MORPHOLOGICAL BASES FOR A NURSING ROLE OF GLIA IN THE TOAD RETINA. ELECTRON MICROSCOPE OBSERVATIONS

ARNALDO LASANSKY. From the Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires, Argentina

Müller cells are glial elements that constitute the sponge-like framework in which retinal neurons are embedded (1, 12). They have their nuclei at the inner nuclear layer and the cell bodies extend across all the retinal layers in between the two limiting membranes. Müller cell processes are present everywhere in the retina, interposed between the other cells, and consequently little, if any, extracellular space is left in this tissue. As a result of this architecture, metabolites reaching retinal neurons probably pass through the cytoplasma of Müller cells, as seems also to be the case for glial cells in the central nervous system (5). It is therefore possible that Müller cells are not simply supporting elements and that they might have a nursing role, as postulated for other glial cells (6, 9, 10).

The present investigations were undertaken to determine whether there are any morphological indications, at the level of the electron microscope, of such a hypothetical nursing function of Müller cells.

Retinas of light-adapted toads (*Bufo arenarum* Hensel) were fixed for 2 hours at 4°C in a solution containing 1 per cent OsO_4, polyvinylpyrrolidone and balanced ions (15). Small pieces of retina were embedded, properly oriented for perpendicular sectioning, in a mixture of 10 per cent methyl methacrylate in *n*-butyl methacrylate. Observations were accomplished in an RCA EMU2E microscope.

When a section of toad retina is inspected, it is seen that the retinal neurons are apparently embedded in a clear matrix which occupies all available spaces in between cells. Bounding the cellular profiles and intervening between them and this low-density substance, there are two dense membranes separated by a clear space about 100 A wide (Fig. 2). At first sight it seems as if retinal cells have a double plasma membrane, but actually one of the membranes is the plasma membrane of the Müller cell, the clear matrix in between retinal neurons being the ground cytoplasm of Müller cell processes.

At the outer nuclear layer where nuclei, perikarya and fibers of the photoreceptor cells are located, Müller cell processes are very thick and show a high concentration of cytoplasmic organelles (Fig. 1). These consist of vacuoles of the endoplasmic reticulum (Fig. 2), an impressive array of long and very tortuous double membranes (Figs. 1 and 2), and ovoid or elongated bodies about 0.5 μ wide and 1 μ long which are bounded by two membranes and have an amorphous matrix of higher density than the surrounding cytoplasm (Fig. 1). These bodies sometimes have in their matrix a few crista-like structures (Fig. 1) which in some instances show continuity with the inner of the two membranes. It seems, therefore, reasonable to assume that they are mitochondria, a conclusion supported by the fact that no other structures resembling mitochondria are found in the Müller cells of the toad retina. These atypical mitochondria can be found also at the level of the inner nuclear and ganglion cell layers, but they are more numerous at the outer nuclear layer, in the thick Müller cell processes interspersed between the photoreceptor cells.[1]

In a survey of this area, it first came to our attention that there is occasionally a close relationship between the mitochondria and the plasma membrane of the Müller cell. When systematic observations were made, it was found that some mitochondria appear to adhere to the plasma membrane of the Müller cell and are thus in close proximity to the plasma membrane of the photoreceptor cell (Figs. 2 to 4).

[1] It should be pointed out that in other vertebrates Müller cells have been reported to have a small number of mitochondria, which are concentrated mainly near the outer and inner limiting membranes (16, 17).

