RAPID DEGENERATION OF AMPULLARY ELECTRORECEPTOR ORGANS AFTER DENERVATION

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ABSTRACT

Electroreceptors (ampullary organs) of the transparent catfish (*Kryptopterus bicirrhus*) lie in the epidermis, and contain spherical receptor cells that receive purely afferent innervation from the lateral line nerve. Section of this nerve causes rapid degenerative changes to occur in the receptors. Fine structural alterations occur in the receptor cell synapses and nerve fiber 6–12 h postoperatively. Disruption of the receptor cells begins by 18 h and most are lost by 48 h. By 72 h supporting cells and secretory cells also show marked degeneration, and by 96 h they may be totally lost. The rapid degeneration of the electroreceptor organs of *Kryptopterus* should make them a useful preparation for analysis of neurotrophic functions.

INTRODUCTION

In many receptors the initial active transformation of the stimulus is carried out by axonless receptor cells that transmit synaptically to the afferent nerve fiber or fibers which in turn conduct impulses to the central nervous system. Receptors of this kind are often termed secondary receptors. They are found in all vertebrates and include the mechano- and electoreceptors of the acousticolateralis system (Bennett, 1971 *a*, *b*; Wersäll et al., 1965), taste receptors (Murray et al., 1969; Ozeki, 1971), and perhaps carotid body chemoreceptors (Eyzaguirre et al., 1970 but see Biscoe, 1971) and some tactile receptors (Iggo and Muir, 1969).

Many secondary receptors require an intact nerve supply for maintenance of their structure, although the degree of dependence on their innervation varies considerably. After nerve section the receptor cells may degenerate and supporting cells and accessory structures may also disappear entirely (Guth, 1969; Zelena, 1967). In mammals and fishes, taste buds degenerate in 2–15 days after section of the afferent nerve (Fujimoto and Murray, 1970; Guth, 1969; Torrey, 1934; Zelena, 1967). Ultrastructural changes in the innervating fibers are seen after only 12 h (Fujimoto and Murray, 1970). In the ordinary catfish *Amiurus*, signs of degeneration in the lateral line organs occur 4 days after section of the lateral line nerve, with complete disappearance of the receptor cells by 16 days (Brockelbank, 1925). In contrast, the lateral line receptors of amphibians are relatively independent of their nerve supply and remain intact for at least 2 mo after section of the lateral line nerve (Jones and Singer, 1969). The process of degeneration can be accelerated when the total environs of the receptors are rendered nerveless by also cutting the incident spinal nerves. There is some degeneration after 3 wk; degeneration is advanced after 2 mo.

Hair cells of the mammalian cochlea survive section of the auditory nerve indefinitely. However,

degeneration of the afferents, which is retrograde, does not affect a few percent of the fibers and it is possible that all the hair cells retain some innervation (Spoendlin, 1971). The results do demonstrate that efferent innervation is not required for maintenance of these receptor cells. Efferent terminals show signs of degeneration 2 days after nerve section (Smith and Rasmussen, 1965); terminals of afferents require longer, about 4 days (Kimura and Wersäll, 1962).

Electrical activity of receptor cells of phasic electroroeceptors in the gymnotid *Eigenmannia* can be recorded 1 mo after nerve section, although the nerves are no longer active and gross degenerative changes have occurred (Bennett, 1967). In the large electroreceptor organs (Knollenorganen) of the African electric fish, *Gnathonemus*, denervation causes receptor cells to degenerate in 2–12 days (Roth and Szabo, 1969). Externally recorded spike potentials, presumably generated by receptor cells (Bennett, 1967), are normal for 1 or 2 days after all propagated activity in the nerve has ceased. In the ampullae of Lorenzini of *Torpedo*, sectioning of the afferent nerve causes ultrastructural changes in the sensory-neural synapse in 3 days, and total lysis of the receptor cells by the 22nd day (Derbin, 1970). Unlike lateral line mechanoreceptors, electroreceptors are known to be innervated only by afferent fibers (Lissmann and Mullinger, 1968; Szamier and Wachtel, 1969, 1970; Wachtel and Szamier, 1966, 1969).

The present paper describes very rapid degeneration of the tonic electroreceptors (ampullary organs) of the transparent catfish, *Kryptopterus bicirrhus*. After section of the innervating lateral line nerve, ultrastructural changes can be observed in the receptor synapses within 6–12 h, and the receptor cells can be completely lost by 48 h. The normal morphology and distribution of the *Kryptopterus* receptors (Wachtel and Szamier, 1969) and some aspects of their function have been reported (Bennett, 1971 *b*). They are distributed over the entire body surface and are even found on the thin and very transparent fins. The posterior lateral line nerve, which supplies all of the region behind the head, is readily accessible for section. Becasue of the speed of degeneration and ready accessibility of both nerve and receptors, this preparation appears particularly suitable for further experimentation. A preliminary report of this work has appeared (Szamier, 1970).

