Fine structure of degenerating taste buds after denervation

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It is well known that taste buds are dependent on an intact nerve supply, and when experimentally denervated they degenerate and disappear (von Vintschgau & Honigschmied, 1877; von Vintschgau, 1880; Griffini, 1887; Meyer, 1897; Olmsted, 1920a, b, 1921, 1922; May, 1925; Whiteside, 1927; Torrey, 1934, 1936; Wagner, 1953; Guth, 1957, 1958, 1963; Beidler, 1962, 1963). Olmsted (1920b) has suggested that the degenerating taste bud cells are cleared away by macrophages invading the epithelium; Guth (1957, 1958, 1963) has demonstrated sloughing of degenerating taste buds from the epithelial surface, and others believe that taste bud cells dedifferentiate to become lining epithelium (Meyer, 1897; Wagner, 1953). Because of this disagreement and because recent evidence for cell turnover in taste buds has indicated that cell death and replacement is a normal occurrence (Beidler, 1962, 1963; DeLorenzo, 1963; Beidler & Smallman, 1965), it is pertinent to study the fine structure of degenerating taste buds in the hope of elucidating the process by which taste bud cells and nerve terminals degenerate.

MATERIALS AND METHODS

Adult Wistar albino male rats were anaesthetized with ether. A 3 cm long incision was made along the ventral aspect of the neck, about 0.5 cm to the left of the midline. The combined lingual and chorda tympani nerve was exposed by retracting the digastric muscle medially and the internal pterygoid muscle posteriorly. The nerve was crushed by applying a small haemostat for 1 min at each of three locations along its length; in other animals a segment of the nerve, 2–4 mm long, was excised. The results of the two types of nerve injury were identical. The right lingual nerve was left intact. The wound was sutured and the animal allowed to recover. Rats were killed with ether at intervals of 3, 6, 12, 18 and 24 h and daily until 7 days after the operation. The anterior half of the tongue was excised and divided longitudinally into thirds of approximately equal width. The middle third, containing the median sulcus, was discarded to eliminate possible confusion arising from taste buds on the denervated

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side which might derive some innervation from contralateral nerves crossing the midline (Hayes & Elliott, 1942). The left (operated) and right (control) sides were minced, fixed in ice-cold phosphate-buffered (pH 7.2-7.5) Karnovsky's mixture (Karnovsky, 1965) for 2 h, rinsed briefly in phosphate buffer and transferred to 1.33% osmium tetroxide in the phosphate buffer for 2 h. Specimens were then placed in 70% alcohol and individual fungiform papillae were dissected away from the surrounding tissue. The papillae were dehydrated in a graded series of alcohols, passed through several changes of propylene oxide, and embedded in Epon 812 (Luft, 1961) or Maraglas (Erlandson, 1964). Thin sections of the taste bud area were made with a diamond knife on a Sorvall MT-1 microtome, and placed on formvar-coated copper grids. The sections were treated with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop b.

The test for acid phosphatase activity was made on calcium-formol fixed frozen sections (50 μ) of denervated tongue in a modified Gomori's incubation mixture (Barka & Anderson, 1963). After 30 min of incubation the specimens were rinsed in distilled water and fixed in buffered 1.33% OsO₄ as above. Those sections containing taste buds were selected and the above methods used for dehydration, embedding, sectioning and examination in the electron microscope.

RESULTS

Before the results of the degeneration experiments are described, it is pertinent to summarize briefly the fine structure of the cells found in normal or control taste buds of fungiform papillae in rats (for more details, see Farbman, 1965b). Within the taste bud proper there are two fully differentiated cell types, the dark cell (type I) and the light cell (type II), and a third, less well differentiated cell, the basal cell, which will not be considered in the present study. The dark cell, which is spindle-shaped, rests on the basement lamina and extends to the taste pore where its apical surface forms several microvilli; microvilli are found in smaller numbers on its lateral surfaces. The dark-cell nucleus is an elongated oval containing dense granular material. The cytoplasm is filled with a fine

Fig. 1. Electron micrograph of several intragemmal processes 3 h after application of a crush injury to the lingual nerve. Some dense bodies (d) are seen within nerve processes. M, Mesaxon. × 20 000.

