OLFACTORY NERVE FIBERS

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Unmedullated nerve fibers physiologically carrying signals in the afferent direction became of interest as a group apart, when it was learned that those in the skin nerves, having their origin in cells of the dorsal root ganglia (d.r.C), possess properties different from those with cells of origin in the sympathetic ganglia (s.C). It has long been known that the fibers of the olfactory nerve are unmedullated. Therefore, the question naturally suggested itself as to whether the olfactory fibers are relatable to the d.r.C fibers. In as much as the fibers in mammals are too short for investigation with the methods used for other fibers, it was decided to start with observation of their dimensions with the aid of the electron microscope. A picture was revealed unlike that of any nerve previously examined: extremely small fibers in great numbers. How such fibers might arise from the bipolar cells of the olfactory mucosa was a problem that could only be answered by an examination of the mucosa itself. In the course of the examination characteristics of the mucosa, as yet undescribed, came into view as a by-product. Descriptions are here given of the fibers in relation to the mucosa; and finally of some observations on the olfactory nerve of the pike, which will show that the fibers do not correlate with the d.r.C fibers.

Although classification of the olfactory fibers with the d.r.C fibers is not permissible each provides the best morphological analogy for the other. Embryologically both arise from thickenings of the ectoderm, placodes by the forebrain for the olfactory fibers, the neural crest for the d.r.C fibers. As the neurons form, an axonal projection is directed toward the central nervous system, and a dendritic projection toward the periphery, there to terminate in free endings of great fineness. Small as are the diameters of the cilia on the olfactory dendrites they are matched by the terminals of the d.r.C fibers in the skin (Gasser, 1954). In a previous paper it was shown that the axonal branches of the d.r.C fibers are much smaller than the dendritic branches, and that they are often connected in bunches to the Schwann membrane by their mesaxons. The pattern first revealed in connection with the d.r.C neurons is

* In the preparation of the electron microscope pictures used in the references to the cytology of the olfactory mucous membrane.

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repeated in an exaggerated form with the olfactory neurons. There is a greater contrast between the dendrite diameters and the axon diameters; and the number of fibers attached through a single mesaxon is many times as large.

Morphology

Methods.—Electron microscopy is more essential for the visualization of olfactory nerve fibers than it is for the ordinary unmedullated fibers. Nothing approximating resolution is obtained at the wave length of light. For the preparation of sections the technique previously described (Gasser, 1955) was employed. Because it is more convenient to study, the portion of the olfactory mucous membrane on the nasal septum was selected for the observations. Where the objective was the fibers, better fixation was obtained if the mucous membrane was removed before fixation. When the objective was the membranes itself it was usually fixed *in situ*. Owing to the weakness of the attachment of the mucosa to the submucosa many preparations were unsuccessful because the mucosal cells had separated from their base and floated away. In attempts to obviate this hazard, in a number of instances there was employed osmic acid buffered, as described by Palade, in the range pH 7.3 to pH 7.8. Where buffered osmic acid was used specific mention of the pH will be made

The Sheathed Axons.—At the line of the basement membrane of the olfactory epithelium the fibers are delivered to Schwann sheaths about 2 micra in diameter in numbers ranging from 15 to 40. These sheaths run in various directions more or less parallel with the basement membrane, converge with others of their kind to form larger bundles, then turn at right angles to assume a vertical course. Through continuation of convergence the nerves are formed, variable in size, some of them reaching a diameter of more than 0.1 mm. The larger nerves are surrounded by a lamellated sheath, thinner than those present in the nerves of the limbs. A sheath with more than one lamella has not been seen. The Schwann sheaths attain sizes much larger than in other nerves. Nageotte has described them as being of the branching syncytial type holding for unmedullated fibers in general; and such observations as have been made by the author are consonant with his description. The diameters of the Schwann sheaths range between 2 and 50 μ.

The appearance of the fibers in cross sections of the nerves headed toward the cribriform plate can be seen in Fig. 1. Since such high magnification is required in order to make the fibers visible, it is possible from the reproductions to get only a limited impression of the large number of fibers in a sheath. For example, the upper print was cut from a field, itself covering but a portion of two sheaths, in which there were four thousand fibers in 300 square micra. The print as reproduced has the area of the axon in a 16 μ medullated fiber. It is seen at a glance that the fibers constitute a homogeneous system. A pattern in the action potential is not indicated. The fibers range in size from 0.1 μ to about 0.4 or 0.5 μ, with a mode not higher than 0.2. In many of the

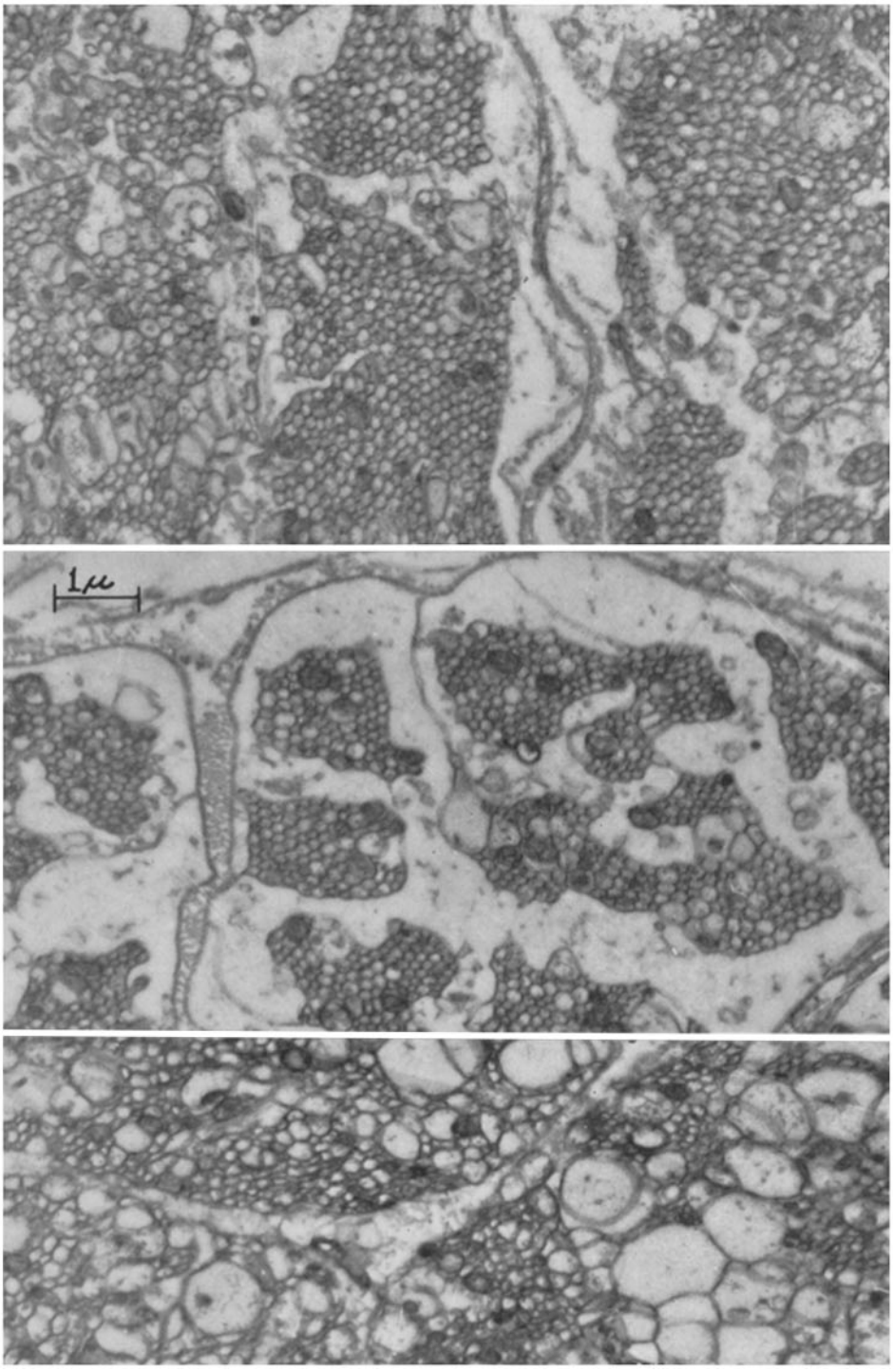


FIG. 1. Samples of cross sections of the olfactory nerve of the pig, fixed in osmic acid in Krebs's solution. All parts of the figure are at × 12,000. Where subject matter and space limitations permit, the same magnification is employed in subsequent figures so that the dimensions of parts of the neuron may be intercomparable.

fibers mitochondria are visible. When longitudinal sections are examined these mitochondria are found to be elongated and practically coextensive with the diameters of the fibers.

