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SUBMICROSCOPIC MORPHOLOGY OF THE INFRARED RECEPTOR OF PIT VIPERS*

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With 9 Figures in the Text

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Introduction

In the facial pit of the crotalid snakes there is an interesting sensory membrane which is highly sensitive to intermediate and long infrared radiation (BULLOCK and DIECKE 1956). According to them, stimulation of this receptor is probably not mediated by a special photochemical mechanism, but depends on a change in temperature of the membrane produced by radiant energy. Physiologically it can be thus considered as a special type of thermoreceptor containing almost a pure population of warm fibers.

BULLOCK and FOX (1957) analyzed the anatomical literature related to this organ and made, with histological methods, a thorough description of the structure of the sensory membrane. This study demonstrated the existence of a large concentration of free nerve endings which was calculated to be about 500 to 1500 per mm^2. The authors described also a single layer of specialized parenchymal cells which were not regarded as sensory elements, but were found to react to degeneration of the nerve fibers.

For BULLOCK and FOX (1957) these two components — nerve endings and parenchymal cells — in between the two layers of extremely attenuated epidermis represent the bulk of the sensory membrane.

The present work was started with the idea that because of the high concentration of nerve terminals such a membrane could be an excellent object for the observation of free endings with the electronmicroscope. It was hoped that the high resolution of this instrument would give information about the intimate structure of nerve endings and of their relationship with the surrounding components, thus permitting a better correlation with the physiological data.

This study has confirmed most of the points described by BULLOCK and FOX (1957) and it has shown that the free nerve terminals, which form a densely packed layer and end among an amorphous connective matrix, contain an enormous concentration of mitochondria. These organoids, packed together in a rather compact mass which occupies almost the entire ending, are apparently formed in the last portion of the myelinated axons by a special mechanism which will be described in another paper.

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The present study gives also an interpretation of the so-called parenchymal cells which is at variance with that of BULLOCK and FOX (1957) and shows other details of the fine structure of the sensory membrane that were not apparent with light microscopy.

Techniques

Five adult specimens of rattlesnake (*Crotalus durissus terrificus* Laurentius) from the Argentine Chaco were used[1]. The venom was extracted and the teeth were cut as a precaution previous to the resection of the sensory membrane. Then the viper was tightly held under a dissecting microscope and the membrane was removed and immediately transfered to the fixative. This was a solution of OsO_4 in "Periston" (Bayer) 1% at p_H 7.4 and at 0—4° C. While being dehydrated in ethanol, the membrane was cut into ribbons of $^1/_4$ by 4—5 mm which were embedded flat in a mixture of butyl-methyl methacrylate (8.5:1.5).

The sections, made with a Porter-Blum microtome, were put on formvar films. Observations were made with a Siemens Elmiskop I at 60 Kv and the micrographs taken at 3000 to 20000×.

Observations

General organization of the sensory membrane

Fig. 1 shows in a diagrammatic form the complex organization of the sensory membrane revealed by the electronmicroscope which is illustrated at low magnification in Figs. 2 and 3. Within a total thickness of only 8 to 16 μ from the outer to the inner surface, the following seven layers can be recognized: 1. Outer epithelium, 2. outer connective layer, 3. layer of the vacuolar cells, 4. layer of nerve endings, 5. layer of myelinated nerve fibers, 6. inner connective layer, 7. inner epithelium.

This subdivision is intended only to facilitate the description by indicating the topography of the different components. It could be made simpler by recognizing the two epithelial layers and in between a connective tissue membrane in which the nerve fibers and their endings, Schwann cells and vacuolar cells are present.

Epithelial layers (Figs. 1—4). Both the outer and the inner epithelia are built of an extremely thin epidermis with cornified cells whose thickness varies with the moulting cycle (BULLOCK and FOX 1957). As recognized earlier by LANGE (1931) this is not a simple cuticle, but a continuous cellular layer covered by corneal elements.

The cellular layer or germinative stratum is in our specimens 0.2 to 1.4 μ thick in the outer epithelium and only 0.2 to 0.3 μ in the inner one. This layer is made of 2—3 strata of flattened cells whose nuclei are seldom observed because they are considerably spaced. At the contact between cells there are interdigitating microvilli and typical desmosomes or adhesion plates (Fig. 4). The deeper cells are rich in ribosomes suggesting a more active protein synthesis. There are also mitochondria and localized sets of parallel membranes corresponding to the Golgi complex. In the more superficial strata there are some large bodies with a single membrane and a heterogeneous dense content which are similar in aspect to those described as queratohyalin granules in the epidermis of the guinea pig (BRODY 1959). In these cells there are less ribosomes and bundles of fine filaments are present.