Fig. 2. Eighteen hours after severance of the lingual nerve. Several dense bodies (d) are evident within dark cells (D), but not within light cells (L). Intragemmal and subgemmal nerve processes are absent. ×4500. Inset: higher magnification of subgemmal area. Portions of Schwann cells, outlined by distinct external laminae (e) are seen, but nerve fibres are absent. ×12 500.

Fig. 3. Micrograph of a portion of a subgemmal Schwann cell from a specimen denervated 12 h before killing. Several degenerating nerve elements are seen. ×15 000.
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filamentous material which gives the cell its 'dark' appearance when examined with the electron microscope. A prominent Golgi apparatus is usually present in the supranuclear position, and a few vesicles and mitochondria are found mostly in the apical half of the cell; in general, there are relatively few organelles. The dark cells usually envelop nerve processes with microvilli which form a mesaxon reminiscent of Schwann-cell mesaxons. The light cell, on the other hand, contains a less dense, more spherical nucleus. The cytoplasm of the light cell is characterized by abundant mitochondria and an abundant agranular endoplasmic reticulum, with many vesicles and a very prominent Golgi apparatus. The plasma membrane of the light cell is fairly smooth, and its relation to nerve processes is simple proximity or contact without any mesaxon-like arrangement.

One of the most striking findings in the experiments on degeneration was the rapid rate at which changes in nerve terminals appeared. Although degeneration did not progress synchronously in all taste buds from a given tongue, in some specimens morphological changes were observed 3–6 h after operation in nerve terminals within the taste bud (intragemmal) and within the nerve plexus immediately subjacent to it (subgemmal). These changes were characterized by the appearance within nerve processes of irregular dense structures, about the same size as mitochondria (Fig. 1). Such changes were not apparent in all denervated specimens at this early time after operation.

The degenerative changes at 12–24 h were more consistently present in all specimens. First, there was a striking reduction in number of viable nerve processes in both intragemmal and subgemmal areas. In the subgemmal region, profiles of Schwann cells identified by a surrounding external lamina were observed without nerve processes (Fig. 2). Secondly, large electron-lucent intracellular vacuoles containing diffuse flocculent material were often present (Fig. 5). Thirdly, membrane-bounded structures (Fig. 3), containing recognizable mitochondria and some vesicles, were observed in the same relation to Schwann cells as that usually associated with unmyelinated nerve fibres.

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Fig. 4. Micrograph of apical portion of a taste bud denervated 24 h before removal. Note dense bodies (d) in dark cells near pore (P). L, Light cell. ×7500.

Fig. 5. Micrograph of the basal portion of a taste bud denervated 24 h before removal. Large, vacuolated areas of cytoplasm are seen. n, Intragemmal nerve process. ×4500.

Fig. 6. Twenty-four hours after severance of the lingual nerve. Several pleomorphic dense inclusion bodies are evident in the dark cell (D). In the uppermost dense body, arrows indicate areas where the double limiting membrane is clearly observed; this material probably represents the phagocytosed remains of a nerve process. L, Light cell. ×17 500.

Fig. 7. Two days after denervation; micrograph showing sites of acid phosphatase activity. The heavy deposits found in most of the dense bodies indicate the presence of the enzyme. ×10 000.

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larly, dense bodies, in the same size range as nerve processes, were seen within dark cells in the taste bud proper (e.g. Fig. 2). Some dense bodies were located near the apical ends of dark cells, i.e. close to the taste pore (Fig. 4), but none were observed in light cells. In both intragemmal and subgemmal locations, these dense membrane-enclosed structures were most numerous 1–2 days after nerve section; thereafter, their number diminished, and none was found in specimens taken 4 or more days after denervation. Morphologically, there was no constancy in their contents. Some contained dense, amorphous material in which no structural details were evident (e.g. Fig. 2); in others, mitochondria were enclosed (e.g. Fig. 3).