The upper print in Fig. 1, selected to illustrate the massing of the fibers, provides no information about how the fibers are held in the sheaths. Not infrequently there are to be encountered locally in the preparations situations, to which vagaries of the technique may be contributory, where the method of suspension of the fibers is made clear. An example is presented in the middle print of Fig. 1. The mesaxons holding the olfactory fibers are branched; and the attachment of an aggregate of fibers can be traced to the Schwann membrane by more than one mesaxon. In the figure the infoldings of six mesaxons from the Schwann sheath membrane are discernible. For the largest group of fibers both branching and a double attachment are illustrated.

That the small structures here described are individual nerve fibers will be supported by information to be brought forward later in the paper. With their description, however, the appearance of the nerve sections is not completely presented. In addition there are to be seen some enclosed areas which do not qualify as representative of nerve fibers, but for which the interpretation is not completely certain. Situations like the one shown in the lower part of Fig. 1 are of frequent recurrence. Although they may come into view in one Schwann sheath in a nerve section and not in the neighboring sheaths (which would resemble the other parts of Fig. 1), there are a number of reasons for not considering them to be nerve fibers. Crucial evidence could be adduced, were it possible to record the conducted action potential. In its absence the action potential of the olfactory nerve of the pike assumes some importance. The latter is characterized by a single elevation. Fibers with other than the small dimensions have not been seen emergent from the olfactory mucous membrane; and at the central end in the olfactory bulb it is large numbers of small fibers that are seen approaching the synapse in the glomerulus. Where the enclosed areas are prominent the fibers are usually found to be compressed. The areas vary greatly in size. Many of them are much larger than the ones illustrated; and the cytoplasm, particularly that of the larger ones, appears to be far too empty to belong to a nerve fiber. A possible interpretation would be that the enclosed areas are presentations arising from artifactitious displacements in the embedding process of the suspending system of the fibers. How such formations might come about is suggested in situations like the one apparent in the upper right corner of Fig. 21. On the basis of this interpretation the centers of the enclosures would be filled with Schwann cytoplasm, which is known to be relatively featureless except in the region of the nuclei.

The description given for the olfactory fibers in the pig appears to be representative of olfactory nerve fibers in general. Fibers with the same characteris-

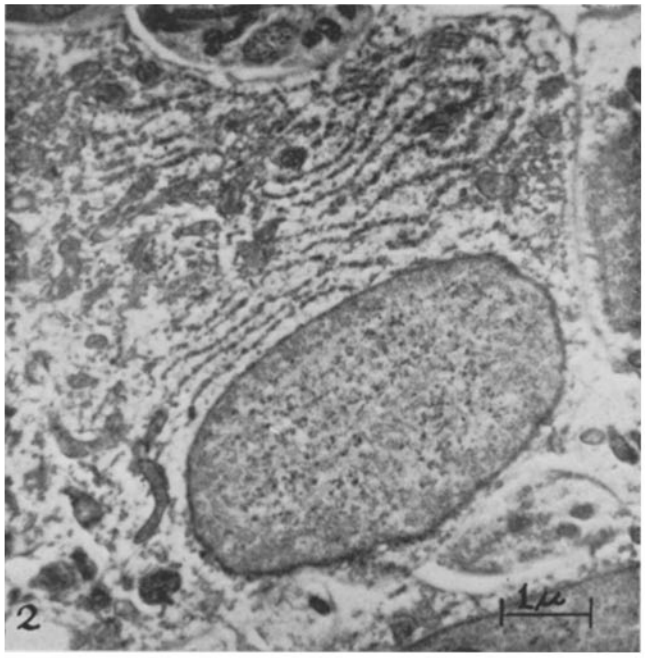
tics have been observed in the cat, rabbit, frog, fish (pike), and in the antenna of the honey bee. Also the fibers in the nerve of Jacobson have similar properties.

The Olfactory Mucous Membrane and the Origin of the Fibers.—Whether the small structures are actually nerve fibers is a question that inevitably will be asked. In anticipation of it, the best way to an answer was considered to be through tracing the derivation of the fibers from their cells of origin. The problem turned out to be an exceedingly knotty one. In addition to the smallness of the structures, which makes them hard to differentiate from organelles (a property which completely frustrated an attempt to interpret the synaptic connection in the glomerulus), three principal difficulties obstructed progress. A start was made under a misleading hypothesis: an inference from the number and smallness of the fibers that a considerable division of the axon leaving the bipolar cell must take place. The hypothesis would have been exploded promptly if it had been possible to find the axon. It so happens that the part of the mucous membrane through which the axons run is so fragile that it was impossible to obtain a preparation in which, in the dimensions under consideration, the structures were not torn apart (Fig. 12 is an example). And in none of the separated parts could the axon be identified. Realization of what the third difficulty was only came with the solution. Prominent in the pictures of the basilar part of the membrane are irregular highly osmiophilic areas which finally were identified as representing the basilar part of the sustentacular cells. The axons are encased in this material, obscured thereby, and only rarely, even with the benefit of hindsight, identifiable with certainty before they emerge.

Two milestones mark the history of the histology of the olfactory epithelium: the differentiation of the bipolar cells from the sustentacular cells in teased preparations, by Max Schulze in 1862; and the proof of the origin of the axons from the bipolar cells with the aid of the Golgi stain, by Cajal in 1889. (A more complete bibliography is given in a recent paper by Bloom.) Advance of comparable significance then had to await modern methods.

A condition considered to be imperative was ability to fit the newer pictures, greatly different in their aspect and covering only parts of the neuron, into the older pictures of the whole neuron. At a stage of the work when this exaction still could not be met, some Golgi preparations, kindly made for the author by Lorente de Nó, turned out to be of assistance. As seemingly no microphotographs of Golgi-stained bipolar cells have been published previously, and in as much as they are useful for reference, two examples are presented in Fig. 5.

A convenient starting point for description of the bipolar cells is at the cell body, though it was not the starting point in the recognition of the parts of the neuron. The order in which the parts were identified is as follows: the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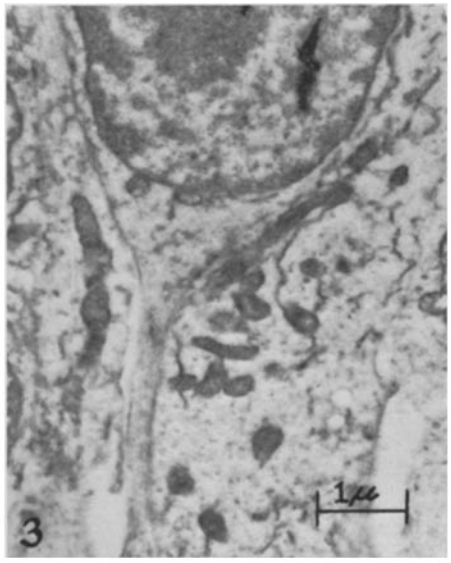
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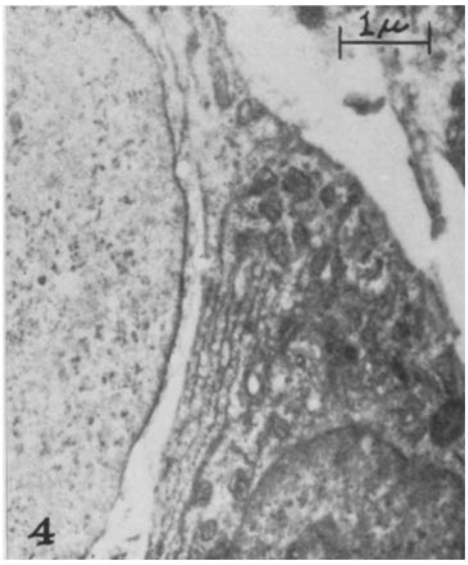
4
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dendrites and adjacent portions of the sustentacular cells, the fiber bundles leaving the inner border, fully formed fibers in the basal cells, the cell body with an emergent axon, the inner portion of the sustentacular cells, and finally the observation which filled in the last gap, delivery of the axon to the basal cells by the sustentacular cells.