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The corneal layer is also made of several strata whose limits become less prominent toward the membrane surface where they assume a rather compact and homogeneous aspect. The corneal layer of the outer epithelium varies

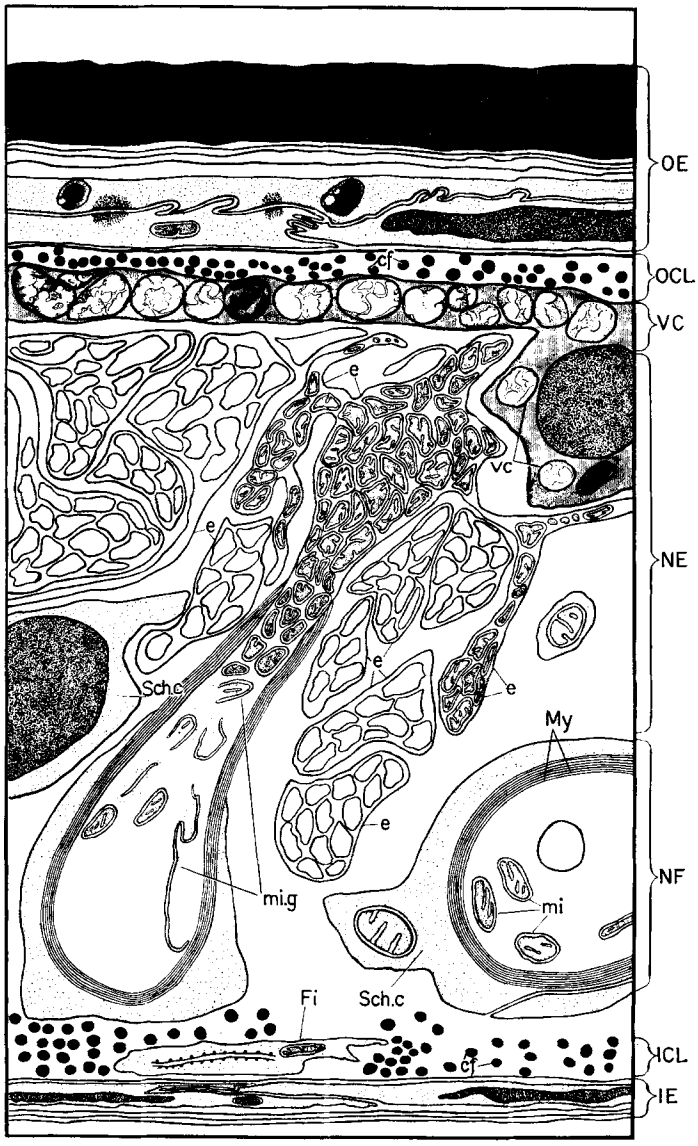

Fig. 1. Diagram showing the general organization of the sensory membrane: *OE* outer epithelium; *OCL* outer connective layer; *VC* vacuolar cells; *NE* nerve endings; *NF* nerve fibers; *ICL* inner connective layer; *IE* inner epithelium; *e* endings; *Sch. c* Schwann cells; *Fi* fibroblast; *cf* collagen fibers; *My* myelin; *mi* mitochondria; *mig.* mitochondrial genesis. (See description in the text)

between 1.4 to 4 μ and that of the inner between 0.1 to 0.3 μ. These measurements probably correspond to certain stages of the moulting cycle and they may vary considerably in the others.