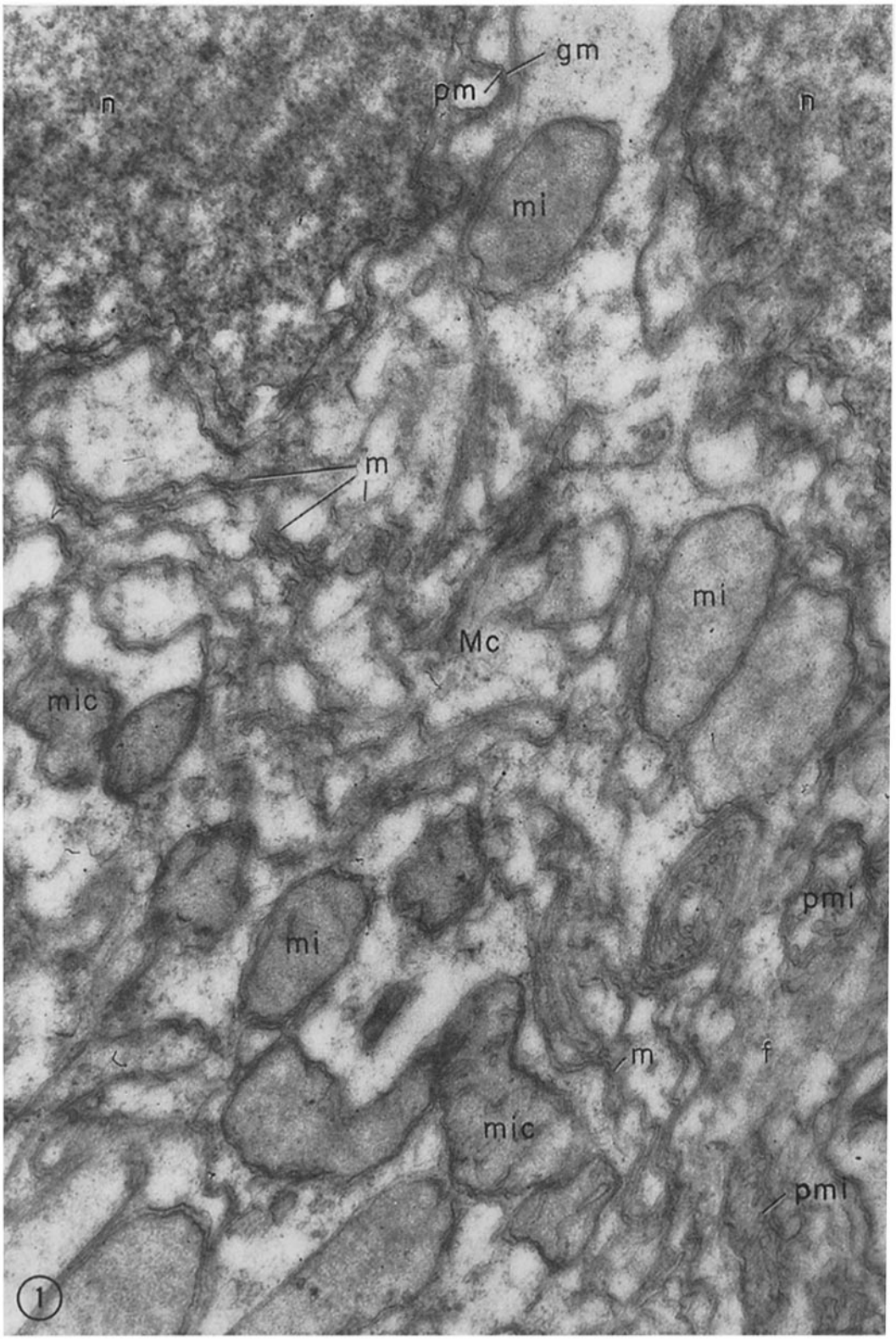

gm
pm
n
n
mi
m
mi
Mc
mic
pmi
mi
m
f
mic
pmi
1

Fig. 2 shows the fiber and synaptic ending of a photoreceptor cell enveloped as usual by the plasma membrane of a Müller cell and surrounded by the cytoplasmic matrix, of low density, of the Müller cell. On the right side, there are two mitochondria of the Müller cell which would seem to be virtually "plugged in" to the fiber of the photoreceptor cell. At the site of contact with the lower mitochondrion there are some radially oriented dense lines that project into the photoreceptor cell fiber. This finding suggests that a change in the organization of the cytoplasmic matrix of the photoreceptor cell has been induced by the proximity of the Müller cell mitochondrion.