That the bipolar cell is asymmetric is clear in the Golgi preparations (Fig. 5). The nucleus is situated close to the axon side. Only a thin rim of cytoplasm lies between the nucleus and the axon. Most of the cytoplasm is on the dendrite side. It has a characteristic cytology, which once learned makes it easy to recognize the cells. The appearance of the dendritic pole of the cell is seen at 12,000 diameters in Fig. 4 and in greater detail in Fig. 16. A series of elongated profiles of endoplasmic reticulum near the edge of the cell is the most useful guide to identification. A quite different arrangement of the profiles is found in the sustentacular cell (Fig. 2). Comparison of Figs. 2 and 4, both at 12,000 diameters, shows that the background is darker in the bipolar cell than it is in the sustentacular cell. The reason for the difference appears in Fig. 16. There are in the bipolar cell a large number of small granules between the mitochondria and the endoplasmic reticulum with circular profiles, wanting in the sustentacular cells.

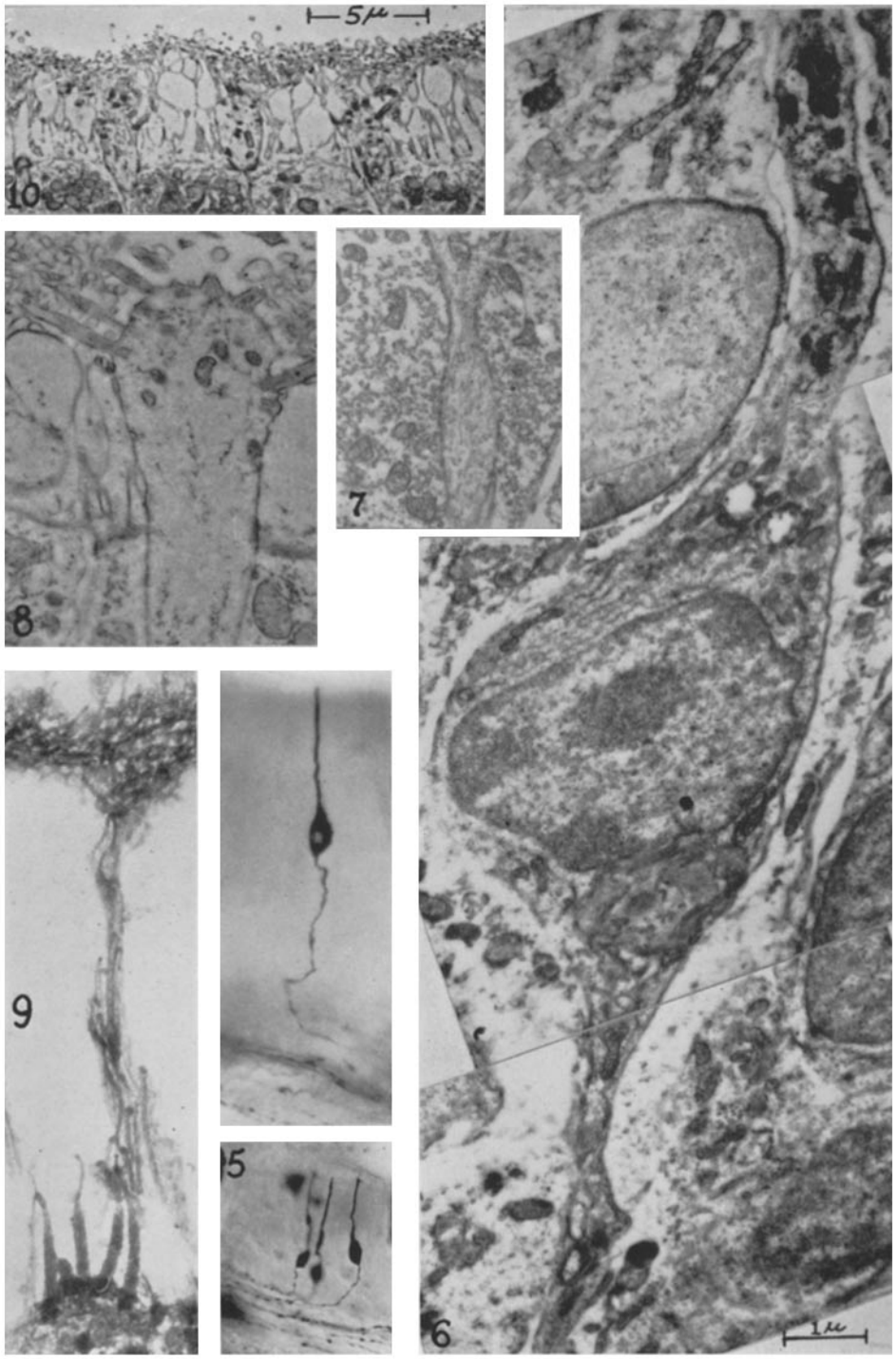
Through pictures of segments of the course of the dendrite in its projection toward the mucosal surface, some of its features are illustrated (Figs. 6, 7, 8, and 10). As found by Bloom, the concentration of organelles is greatest at the proximal and distal ends. Thus it is possible in the midregion to obtain a longitudinal section in which there are no organelles in the field (Fig. 7). At the distal end endoplasmic reticulum is extremely scarce. Mitochondria, however, are regularly present, in some instances in far greater concentration than

FIGS. 2, 3, and 4. Three prints presented at a magnification corresponding to that used for the nerve fibers in Fig. 1 (× 12,000), to show the features useful in the identification of the mucosal cells. Top of the figures oriented toward the external surface of the mucosa. Rat; fixation 2 hours at pH 7.5, in later figures referred to as rat A. (Palade preparation.)

FIG. 2. Sustentacular cell. Nucleus with adjacent cytoplasm directed obliquely toward the upper left corner. Shows the disposition of mitochondria, and of endoplasmic reticulum in circular and elongated profiles. Part of a proximal dendrite is visible at the top.

FIG. 3. Axonal pole of a bipolar cell. Adjoining the cell body and axon are cell borders of sustentacular cells.

FIG. 4. Dendritic pole of a bipolar cell. The endoplasmic reticulum near the surface arranged in a series of elongated profiles, is a good guide to identification. The dark appearance of the cytoplasm at this magnification is due to the large number of small granules, and is in contrast with the appearance of the sustentacular cell which has few small granules in the region shown. At the left, part of a sustentacular cell nucleus.


5μ
10
8
7
9
5
6
1μ

might be inferred from the illustrations presented. For the variation there is no accounting.

At its protrusion into the nasal cavity the dendrite undergoes a head-like enlargement without transitional features in its cytology (Fig. 8). The enlargement is not a vesicle, as the text-book designation implies, but it does have the property, unusual for a neuron, of bearing cilia. These have been described in the frog by Bloom, who was able to count in their interior the number of fibrils characteristic of cilia. Additional information about the structures was obtained in a fortunate distortion observed in a trial of celloidin-paraffin as an embedding medium. In a stretched out cilium (Fig. 9) it is seen that the cilium soon divides into streamers of great fineness (0.07 to 0.1 μ, measured on high magnification versions of Fig. 10). The techniques of electron microscopy are not suitable for determining the length; but it is known from older observations on fresh preparations that the over-all length must be more than 100 μ. It is these streamers that normally turn laterally to form

FIG. 5. Two microphotographs of bipolar cells in the olfactory mucosa of a 5 day old mouse, stained with the Golgi technique by Lorente de Nó. In order to have the axon in focus in its changing levels in the thick 80 μ sections, microphotography at low magnification was necessary. The light spot in the upper picture shows the position of the nucleus.