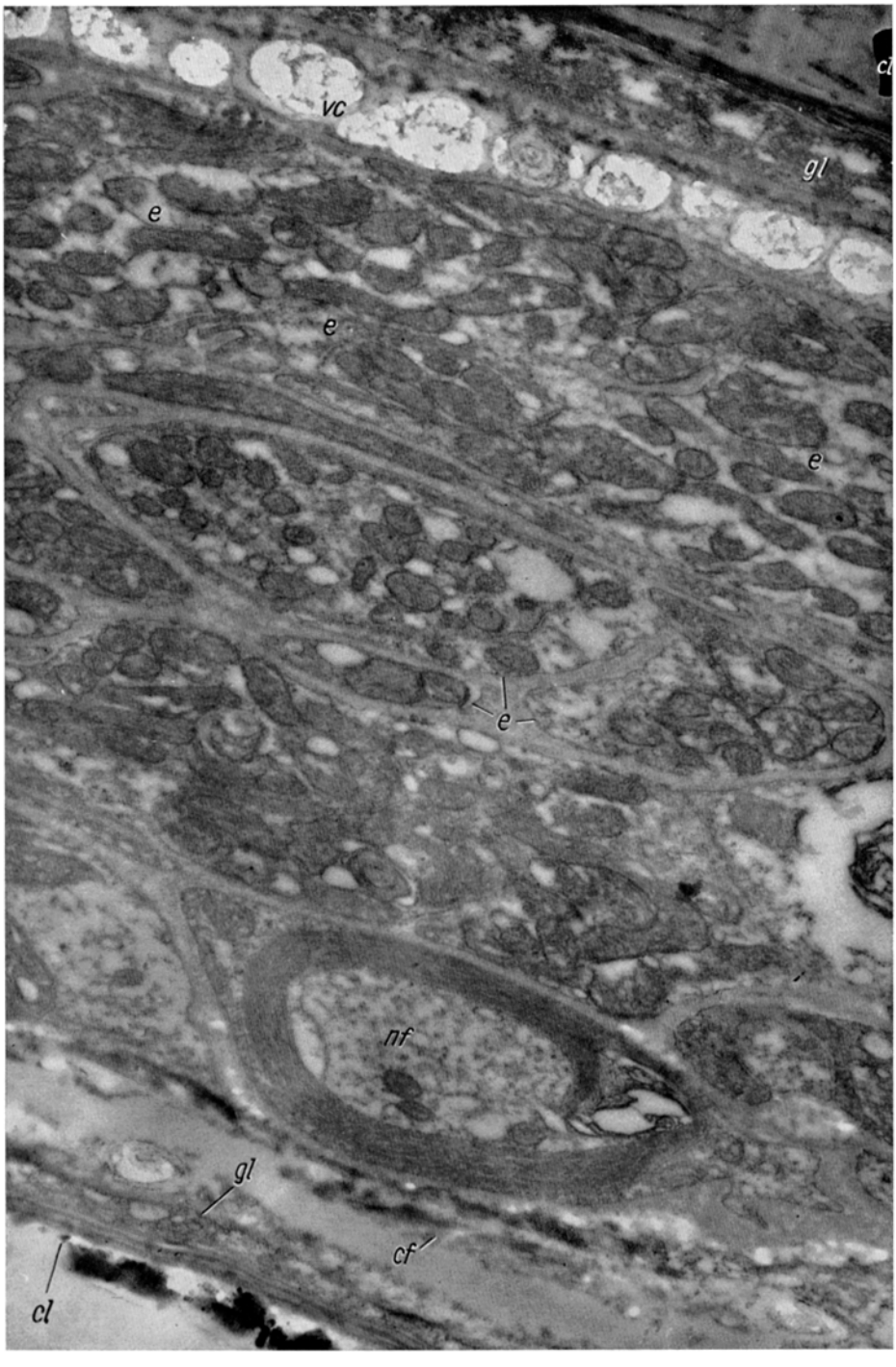

Fig. 2. Electronmicrograph of a section across the whole thickness of the sensory membrane. *cl* corneal layer; *gl* germinative layer; *vc* vacuolar cell; *e* nerve endings; *nf* nerve fiber; *cf* collagen fibers. 24 500 ×

Outer and inner connective layers (Figs. 1—4). These two layers are differentiated from the rest of the connective tissue that fills the sensory membrane by the

Fig. 3. Electronmicrograph of the outer layers of the sensory membrane. *cl* corneal layers; *cf* collagen fibers; *vc* vacuolar cell; *e* nerve endings. 30000 ×