Fig. 3 demonstrates another striking example of a "plugged in" Müller cell mitochondrion. This figure serves also to illustrate the fact that, in the toad retina, the photoreceptor cells have mitochondria within the fiber connecting with the synaptic ending, in addition to the mitochondria located at the ellipsoid body. This is an interesting feature, since the visual cells of other vertebrates have all mitochondria confined to the inner segment, at the ellipsoid body (2, 3, 14). In the rat, a mitochondrion can also be found at the synaptic ending of the rod cells (11).

In Figs. 2 and 4, the structural arrangement at the points where Müller cell mitochondria are contiguous with photoreceptor cells can be analyzed further. It can be seen that at the area of contiguity the plasma membrane of the Müller cell appears to be fused (marked with an arrow) with the outer mitochondrial membrane.

Müller cell mitochondria were found to be contiguous with the perikarya and fibers of the photoreceptor cells. No close relationship between Müller cell mitochondria and bipolar or ganglion cells has been detected so far, despite the fact that Müller cell mitochondria are also present in the neighborhood of these elements.

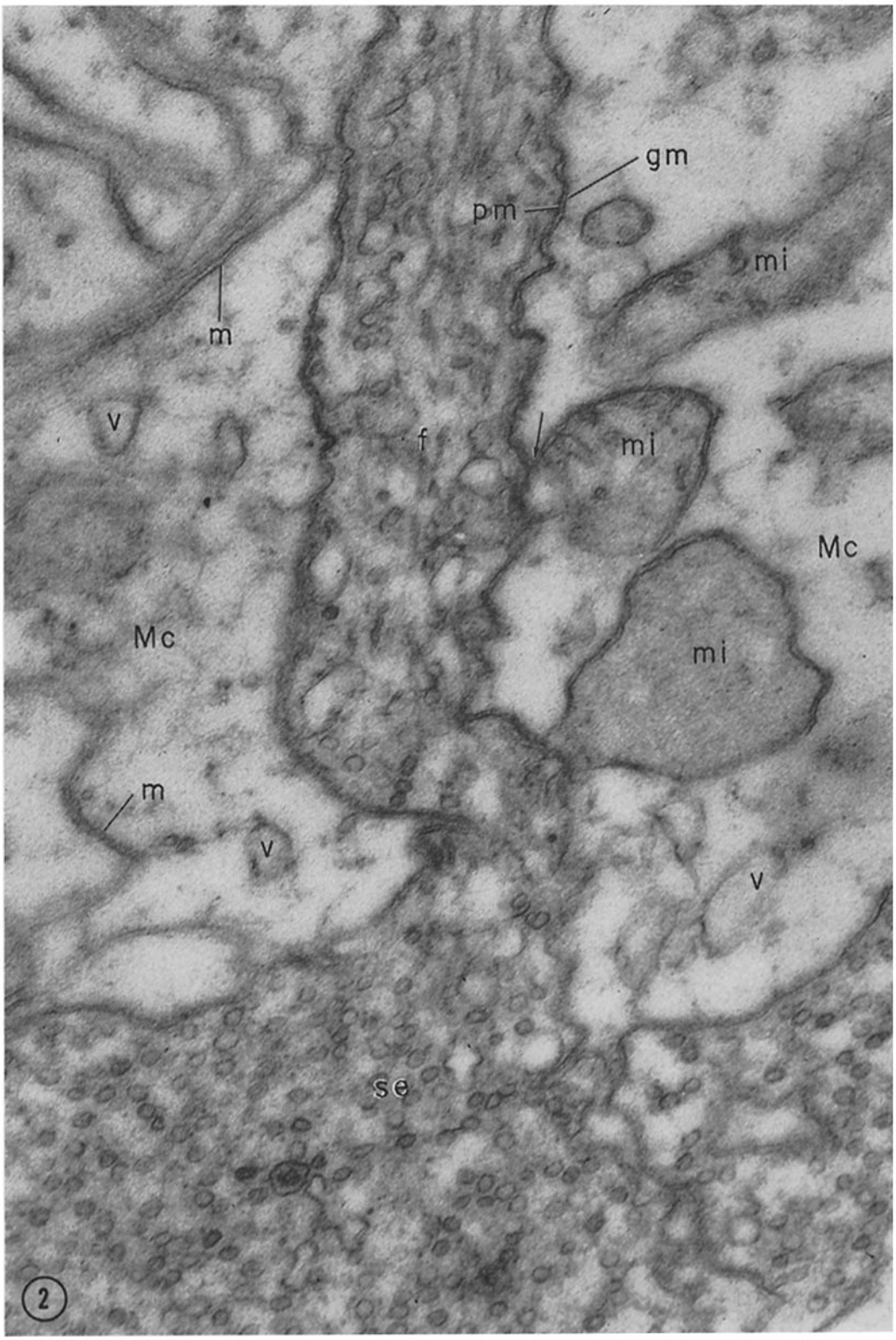
The presence in Müller cells of mitochondria attached to the plasma membrane and very intimately related to the photoreceptor cells suggests the existence of a direct functional interaction between Müller and photoreceptor cells, perhaps based upon a transference of metabolites at the sites where Müller cell mitochondria are contiguous with the plasma membrane of the photoreceptor cell.

