Electron Microscopic Observations on the Taste Buds of the Rabbit*

BY A. J. de LORENZO, Ph.D.

(From the Department of Anatomy and the Beaumont-May Institute of Neurology, Washington University School of Medicine, St. Louis)

PLATES 69 TO 75

(Received for publication, October 4, 1957)

ABSTRACT

An examination of the fine structure of the taste buds in the rabbit was undertaken. Gustatory epithelium was fixed in OsO$_4$ or 1 per cent KMnO$_4$ solution, containing polyvinylpyrrolidone (PVP). Thick sections were examined in the phase microscope and contiguous sections prepared for the electron microscope.

The bud contains two types of cells, gustatory receptors and sustentacular cells. The receptors are characterized by a dark nucleus and densely granular cytoplasm. The apical processes bear numerous microvilli which extend into the taste pore. Imbedded between the microvilli there is a dense substance, which is also present in the apical cytoplasm of the receptors. The sustentacular cells contain a large pale nucleus and less dense cytoplasm. Their basal surfaces rest upon a basement membrane.

The subepithelial nerve plexuses comprise the fibers which innervate the gustatory receptors. The nerve fibers vary in diameter from 500 Å to 0.3 µ, and are ensheathed by Schwann cells. The intragemmal fibers enter the taste bud between adjacent cells, and are ensheathed by the plasma membranes of the supporting cell until they synapse upon the gustatory cell. The synaptic terminals contain synaptic vesicles, which at this junction reside in the postsynaptic element. This observation is discussed with reference to synapses described elsewhere in the nervous system.

The problem of the relation of structure to function in the nervous system is one that has occupied neurologists for many years. In recent decades, morphological investigations have failed to keep pace with physiological and chemical advances, primarily because of the limited reliability of silver impregnation and methylene blue staining methods and the limited resolving power of the light microscope. This has been especially true in the general area of sensory receptors, where, morphologically, the neural basis of sensory discrimination is not much better understood today than it was fifty years ago. However, it is now possible with the electron microscope to examine more intimately the relation between structure and function and some progress has been made (22).

* This investigation has been aided in part by Grants B-425 and RG 3784 from the United States Public Health Service, National Institutes of Health.
pore, the "sensory hairs" of the gustatory cell, and the relation of the sensory nerves to the gustatory cells, despite a preliminary study by Engström (10 a). These problems lend themselves particularly well to an examination with the electron microscope. Preliminary to other planned studies, the fine structure of the taste buds and the relationship of the sensory nerves to them have been investigated and are here described.

Materials and Methods

Adult rabbits were anesthetized with nembutal and the papillae foliatae were located on the posterior lateral sides of the tongue. Chilled fixative was then dripped upon this area. The animals were killed, and the papillae were removed, cut into small pieces, and fixed for 1 or 2 hours in 1 per cent OsO₄ as recommended by Dalton (5), or in a KMnO₄ solution (16). Permanganate fixation results in excellent visualization of cellular membranes, whereas certain cytoplasmic granular material is not preserved (6, 16). The KMnO₄ solution consists of 1 per cent KMnO₄ buffered to pH 7.6 with acetate-veronal (18). Mitochondria of certain cells tend to be swollen following this procedure, but this can be reduced by increasing the osmolarity of the fixative with 1 per cent polyvinylpyrrolidone (PVP). Dehydration, imbedding, and polymerization were carried out by the standard methods. Sections were cut on a Porter-Blum (Servall) microtome and examined in an RCA EMU-2E electron microscope.