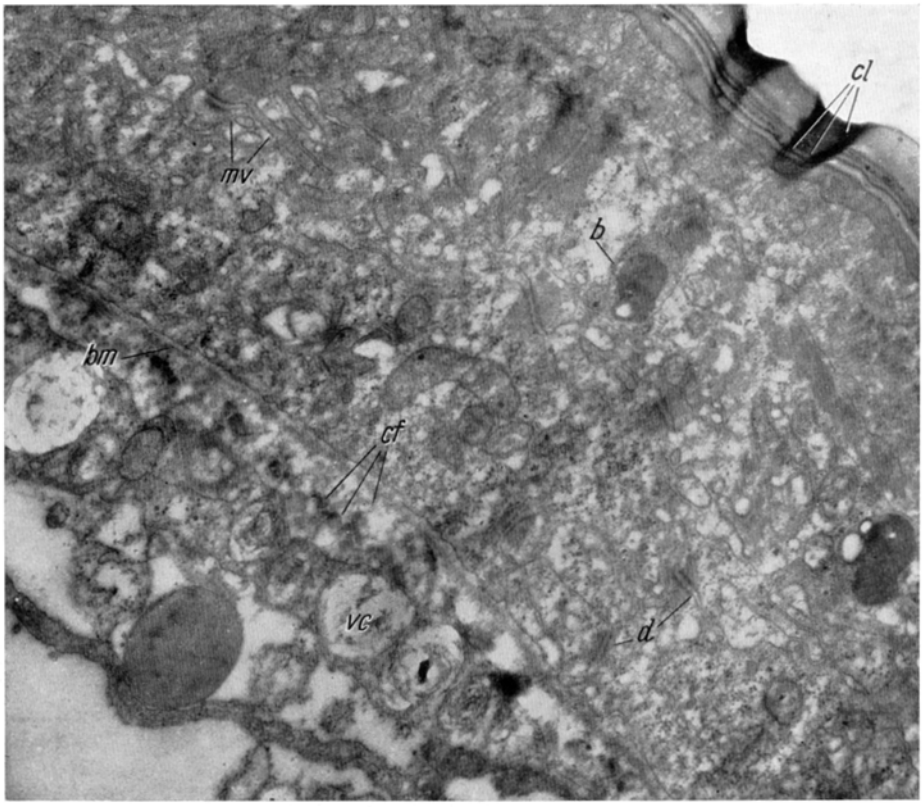

Fig. 4. Electronmicrograph of the outer epithelium. *cl* corneal layers; *b* epithelial body; *mv* microvilli; *bm* basal membrane; *cf* collagen fibers; *d* desmosomes; *vc* vacuolar cell. 50 000 ×

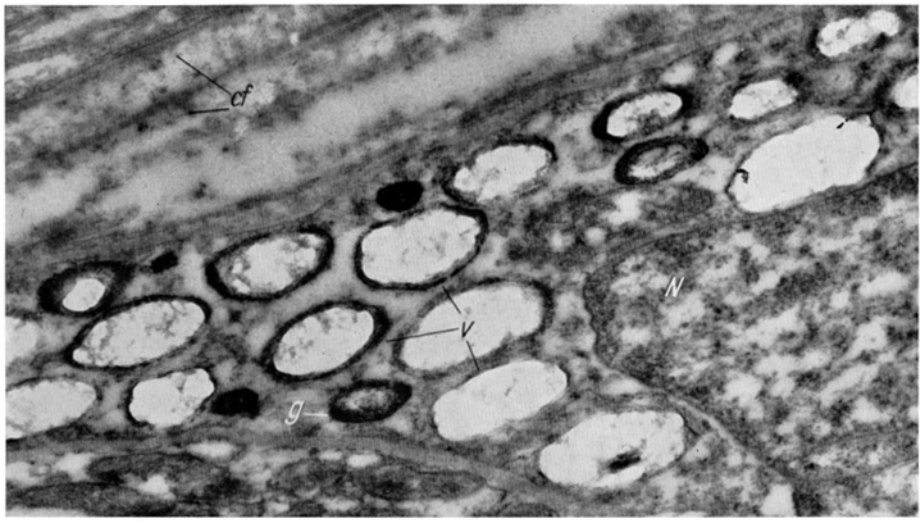

Fig. 5. Electronmicrograph of a portion of a vacuolar cell. *N* nucleus; *v* vacuoles; *g* granule; *cf* collagen fibers. 30 000 ×

higher concentration of collagen fibers. We confirm in this respect the interpretation of BULLOCK and FOX (1957) who observed that under both epidermal layers there was a stratum staining differentially with Masson trichrome. In our sections the outer layer varies in thickness between 0.07 and 2 μ and the

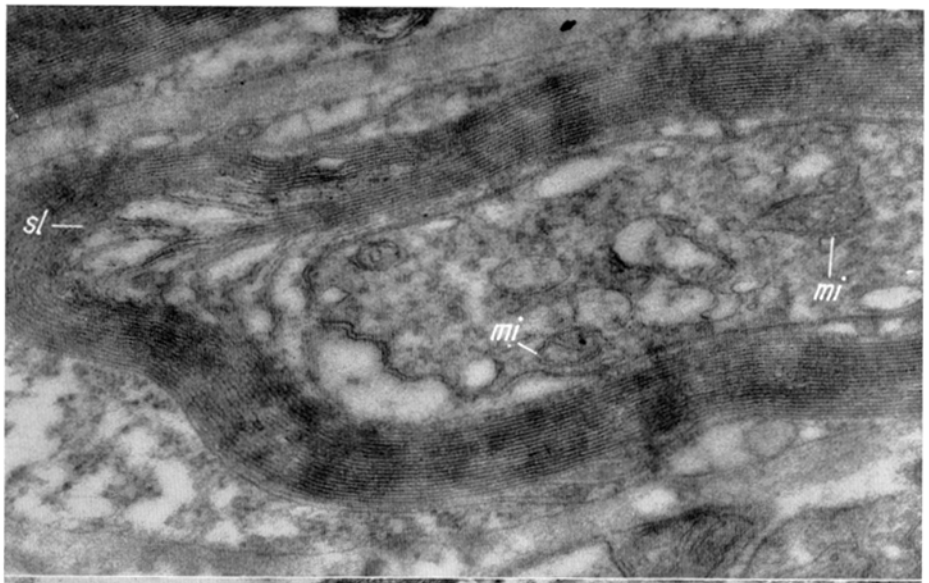

Fig. 6

Fig. 7

Fig. 6. Electronmicrograph of a myelinated fiber approaching the palmate ending. *sl* Schmidt-Lantermann incisure; *mi* mitochondria. 60 000 ×

Fig. 7. Electronmicrograph of a myelinated fiber (*nf*) closer to the palmate ending than that of Fig. 6. *e* nerve ending. 52 500 ×

inner one is generally thicker. Occasionally one may observe isolated cellular elements resembling fibroblasts and macrophages, but the main component is represented by bundles of collagen fibers in an amorphous matrix. At the limit with these connective layers the epithelia have definite basal membranes.