MATERIALS AND METHODS

Kryptopterus bicirrhus, 3–5 cm long, were maintained in aquaria at 22°–24°C. The lateral line nerve whose cell bodies are located intracranially, was sectioned at a point just distal to where it enters the cranium. Here the nerve runs immediately beneath the skin allowing the operation to be performed with minimum trauma and bleeding. The fish were anesthetized with Finquel (Ayerst Laboratories, New York) 1:5000.

In most cases the nerves were sectioned bilaterally and a 1 mm segment of each nerve was removed. Some fish were sectioned unilaterally and the unoperated side served as a control. In these cases, operated and control organs from the anal fin could be compared in the same section.

Fish were sacrificed by decapitation. Segments of the trunk were immersed in cold 2% OsO_4 in $\frac{1}{15}$ M phosphate buffer at pH 7.3 for 1 h, washed in cold distilled water, and dehydrated in ethyl alcohol. While in alcohol, small pieces of skin were dissected from the trunk. Embedment was in Epon 812 (Luft, 1961) or D.E.R. (Spurr, 1969). Sections were stained for light microscopy with aqueous toluidine blue and for electron microscopy with lead citrate (Reynolds, 1963). Thin sections were examined with a Philips EM 200.

RESULTS

The receptors are composed of a small ampulla that opens directly to the exterior by way of a short canal (Fig. 1 A). Spherical to oval receptor cells and columnar supporting cells are clustered together opposite the ampullary opening to form a "sensory hillock." A cluster of secretory cells, the "secretory hillock," lie between the sensory hillock and opening and apparently supply the gelatinous contents of the lumen. Microvilli project from the receptor cells in the apical region which abuts the ampullary lumen (Fig. 1 B). A single myelinated fiber from the lateral line nerve innervates each receptor. Nonmyelinated branches of this fiber pass between the supporting cells to terminate in irregular indentations along the bases and lateral borders of the receptor cells, between the receptor cells and the supporting cells. Narrow projections of the receptor cells extend into the nerve terminals at areas of synaptic contact and contain presynaptic rods surrounded by vesicles (Figs. 1 B, 2). The presynaptic rods are about 1 μm in length and 0.3 μm in diameter at their bulbous ends.

After section of the lateral line nerve the onset of the degenerative process can be observed in the

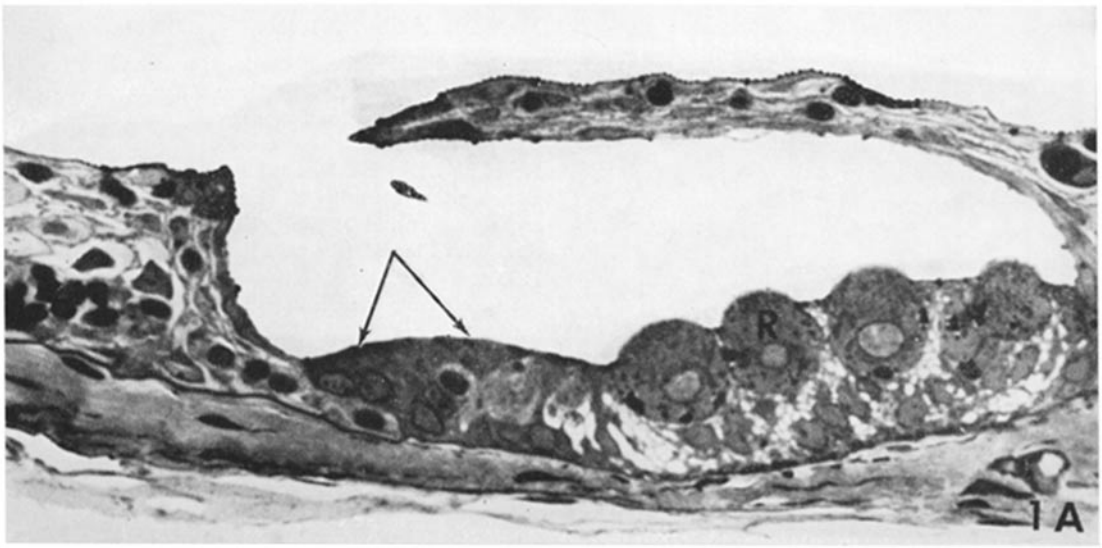

FIGURE 1 A Light micrograph of an ampullary organ from the unoperated control side of *Kryptopterus*. Four rounded receptor cells rest on a layer of supporting cells. Together these cell types constitute the sensory hillock. The layer of columnar cells nearer the pore make up the secretory hillock (arrows). × 840.

innervating fiber and its terminal between 6 and 12 h postoperatively (Fig. 3). Some mitochondria are swollen and their cristae disrupted. Numerous multivesiculate bodies are present. There are changes in the synaptic relations between receptor cells and nerve fiber. The cleft between the synaptic membranes becomes irregular and variable while the projections of the receptor cells and the corresponding depressions in the nerve terminal are reduced in size or absent. The presynaptic rods lose their normal shape and appear as irregular masses surrounded by synaptic vesicles. The remainder of the organelles in the receptor cell cytoplasm are unchanged.

