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ULTRASTRUCTURE OF THE HUMAN VOMERONASAL ORGAN

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Summary—Virtually all vertebrates have a vomeronasal system whose involvement in pheromone detection plays a crucial role in reproduction. In humans, the vomeronasal organ has been assumed to be vestigial or absent and without functional significance. In the present study involving over 400 subjects, vomeronasal pits were observed in all individuals except those with pathological conditions affecting the septum. Electron microscopy of the adult human vomeronasal organ indicates the presence of two potential receptor elements in the pseudostratified epithelial lining: microvillar cells, and unmyelinated, intraepithelial axons. In addition, unmyelinated axons are common in the lamina propria surrounding the organ. They appear to constitute the components essential for a functional chemosensory system, and may thus provide the basis for a pheromone detection system as in other animals.

INTRODUCTION

It is well known that the vomeronasal organ arises from the medial olfactory placode in the early stages of fetal development as a separate and anatomically distinct receptor component of the olfactory system. This sack-like olfactory organ was discovered in the nasal septum of animals by Jacobson [1] in 1811 and in humans by Potiquet in 1891 [2]. It is now known to constitute a pheromone detection system [3–8]. Embryologic studies universally reveal a well developed tubular primordium in all human and animal specimens studied to date [7, 9–17]. Thus, during the first half of gestation this olfactory derivative is a prominent and well-defined entity [9, 12, 16, 18, 19].

On the other hand several early studies of central nervous system components of the vomeronasal system in man cast doubt on the persistence of receptors in the vomeronasal organ and on the existence of a clearly delineated accessory olfactory bulb in the adult [7, 18, 19, 20–27]. A recent study of the adult human nasal septum by Johnson *et al.* [28] has attempted to resolve this issue. They found a vomeronasal organ in most of the twenty-seven specimens subjected to microscopic analysis, but they describe the mature organ as a "blind diverticulum" with "no neuro-epithelium or nerve endings", and lacking "a concentration of erectile vasculature". Although in their view the organ may be non-functional, only light microscopy and conventional staining techniques were employed. Thus, these investigators were not able to conclusively document either the presence of nerve endings or the existence of receptor cells, features which if present would greatly strengthen the case for the functional status of the organ.

Surprisingly, no ultrastructural studies of either the fetal or adult human vomeronasal organ have been carried out. Numerous electron microscopic investigations of the human olfactory epithelium reveal both intraepithelial axons and an abundance of bipolar receptor neurons. Furthermore, the existence of both ciliated receptor neurons and non-ciliated microvillar receptor cells in human olfactory mucosa is consistent with the notion of multiple classes of chemosensory elements [28–33]. The more recently discovered microvillar cell is a prominent element not only in the olfactory epithelium but also of several other strategic sites in the respiratory system of mammals where chemoreception may be important [15, 28, 29, 34, 35]. Most importantly, the microvillar cell is

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the dominant receptor cell in the vomeronasal organ of all lower vertebrates where it is believed to be the sensor of non-volatile pheromone stimuli [6, 11, 28–30, 36, 37].

In the present electron-microscope study, intraepithelial axons and microvillar cells were found to be the principal constituents of the adult human vomeronasal organ. The fact that numerous unmyelinated axons also occur in the underlying lamina propria indicates that connections persist between the human vomeronasal organ and the brain and that the neural components constitute elements of a functional chemosensory system which may act as a pheromone detection system [3–5, 7, 8, 38–40].

EXPERIMENTAL

The nasal septum of 410 normal candidates for plastic surgery was examined for the presence of vomeronasal pits in the ventral nasal septum. In accord with methods described by Johnson *et al.* [7], the pit was located by means of a headlight and nasal speculum, but there was no application of topical histamine to the nasal septum. The incidence and pathological changes affecting the vomeronasal pit were enumerated for each individual. In addition, 108 serially-sectioned human fetal specimens from the Yakovlev Collection at the Armed Forces Institute of Pathology in Washington, DC were also examined for the presence of a vomeronasal organ.