On the basis of their size, number and location, many of the dense bodies have been identified as remnants of degenerating nerve processes. The morphological criterion used was the presence of a double membrane around the dense body (Figs. 6, 9). The outer membrane presumably belongs to the taste bud cell, and the inner one to the degenerating nerve process. Where the dense body had only a single limiting membrane, it was more likely to be a member of the lysosome family. In either event, many dense bodies, bounded by one or two membranes, showed a positive reaction when tested for acid phosphatase (Fig. 7).

The relation of degenerating nerve processes to dark cells in taste buds may be contrasted with the typical nerve-cell relationship in taste buds from untreated animals (e.g. DeLorenzo, 1963; Farbman, 1965a, b; Gray & Watkins, 1965; Scalzi, 1967; Murray & Murray, 1967). As stated previously, in the latter the dark cells enclose nerve processes by overlapping, cytoplasmic extensions, thus forming a mesaxon (cf. Fig. 1). Mesaxons were not observed in the denervated specimens after 24 h. The degenerating nerve fragment was a true inclusion within the dark cell.

The dense bodies, described above, were morphologically distinct from the structures which are typically found in the dark cells of the fungiform papillae of untreated rats, but which are not found in buds from circumvallate or foliate papillae of rats or other mammals (Farbman, 1965b, 1967). In Fig. 9 the two types of inclusions are seen in the same dark cell. Neither recognizable nerve elements nor inclusions bounded by a double membrane were present in specimens taken 4 or more days after denervation, but the cytoplasm of the dark cells contained lamellar inclusions.

In addition to the small (1–3 μ) dense bodies, larger membrane-enclosed dense structures, up to 6–8 μ, were observed within dark cells (Fig. 8) as well as between cells (Fig. 10). In view of their size, and the frequent presence of a dense 'nucleoid', some of these larger structures were tentatively identified as degenerating taste bud cells or parts thereof.

Four to five days after interruption of the nerve the number of taste bud cells was much diminished, and, as mentioned previously, no intragemmal nerve elements were found (Fig. 11). Significantly, the number of dark cells remaining
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at this stage was about equal to that of light cells. This is in contrast to the ratio of about two or more dark cells for each light cell in control taste buds. Aside from the loss in total number of cells and the absence of nerve processes, the cells remaining at this stage of degeneration were morphologically indistinguishable from those in control taste buds.

Fig. 8. Micrograph illustrating some of the large dense bodies seen in degenerating taste buds 24 h after interruption of the lingual nerve. × 7500.

Fig. 9. Micrograph showing two types of dark cell dense bodies. The upper one is of a type usually seen in dark cells from untreated taste buds. The lower bodies, both bounded by a double membrane, are typically seen in dark cells of degenerating taste buds. × 24000.

Fig. 10. Micrograph of part of a degenerating taste bud from a rat 3 days after lingual nerve was severed. Intracellular dense inclusion bodies (d) are abundant in dark cells. Note portions of two degenerating cells (C); in the degenerating cell on the left a portion of the nucleus is shown. L, Light cell. × 5000.
Fig. 11. Micrograph of a cross-section through a taste bud 4 days after lingual nerve interruption. Typical light cells (L) and dark cells (D) are present but in reduced number. No nerve processes are visible. ×4500.

Fig. 12. Micrograph of a taste bud 7 days after denervation. Typical dark and light cells are absent. Of the cells present, those labelled × resemble light cells in many respects. The others are similar to stratum basale cells. ×6000.
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One week after section of the nerve, the familiar rounded bud-like bulge of epithelium into connective tissue was still visible in sections of fungiform papillae at low magnifications, but careful examination of these areas failed to reveal any typical light or dark cells. The cells in the middle of the rounded bulge of epithelium could, however, be distinguished from the ordinary keratinizing epithelium of the fungiform papillae by the following features: their more nearly spherical nucleus, less dense ground cytoplasm, more regular profile (i.e. few microvilli), fewer tonofilaments and fewer desmosomes (Fig. 12). In other respects, the population of organelles was comparable to that found in stratum basale cells, namely, abundant free ribosomes, little endoplasmic reticulum, a small Golgi apparatus and small, rod-like mitochondria.