Degenerative changes in the nerve fiber are well advanced by 18 h (Fig. 4). Only remnants of cellular organelles are recognizable in the condensed axoplasm. The myelin sheath is now disrupted, and there is an increase in the number of lysosomes in the Schwann cells. Nerve terminals are greatly reduced in size (Fig. 5) and extracellular spaces are found where the terminals appear to have retreated from their previous locations. In the receptor cells, presynaptic rods adjacent to the spaces appear much as they did a few hours previously.

Nerve terminals are no longer found on the receptor cells by 24 h (Fig. 6) but extracellular spaces are present that apparently mark their former positions (Fig. 7). The nonmyelinated branches of the nerve are shrunken and contain only remnants of organelles. The receptor cell mitochondria are disrupted, large vacuoles are numerous, and a hyaline material fills the lumenal border of the cell, perhaps as a result of swelling of the cytoplasm (Fig. 6).

All cells in the sensory hillock are in an advanced state of degeneration by 48 h. Most receptor cells are gone and presumably have been sloughed into the ampullary lumen where occasional cellular remnants are seen (Fig. 8). However, a few degenerated receptor cells containing dense bodies which are the remains of the presynaptic rods do remain at 48 h. These dense bodies are occasionally surrounded by vesicles. Supporting cells have severely disrupted mitochondria and increased vacuolization and in many cases the plasma membrane is ruptured.

Secretory cells are the last cells to degenerate. Nuclear swelling and mitochondrial disruption have occurred by 72 h (Fig. 9). Almost all of the cells associated with the receptor have been lost at 96 h. In some cases an empty cavity remains (Fig. 10), but often the cavity disappears entirely.

There is some variability in the rate of degeneration and receptor cells in advanced stages of degeneration are seen as early as 24 h. However, the time course described here is the usual one.

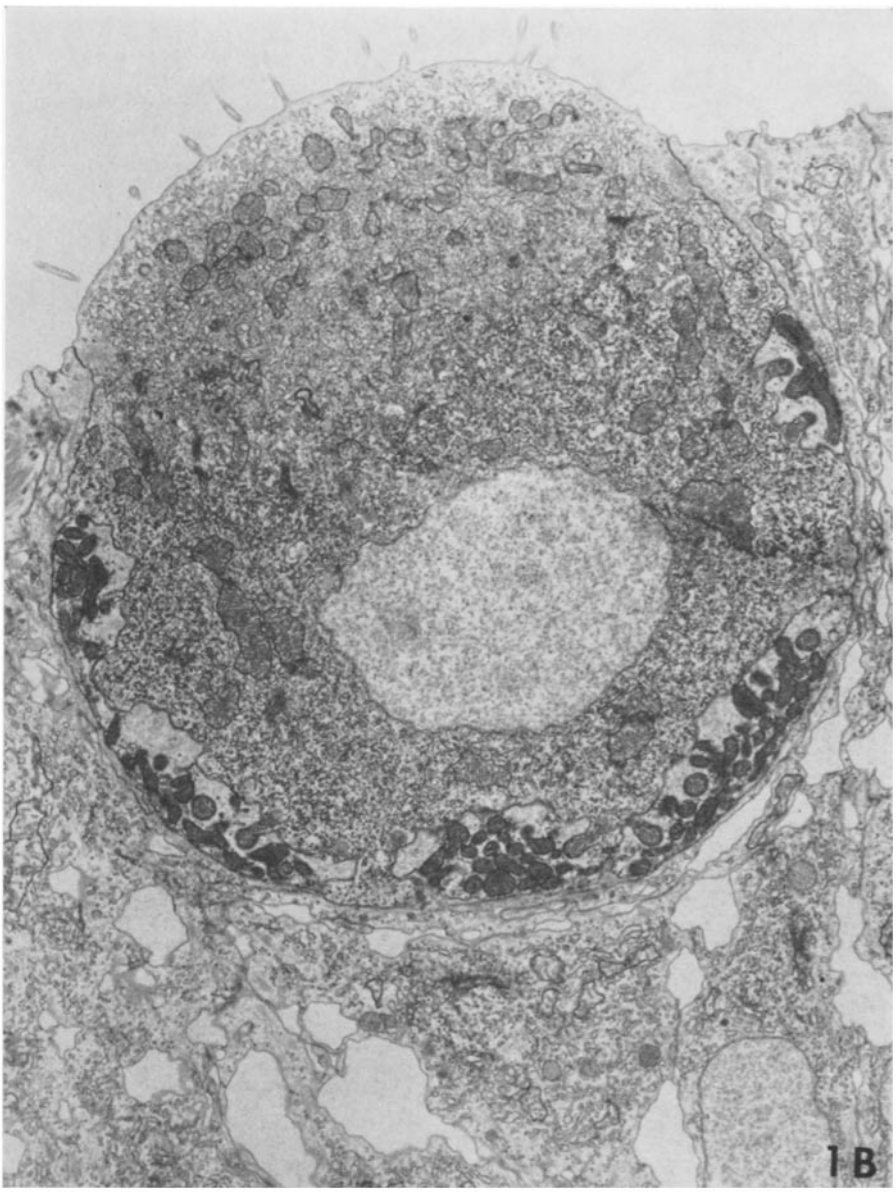

FIGURE 1 B Receptor cell of an electroreceptor from the unoperated control side of *Kryptopterus*. Irregular nerve terminals crowded with mitochondria line the lateral and basal surface of the receptor cell. Large extracellular spaces are present among the supporting cells. × 7000.