OBSERVATIONS

Light Microscopy

Thick sections were cut from the OsO₄-fixed methacrylate-embedded tissue for study in the phase microscope. Text-fig. 1 is a drawing of a region of a papilla foliata containing two taste buds, as seen in the phase microscope. The taste buds are usually found in groups, the "sensory fields" of Heidenhain (12). The taste buds are crowded together, but separated by small septa of epithelium. The bud itself is surrounded by a row of dense, elongated cells, and these gutter cells form the wall of the inner taste pore at the distal end of the bud. The outer taste pore is a cavity penetrating between the cornified cells of the gustatory epithelium. Within the taste bud itself, at least two different types of cells are encountered. The sustentacular cells described by Heidenhain (12) and Retzius (26) are pale in appearance. They contain large round nuclei and vacuolated cytoplasm, but no large granules. Contrary to the opinion of early workers (9, 12), these cells are not restricted to the periphery of the bud, but are seen throughout. The gustatory cells are rod-like in shape and contain elongated oval nuclei which are denser than those of the supporting cells. Their cytoplasm is densely granular. The apical ends of these cells terminate in cuticular processes from which dense "hair-like" threads extend into the pore canal. An occasional eosinophilic leucocyte is seen between the other cells of the bud. There is little evidence of a basal cell type distinct from the supporting cells. Beneath the basement membrane of the taste buds lies the connective tissue of the papilla.

Electron Microscopy

The taste bud is surrounded, as noted above, by a peripheral row of elongated cells, which have been described as enclosing the bud like the staves of a barrel. These gutter cells differ from the remaining epithelium of the tongue in several respects. They are much denser and vary in shape from flattened to ellipsoidal. They form a layer about 2 to 3 μ wide which encloses the bud (Fig. 5). Distally they narrow to sharp points which circumscribe the inner taste pore. Overlying the distal portions of these cells is a layer of cornified epithelium through which the outer taste pore communicates with the oral cavity. In electron micrographs of this area (Figs. 2 and 5) the gutter cells are seen to have an extremely dense cytoplasm filled with ergastoplasmic membranes and granules. Mitochondria and some vesicles are also evident. A small space about 100 to 150 A wide separates the plasma membrane of the epithelium from the cell membrane of the taste bud cell. However, at the basal regions of the taste bud the space widens considerably and approaches 0.1 μ. The plasma membrane of the gutter cell frequently interdigitates with that of the adjacent bud cell. Although perigemmal nerve fibers are observed, endings on epithelial cells have not been noted.

Two cell types are clearly recognized in electron micrographs of the taste buds. By reference to contiguous thick sections (0.1 μ) examined in the phase microscope, these cells can be identified as the gustatory and the sustentacular cells.

The Gustatory Cell:

General Appearance.—The taste receptor cells are seen throughout the bud, freely intermingled with the sustentacular cells. They are characterized by a basally located, elongated nucleus in which a nucleolus is usually present. The karyo-
plasm is uniformly dense following OsO₄ and KMnO₄ fixation. This is to be contrasted with the large, round, and less dense nucleus of the supporting cell (Fig. 1). The nuclear membrane of the taste receptor cell consists of an inner membrane about 75 Å thick, a less dense area about 100 Å wide, and an outer membrane of the same dimension as the inner. Occasionally the outer membrane may be seen to extend as a loop into the cytoplasm. Cytoplasmic granules lie along the outer membrane. Nuclear pores are occasionally present. For descriptive convenience the gustatory cell can be divided into three parts, the basal, middle, and apical regions.

The Basal Region.—This part of the cell contains the nucleus and is surrounded by glossopharyngeal nerve fibers, which terminate upon the taste cells. The details of the nerve endings are described below. Mitochondria, and a few scattered membranes of the endoplasmic reticulum, are present in the cytoplasm. The mitochondria contain few cristae in a dense matrix. The membranes encountered in the cytoplasm are associated with granules; a Golgi region is not prominent. A well organized ergastoplasm is likewise lacking, and instead, the cytoplasmic granules are distributed throughout the cytoplasm in rosettes and clusters. Vesicles of various sizes (200 to 1000 Å) are present. The proximal processes end upon a basement membrane, beneath which lies the connective tissue of the papilla (Fig. 9).

The Middle Region.—The usual formed elements of the cytoplasm are seen in this region of the cell. The most conspicuous component is a dense granule that measures about 0.1 μ in diameter and occurs in large numbers (Fig. 2).