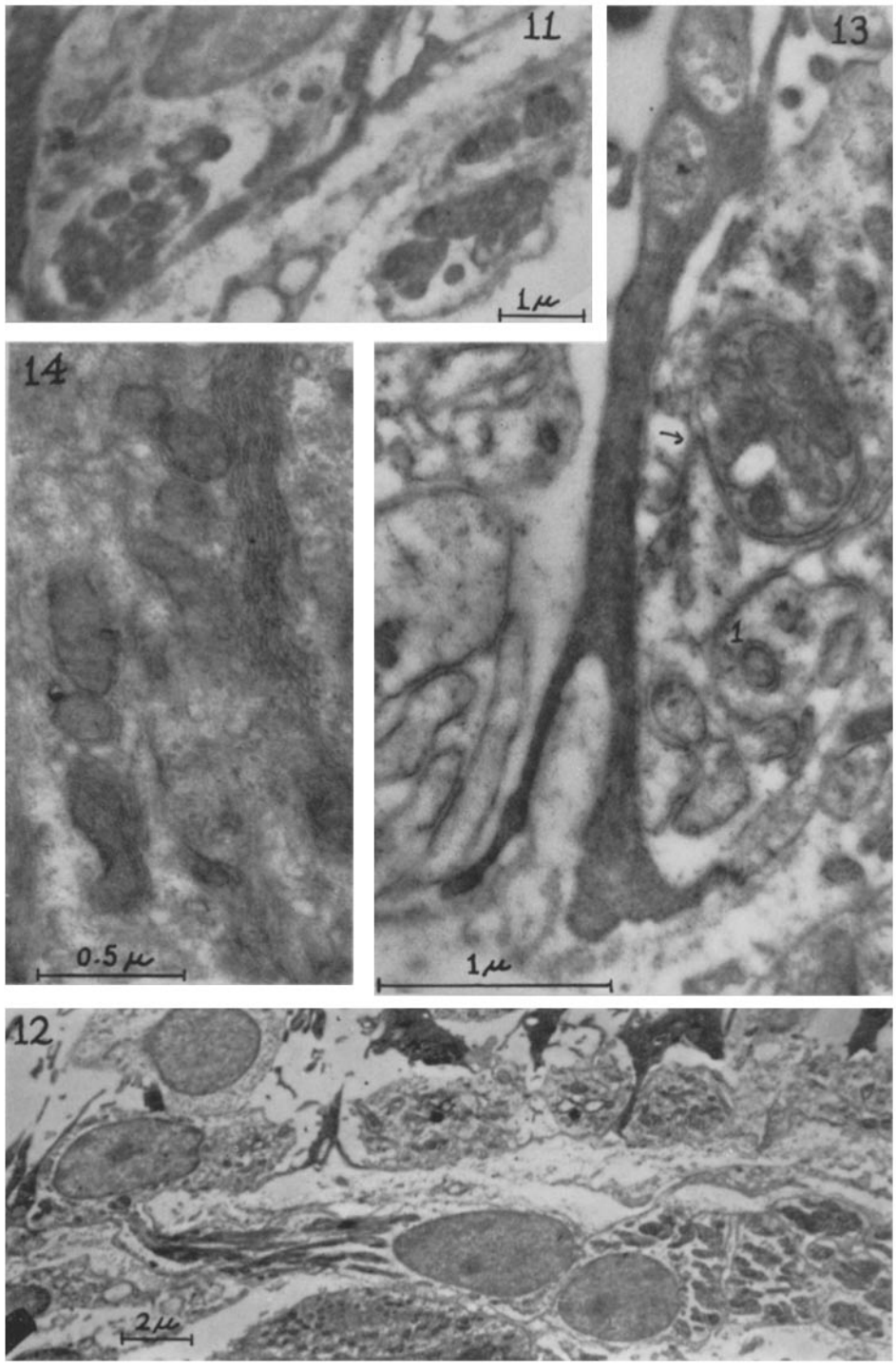
FIG. 6. Mounting prepared from three negatives obtained from rat A. The section was made by Dr. Palade and is the only one in the whole collection which chanced to pass through a bipolar cell in such a way as to include the proximal portions both of the dendrite and the axon. Dendrite, toward the top; axon toward the bottom. The part of the figure not occupied by the neuron belongs to the ensheathing sustentacular cells. Parts of three sustentacular nuclei are in the field. The calibration line serves also for Figs. 7 to 9. × 12,000.

FIG. 7. Segment of a dendrite in the midregion in which there are few organelles. The section appears to have passed somewhat to the side of the axis. In the adjoining sustentacular cytoplasm, mitochondria and vesicular endoplasmic reticulum. The light background in this cytoplasm reflects the absence of small granules. Cat; fixation at pH 7.5. (Palade preparation.) × 12,000.

FIG. 8. Head of a dendrite. Bases of cilia arising from the head can be seen. In the longer ones there is evidence of the fibrillae better seen at higher magnification. Cross sections of streamers are to be noted in the upper left hand corner. Pig; fixation in osmic-Krebs's. × 12,000.

FIG. 9. Cilia on a dendrite, showing branching. Pig; fixation in osmic-Krebs's; embedding, celloidin-paraffin. The section was thicker than those cut from methacrylate blocks. × 12,000.

FIG. 10. External border of the olfactory epithelium showing the protrusion of the heads of three dendrites above the line of the external surfaces of the sustentacular cells, at the bottom. At the top is a cross section of a feltwork made up of streamers from the cilia on the dendrites of the bipolar cells. The axes of the streamers are at right angles to the axes of the dendrites. Pig; fixation in osmic-Krebs's. × 3,300.


11
1μ
13
14
0.5μ
1μ
12
2μ

the feltwork cover of the epithelial surface (Fig. 10). In the pig at least the apparatus for motility does not appear to extend much beyond 1 μ.

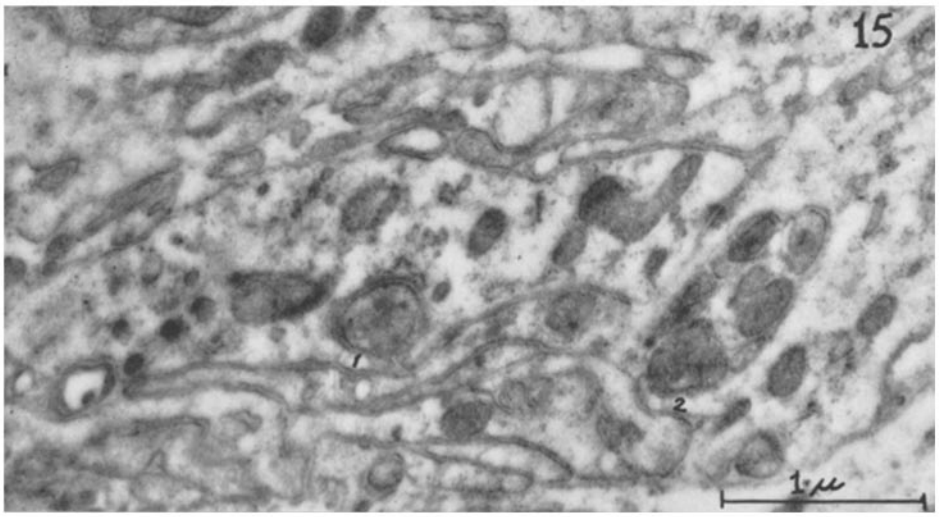
On the central side of a bipolar cell, the likelihood of seeing the emerging axon is small, about one chance in sixteen where the cell body is 4 μ in diameter, and the axon around 0.25 μ. The odds against the plane of the section passing through the axis of an axon are much higher, and against its passing through both the dendrite and axon much higher still. An instance of the latter occurred but once. It is illustrated in Fig. 6. Only a few sections cut into

FIG. 11. Part of a basal cell, nucleus at the top. Flanking the cell on each side are centrally extended projections of sustentacular cells, with feet on the basement membrane. In the cytoplasm, a bundle of ensheathed nerve fibers, ready for delivery to a Schwann sheath in the manner shown in Fig. 12. The line of the basement membrane passes obliquely through the figure from lower left to upper right. Below it at the lower right, two Schwann sheaths containing bundles of fibers that have emerged from other basal cells. Cat; fixation 3 hours at pH 7.8, called cat A in subsequent references. × 12,000.

Fig. 12. Transition from the olfactory mucous membrane to the submucosa. Just above the middle of the picture there runs a row of basal cells. In the left hand cell the section passes through the nucleus. Black areas at the top, central ends of sustentacular cells. The fragmentation of the preparation in this zone is one commonly experienced. In the basal cell with the nucleus the plasma membrane can be seen to be continuous with that of the Schwann cell forming the Schwann tube. Emerging from the basal cell to pass through the tube is a bundle of fibers. Toward the right the tube joins similar collecting tubes, making a right-angled turn as it does so. Two Schwann nuclei are prominent. Their similarity in appearance to basal cell nuclei is to be noted. At the bottom of the picture the edge of a gland of Bowman is protruding. Cat A. × 4,740.

FIG. 13. Vertically prominent in the middle of the print is a central projection of a sustentacular cell which divides as it forms terminal feet on the basement membrane. Submucosa extends into the cleft. To the right of the larger foot there can be seen two axons pushing back the plasma membrane of a basal cell, the first step in the picking up of their sheaths. Farther to the right (at 1) another axon is nearly surrounded by a sheath which connects with a short mesaxon-like double membrane to a channel, with one wall of which it is continuous. If this wall be followed upward it will be noticed that it evaginates two other loops to form the sheaths of two more axons. Higher up in the figure an invagination of the basal cell membrane forms a channel toward a small group of axons. When the membranes forming the channel reach the group of axons they spread apart (at the arrow) to become continuous with the outer of two osmiophilic lines about the group. Cat A. (Palade section.) × 33,000.