The nasal pit within the nasal septum of several adult specimens was identified at autopsy soon after death, and the underlying vomeronasal organ was fixed using a thin (0.7 m), polyethylene catheter which was carefully introduced for a distance of 4 to 5 mm. The pigmented fixative consisted of equal parts of phosphate buffer (0.1 M), glutaraldehyde (25%), paraformaldehyde (37%), methylcellulose (5%), and India ink, and it marked the location of the vomeronasal organ. After removal of the nasal septum, the entire specimen was additionally fixed in a solution containing 0.1 M phosphate buffer, 2% glutaraldehyde, 2% paraformaldehyde, 0.01% calcium chloride at 4°C for at least 18 h, and a map was prepared showing the location of the vomeronasal organ which appeared gray when transilluminated. The portion of the septum containing the organ was cut out, washed in 0.1 M phosphate buffer containing 10% sucrose, post-fixed in osmium, washed in phosphate buffer, dehydrated in alcohol and embedded in epoxy resin. Blocks of tissue through the vomeronasal organ were prepared to provide coronal (transverse) sections. Semithin sections were cut on a Reichert OM-2 ultramicrotome and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate, and photographed with a Hitachi 7000 electron-microscope operated at 60–80 kV.

RESULTS

Vomeronasal pit and duct

Bilateral vomeronasal pits were visible in 380 of 410 consecutive subjects interviewed for plastic surgical procedures. In 30, pathological conditions affecting the nasal septum were observed which compromised the respiratory mucosa lining the nasal cavity so that the small vomeronasal pit could not be located. Of the 108 fetal specimens of the Yakovlev Collection, all had pits leading to prominent vomeronasal organs. There were also characteristic aggregates of nervous terminalis ganglion cells and vomeronasal nerves connecting the vomeronasal organ to the brain. The vomeronasal duct arising from the nasal pit of an adult specimen is shown in Fig. 1(a). It is situated superficially in submucosal connective tissue of the nasal septum. In contrast to the nearby stratified respiratory epithelium, the duct consists of a pseudostratified, columnar epithelium whose oval lumen is <1 mm in diameter. Three types of cells can be distinguished. Basal cells are dark, compact elements situated at the base of the epithelium; they constitute approx. 20% of the total cell number. Microvillar cells are tall, columnar, and lightly staining; they are the predominant element forming 60 to 70% of the total. Sustentacular cells are elongate elements with dark nuclei, thick processes and darkly staining cytoplasm. They are the least numerous and represent only 10 to 20% of cells in the duct.

Light microscopy of the vomeronasal organ

The mature vomeronasal organ is distinguished from the duct, with which it is continuous, by its larger size and elongate profile in cross-section [Fig. 1(b)]. It is situated within the ventral septum having an anterior to posterior orientation. The tubular organ extends from the epithelial surface of the septum through a thick layer of submucosal connective and glandular