DISCUSSION

Electron-microscopic examination of denervated taste buds in the rat has clarified some of the cytological events that occur during degeneration and has given some insight into what may happen during normal turnover of the cell population. One of the major findings in the present study was the relatively rapid reduction in the number of dark cells in the taste bud after denervation. The ratio of dark to light cells declined from approximately 2:1 in control taste buds to 1:1 in 4-5 days after denervation (cf. Kitamura, 1965); 1 week after denervation, dark cells were completely absent but altered light cells remained. These observations demonstrate that dark cells disappear more rapidly than light cells in the degenerating taste bud.

The significance of these findings must be considered in the light of recent autoradiographic experiments showing that taste bud cells in both rats and rabbits undergo continuous cell renewal (Beidler, Nejad, Smallman & Tateda, 1960; Beidler, 1962, 1963; DeLorenzo, 1963; Beidler & Smallman, 1965). The estimated life-span of the ‘average’ taste bud cell in the rat fungiform papilla was 250 ± 50 h, with the qualification that some cells had much shorter and others much longer lives (Beidler & Smallman, 1965). However, this estimate was based on the assumption that there is an ‘average’ cell, i.e. that there is only one basic cell type. Recent electron-microscopic investigations do not support this assumption (Iriki, 1960; Nemetschek-Gansler & Ferner, 1964; Farbman, 1965a, b, 1967; Scalzi, 1967; Murray & Murray, 1967) and indicate that there are at least two well-differentiated cell types. In the light of this evidence the autoradiographic data on cell renewal must be reinterpreted. It is possible to interpret the data by supposing either (1) that only one of the two cell types is undergoing continual replacement, or (2) that both are replaced, but at different rates (Farbman, 1967; cf. also Robbins, 1967). If either of these suppositions is correct, it would account for the wide range of values obtained in the estimated life of taste bud cells (Beidler & Smallman, 1965). The results of the present study are consistent with both of the above suggestions.
The time elapsing between denervation and complete disappearance of dark cells was 6–7 days in the present study. If this period is assumed to be an index of the life-span of these cells under normal conditions, then it is consistent with the lower ranges of values obtained by the autoradiographic studies cited above, and also with the period found to be required for the complete disappearance of taste buds in other denervation experiments (Guth, 1957; Zelena, 1964).

After the dark cells had vanished, the remaining cells resembled altered light cells in some respects. This suggests that this cell type may have a longer life than the dark cell and that it undergoes 'dedifferentiation' after denervation (Meyer, 1897; Wagner, 1953). Recent unpublished autoradiographic data on the renewal of epithelial cells in mouse tongue have shown that the nuclei of some cells within taste buds remained unlabelled after 30 days' continuous exposure to tritiated thymidine (Cameron, 1968, personal communication). This indicates that these cells had not engaged in DNA synthesis for at least 30 days, and therefore must be more than 30 days old.

From the foregoing brief discussion on the cell dynamics of taste buds, it is apparent that none of the available data conflict with either of the above suggestions concerning the differences in replacement rate of the two cell types. It is no longer possible to consider the population of taste bud cells as being members of one species which differ in appearance only because they have reached different stages of development. It may be concluded that dark cells are more dependent on nerve processes for their survival than light cells. Moreover it is likely that dark cells have a shorter life-span and a more rapid turnover rate than light cells.

With respect to the events during degeneration of the taste bud, the first one of major importance is the striking morphological change in the intragemmal nerve terminals that occurs within 12 to 24 h of injury. The onset of early degenerative changes in the nerve terminals agrees with that recorded in the denervated taste buds of fish (May, 1925) and in various other experimental systems: at synapses of the guinea-pig acoustic ganglion after removal of the cochlea (DeRobertis, 1956), in the superior cervical sympathetic ganglion after preganglionic denervation (Taxi, 1959; Quilliam & Tamarind, 1967) and in early Wallerian degeneration (Vial, 1958; Webster, 1962).