FIG. 14. Fine structure of the cytoplasm of the centrally projected portion of a sustentacular cell. At right, bundle of extremely fine elongated profiles oriented in parallel with the long axis of the cell. Mitochondria at left. Endoplasmic reticulum in circular profile scattered through the cytoplasm against a background richly populated with small granules. Cat; fixation 2 hours in osmic-Krebs's. (Palade preparation.) × 41,6000.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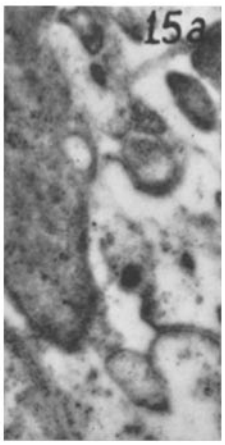
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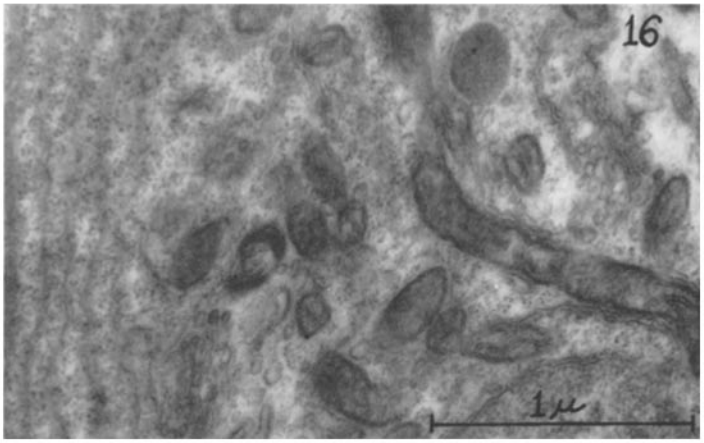
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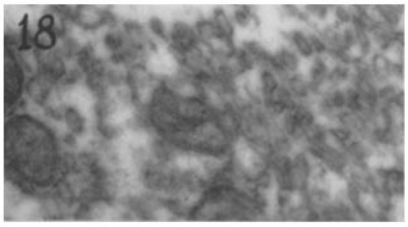
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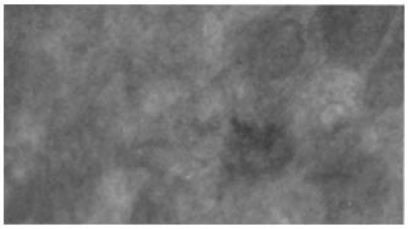

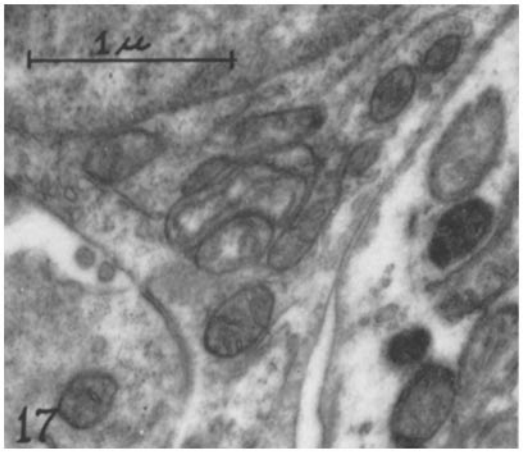
1 μ
17

the exit of the axon. One is shown at 12,000 diameters in Fig. 3, in the assembly for size comparison; and another at greater magnification in Fig. 17. No generalization is admissible from the number of mitochondria appearing in the latter (compare Fig. 3). It happens to be derivative from a preparation that also contained many mitochondria in the outer portions of the dendrites.

Beside the cell body and the axon in all three of the above mentioned figures the abutment of parts of sustentacular cells is apparent. More frequent in occurrence is the meeting of the borders of the sustentacular cells, showing that the plane of the section has just missed the axon. In the central continuation of the sustentacular cells the cytoplasm becomes so osmiophilic that routinely it takes on a silhouette-like blackness. Once the axon has been engulfed by the sustentacular cells it is lost to view. A glance at the Golgi prints shows that, even if the axon could have been picked up easily, it would have been impossible to follow it from its cell of origin to the basement membrane. Owing to the tortuous course of the axon about other cells it would soon have passed out of the plane of the section. Another point, revealed by the Golgi prints, is the probability that the axon will cross the basement membrane, in terms of electron microscope dimensions, at enormous distances from orthogonal projections of cell bodies.

The axons are next seen where they leave the sustentacular cells at the contact of the latter with the basal cells, in the places in which projections of the sustentacular cells pass between the basal cells to end with feet upon the basement membrane. Through reference to Figs. 13 and 15 *a*, and their legends, it can be ascertained how the axons push back the plasma membrane of the basal

FIG. 15. Part of a basal cell showing channels formed by invaginations of the cell's plasma membrane. Some details of the ensheathing of the axons are visible. At 1, the membrane on one side of a channel evaginates to form the sheath of an axon. At 2, a membrane turns out to a group of fibers. The section has not permitted resolution of the membrane from the opposite side. Fig. 15 *a*. Sustentacular foot in juxtaposition with a basal cell. Two axons are pushing back the cell's plasma membrane. Cat A. (Palade section.) × 26,600.

FIG. 16. Cytology of part of the proximal portion of a dendrite. Nucleus under the calibration line. At left, the characteristic endoplasmic reticulum with elongated profiles. The disposition of small granules is evident. Rat A. (Palade preparation.) × 34,600.

FIG. 17. Cytology at the axonal pole of a bipolar cell. Nucleus at top. At the sides, portions of sustentacular cells. Rat A. (Palade preparation.) × 26,800.

FIG. 18. Contrast between the fine structures in the outer and inner portions of a sustentacular cell. The samples are cut from prints at the same magnification from plates made of a single set of sections. Upper sample, at a level just below that of the dendrite heads. Lower sample, from a foot. Here the number and extreme fineness of the small granules cause the profiles of the endoplasmic reticulum to fall just short of being obscured. Rat A. (Palade preparation.) × 34,600. Calibration line on Fig. 16.