Direct metabolic interactions between animal cells have been reported in several tissues (see reference 7), and a possible role of neuroglia cells in feeding nerve cells with nutritive materials has been already indicated (6, 9). Recently, Hydén and Pigon (10), working with isolated nerve and glia cells, have produced data which they interpret as evidence that nerve cells are linked with oligodendrocytes in an energy relationship. These authors have hypothesized that neuroglia cells transfer energy-rich compounds to the nerve cells, the electron transport system of the latter being given priority when functional demands are increased. The idea of such a nutritive function of neuroglia is also supported by morphological evidence indicating in some instances a very intimate relationship between glia and nerve cells (8, 13).

The possibility that the retinal photoreceptor cells need the metabolic support of neuroglia cells is not surprising. Visual cells are highly specialized elements engaged in a variety of activities related to the reception, conduction, and transmission of the light stimulus. It is, therefore, not unlikely that in order to perform these tasks they have to delegate important functions to satellite cells. This is probably the situation in the course of the rhodopsin cycle, in which vitamin A formed from retinene during light adaptation moves into the pigment epithelium, probably to undergo isomerization, the direction of the movement being reversed during dark adaptation (4). At present, however, little can be said about the nature of

FIGURE 1

Electron micrograph of a Müller cell process (*Mc*) at the outer nuclear layer of the toad retina. At the upper corners two nuclei of photoreceptor cells (*n*) surrounded by narrow perikarya are seen. The plasma membrane of the photoreceptor cells (*pm*) is separated from the plasma membrane of the Müller cell (*gm*) by a narrow clear space. Within the Müller cell process there are long and tortuous paired membranes (*m*) and conspicuous mitochondria (*mi*). Most of these mitochondria lack cristae but two of them that have several cristae can also be observed (*mic*). At the lower right there is a fiber of a photoreceptor cell (*f*) which contains mitochondria (*pmi*). × 50,000.


gm
pm
mi
m
v
f
mi
Mc
Mc
mi
m
v
v
se
2

the relationship between Müller and photoreceptor cells. In order to solve this problem, it is necessary to have data on the metabolic properties of Müller cells and to identify the functional features of the peculiar mitochondria found in the Müller cell of the toad retina.

The author wishes to thank Prof. E. De Robertis for reading the manuscript.

This work was supported by research grants from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, and from the United States Air Forces Office of Scientific Research (5-60).

Received for publication, March 6, 1961.