Layer of vacuolar cells (Figs. 1—5). Immediately behind the outer connective layer and in front of the nerve endings there is a stratum of about 0.3 μ made of flat processes of some cells whose nuclei are very sparse and lie deeper in the membrane (Fig. 5). These processes do not cover the entire surface of the mem-

brane and may be absent in certain parts (Fig. 3). Furthermore some similar processes may more seldom be found deeper into the sensory membrane and even covering part of the inner connective layer. The main characteristic of these cells is the presence of large vacuoles with a thin limiting membrane whose interior has an empty aspect because of its extremely low electron density. This may result of extraction of some material by the technical procedures. Some of these vacuoles contain strands of a rather amorphous material, others are filled with a dense content and there are also smaller and denser granules (Figs. 4 and 5). All these different inclusions suggest the existence of a cycle within these vacuolar cells. In addition these cells contain a few mitochondria, and intracellular membranes and ribosomes.

This layer is in contact with the collagen fibers of the outer connective layer on one side and is separated from the nerve endings by a space of about 500 Å filled with an amorphous matrix.

Layer of nerve fibers (Figs. 1, 2). The rich innervation of the membrane was already noticed by several investigators of the XIX century and led to its interpretation as a sensory organ (see LYNN 1931). In whole mounts of the membrane observed under the light microscope numerous myelinated nerve fibers grouped into trunks are seen to penetrate from the outer edge into the sensorial membrane (LYNN 1931; BULLOCK and FOX 1957).

Using silver impregnation, BULLOCK and FOX (1957) found that the myelinated fibers in their transit within the membrane became isolated from the nerve trunks and formed nerve endings. The nerve fibers were seen to loose their myelin, to taper down, and then to expand into flattened structures like palms with finger-like expansions which were called *palmated* endings.

In our electronmicroscope observations the myelinated fibers are concentrated in some thicker regions of the membrane and more widespread in the thinner ones. In all cases they occupy the deeper portion of the sensory membrane near the inner connective layer. They have the typical organization, with a thick myelin sheath, surrounding Schwann cells and an axon which varies considerably in structure according to the region. In the nerve trunks the axons have no special features and contain few mitochondria and numerous neuroprotofibrils. When they approach the endings, the neuroprotofibrils tend to disappear, the axoplasm becomes denser and the axons are filled with numerous membranes and mitochondria (Fig. 6). At a certain portion the axon approaching the terminal the number of mitochondria increases considerably reaching a population density almost similar to that of the palmated endings (Fig. 7). In between the mitochondria there are also vacuoles with a clear content and a definite membrane. Very soon the myelin sheath becomes thinner with less unit membranes and finally disappears to give rise to the palmate ending. A more detailed description of this last portion of the nerve fiber together with the mechanism of mitochondriogenesis, in which both the axon membrane and the ground matrix of the axon appear to be involved, will be described in a separate paper.

Layer of nerve endings (Figs. 1, 2, 3, 8 and 9). The layer of nerve endings represents by far the thicker portion of the sensory membrane (Fig. 2). The size and shape of these nerve endings show great variations depending of the region cut and the incidence of the section. The profiles observed may correspond

to transections through the palms, processes or the minor branches observed by BULLOCK and FOX (1957) in silver staining preparations. They have a rather