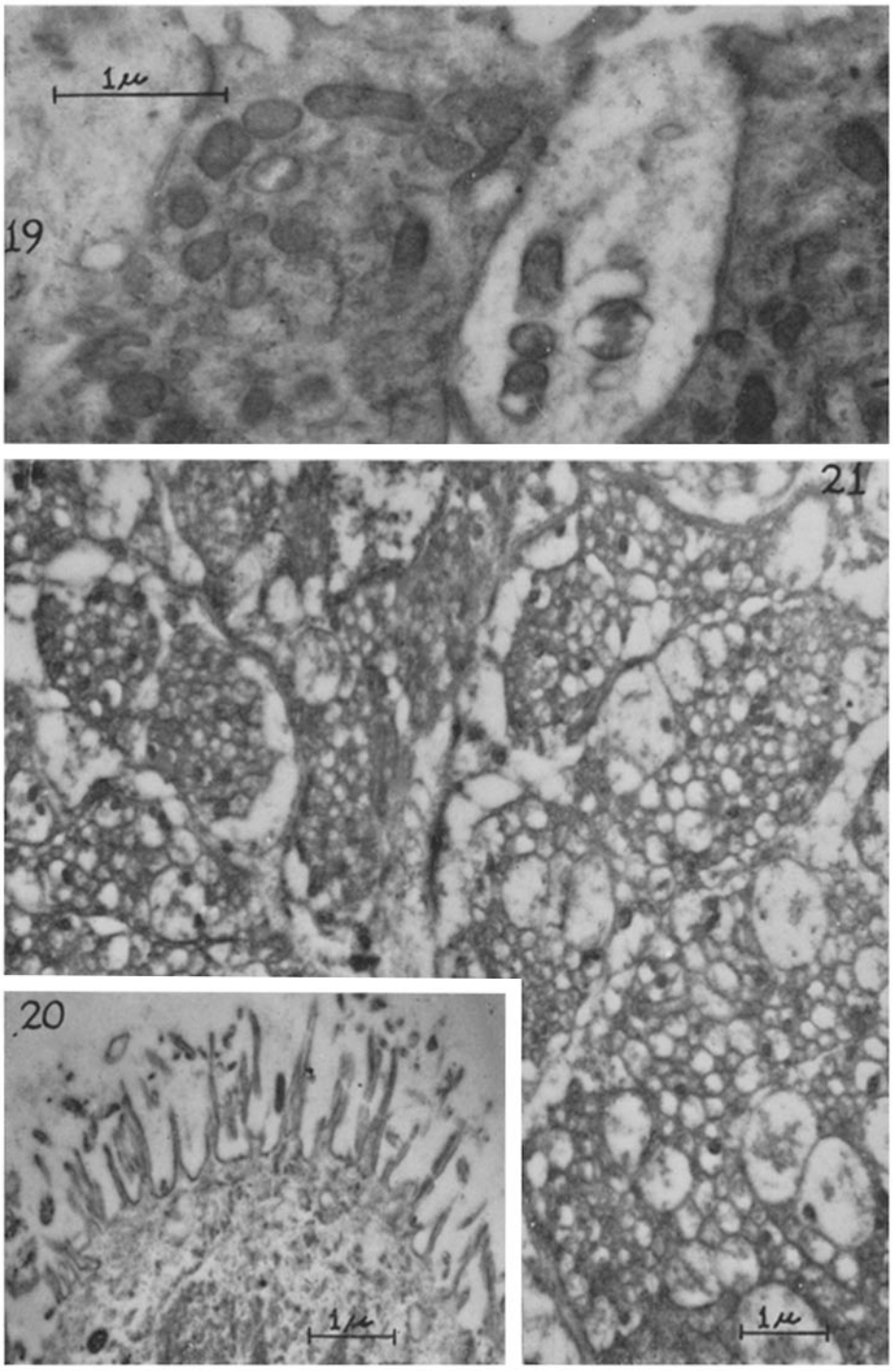

FIG. 19. Outer border of the olfactory epithelium. A dendrite head (lighter area) is between two sustentacular cells containing a wealth of organelles. Part of another head at the left. Cat; fixation 2 hours in osmic-Krebs's. (Palade preparation.) × 23,600.

FIG. 20. Outer border of a sustentacular cell. Same preparation as for Fig. 19. × 12,000.

FIG. 21. Cross section of the olfactory nerve of the pike, including parts of two Schwann sheaths. Fixation, osmic-Ringer's × 12,000.

cells. Loops of the membrane enfold the axons to form sheaths. The axons are now leaving the protection of the sustentacular cells on their way to the sheath system of the Schwann tubes, which protects them until they are translated to the glial system in the olfactory bulb. There is a striking analogy between the first stage of the sheath formation of the olfactory fibers, the sheath structure of individual unmedullated fibers in peripheral nerves (Gasser, 1955), and the embryological first step in the formation of the myelin sheath (Geren).

The subsequent events in the sheath formation are highly complex. Derivative from the figures only glimpses of some steps in the process are obtainable. Where the axons appear singly in the interior of the basal cells they are found along channels, the walls of which obviously are continuations of the infoldings of the plasma membrane from the surface. An evagination of one membrane of the channel can be seen to envelop the axon as a sheath (Fig. 15), and a series of sheaths may be so formed along one membrane (Fig. 13). What happens next is conjectural, but not unintelligible. By approximation of the ends of the membrane and shortening of the distances a group of axons would be aggregated into a small bundle attached to a single mesaxon. By some such process the small groups in Figs. 13 and 15 would be formed. These small groups are not yet at the end of preparation for delivery, however. A fully developed bundle, ready to be passed on to a Schwann tube by a basal cell, can be seen in Fig. 11. Clearly a number of small groups have come together in the completion of the sequence. The actual delivery of a bundle can be seen in Fig. 12. At the delivery point the Schwann membrane becomes continuous with the plasma membrane of the basal cell. That the delivery is in bundles need not be considered as an incompatibility with the Golgi pictures. The capriciousness of the stain, which gives it its selective property generally so useful, in this instance would be conducive to a failure of the multiplicity feature to receive attention. If one search a Golgi preparation with an oil-immersion lens one can, indeed, find places at the delivery points where more than one fiber is resolvable.

Heretofore the function of the basal cells has been unknown; and speculation about the cells has failed to include the one they possess. The basal cells are the primary cells of the sheath system. They provide the axons with the sheaths they carry in continuity throughout their traverse of the Schwann tubes.

Some observations on the sustentacular cell environment of the bipolar cells now remain to be brought together. The border of a sustentacular cell exposed on the outer side of the olfactory mucosa is fimbriated (Fig. 20). In the subjacent cytoplasm there is a dense population of circular profiles of endoplasmic reticulum (Fig. 19). Also there are many mitochondria. Unless the fixation is good the latter may not be recognizable as such. There is no

evidence whatever for the presence of secretion granules. Between the outer and inner ends of the cell there is a striking differential in the content of small granules of the type recently described by Palade. So sparse are the granules between the outer border and the nucleus that the background is clear. Somewhat below the nucleus a change takes place; and the granules become so numerous that except at high magnification of very thin sections nothing is resolvable at all. A sample of the cytology of the central projection is shown in Fig. 14; and an illustration of the contrast between areas, one in the outer part of the cell and the other in a black foot, is given in Fig. 18. The abundance of fine structure in the sustentacular cells must have significance. When one compares the wealth of endoplasmic reticulum in the parts of the cells adjacent to the dendrites with the poverty of the latter with respect to the same organelles, the suggestion becomes strong that the sustentacular cells have much more important functions than their name implies.

Tracing of the axons to the bipolar cells has provided sufficient proof that the small structures in the nerve pictures represent individual nerve fibers. Anteriorly, however, evidence, pointing to the outcome and encouraging continuation of the search, was derived through another approach: comparison of the number of bipolar cells with the number of fibers emanating from them.

The comparison was made possible by a transverse section through the olfactory epithelium of the pig, of sufficient merit to permit counting of the dendrites. Fortunately the section had passed through the outer 8 μ of the epithelium at such an angle that all the cross sections of the dendrites were round. In this zone the total contribution of dendrites by the bipolar cells would have been complete. From the appearance of the section, it must have been nearer to the line of cells than to the surface. The count was 264 dendrites in 4281 square micra, which through calculation indicated 6,180,000 bipolar cells per cm.^{2} of epithelium. Allison and Warwick reported a population density about twice as large in the rabbit.

As supplementary information there was needed the area of the olfactory epithelium, the area of the nerves supplied by it, and the number of fibers per unit area of the nerves. In order to obtain the first two parts, the mucous membrane was removed from the two sides of the septum of a pig. After trimming the pieces to the apparent sizes of the olfactory regions the areas were measured with a planimeter on tracings of projections of the fresh pieces at a known magnification in a photographic enlarger. The pieces were then fixed in osmic acid and embedded in paraffin. After obtaining sections from the upper border, just below the line at which the mucous membrane turns on to the turbinates, sections were made from the lower border in order to determine the validity of the area obtained after the original trimming. A small correction was necessary for one of the pieces. On both of them, however, an adjustment had to be made for the amount of mucous membrane removed in order to get the upper sections. There was considerable asymmetry between the two sides. The valid areas were respectively 1.83 and 0.66 cm.^{2}. After obtaining the upper sections the corresponding net values became 1.76 and 0.627 cm.^{2}.

Enough microphotographs were taken to cover completely the sections at the upper border, and on enlargements of them the sizes of the nerves were measured in the manner one would use for nerve fibers. The sums of the areas of the individual nerves yielded the needed information about the total fiber-bearing areas. The latter were respectively 793,000 and 453,000 μ^2.

To obtain the number of fibers per unit area counts were made on prints at 12,000 diameters, of the sort illustrated in Fig. 1. Eight fields were selected, differing in appearance, with the idea of making allowance for histological variations, space between the Schwann sheaths, and the presence of Schwann nuclei in the field. A count of 16,709 fibers yielded a value of 9.94 fibers per μ^2. Between the several fields there was a 2.77-fold variation in the number of fibers per unit area.

In the olfactory mucous membranes on the two sides of the septum in the pig the ratios between the numbers of cells and fibers were found, on the basis of the evaluations above set forth, to be: on one side, 7,882,000 fibers and 10,876,000 bipolar cells, with a ratio of 0.84; and on the other, 4,503,000 fibers and 3,875,000 cells, with a ratio of 1.16. It will be noticed that the ratios, while not in agreement, are close to and on opposite sides of one. They are incompatible with any interpretation other than that of a correspondence in number between the nerve fibers and the bipolar cells.

Action Potentials

For a study of the properties of the nerve fibers, in as much as the olfactory nerves of mammals have an inadequate length, it was necessary to turn to a form in which this shortcoming could be circumvented. Fortunately a suitable preparation has long been known in physiology: the olfactory nerve of the pike. Its usefulness was first pointed out in 1880 by Kühne and Steiner. And at one time a considerable portion of what was known about nerve potentials had been derived from it. As long as the capillary electrometer was the instrument of choice, the long time constants of the nerve caused it to be the one most favorable for investigation under the limitations set by the time constant of the instrument. Garten's observations, as set forth in his monograph, still stand as the most important source of information about the nerve.