REFERENCES

1. CAJAL, S. R., Textura del sistema nervioso del hombre y de los vertebrados, Madrid, 1904, **2,** 585.
2. DE ROBERTIS, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 319.
3. DE ROBERTIS, E. and LASANSKY, A., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 743.
4. DOWLING, J. E., *Nature*, 1960, **188,** 114.
5. GERSCHENFELD, H. M., WALD, F., ZADUNAISKY, J. A. and DE ROBERTIS, E., *Neurology*, 1959, **9,** 412.
6. GLEES, P., *in* Biology of Neuroglia, (W. F. Windle, editor), Springfield, Illinois, C. C Thomas, 1958, 235.
7. HERRMANN, H., *Science*, 1960, **132,** 529.
8. HESS, A., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 731.
9. HYDÉN, H., *Proc. 4th Internat. Congr. Biochem.*, 1959, **3,** 64.
10. HYDÉN, H., and PIGON, A., *J. Neurochem.*, 1960, **6,** 57.
11. LADMAN, A. J., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 459.
12. POLYAK, S. L., The Retina, University of Chicago Press, 1941, 343.
13. SCHEIBEL, M. E., and SCHEIBEL, A. B., *in* Biology of Neuroglia, (W. F. Windle, editor, Springfield, Illinois, C. C. Thomas, 1958, 5.
14. SJÖSTRAND, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42,** 45.
15. TRUJILLO-CENÓZ, O., *Z. Zellforsch.*, 1957, **46,** 272.
16. VILLEGAS, G. M., *J. Gen. Physiol.*, 1960, **43,** 15.
17. WALD, F., and DE ROBERTIS, E., to be published.

FIGURE 2

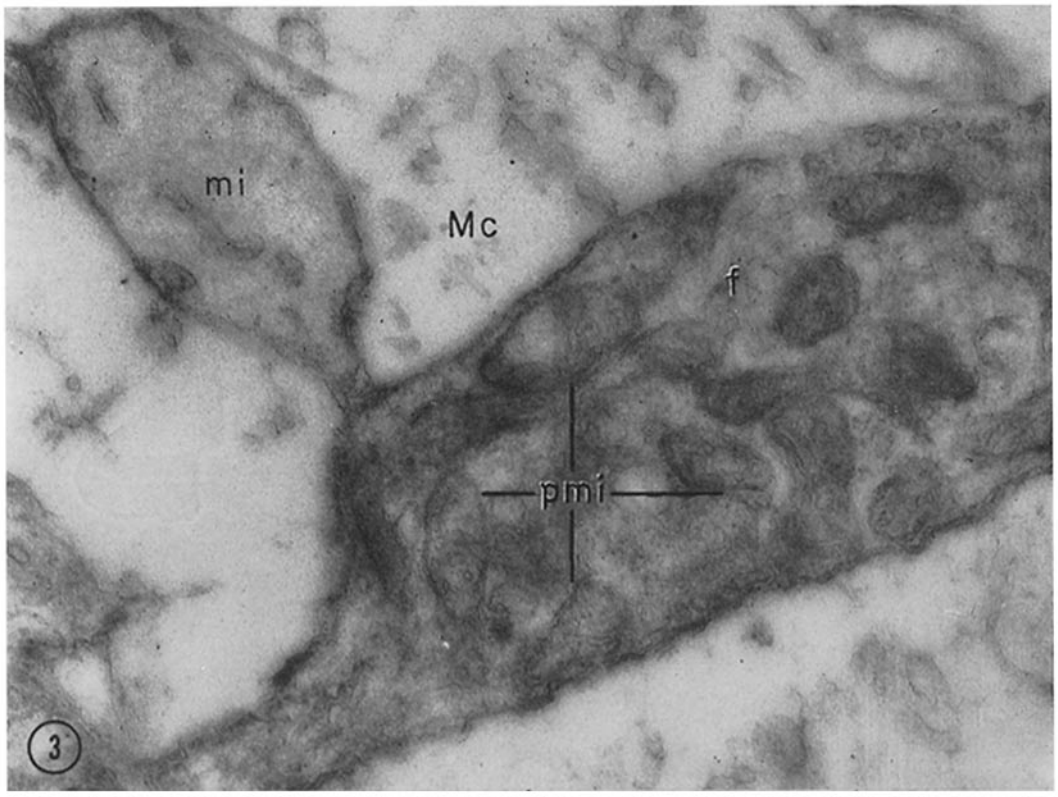
Electron micrograph of toad retina showing the terminal segment of the fiber (*f*) and part of the synaptic ending (*se*) of a photoreceptor cell. The clear matrix on the sides is the cytoplasm of a Müller cell (*Mc*) which contains several paired membranes (*m*) and endoplasmic reticulum vacuoles (*v*). The plasma membranes of the photoreceptor cell (*pm*) and Müller cell (*gm*) are separated by a clear space about 100 A wide. On the right side there are two Müller cell mitochondria (*mi*) that seem to be "plugged in" to the photoreceptor cell fiber. At the area of contact with the lower mitochondrion some radially oriented dense lines can be seen within the fiber. The plasma membrane of the Müller cell appears to be fused with the outer membrane of the upper mitochondrion (arrow). × 50,000.