The Apical Region.—The apical end of the cell presents several interesting structural features (Figs. 2 and 3). The formed elements of the cytoplasm do not penetrate into the extreme end of the cell, but terminate instead some distance from the tip. Many cytoplasmic vesicles and a few small granules, however, are seen in the region of the taste pore (Fig. 4). This particular distribution of cytoplasmic elements is also characteristic of the olfactory receptors (6). The region represented in Fig. 4 corresponds to the cuticular zone described in the light microscope (12, 25). In Figs. 2 and 4 the gustatory cells can be observed extending to the inner taste pore. The pore can be seen in Fig. 2 to be bounded on either side by the tips of the dense epithelial cells surrounding the taste bud. Fig. 4 represents an oblique section through the region of the inner taste pore. Epithelial cells (E) are evident on either side of several gustatory cells, which are characterized by the dense granules described above. Terminal bars (TB) are present between adjacent epithelial cells. It is interesting

Text-Fig. 1. Drawing depicting two taste buds as they appear in the phase microscope. A dense epithelium surrounds each of the buds and also provides a thin septum between them. The taste buds rest upon a basement membrane, beneath which lies the connective tissue of the papilla and the nerve plexuses. Two types of cells are apparent, one of which possesses an elongated dense nucleus, and another whose nucleus is round and light in appearance. At the distal end of the bud, the gutter epithelial cells constrict to form the margins of the inner taste pore. The outer taste pore margins are composed of the superficial cornified cells of the gustatory epithelium. In the taste pore dense "hair-like" processes extend into the pore canal.
to compare the appearance of the epithelium in Fig. 4 (KMnO₄ fixation) with that of Figs. 2 and 3 (OsO₄ fixation). The plasma membrane at the peripheral tip of the cell continues over the surfaces of numerous microvilli arranged as a striated cuticular border (Figs. 2 and 3). An extremely dense substance occupies most of the spaces between the microvilli, often obscuring the cell membranes (Fig. 2). This opaque material which appears structureless is probably the "mucoid substance" of Ranvier (25). It is located between the cells as well as in the cells, as can best be seen in Fig. 2. Whether or not it plays a role in chemoreception is not known. There is no evidence of terminal sensory hairs nor of cilia extending from the taste cells. Basal bodies are likewise absent. An interdigitation of the plasma membranes of adjacent taste cells is prominent in the apical regions (Fig. 2).

The Sustentacular Cell:

This cell is characterized by its large pale nucleus, adequately described in light microscopy (9). Its cytoplasm contains the usual complement of formed elements. The mitochondria are similar in structure to those seen in other tissues (21, 30). In contrast to the taste receptor, the supporting cell contains large distended ergastoplasmic sacs (Fig. 1). Small granules and vesicles are also evident. The peripheral processes do not extend to the taste pore, but end short of this area. The cytoplasm is homogeneous in composition throughout the cell.

Perhaps the most striking feature of this cell type is the relation of its plasma membrane to the penetrating nerve fibers. In Figs. 5 and 6, nerve fibers, which form the first order neurons in the taste pathway, are distributed throughout the buds and synapse upon the gustatory cells. The fibers are ensheathed by the supporting cell plasma membrane in a fashion entirely analogous to that seen in the olfactory mucosa (6), and thus mesaxons are provided for the nerve fibers by the plasma membranes of the sustentacular cells.

The basal end of the cell lies upon a thin basement membrane which separates the taste bud from the underlying papilla. In this region are found the gustatory nerve plexuses (Fig. 7).

Nerve Fibers

The taste buds are supplied by branches of the glossopharyngeal nerve which terminate as intragemmal and perigemmal endings (9). Fig. 5 shows a restricted portion of the lateral side of a single taste bud. The elongated gutter epithelial cells (E) can be easily recognized. In the taste bud numerous fibers can be seen intermingled with the taste bud cells. Mitochondria and some vesicles are present in the axoplasm of these fibers. Fig. 6 represents the basal region of a taste bud. To the lower left the connective tissue space (CT) is visible. Numerous nerve fibers are seen throughout the bud.