Fish may have either short olfactory nerves and a long olfactory tract, as does the carp, or long olfactory nerves and a short olfactory tract, as in the genus *Esox*. The nerves used were from *Esox estor*, generally known as the northern pike. Isolation of the nerve for experimentation has been described by Sowton, along with an excellent picture of the dissected preparation. For about two-thirds of its course between the olfactory mucous membrane and the olfactory bulb the nerve runs in a tube of cartilage. Around the other third there is a dense sheath of connective tissue which cannot be removed, and which limits the usefulness of this stretch of the nerve to facilitation of handling and to protection of the other part. After some practice shaving off the top of the cartilaginous tube can be accomplished without great difficulty in large specimens. It is much harder in small specimens. After the tube was well opened

strands from silk thread were tied about the ends of the nerve, and the connections severed. By the strand on the peripheral end the nerve was then lifted from its bed and floated on Ringer's solution in a trough in the nerve box. Next, the two silk strands were fastened in the clips, and the electrodes, below the nerve in the solution, were moved to their proper positions. Once this stage was reached the danger of injury had been passed; and the preparations were found to be durable. When everything was ready for recording, by lowering the trough the nerve was brought to rest upon the electrodes as they emerged from the solution. The nerve was oriented so that the leads were from the central end. Recording was differential with the stimulating cathode at earth potential. Amplification was D.C.; and the shocks were square waves from a Grass stimulator set for durations of 1 msec. To achieve monophasicity cocaine was applied with great care to prevent spread. The desired effect was obtained almost instantly.

The observations here recorded have been derived from fewer experiments than would have been the case were it easier to transport the fish to New York City and maintain them in an improvised aquarium. They suffice, however, to supply a definite answer to the question primarily motivating their initiation: whether as unmedullated fibers with afferent function the olfactory fibers are to be classified with the unmedullated fibers arising in dorsal root ganglia, also afferent in function. In addition, the experiments afford information, qualitative or roughly quantitative though it may be, about some aspects of the fibers' activity not previously described.

Fig. 21 establishes the validity of considering the pike nerve as representative of olfactory nerves in general. There is no obvious difference, either in the size of the fibers or in their sheath system, to distinguish them from the olfactory nerves of other vertebrates. Description of the electrophysiological experiments can be brief, as all that is required is a comparison of the properties of the fibers with the same properties of other fibers.

Most obvious among the characteristics of the action potential is its simplicity, a feature anticipated from the homogeneity of the fiber sizes. That the simplicity is not merely an apparent one resultant from the short conduction distances (*e.g.* 10 mm.), imposed by the nature of the preparation, follows from the fact that after 7 mm. of conduction a complex sequence of elevations can be seen in the action potential of the C fibers in the sciatic nerve of the frog. Irregularly present ahead of the main wave in the pike olfactory action potential is a very small wave, seen in Fig. 22 but not in Fig. 24. When present it appears as the first response to a stimulus (Fig. 22), and does not grow in height or duration as the strength of the stimulus is increased. The most likely interpretation would be that there are a few fibers conducting impulses at about three times the velocity of those responsible for the front of the main wave. But the fibers postulated have not been identified. If the enclosed areas, described in connection with the histological picture, were interpreted as fibers, too rapid velocities and far too large an area would be predicated.

A time, called the spike duration, is often used in the description of nerve fibers, though the term lacks precise physiological meaning on account of the overlapping of the spike and after-potentials. Conventionally the reference is to a value yielded alike by each of two operations of measurement. One is measurement of the duration of the spike up to the point, late in the falling phase, at which the slope of the negative tangent undergoes a sharp decrease.

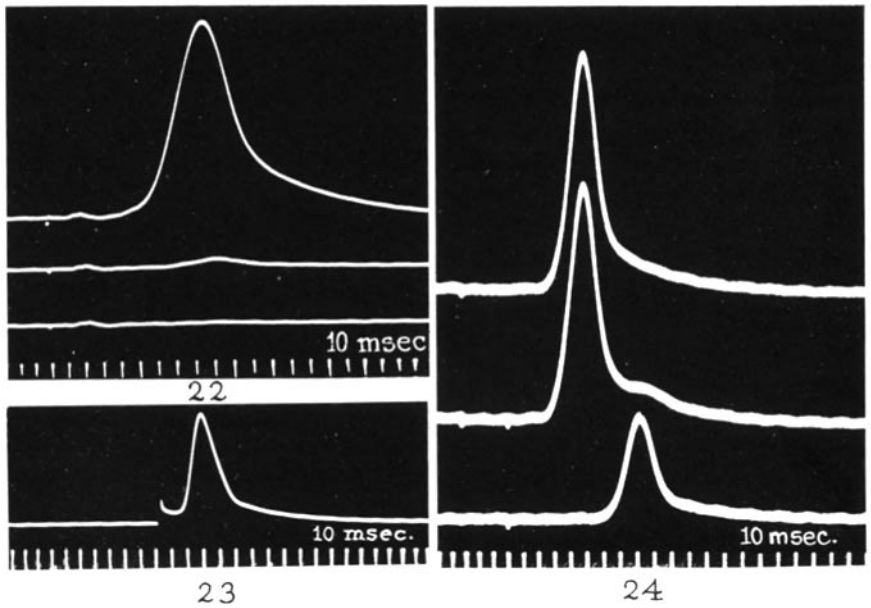

FIG. 22. Action potential of the olfactory nerve of the pike at three strengths of stimulation. Conduction distance, 6.5 mm., 21°C.

FIG. 23. Action potential of the olfactory nerve of the pike. Conduction distance, 5.7 mm.

FIG. 24. Olfactory nerve of the pike. The middle curve shows a second response in the early period of recovery. Bottom curve, response to the testing shock in isolation. Conduction distance, 9 mm., 21°C.

The other is the determination of the end of absolute refractoriness. For the olfactory nerve the first of these procedures is poorly applicable. Owing to the nature of the sheath system there is no possibility of isolating single fibers or even small bundles of fibers. Threshold shocks, as for other unmedullated nerves, are not selective of the fibers with the fastest velocity; and they have long utilization periods varying among the fibers. Thus one does not obtain a shorter spike at threshold than one does with a strong shock of brief duration. The spike shown in Fig. 23 lasts between 30 and 40 msec., that in Fig. 24, 50 msec.

Because the nerves are so short the absolute refractory period had to be

determined with the conditioning and testing shocks applied through the same electrodes, not the preferred method. In an experiment in which both shocks were strong, refractoriness lasted 28 msec. In the experiment illustrated in Fig. 24, in which the testing shock was weak, the excitability was found to be emerging from absolute refractoriness at 31 msec. A good approximation for the spike duration would be 30 msec.

The fastest velocity of conduction in the main wave was found to be 0.2 m./sec. (average from six preparations in which the readings were made with conduction distances of 1 cm. or more, variation 0.18 to 0.217), a value coinciding with the one given by Garten. With respect to the slowest velocity little accuracy is possible because of the mergence of the spike with a negative after-potential. If 30 msec. be taken for the length of the spike it follows that there hardly can be more than a twofold variation within the total range of velocities from the fastest to the slowest. For a comparison, frog C fibers are the ones most available. As reliable measurements of the component velocities in the C action potential were lacking, some experiments were performed upon the frog sciatic nerve. It was found that the C action potential is characterized by a series of elevations having a considerable similarity to those in the saphenous nerve of the cat. The values of the velocities for the first and last elevations were 0.69 and 0.206 m./sec. When compared with the corresponding values for the cat saphenous, 2.3 and 0.72 m./sec., it is seen that the ratios are alike, and that the frog velocities are about 0.3 of those in the cat. With a similar range of fiber sizes and the last elevation in the cat known to start at 0.4 μ (Gasser, 1955), it may be supposed that the last elevation in the frog would also start at about this size. The last elevation in the frog has a velocity of 0.2 m./sec., the same as that for the pike olfactory fibers. Hence the inference is that the velocities in the olfactory fibers approximate the expectation for their dimensions.