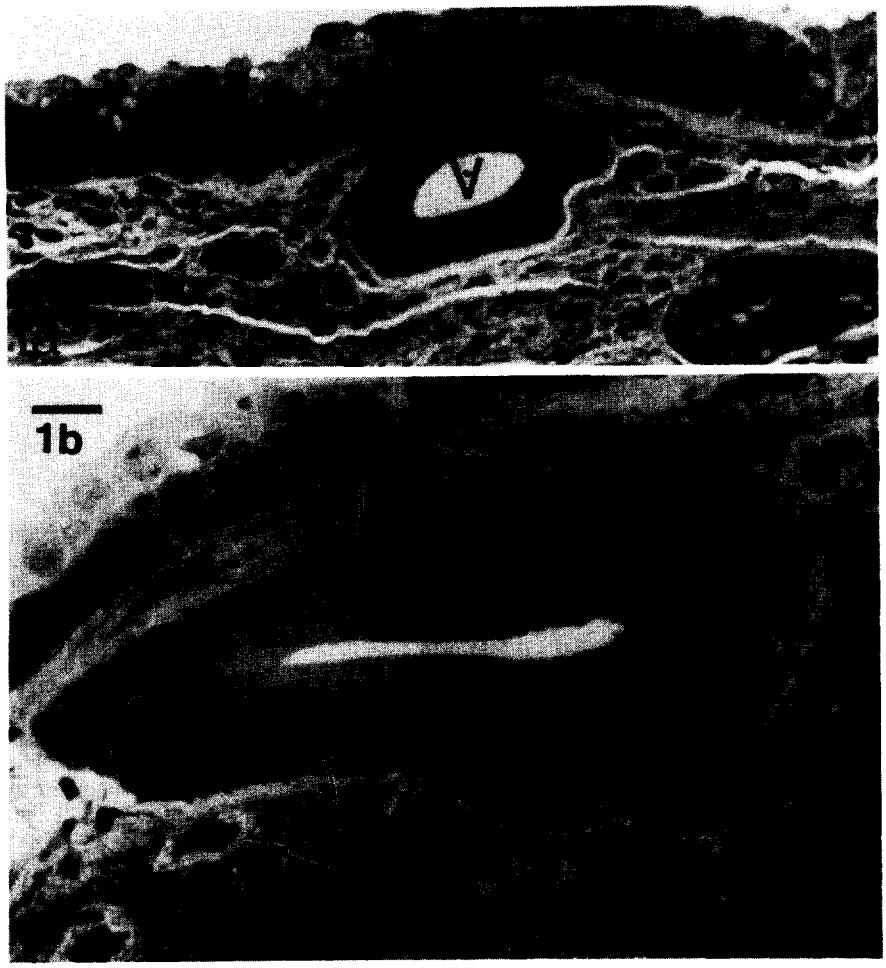

Fig. 1. The vomeronasal duct (V) in a serial, semithin section showing close proximity to the surface epithelium (E). Scale bar = 10 μm. (b) The vomeronasal organ of the same specimen in a serial, semithin section at a deeper level. Numerous lightly-staining microvillar cells are interspersed among darker, basal cells (*), and supporting cells (←). Note the absence of ciliated and goblet cells. Lightly stained areas in submucosal connective tissue (⬅) probably represent unmyelinated axons ensheathed by Schwann cells. Scale bar = 10 μm.

tissue to lie near the perichondrium of the septum. Based on its appearance following injection of the India ink-fixative, but prior to osmication, it was estimated to range in length from 5 to 10 mm, although this was an approximation and may underestimate its true length due to incomplete filling with the pigmented mixture. It is easily distinguished from the ducts of glands, some of which are confluent with the vomeronasal organ, by its large size and proximity to the septal cartilage. The organ consists predominantly of pseudostratified columnar epithelium, and it contains similar types of cells to those observed in the duct. However, both the character and arrangement of cells of the vomeronasal epithelium differ greatly from cells of the nearby respiratory mucosa. As shown in Fig. 1(b), lightly staining microvillar cells outnumber the darker supporting and basal elements. Ciliated and goblet cells also occur within some portions of the epithelium of all specimens, either singly or in groups. The patchy, irregular occurrence of such cells appears similar to transitional regions described in the human olfactory mucosa [30]. A highly vascular lamina propria surrounds the organ, and it contains numerous, lightly-staining sites with the appearance of small nerves [Fig. 1(b), arrows]. Myelinated axons were rarely seen in proximity to the vomeronasal organ.