Fig. 7 is a higher magnification of a restricted portion of the basal area of a bud. The sustentacular cells (S) rest upon a basement membrane (BM). Beneath the basement membrane lies the connective tissue in which a Schwann cell with its nucleus (SN) is located. To the left are a number of non-myelinated nerve fibers (NF) ensheathed by membranes of a Schwann cell, whose nucleus is not in the field. In Fig. 8 a group of small unmyelinated nerve fibers is depicted as they exist in the subepithelial connective tissue space. These fibers are ensheathed with mesaxons in a fashion entirely analogous to those seen elsewhere (11), but some variation is apparent: for example, the fiber labelled A possesses two distinct mesaxons, and the fibers labelled B and C share a common mesaxon. These variations may represent a terminal overlapping of Schwann cell processes at a point where the fibers are about to be delivered to the taste bud. That some of these fibers are near their point of delivery from the Schwann investment is evidenced in the fiber labelled D, which is only partially ensheathed and contains small vesicles. In addition, the large fiber in the upper right (arrow) has all but lost its Schwann investment. Of particular interest is the extremely small fiber (E) which is clearly ensheathed by a mesaxon. The average size of the fibers in this figure is about 0.3 μ; however, fiber E has an over-all diameter of less than 0.1 μ, and the smallest such fiber encountered measures about 0.05 μ in diameter. Such fibers are seen at the regions designated by the arrows. That these structures are nerve fibers can be inferred from their morphological similarity to fibers C and E, which are clearly ensheathed by the membranes of the Schwann cells.

The nerve fibers gain access to the taste bud by passing between the plasma membranes of adjacent cells. In Fig. 9, at least two such fibers,
which have crossed the level of the basement membrane, are seen at the left. The plasma membrane of the sustentacular cell (S) is reflected back to accommodate the fibers. Mesaxons (M) are provided by the supporting cell membrane. Groups of small fibers similar to those described in the preceding figure are also seen between the plasma membranes of adjacent cells (see arrows). The larger fiber at left center contains vesicles, although an ending is not apparent.

Synapses usually appear as terminal enlargements on the superficial portion of the taste cells (9). Figs. 10 and 11 show two such endings. A few mitochondria, vesicles, and small granules are present in the synaptic terminal (Fig. 10). The vesicles and granules are densely packed, giving a somewhat different appearance than that of synaptic junctions seen elsewhere. The vesicles vary in diameter, from 300 to 600 A. Fig. 11 represents a cross-section through the apical portion of a taste bud. The synaptic ending (S) is in contiguity with several apical processes of gustatory cells (G), which are characterized by their cytoplasmic granules (described above). An interdigitation frequently occurs between the gustatory cell processes and the endings. A space about 150 to 200 A separates the membranes at the point of contiguity.

Discussion

Examination of the taste buds of the papillae foliata of the rabbit with the electron microscope has made possible several interesting observations. Two cytologically different cells, the gustatory receptor and the sustentacular cell, can be clearly distinguished. A basal cell distinct from these cells is not apparent.

The gustatory cell shows at its apical tip an interesting structural modification that is somewhat analogous to the olfactory receptor (6). The terminal process possesses microvilli, which in section appear as foldings of the plasma membrane of the gustatory cell. The cytoplasm of the terminal process contains a paucity of formed elements, although cytoplasmic vesicles are numerous in this region. This situation is similar to that encountered in the olfactory receptor. The apical end of the cell also contains many dense granules of various sizes which are exclusive to this part of the cell. The spaces between the apical ends of the cells are filled with a dense substance which also extends inside the cells.

Whether the vesicles, the microvilli, and the dense material play a role in the mechanism of taste remains to be determined. Although sensory hairs, cilia, and basal bodies have been described in the tips of the taste cells (2, 9, 12), no such structures are seen in the electron microscope. However, the dense mucus-like substance which is seen in the taste pore frequently assumes long, tenuous shapes which could easily be interpreted as sensory hairs with the light microscope.