Degeneration proceeds at apparently constant rates in different regions of the fish, although considerably different distances from the point of section are involved.

Receptor cells of lateral line canal organs are more resistant to denervation and are unaffected 96 h after nerve section, although afferent and efferent nerve terminals are totally degenerated by 48 h.

DISCUSSION

The initial morphological changes we report here occur simultaneously in the nerve ending and presynaptic cell 6–12 h after nerve section,

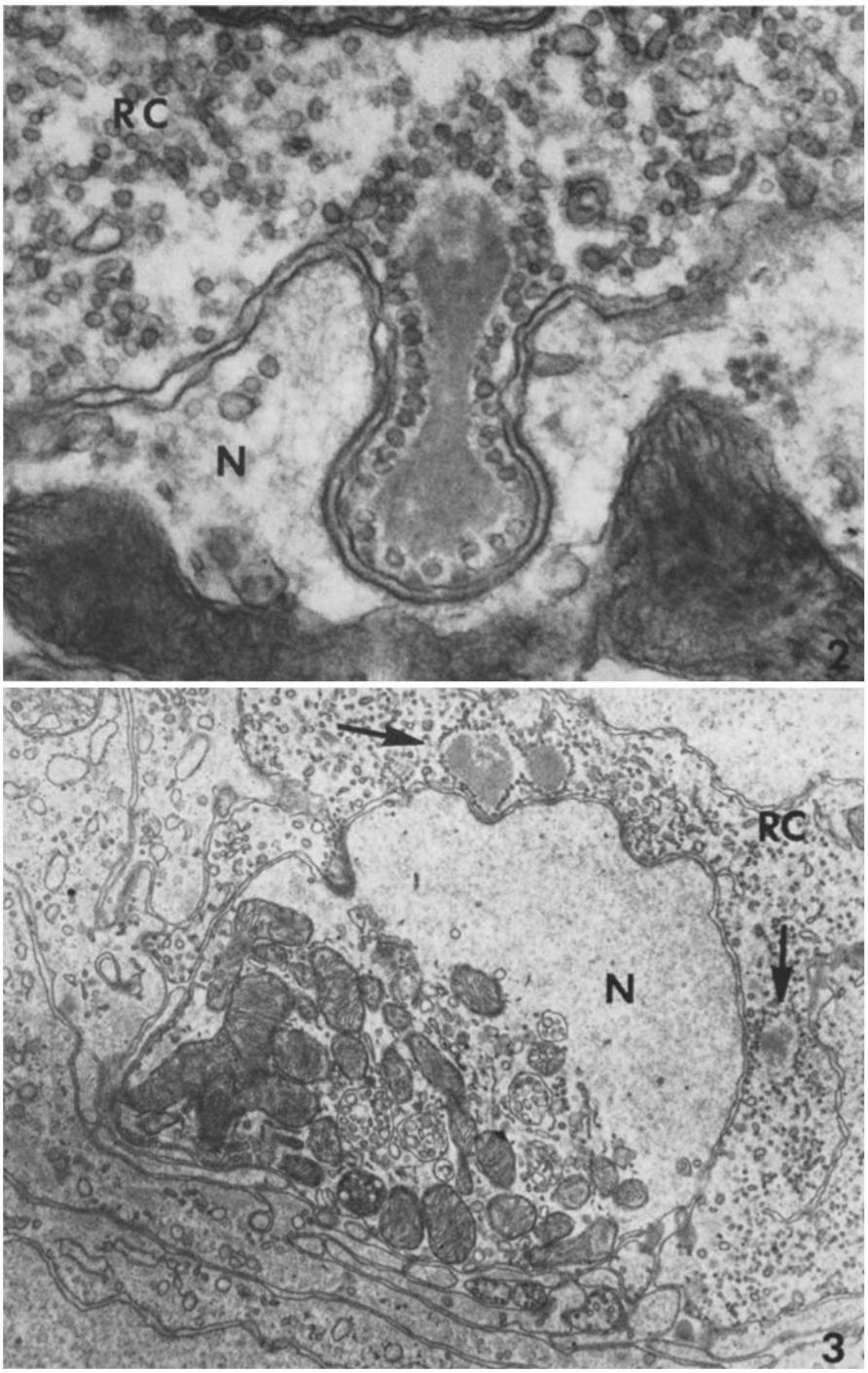

FIGURE 2 A synaptic projection in a control receptor cell (*RC*) extends into a corresponding depression in the nerve terminal (*N*). A dense presynaptic rod, surrounded by vesicles, lies within the projection. × 66,000.