An electron-micrograph of luminal epithelium of the adult vomeronasal organ is shown in Fig. 2(a). It consists primarily of lightly staining microvillar cells. Apical portions of these columnar elements are united by junctional complexes, and zonulae adhaerens predominate within the epithelium where the cell surface is uncomplicated by numerous, small,

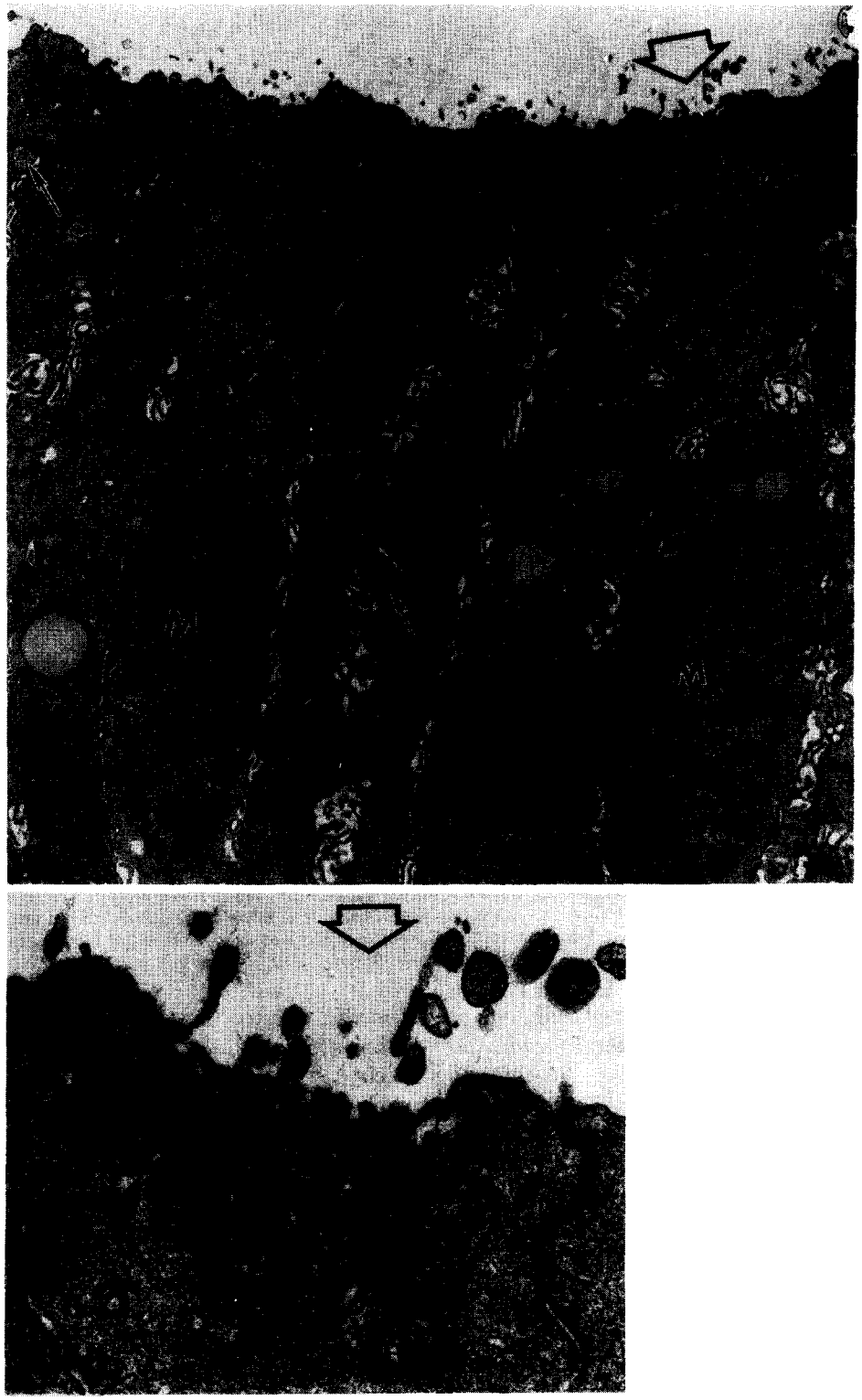

Fig. 2. Apical portion of cells lining the vomeronasal organ. (a) A regular array of microvillar cells (M) surround a few intraepithelial axons (←). An open arrow indicates the microvillar cell shown at high magnification in Fig. 2b. (b) The luminal surface of a microvillar cell is characterized by short microvilli, junctional complexes (⇧), and numerous coated vesicles (↑) and mitochondria (m). Scale bar: A = 1 μm; B = 0.1 μm.