Concerning the distribution of nerve fibers and their relation to the cells of the taste bud, several interesting features are apparent. In the subepithelial plexus, the unmyelinated nerve fibers are ensheathed by the Schwann cells via mesaxons entirely analogous to those seen elsewhere. These fibers apparently are delivered to the taste bud by entering between adjacent sustentacular cells in the basal region of the bud. There they become ensheathed by the supporting cell plasma membrane until they synapse upon gustatory cells in the bud. The sustentacular cells of the olfactory mucosa, likewise, ensheath both the dendrite and the axon of the primary olfactory neuron (6). In this respect, these situations are analogous. The exact locus at which any one fiber crosses the level of the basement membrane and is delivered from the Schwann cell to the sustentacular cell has not been observed. The fibers are tenuous and tortuous in their path, thus rendering the probability of seeing such a region in sections 200 to 300 A thick statistically remote. Only the most favorably oriented section would pass through such a location, and, unfortunately, such a section has not been obtained. The nature of the small fibers described remains uncertain, but since they are ensheathed by mesaxons in Schwann cells they would appear to be true nerve fibers.

Although Ebner (9) described many of the fibers of the plexus as perigemmal, only a few were observed in this study, and in no case were endings (synapses) seen upon the epithelial cells. However, the extreme density of these cells may obscure these regions. In the taste bud itself many synapses occur on the gustatory cells, mostly in the apical regions of the taste cell. In these regions the gustatory cells prevail, since the majority of supporting cells do not reach the surface of the bud. The fiber makes contact with the receptor after having lost its sheath in the more basal regions of the bud. Although it has
been suggested that endings occur upon sustentacular cells as well (9), no such endings were observed, unless the vesicle-filled fibers running through their sustentacular sheaths could be so regarded.

The question of whether the nerve fibers which terminate in the buds are sensory or motor is a critical one. In most synaptic regions investigated with the electron microscope clusters of minute vesicles 200 to 700 Å in diameter are seen, usually occurring in the presynaptic element of the junction (8, 20, 27). Mitochondria are also usually present in the presynaptic terminals, although this feature is apparently not essential, as has been shown in the rabbit retina (7). In the taste bud, the vesicular bodies reside on the postsynaptic side of the junction. This is not an entirely new observation, however, since in at least two other neural junctions, the hair cell–vestibular nerve (32, 34) and the hair cell–acoustic nerve (10), the synaptic vesicles are likewise in the postsynaptic element. In the taste bud the evidence is strongly in favor of most of the fibers’ being sensory, as shown by the thorough studies of Retzius (26), Jacques (13), von Lenhossek (14), and Arnstein (1). As such, they constitute the first order neuron in the taste pathway. Cutting the glossopharyngeal nerve results in degeneration of the taste receptor (17, 19, 33), and suturing the sectioned gustatory nerves results in taste bud regeneration (19). Since all the recognizable endings seen in the taste bud are similar in fine structure, and since the vast majority if not all of the fibers are sensory, then the synaptic vesicles are indeed in the postsynaptic element, and the taste bud must be likened to the inner ear in this respect. It thus appears that this characteristic may be common in certain junctions between specialized sense receptors and sensory nerves.

Addendum.—After this manuscript was submitted for publication, a paper appeared entitled Electron microscope study of the rabbit gustatory bud, by O. Trujillo-Cenoz (Z. Zellforsch., 1957, 46, 272), which duplicates some of the observations made in this study.

References

27. Robertson, J. D., J. Biophyseal and Biochem. Cytol., 1956, 2, 381.
EXPLANATION OF PLATES
PLATE 69

Fig. 1. Low magnification of a taste bud in which the sustentacular and receptor cells are conspicuous. The gustatory cell is characterized by a dense, elongated nucleus (GN); the cytoplasm (G) contains mitochondria, vesicles, and numerous granules. The supporting cell possesses a larger round and less dense nucleus (SN) and cytoplasm (S), which is much less granular and characterized chiefly by distended ergastoplasmic sacs. A nerve ending (Se) is seen in relation to a gustatory cell. OsO₄ fixation; X 7,500.
(de Lorenzo: Fine structure of taste buds)
PLATE 70

Fig. 2. A section through the taste pore area of a bud. The gustatory cells are seen ending in the inner taste pore. The plasma membrane of the cell shows microvilli which are partially obscured by the dense osmiophilic material which lies in the spaces between cells. The cytoplasm of the gustatory cell contains mitochondria, vesicles, and large round granules which do not extend to the tip of the process. The relationship of the epithelium (E) to the inner taste pore and the outer taste pore (OP) is shown. OsO₄ fixation; X 10,000.