FIGURE 3 A nerve terminal (*N*) at the base of a receptor cell (*RC*) 12 h after denervation. The nerve terminal is rounded and contains a large area devoid of cellular organelles. Numerous multivesiculate bodies are present in the nerve terminal. The presynaptic rods have become irregular rounded masses surrounded by vesicles (arrows). The presynaptic projections and corresponding depressions in the nerve terminal are reduced or absent. Part of the nucleus of the receptor cell is in the upper right. × 5500.

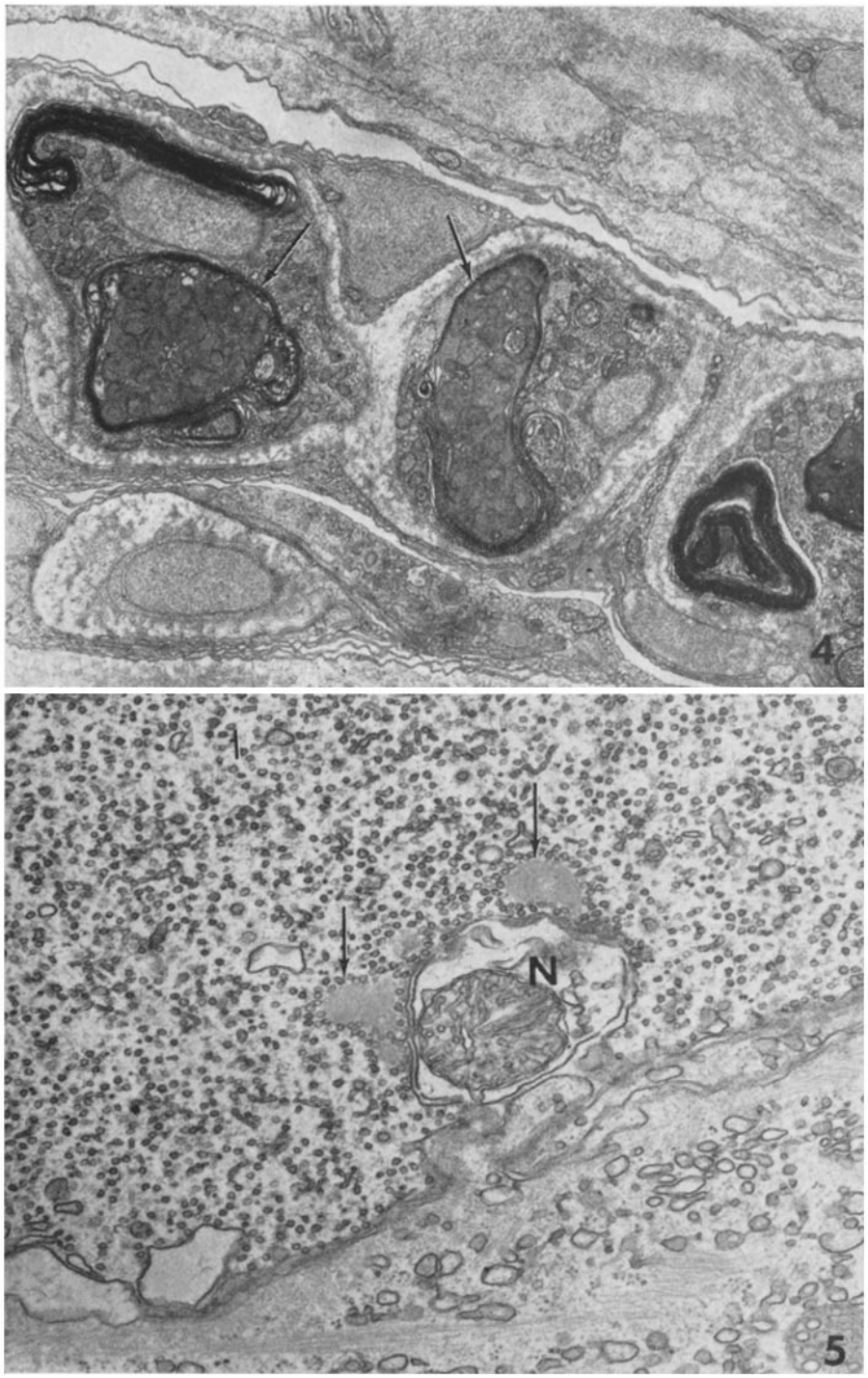

FIGURE 4 Portions of the myelinated afferent nerve fiber 18 h after denervation (arrows). The fiber bends in and out of the plane of section beneath the capsule of the organ. The myelin is disrupted and the dense axoplasm contains remnants of cellular organelles. × 9000.

FIGURE 5 A nerve terminal 18 h after denervation. The terminal (*N*) is greatly reduced in size. The presynaptic dense bodies (arrows) remain surrounded by vesicles adjacent to the terminal. A supporting cell process is in the lower right. × 35,000.