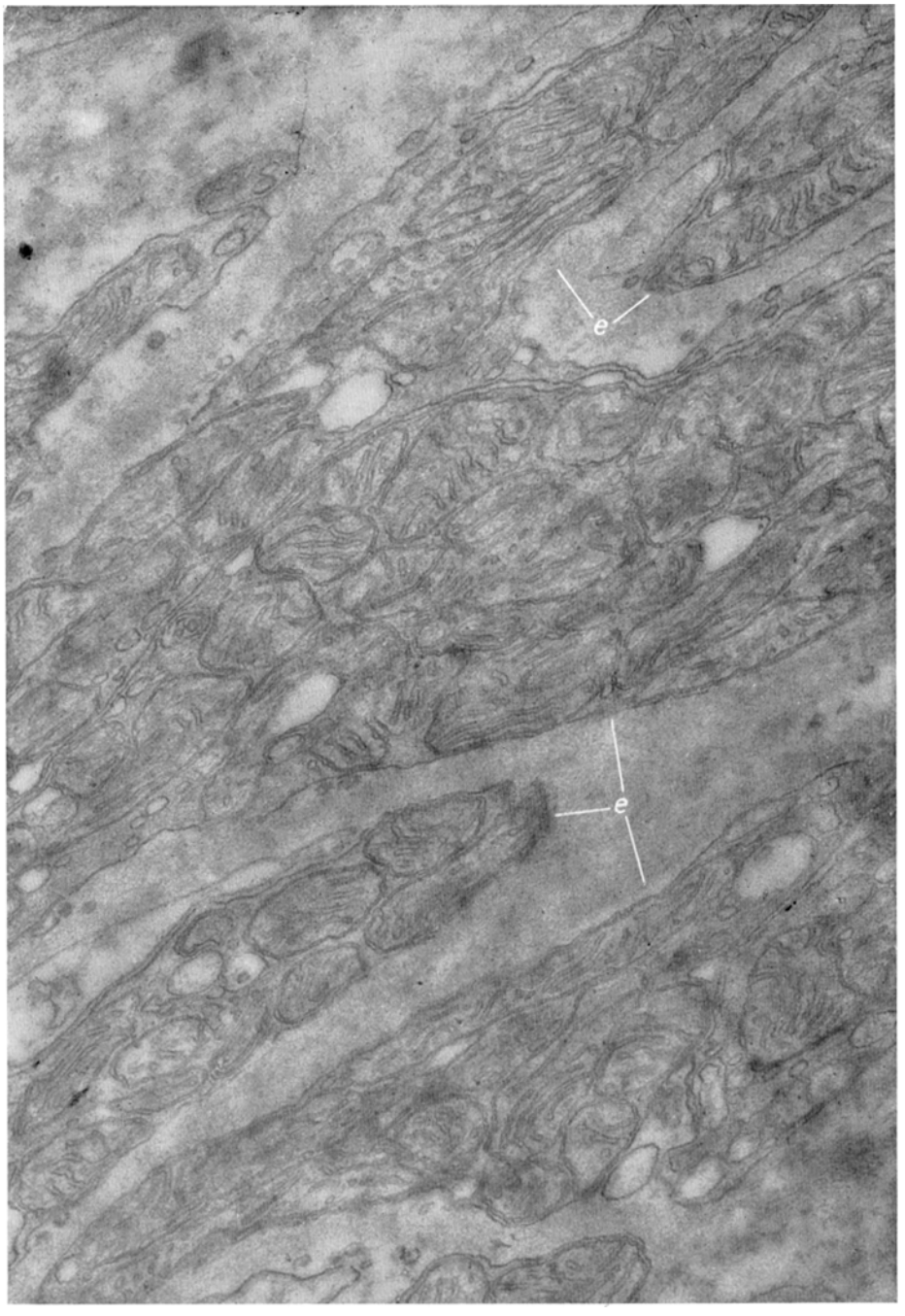

Fig. 8. Electronmicrograpα of nerve endings of the sensory membrane. Notice the high concentration and dense packing of mitochondria. *e* ending. 60000 ×

irregular outline as in a puzzle game. The smaller branches of these endings are entirely free and the axon membrane is in direct contact with the abundant

