receptor cells appear to become more and more complex. In the supporting cells, there is a reduction of ciliated cells and secretion granules, culminating in an absence of ciliated cells in the vomeronasal organ of slow loris.

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