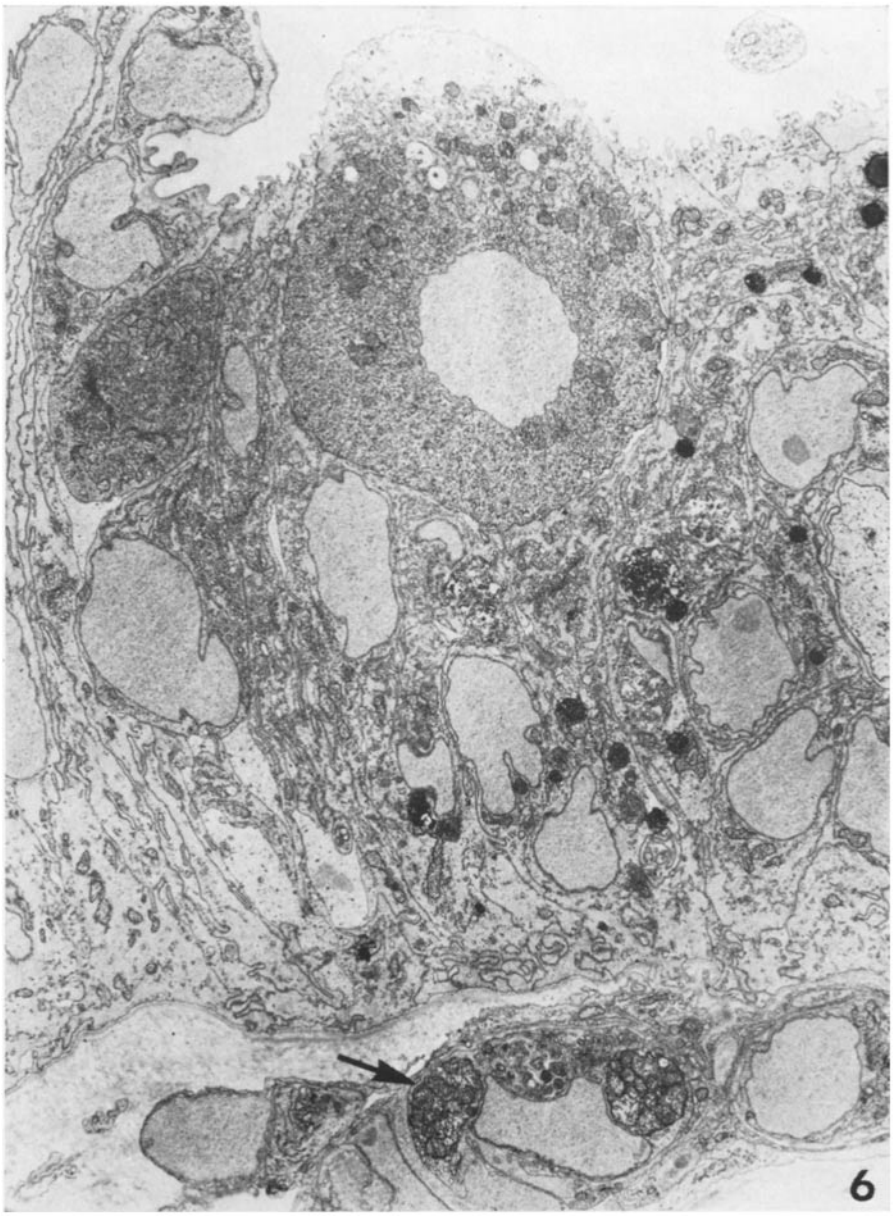

FIGURE 6 24 h after denervation the apical region of the receptor cell is filled by hyaline material. Microvilli are reduced in size and number. Nerve terminals are no longer present on the receptor cells and the afferent fiber (arrow) contains only remnants of organelles. Dense lipid droplets are present in the supporting cells. × 2200.

which is as early as or earlier than reported for any other vertebrate receptor. These changes are flattening out of the synaptic projections from the receptor cells into the nerve, flattening out of the corresponding indentations in the nerve terminals, and rounding up of the presynaptic dense bodies. Any one of these changes could initiate the other ones. Similar changes in the synaptic interdigitations are seen after denervation of the ampulla of Lorenzini of *Torpedo* in which, however, the time