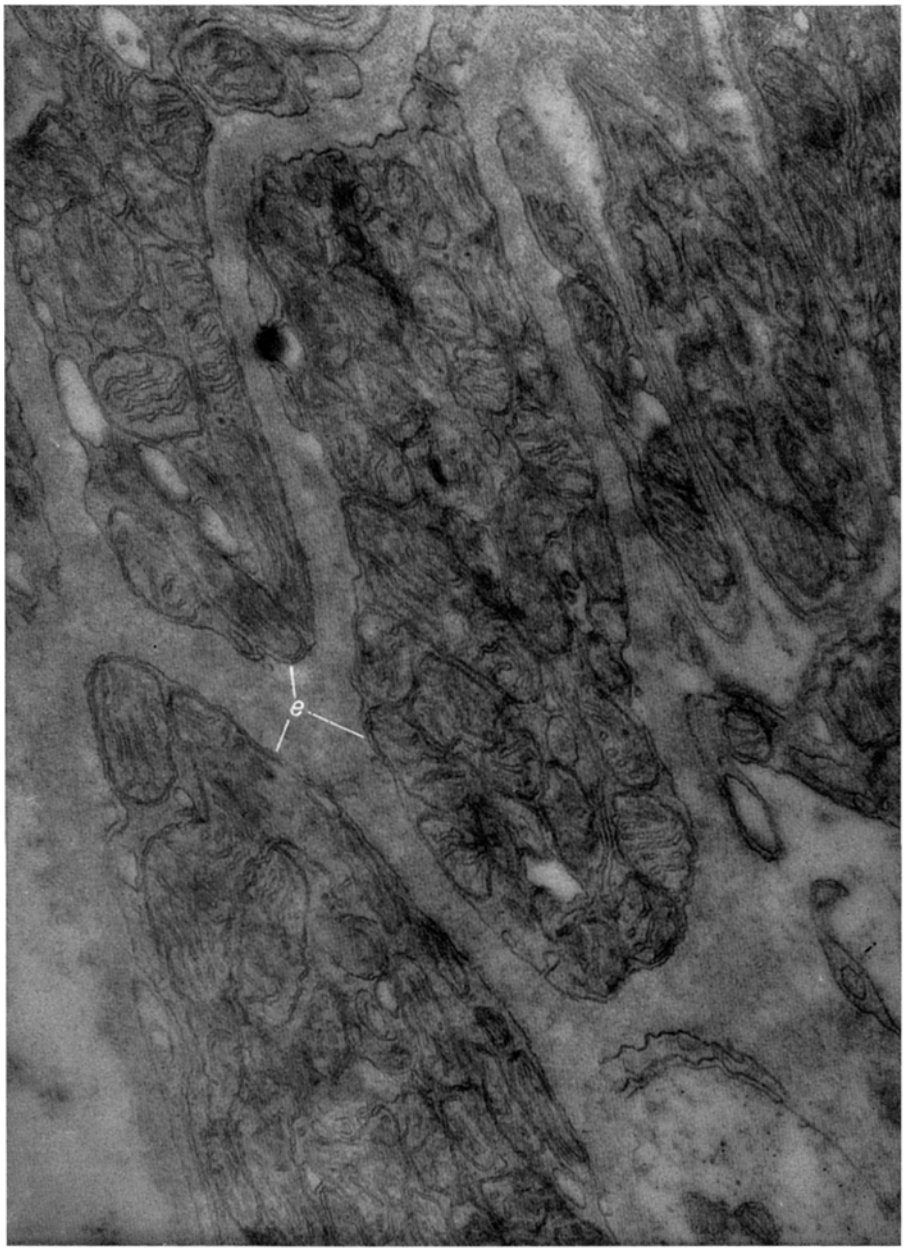

Fig. 9. Similar description to that of Fig. 8. 52500 ×

amorphous matrix that fills the intercellular spaces and is continuous throughout the sensory membrane. However some portions of the palmate endings are seen covered by thin processes of the Schwann cells. Some of these cells are found

in between groups of nerve endings and show an infolded nucleus with irregular outline and short cell processes.

The inner structure of the nerve endings is remarkable because of the dense packing of mitochondria that occupies practically all the axoplasmic matrix. These mitochondria show the typical structure with the two membranes and the inner crests that are frequently oriented along the axis (Figs. 8 and 9). In the narrow spaces in between mitochondria a few vacuoles can be observed.

Discussion

The ultrastructure of the sensory membrane

The above electronmicroscope observations on the sensory membrane of pit vipers confirm in general lines the light microscope findings of BULLOCK and FOX (1957). However some discrepancies arise regarding the interpretation of the so-called "parenchymal cell layer" and the space occupied by the nerve endings within the membrane. The higher resolution of the electronmicroscope permits a better definition of these structures and to clarify these two points.

BULLOCK and FOX (1957) interpreted that in a section of the membrane about 4—12 μ in thickness were occupied by a single cell layer. They described these "parenchymal cells" as having an irregular nucleus and an "abundant cytoplasm which is coarsely reticular, almost granular and stains darkly". Comparing this description with our observations on the layer of nerve endings, the correspondence seems evident. The irregular nuclei are similar to the Schwann and vacuolar cell nuclei observed by us which have little cytoplasm. The "abundant cytoplasm" probably corresponds to some of the branches of the palmate endings that surround Schwann cells and are filled with mitochondria. That this interpretation is correct, is also proven by the fact that no other cellular elements, besides the Schwann nuclei and those of the vacuolar cells, are present in the same layer. Furthermore the profiles filled with mitochondria around the nuclei are certainly of nervous nature since we have seen all the intermediary stages of the transformation of the myelinated nerve fibers into the palmate endings filled with mitochondria.

This brings us to the other point, that of the space occupied by the nerve endings in the sensory membrane. The electronmicroscope observations show that at least half of the total thickness of the membrane is occupied by the free nerve endings which are packed rather tightly and immersed in a continuous amorphous connective matrix. This finding contrasts to some extend with the images of the whole mounted silver stained preparations of BULLOCK and FOX (1957), which although showing a considerable density of nerve endings (about 520 palmated endings per mm^2), have a less compact organization than that observed with electronmicroscopy.

This apparent discrepancy may have several explanations, one of which may be an incomplete staining of the endings. It is well known that in other tissues the silver methods may leave unstained a certain number of endings, but this is not the case with electronmicroscopy. In addition the stainability with silver may vary along the fiber and the terminal portion may remain unstained. In a recent work by BOYCOTT et al. (1961) this problem is discussed for the CNS

and the conclusion is reached that the pattern of silver staining corresponds to the neurofibrillar portion of the axon and not to the ending proper, containing mitochondria and synaptic vesicles, where neurofilaments are generally lacking. Another contributing but secondary factor could be a retraction of the material by the technical handling of the silver staining, which is more drastic on the tissue than the treatment for electronmicroscopy.