For interpretation of a negative potential after a spike as a negative after-potential, or as containing a negative after-potential fraction, the condition of supernormality must be met. In one experiment in which the conditions were satisfactory a definite though small supernormality was observed. From the readings a sketch of the dimensions of the early part of the excitability cycle could be drawn. Following the end of absolute refractoriness at about 30 msec., the excitability rose through a period of relative refractoriness ending at about 240 msec., and then passed into supernormality. At the last reading (400 msec.) the supernormality had not yet begun to abate. A true negative after-potential should be followed by a positive after-potential. For evidence on this point it is necessary to turn back to Garten. He found positive after-potentials after a tetanus, and after single responses in nerves previously conditioned by a tetanus. Measurements on his records of the latter show the

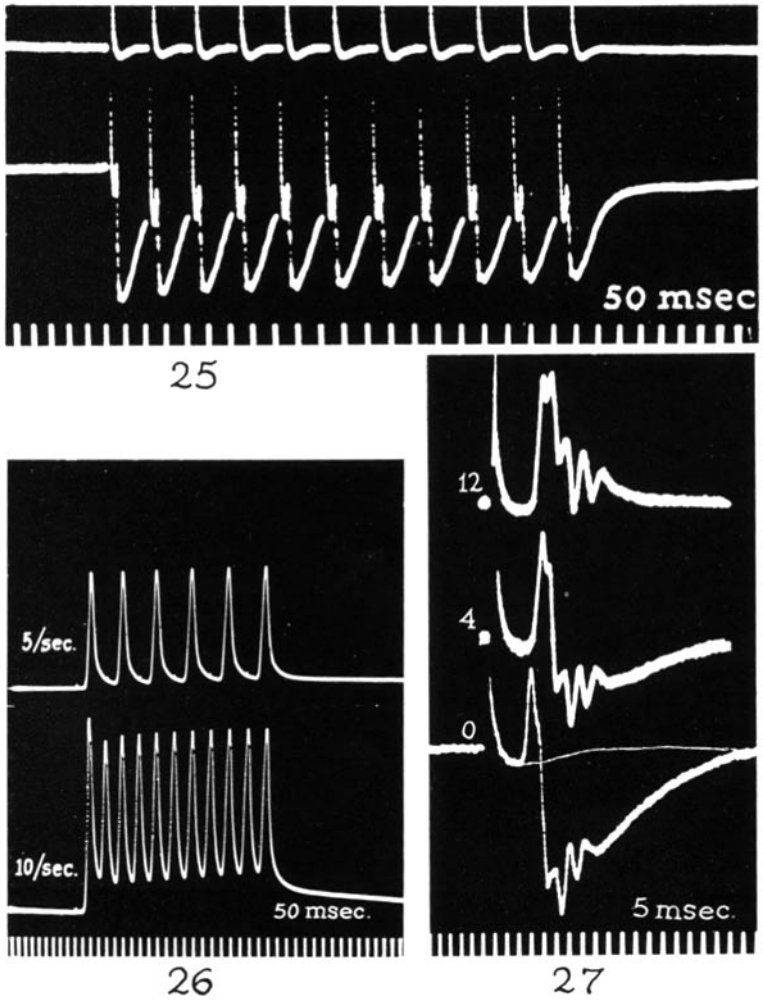

FIG. 25. Saphenous nerve of the cat. Lower curve, eleven responses of the C fibers in a tetanus at 10 per sec. Upper curve, base line for the tetanus determined by the after-potentials of the A fibers. D.C. amplification, monophasicity produced by cocaine. Conduction distance, 3 cm., 37.1°C.

FIG. 26. Tetani, olfactory nerve of the pike. Conduction distance, 12 mm.

Fig. 27. Saphenous nerve of the cat. Changes in the form of the responses in the course of a tetanus at 10 per sec. 0, form of the response in the rested state. The added line shows the base line determined by the A after-potential. 4 and 12, forms of the responses 4 and 12 sec. after the start of the tetanus. D.C. amplification, monophasicity produced by cocaine. Conduction distance, 29 mm., 38°C.

positivity starting at about 0.8 sec. and reaching its maximum at 1.5 sec. A test for subnormality has yet to be made.

From the foregoing survey it follows that the olfactory nerve fibers conform in their pattern of behavior to that of the generality of nerve fibers: a spike continued by a negative after-potential followed by a positive after-potential; and an excitability cycle extending through relative refractoriness to supernormality and then by inference to subnormality. Such differences as there are are quantitative, rather than qualitative, a longer duration of all parts of the action potential than any known for other vertebrate fibers.

It is the d.r.C fibers that do not conform to the generalized schema of nerve action potentials. They are set apart by the large positivity following the spike, and by the absence of a negative after-potential and a supernormal period. When the changes taking place in the successive responses of the d.r.C fibers during a tetanus come to be worked out another contrast with the olfactory fibers will be delineated. Considering the constants of the olfactory fibers, the ability of the latter to carry a tetanus is excellent. Fig. 26 shows 1 sec. tetani at 5 and 10 per sec. The configurations are so well known in neurophysiology that they require no description. Only the time parameters are of interest. At 10 per sec. the intervals are hardly half as long as the time for threshold recovery, and fall somewhat short of the time necessary for restoration of the ability to yield a full sized spike.

In order to illustrate the contrast with the d.r.C fibers, in Fig. 25 there is presented a record of a 1 sec. tetanus at 10 per sec., obtained from the saphenous nerve of the cat. Although in this nerve the duration of the C spike is less than one-tenth of the spike duration in the olfactory nerve of the pike, at this frequency the configuration of the successive responses in the tetanus undergoes a progressive change characterized by increasing inability to produce post-spike positivity. The trend of the changes, the start of which can be seen in the figure, can be better understood through reference to a longer tetanus at the same frequency recorded from another nerve in an experiment in which a different procedure was employed. The lowest record in Fig. 27 shows the shape of the C action potential after a period of rest. After the record was made a tetanus was started at 10 per sec.; and at various times thereafter samples of the action potential were taken, with an attempt to have as few sweeps as possible in an exposure. The records taken after 4 and 12 sec. were legible, and are reproduced in the figure.

DISCUSSION

In spite of the fact that the olfactory nerve fibers and the unmedullated nerve fibers arising in dorsal root ganglia have much in common in their morphology, have similar embryological origins, and are both afferent in function, they differ widely with respect to the physiological properties of their axons. Among unmedullated fibers uniqueness of behavior is not a characteristic

tied to afferent function. If the d.r.C fibers were like the olfactory fibers it would not have been necessary to create a C subgroup in which to classify them.

New information has always been upsetting to any antecedent classification of nerve fibers. A few years ago, in order to remove an impasse that appeared at the time, two subdivisions of the C group were created: dorsal root C (d.r.C) and sympathetic C (s.C). The properties of the olfactory fibers exclude them from d.r.C. They would not exclude them from s.C; but this subgroup is inappropriate, both on account of its name and the implication of motor function. There is a simple way out, however. The olfactory fibers have qualities rendering them admissible to the C group. There, as long as they are unmatched, they can constitute a subgroup by themselves. Such a subgroup could appropriately include the fibers in the nerve of Jacobson.

SUMMARY

Cross sections of olfactory nerves present a unique appearance. They indicate the presence of large numbers of very small nerve fibers, with a modal diameter of about 0.2 μ and a narrow range for their size variation. From one side of the nasal septum of a pig the yield of fibers was estimated at 6,000,000; the number arising from the turbinates would be considerably larger. The fibers are attached to the membranes of the Schwann sheaths in large bundles through mesaxons longer and more branched than those that have been seen in other nerves.

Continuity of the axons between the nerves and the bipolar cells was traced in an examination of the olfactory mucous membrane; and the indication of a one-to-one relationship between cells and axons was reinforced by a comparative count. After the axons leave the bipolar cells they become incased in the central projections of the sustentacular cells. Where the latter come into contact with the basal cells the axons emerge to push back the plasma membranes of the basal cells in the first step in acquiring their nerve sheaths. Later steps are described. When the axons are delivered by the basal cells to the collecting Schwann tubes, they are already aggregated into small bundles with sheaths fundamentally the same as those they will possess until they are delivered to the glia in the olfactory bulb.

Some of the aspects of the cytology of the bipolar cells and adjoining sustentacular cells are described.

A survey of the physiological properties of olfactory nerve fibers was made in some experiments on the olfactory nerve of the pike. Almost all of the action potential is encompassed within a single elevation, manifesting at its front a conduction velocity of 0.2 m./sec. For a comparison, the last elevation in the C action potential in the sciatic nerve of the frog is cited as an example of conduction at the same velocity.

Though expressed through long time constants, the properties of the pike

olfactory fibers conform to the generalized schema for properties of vertebrate nerve fibers. This conformity signalizes that they differ from the exceptional properties of the unmedullated fibers of dorsal root origin.

An afferent function for unmedullated nerve fibers does not imply that the fibers concerned are alike in their physiological properties.

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