Fig. 3. The inferior portion of the vomeronasal epithelium consists of basal cells (B), dark processes of supporting cells (S), and numerous electron-lucent microvillar elements (M). Intraepithelial axons are indicated by arrows. Note the protruding processes of microvillar cells and irregular lamina propria (outlined). Scale bar = 1 μm.

intercellular interdigitations characteristic of these elements. Mitochondria are clustered in the cytoplasm near the luminal surface; they are less numerous in the perinuclear cytoplasm where they are interspersed with lipid inclusions and endoplasmic reticulum. Short microvillar processes arise from each microvillar cell at the luminal surface. As shown at high magnification in Fig. 2(b), the processes of microvillar cells lack a basal body, and their surface is coated with a flocculent material which may arise from nearby vesicles whose contents are released at the surface. Such vesicles commonly occur at the base of microvillae in proximity to aggregates of rough endoplasmic reticulum and mitochondria [Fig. 2(b)]. Secretory and vesicular constituents characteristic of the Clara cell which occur in the nearby respiratory epithelium were absent in the vomeronasal organ.

A basal portion of the vomeronasal epithelium and its highly cellular lamina propria is shown in Fig. 3. Electron-dense basal cells are surrounded by numerous supporting and microvillar cells; all rest on a prominent but irregular basal lamina. The lower surface of the epithelium lacks junctional complexes characteristic of the luminal surface, and the microvillar cells are united by zonula adhaerens and numerous, small intercellular interdigitations. In contrast with large basal appendages of the electron-dense supporting cells, processes of the microvillar cells taper as they approach the basal lamina, and they lose the mitochondria and other organelles characteristic of perinuclear cytoplasm. Some of these elements resemble profiles of unmyelinated axons in the submucosa, and they form groups which displace the basal lamina in a manner suggesting continuity with nearby axons in the submucosa.

Electron-lucent profiles of intraepithelial unmyelinated axons occur at all levels of the vomeronasal epithelium [Figs 2(a) and 3]. None have been observed which reach the luminal surface. All are $<2\,\mu$m in diameter and most contain cytoplasmic filaments. Aggregates of vesicles and membrane specialization characteristic of synapses were not observed. The distinctive electron-lucent cytoplasm of such intraepithelial axons is unlike that of any epithelial cell, and it therefore appears unlikely that they arise as collaterals from microvillar elements. On the other hand, they resemble unmyelinated axons in the lamina propria which