Fig. 3. Higher magnification of a restricted region of the inner taste pore. The microvilli, which appear as foldings of the gustatory plasma membrane, can be clearly seen. In the region designated by the arrow, the microvilli are cut in cross-section. Note the dense mucus-like substance described above. Similar material is likewise seen inside of the cell process (Fig. 2). OsO₄ fixation; X 42,000,
PLATE 71

Fig. 4. An oblique section through the region of the taste pore (P), following KMnO4 fixation. Note the gustatory epithelium (E) bounding both sides of the pore in which terminal bars (TB) are evident. At the bottom of the figure, several large dense granules are seen which are characteristic of the apical region of the gustatory cell. The terminal processes contain a vast array of cytoplasmic vesicles (V) of various sizes. × 28,000.
(de Lorenzo: Fine structure of taste buds)
Fig. 5. Low magnification of the lateral side of a taste bud, showing both intragemmal and perigemmal nerve fibers. Two epithelial cells (E) are seen at the bottom which constitute the gutter cell lateral margin of the bud. OsO₄ fixation; X 9,500.

Fig. 6. Section through the basal region of a taste bud, demonstrating the distribution of nerve fibers in this area. To the left can be seen the connective tissue space (CT) and above it the proximal processes of the septal epithelial cells. OsO₄ fixation; X 10,500.
Fig. 7. Section through the basal portion of a taste bud, showing the sustentacular cells (S) resting upon a basement membrane (BM), and the subepithelial connective tissue containing a Schwann cell nucleus (SN) and several unmyelinated nerve fibers (NF). KMnO₄ fixation; X 18,000.

Fig. 8. High magnification of a group of nerve fibers in the connective tissue space immediately below the basement membrane (BM). The nerve fibers are ensheathed by membranes of the Schwann cell in a manner analogous to those seen elsewhere. Some variation, however, is apparent; for example, fiber A has two clearly defined mesaxons, whereas fibers B and C share a common mesaxon. That some of these fibers are about to be delivered from their Schwann investment is apparent in the case of fiber D, which is only partially ensheathed by the membranes of the Schwann cell and contains vesicles, and in the large fiber at the upper right (see arrow), which completely lacks a sheath. Note particularly fibers C and E, which are clearly invested by mesaxons. Similar fibers are seen at the locations designated by arrows. Note the relative sizes of these fibers from the scale at the lower left, which represents 1 micron. KMnO₄ fixation; X 45,500.
(de Lorenzo: Fine structure of taste buds)
FIG. 9. Section demonstrating how the nerve fibers apparently gain access to the taste bud. In the center left of the micrograph, two large fibers can be seen lying between the plasma membranes of a sustentacular cell (S) to the left, and a gustatory cell with prominent nucleus (GN). The proximal processes of both these cells rest upon a basement membrane (BM). Note that the plasma membrane of the sustentacular cell (S) to the left is reflected back, providing mesaxons (M) for the two large fibers, and that the larger fiber contains vesicles (see text). At the regions designated by the arrows, more of the minute nerve fibers described in Fig. 8 are seen lying between the two cell membranes. KMnO₄ fixation; X 45,500.
(de Lorenzo: Fine structure of taste buds)
PLATE 75

Fig. 10. Synapse (Sv) seen in the basal region of a taste bud. Present in the synaptic terminal are mitochondria, numerous vesicles, and granules. GN designates gustatory cell nucleus. OsO₄ fixation; X 19,000.

Fig. 11. Synaptic ending (Su) seen in the apical region of a taste bud. The apical processes of gustatory cells (G) are clearly recognized, because of the dense granules which are restricted to the distal processes (see text). The ending (Su) contains numerous vesicles 200 to 600 Å in diameter. Note that this ending is in contiguity with several taste cell processes, and that curious hook-like invaginations occur between these processes and the ending. OsO₄ fixation; X 27,500.
(de Lorenzo: Fine structure of taste buds)