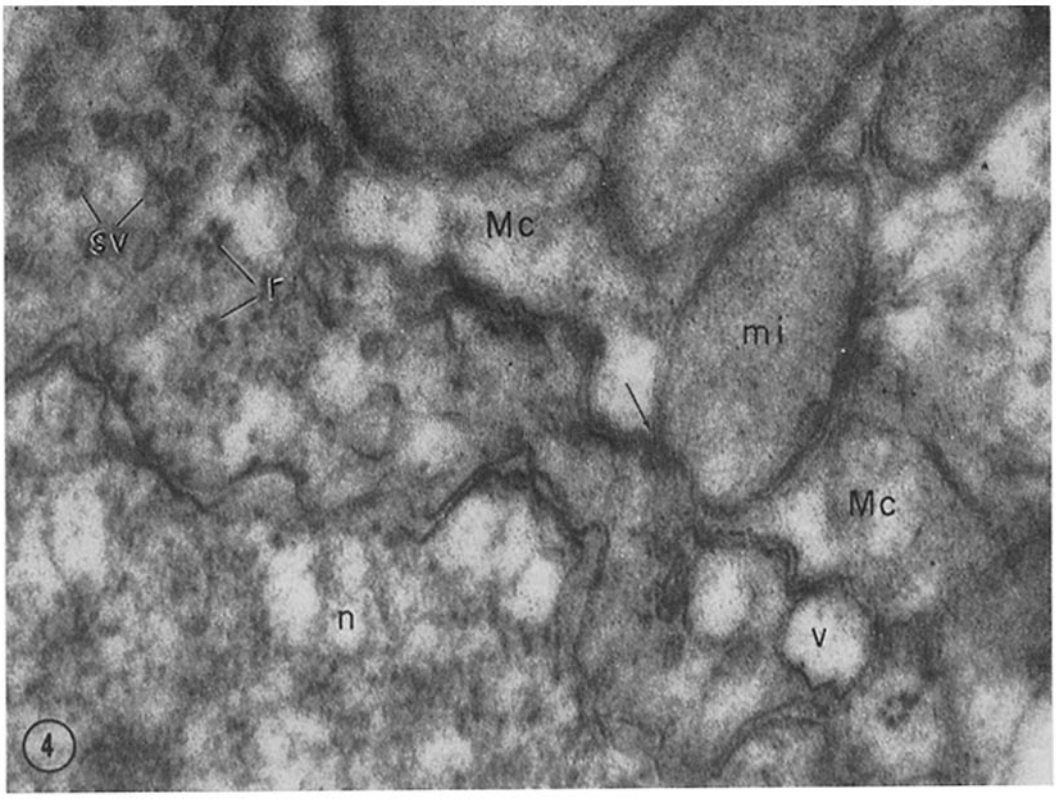
FIGURE 3

Electron micrograph showing a Müller cell mitochondrion (*mi*) apparently "plugged in" to the fiber of a photoreceptor cell (*f*). The fiber is surrounded as usual by the cytoplasm of the Müller cell (*Mc*). Within the fiber numerous photoreceptor cell mitochondria (*pmi*) are observed. × 50,000.

FIGURE 4

Part of the nucleus (*n*) and perikaryon of a photoreceptor cell surrounded by the Müller cell cytoplasm (*Mc*) of low density. Within the cytoplasm of the photoreceptor cell are seen endoplasmic reticulum vacuoles (*v*), ribosomes (*r*), and synaptic vesicles (*sv*). Near the photoreceptor cell there are several Müller cell mitochondria and one of them (*mi*) is attached to the plasma membrane. At the point marked with an arrow the outer mitochondrial membrane seems to be fused with the plasma membrane of Müller cell. × 92,000.


mi
Mc
f
pmi
3


sv
r
Mc
mi
Mc
n
v
4