BULLOCK and DIECKE (1956) in analyzing the properties of the sensory membrane concluded that its high sensitivity depends mainly on anatomical adaptations. One of them is represented by the high concentration of nerve endings and their palmate shape, which may lower the threshold by simultaneous stimulation of several receptor units. Another adaptation is the thinness of the membrane and the closeness of the nerve endings to the surface. BULLOCK and FOX (1957), although acknowledging the difficulty of obtaining accurate measurements, postulate that the average depth of the endings is no more than 5 μ and possibly only 2 μ, from the outer surface. These figures are confirmed here by showing that the thick layer of nerve endings is only within a minimal distance of 2 μ from the outer surface thus providing little absorbing material in between for the infrared radiation.

Another point which is confirmed is the apparent lack of cellular elements that could be truly sensory in nature. If we discard the Schwann cells, only the layer of vacuolar cells with the clear inclusions might have some function in reception. These cells are generally in the way of the stimulus impinging upon the nerve endings and might have an accessory function in reception. However the fact that they do not form a continuous layer and the lack of a direct contact with all nerve endings seems to preclude that they have an essential role in the mechanism of activation of the sensory endings.

Probable significance of mitochondria in the nerve endings

At first sight the electronmicroscope observations seem to rule out the existence of a morphological differentiation at the nerve endings that may act as a specific receptor organ. Nothing comparable to the outer segments of the photoreceptors, with the compact system of membranes, has been observed in these endings. Instead what strikes one here is the enormous concentration of mitochondria that, originating in a special mitochondriogenic region of the myelinated nerve fiber, fill the entire palmate ending.

The population density of these mitochondria is so great that practically all the nerve ending is occupied by these organoids and we can say without exaggeration that about half the volume of the sensory membrane is made of compact masses of mitochondria. This leads one to speculate if these organoids could be involved in the transducing mechanism of temperature change into neuronal activation.

The production of receptor and generator potentials, with the active transport mechanism involved, requires a great deal of energy and this could be provided by the mitochondria present at the ending. Concentrations of these organoids have been observed in the nerve endings of the Paccinian corpuscle (PEASE and QUILLIAM 1957), the Meissner corpuscle (CAUNA and ROSS 1960) and the neuromuscular spindle (MERRILLEES 1960). Although these findings could be indicative

of a non specific function of mitochondria one may still wonder if the exceptionally high concentration of these organoids could not have a more special significance.

The physiological observations of BULLOCK and DIECKE (1956) tend to rule out the existence of a photochemical mechanism in the sensory membrane. The spectrum of activation is apparently not suggestive of a specific absorption, as in photoreception, but of a change in temperature of the membrane produced by infrared radiation. One may hypothesize that the changes in temperature of the membrane may influence the enzymatic and electron transport systems present in mitochondria. These changes might in turn influence, by electron transfer, the conductivity properties of the surface membrane thus modifying the spontaneous activity of the ending. BULLOCK and DIECKE (1956) although they did not record prepotentials assumed that the initiation of nerve impulses in the free endings might be similar to those recorded in certain dendrites and that the action potential proper might start at the beginning of myelinated fiber. Only a microphysiological investigation by fine microelectrodes implanted in the different layers of the sensory membrane could give information about the generator potentials and might permit a test of the hypothesis that we postulated above. However because of the small size of the nerve endings and the hardness of the corneal layers such a study seems for the moment technically difficult.

Summary

The fine structure of the infrared receptor membrane of pit vipers has been studied under the electronmicroscope. From the outer to the inner surfaces, within a total thickness of only 8 to 16 μ, the following seven layers were recognized: 1. Outer epithelium, 2. outer connective layer, 3. layer of vacuolar cells, 4. layer of nerve endings, 5. layer of nerve fibers, 6. inner connective layer, 7. inner epithelium.

The nerve endings, which form a densely packed layer, represent the most prominent component of the sensory membrane. Their inner structure is remarkable because of the high mitochondrial concentration. The population density of these organoids is as great as virtually to occupy the entire ending. Almost half of the volume of the sensory membrane is thus made of compact masses of mitochondria.

The structure of the myelinated nerve fibers entering the sensory membrane, was analyzed together with the stages of transformation into nerve endings.

This study revealed that there is a special region of the nerve fiber in its transition toward the nerve ending where mitochondriogenesis is very active, permitting the analysis of the mechanism of formation of these cell organoids.

Some physiological implications inferred from the particular structure of the sensory membrane are discussed. Special emphasis is put on the enormous mitochondrial concentration at the nerve endings. The hypothesis is advanced that these organoids might in some way be involved in the mechanism of transducing temperature changes into nerve impulses.

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