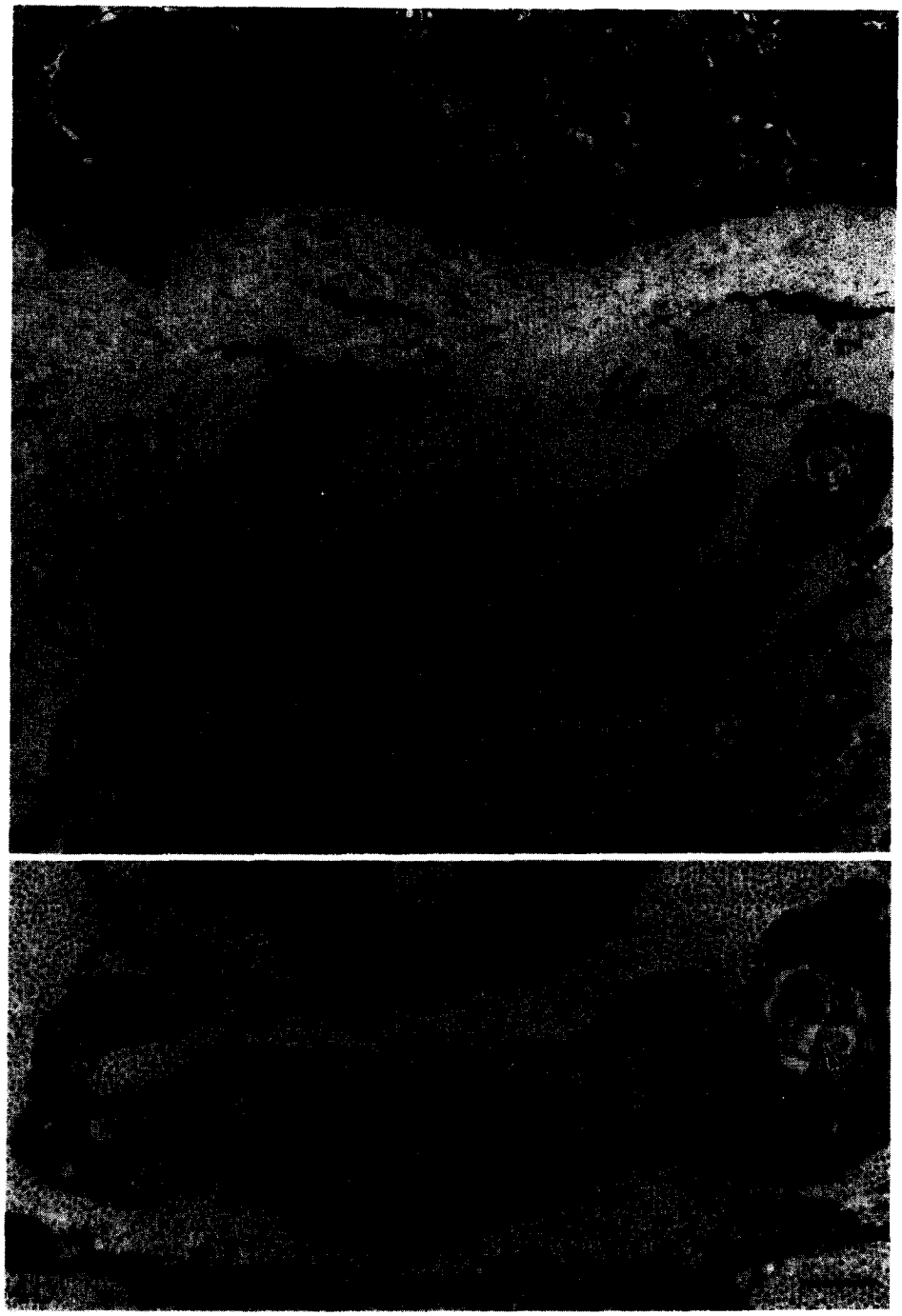

Fig. 4. (a) Unmyelinated axons are common in the lamina propria beneath the vomeronasal organ. Axonal ensheathment is provided by Schwann cells (S) with characteristic dark nuclei, but perineurial cells are absent. Basal cells (B). Scale bar = 1 μm. (b) Diversity in the cytology of axons at high magnification: large profiles with dark cytoplasm (*) resemble processes of microvillar cells; small electron-lucent profiles are similar to intraepithelial axons; arrows indicate axons which may be undergoing autoysis. Scale bar = 1 μm pa.

are particularly numerous in proximity to the vomeronasal organ [Fig. 4(a) and (b)].

Figure 4 depicts unmyelinated axons which are a characteristic feature of connective tissue near the vomeronasal organ. Ensheathment is provided by Schwann cells having a thin basal lamina. A striking feature is the absence of perineurium surrounding the nerve elements and delineating a fascicle. Similarly, fine-caliber collagen fibrils characteristic of endoneurial connective tissue are also absent. Such deficiencies in the fundamental character of a

peripheral nerve are unusual, and they suggest that axons associated with the vomeronasal epithelium do not acquire a fascicular organization until they join a large nerve trunk. The incidence of unmyelinated axons in the lamina propria is much higher for the vomeronasal organ than nearby respiratory mucosa. It appears that their number is commensurate with the incidence of microvillar cells in the vomeronasal epithelium. Large nerve fascicles containing a combination of myelinated and unmyelinated axons occur in the vomeronasal-terminal nerve and were only observed in proximity to the caudal, blind end of the vomeronasal organ.