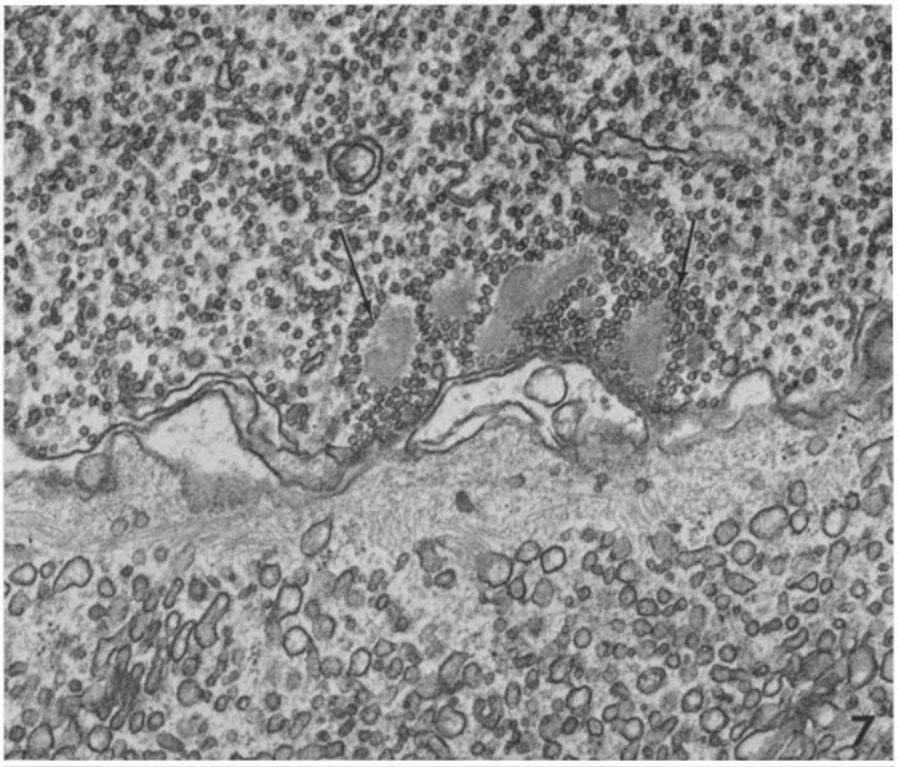

FIGURE 7 Presynaptic dense bodies 24 h after denervation. The dense bodies (arrows) are present in the receptor cell (upper half) adjacent to large extracellular spaces and are still surrounded by vesicles. The supporting cells (lower half) contain numerous large vesicles. × 40,000.

FIGURE 8 Light micrograph of a receptor 48 h after denervation. No intact receptor cells are present. Most receptor cells presumably have been sloughed into the ampullary lumen and some apparent cellular debris is present in the lumen. × 1000.