In addition to the relatively early appearance of degenerative changes within the nerve terminals, there was a notable change in the morphological relationship between intraepithelial nerve processes and the dark cells of the taste bud. In specimens taken one or more days after denervation, the degenerating nerve processes appeared as true inclusions of dark cells. This confirms the often reported and well-known phenomenon of fragmentation of nerves undergoing secondary (Wallerian) degeneration. Moreover, it indicates that the dark cell has performed a function not usually associated with epithelial cells, namely phagocytosis. Although this function is usually assigned to macrophages and reticulo-endothelial cells, it has been shown that some epithelial cells can per-

The absence of recognizable organelles in phagocytosed neurites as early as 1 or 2 days after operation indicates their rapid breakdown; this agrees with observations made by others on degenerating peripheral nerve processes (e.g. Vial, 1958; Ohmi, 1961; Webster, 1962; Lee, 1963; Nathaniel & Pease, 1963; Holtzman & Novikoff, 1965; Quilliam & Tamarind, 1967; and many others). Moreover, the absence of inclusions resembling fragments of nerve processes in the reduced population of dark cells 4-5 days after operation suggests that these cells disposed of the phagocytosed fragments rapidly and with virtually no trace.

Finally the nature of the neural influence on the epithelial cells of taste buds should be briefly considered, i.e. whether the nerve provides a continuous trophic influence for the maintenance of the taste bud (Olmsted, 1920a, b; May, 1925; Torrey, 1934; Robbins, 1967) or whether a short-acting, inductive influence operates at a critical time during cytodifferentiation to determine the specific fate of the cell (Torrey, 1940; cf. also Guth, 1957). In the present study, it was shown that dark cells survived for at least 2 days after all trace of healthy or degenerating nerve had vanished, and the light cells presumably survived longer. This indicates that the two cell types differ in their degree of dependence on nerve. With respect to the dark cell, the neural influence upon it may have been exerted for a short period during its early life, or the cell might have obtained enough of whatever it needed from the nerve by phagocytosing a degenerating fragment. The light cells, on the other hand, seemed able to survive longer without innervation. Any conclusions concerning the nature of the nervous influence must await further experiments.

SUMMARY

1. The fine structure of degenerating taste buds in the rat was examined at intervals of from 3 h to 7 days after denervation. Striking morphological changes were evident in 12-24 h. These included disruption and reduction in number of both intragemmal and subgemmal nerve processes and engulfment of degenerating intragemmal nerve fragments by dark cells.

2. Four days after nerve section, virtually no nerve elements were observed in the taste-bud area. At this stage, the number of dark cells in the taste bud was significantly reduced although the number of light cells seemed unchanged.

3. Within 7 days, all dark cells had disappeared. Some of the cells remaining in the taste bud area at 7 days resembled altered light cells. The findings suggest that the life span of the dark cell is shorter than that of the light cell.
RÉSUMÉ

Structure fine de bourgeons gustatifs en dégénérescence après dénervation

1. On a examiné la structure fine de bourgeons gustatifs en dégénérescence chez le rat, à des intervalles de 3 h à 7 jours. Des modifications morphologiques frappantes étaient évidentes après 12 à 24 h. Elles comprenaient: la rupture et la réduction en nombre des prolongements nerveux à la fois intragemmaux et subgemmaux et l'absorption de fragments de nerfs intragemmaux en dégénérescence par des cellules sombres.

2. Quatre jours après la section du nerf, on n'a observé virtuellement aucun élément nerveux dans la région du bourgeon gustatif. A ce stade, le nombre de cellules sombres dans le bourgeon gustatif était significativement réduit, quoique le nombre de cellules claires paraissait inchangé.

3. En 7 jours, toutes les cellules sombres avaient disparu. Quelques-unes des cellules restant alors dans la région du bourgeon gustatif ressemblaient à des cellules claires altérées. Les observations faites suggèrent que la durée de vie de la cellule sombre est plus courte que celle de la cellule claire.

This research was supported by grant no. R01 NB-06181 from the National Institutes of Health, and by a grant from The Dysautonomia Society, Inc., New York.

The author is in receipt of a Research Career Development Award no. K3-DE-17,654 from the National Institutes of Health.

The author is indebted to Dame Honor Fell, F.R.S., for her helpful criticism of the manuscript, to Mrs Katherine Walsh for technical assistance and to Mr Roland Sinclair for preparation of the photographic prints.

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(Manuscript received 18 November 1968)