DISCUSSION

In the olfactory system a distinction is traditionally made between anosmatic animals in which the olfactory bulb and sensory epithelium are absent, microsmatic animals in which the olfactory components are small relative to the size of the brain, and macrosmatic animals in which all olfactory components are large [4, 6]. Primates, particularly old world monkeys and man, are the usual examples of microsmatic animals. The present investigation indicates this distinction may also apply to the vomeronasal system. In man the vomeronasal organ is not small in absolute terms, since it has a length of about 1 cm, but it consists of a simple epithelium lacking association with submucosal erectile tissue. On the other hand the issue of whether the human organ has functional chemosensory capabilities depends on the existence of receptor elements having projections to the brain. The principal findings of the present study are that two types of potential receptors (i.e. microvillar cells and intraepithelial axons) can be distinguished by means of electron-microscopy, and that numerous unmyelinated axons are common in the underlying connective tissue of the lamina propria. Together they appear to constitute the essential components of a functional chemosensory system, and they support the notion that the vomeronasal organ of the human may provide the basis for a pheromone detection system despite its relatively simple morphology [6, 8].

Recent ultrastructural studies of human olfactory epithelium have identified the microvillar cell as a second type of receptor element interspersed with ciliated bipolar neurons [28, 30, 32, 34]. Clear cytoplasm, proximity to the nasal cavity, and a non-ciliated microvillar surface distinguish them from the traditional bipolar olfactory neuron; like the bipolar neuron, they give rise to an elongate, thin basal process resembling an axon. Although their function in the human has yet to be demonstrated, Moran *et al.* [28, 29] speculate they maybe chemoreceptors based on the fact that similar microvillar cells are the dominant receptor element within the vomeronasal system of lower animals where they play an important role in pheromone detection and reproductive function. Microvillar cells with morphological characteristics of neurons have also been reported to occur in the epithelium of the nasal cavity in a wide variety of animals [28–30]. Thus, microvillar cells emerge as a distinct cellular element of chemoreception, in both the human and animal olfactory mucosa. While the incidence of microvillar cells in the human olfactory epithelium is considerably less than other cell types [30], they are the dominant cellular element in the human vomeronasal organ, and they appear to be major determinants of its limited overall thickness. Goblet and ciliated cells typical of respiratory epithelium occur singly and in patches among the microvillar elements in portions of the organ, but they are not its principal element in the specimens prepared for the present study. Furthermore, Clara cells are entirely absent within the vomeronasal epithelium.

The high incidence of normal individuals in which it was possible to identify the vomeronasal pit, and the occurrence of vomeronasal organs in all autopsy specimens indicates that the vomeronasal system is a universal feature of the adult human nasal cavity. This also appears to be true for the vomeronasal organ of fetal specimens of the Yakovlev Collection in which all of the 108 specimens had a prominent pair of organs and well delineated vomeronasal nerves leading to the brain. In adult specimens we have confirmed the existence of regional differences in epithelium lining the vomeronasal organ which appear to be related to the age of the individual and to the unpredictable distribution of goblet and ciliated elements within the organ [7]. A similar diversity of elements has been observed in the human olfactory mucosa where an irregular, patchy distribution of olfactory receptors occurs both within respiratory epithelium, and along transitional "border" zones [30]. Recent ultrastructural studies therefore reveal an unexpected diversity in the character and distribution of receptor neurons, and they collectively

support the notion that there are subsets of receptors with distinctive functional properties. We conclude that the human vomeronasal system should not be considered a rudimentary or vestigial organ because of its relatively small size. Rather, the organ is a ubiquitous feature of the nasal cavity of all terrestrial mammals where it plays a critical role in pheromone-induced reproductive behavior.

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