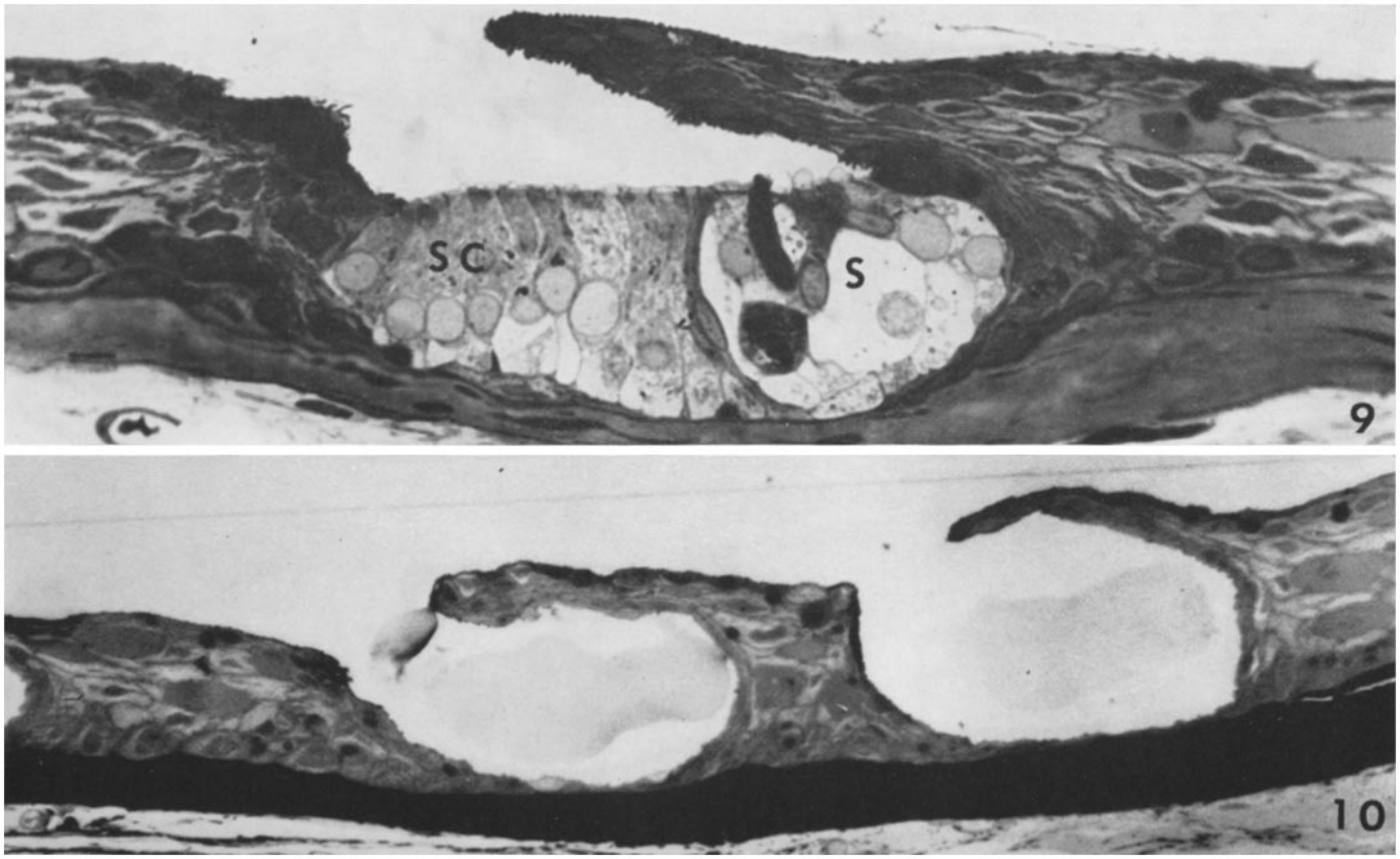

FIGURE 9 Light micrograph of a receptor 72 h after denervation. Most of the cells of the sensory hillock (*S*) have degenerated and large extracellular spaces are present. Secretory cells (*SC*) have begun to degenerate. × 10,000.

FIGURE 10 Light micrograph of two receptors 96 h after denervation. Most of the cells have been lost leaving empty cavities. × 2800.

required is about 3 days (Derbin, 1970). In this preparation, the presynaptic dense bodies are in the form of ribbons, which do not round up in the course of degeneration. In both *Kryptopterus* and *Torpedo* recetors, some vesicles remain in close association with the presynaptic dense bodies during degeneration, but in the latter case the vesicles swell markedly.

The composition and function of the presynaptic dense bodies are unknown. Presynaptic dense bodies in both tonic and phasic electroreceptors and the presynaptic ribbons in the photoreceptor terminals of the frog retina are composed in part of protein (Bunt, 1970, Szamier, 1972). One suspects that they are involved in the process of transmitter release, and in this respect it is interesting that they occur in a number of synapses at which the presynaptic depolarization is very small compared to the presynaptic action potential of ordinary interneuronal synapses (Bennett, 1970, 1971 *a*). A further possible correlation is that many of these synapses appear to tonically release transmitter in the absence of stimuli and this release is modulated up and down by stimuli of opposite sign.

The variations in the time course of degeneration of *Kryptopterus* electroreceptors did not correlate with distance from the site of nerve section. No obvious differences in the onset or rate of degeneration were noted between receptors in the anterior and posterior of the body. One explanation of the lack of latency difference is that the signal or disturbance passing down the nerve as a result of section covers the distance in a short time compared to the time for degeneration to begin. The difference in distance from the site of section for anterior and posterior receptors was about 2 cm in our experiments. The fastest reported rates of axoplasmic transport are about 2 cm/h (Graftein, 1969; Lasek, 1970). Thus our failure to observe a latency difference in degeneration is consistent with the time for a neurotrophic substance to pass down the axon by fast axoplasmic transport. Of course other kinds of signal such as progressive depolarization of the nerve fiber cannot be excluded by present data. In the catfish *Amiurus*, longer lengths of nerve between sites of section and taste bud did lead to slower degeneration (Torrey, 1934). The rates of apparent movement along the fibers were in the range of millimeters per day (calculated on the assumption of a modest size catfish, i.e., about 15 cm in length). Dependence upon length of intact nerve has also been noted in denervation of muscle. The apparent velocities are in the range of a few millimeters per hour (Albuquerque et al., 1971) to about 1.5 cm/h (Miledi and Slater, 1970).

Large numbers of postsynaptic vesicles are clustered in the nerve terminal around the synaptic projections of many electroreceptors (Lissman and Mullinger, 1968; Szamier and Wachtel, 1969, 1970), and some of these though fewer in number are seen in the nerve terminals of *Kryptopterus* (Wachtel and Szamier, 1969). Their function is unknown but they perhaps are involved in secretion of trophic substances necessary for the maintenance of the receptor cells.

A matter that has been of some dispute is whether neurotransmitters act as trophic substances in addition to their role in rapid signaling. In *Kryptopterus* as well as other electroreceptors, the transynaptic degeneration proceeds oppositely to the normal direction of transmitter movement from receptor cell to nerve terminal which would support the idea of a separate trophic substance. Although there is no efferent innervation the physiological data do not yet exclude recurrent inhibitory transmission from afferent fibers to receptor cells (cf. Murray, 1965). It is relevant in this respect that the supporting cells and secretory cells are dependent on the nerve supply, although they are not directly innervated. The experiments demonstrating a trophic influence of segmental nerves on lateral line receptors would also indicate a trophic action at some distance from the nerve (Jones and Singer, 1969). These data certainly strengthen the view that trophic substances, whether or not they are neurotransmitters, need not act upon sites involved in the generation of conventional postsynaptic potentials.

Receptor cells in the lateral line canal organs of *Kryptopterus* failed to degenerate within 4 days after nerve section, although nerve terminals were lost in 2 days. These data are consistent with earlier experiments on the catfish *Amiurus* in which the receptors degenerated in 4–16 days (Brockelbank, 1925). We have no more explanation for the difference between different kinds of receptors in the same species than we do for the difference between the same kind of receptors in different species. Although we do not understand the rapidity of electroreceptor degeneration in *Kryptopterus*, this speed makes the preparation an attractive one for further analysis of neurotrophic functions.

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