THE EXTRACELLULAR SPACE IN THE
TOAD RETINA AS DEFINED BY
THE DISTRIBUTION OF FERROCYANIDE

A Light and Electron Microscope Study

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ABSTRACT

Measurements of the uptake of compounds that ordinarily do not penetrate into cells have been a source of data on the size of the extracellular space in nervous tissue. The distribution of one such compound, ferrocyanide, has been studied in the toad retina by means of the light and electron microscopes. At the level of the light microscope, ferrocyanide, detected as Prussian blue, appears to penetrate predominantly within the inner processes of Müller cells. A diffuse background staining by Prussian blue can be noticed also at the inner retinal layers. At the level of the electron microscope, Müller cells exhibit an extensively developed system of channels which are formed by infoldings of the plasma membrane. Ferrocyanide, detected as copper ferrocyanide deposits, is found occupying the lumina of these channels and in the narrow intercellular gaps of the retina. These observations indicate that in the toad retina the extracellular medium includes the intercellular spaces plus a glial compartment formed by the infoldings of the plasma membrane of the Müller cells.

Electron microscope studies of the central nervous system have shown that in nervous tissue the cellular elements are so tightly packed that the intercellular space is reduced to gaps of about 120 to 250 A between the plasma membrane of adjacent cells (7, 8, 13, 18, 22, 25, 32, 34, 42, 43). Horstmann and Meves (22) consider that these narrow interspaces account for an extracellular space in central nervous system of about 5 per cent of the total volume. However, in the view of other authors, the intercellular gaps do not represent real spaces since they would be occupied by a cementing material or by structural components of the cell membranes (see 35). Accordingly, the only diffusion route available to water, ions, metabolites, and so forth, should be traced through the cytoplasm of glial elements (27) that therefore

would behave as the functional equivalent of the extracellular space of other tissues (10, 18, 34, 35).

On the other hand, physiological data on the compartmentation of the brain and retina have consistently indicated the existence of an extracellular space in nervous tissue (see 10). Measurements of the uptake of compounds that ordinarily do not penetrate into cells have been an important source of data on the size of this extracellular space (1, 2, 5, 29, 41), and values higher than those obtained for skeletal muscle have been reported by some authors (1, 2, 5). These values could have a greater significance if it were demonstrated that compounds such as ferrocyanide, inulin, iodide, etc., remain extracellular in nervous tissue as in other tissues. In the case of ferrocyanide such demonstration can be attempted by histo-

logical means using the Prussian blue reaction. With this technique Weed (40) reported the interstitial distribution of ferrocyanide injected into the subarachnoid space of living animals. Allen (1) confirmed this observation in brain slices after incubation periods no longer than 30 minutes, and hence assumed that the ferrocyanide space correctly defined the extracellular space.

In the present work the distribution of ferrocyanide has been studied in the retina which, because of its simpler architecture, was considered a better object for this purpose than the brain. At the electron microscope level the retina has as little extracellular space as the brain, the spaces between neuronal elements being filled by the processes of Müller cells (9, 24, 24, 39), analogues of cerebral glia cells. In addition the retina provides a thin layer of tissue which can be removed easily without injury. Its functional state, moreover, can be assessed *in vitro* by measuring the electrical response to light stimulation.

Ferrocyanide was detected at the level of the light and electron microscopes as Prussian blue and copper ferrocyanide deposits, respectively. Prussian blue stained predominantly the Müller cells, but a diffuse background staining was also observed as a consequence of ferrocyanide penetration into the intercellular spaces. Copper ferrocyanide deposits were found in the 100 Å intercellular gaps and within a complex system of channels formed by infoldings of the plasma membrane of the Müller cells. These findings define the retinal extracellular space in the toad as composed of an intercellular and a glial compartment.

TECHNIQUES

Retinas of the toad *Bufo arenarum* Hensel were used in this study. The retinas were carefully detached from the pigment epithelium and placed for 1 to 60 minutes at room temperature (about 20°C) in an oxygenated ferrocyanide medium similar in composition to that used by Allen (1). Isotonic solution of sodium ferrocyanide was added to the saline medium of Boyle and Conway (3), one part of the ferrocyanide solution to three or nine parts of the saline medium. An alternative technique which was usually preferred for electron microscope studies consisted of cutting the excised eye at the equator and placing the ferrocyanide medium into the ocular cavity after removing most of the vitreous humor. In some experiments the ferrocyanide medium contained 5 mM per liter of cyanide or iodoacetate.

The electroretinogram (a and b waves) in response to a brief flash of light (Grass photostimulator) was recorded before and after the immersion of the retina into the ferrocyanide medium using a technique similar to that of Furukawa and Hanawa (17). When the ferrocyanide medium was flushed into the ocular cavity, the electroretinogram was continuously monitored by simply placing one electrode in contact with the posterior pole of the eye and another (platinum wire) into the medium.

For light microscope observations the retinae were removed from the ferrocyanide medium or detached from the posterior half of the eye, briefly rinsed in saline solution and fixed in 10 per cent formalin with 1 per cent ferric ammonium sulphate and hydrochloric acid (1). In some cases the retinae were washed in saline solution for several minutes prior to fixation. Paraffin sections were studied with or without counterstaining by neutral red.

For electron microscope observations the procedure had to be modified because ferric ferrocyanide deposits require, in order to remain insoluble, a very low pH in the fixative and this is not compatible with the preservation of tissue structure. Therefore the fixative was prepared by mixing 1 cc of a 5 per cent OsO_4 solution with 4 cc of a 4 per cent solution of copper sulphate (pH 3.5) or acetate (pH 5.5). The buffer was omitted from the ferrocyanide medium in order to prevent obtaining precipitates of copper phosphate or carbonate together with those of copper ferrocyanide. Adequate amounts of chloride were added to the saline solution in order to replace phosphate and bicarbonate. After removal from the medium the retinae were fixed for 1 hour at 4°C in the OsO_4–copper sulphate solution. For control purposes retinae were also fixed in this solution either without any previous treatment or after immersion in a ferrocyanide-free unbuffered saline solution. In order to study the fine structure of Müller cells, toad retinae were detached from the pigment epithelium and fixed for 1 hour at 4°C in a solution containing 1 per cent OsO_4, polyvinylpyrrolidone and balanced ions (37). After fixation in either of the solutions small pieces of retina were embedded, properly oriented to obtain perpendicular sections, in a mixture of 10 per cent methyl methacrylate in butyl methacrylate. The observations were accomplished in a Siemens Elmiskop I and the pictures taken at original magnifications of 10,000 to 20,000.

OBSERVATIONS

Light Microscopy

Figs. 1 and 2 show that ferrocyanide penetrates into the retina in a characteristic fashion, the Prussian blue deposits filling the inner processes of the Müller cells. In Fig. 1 the staining is confined
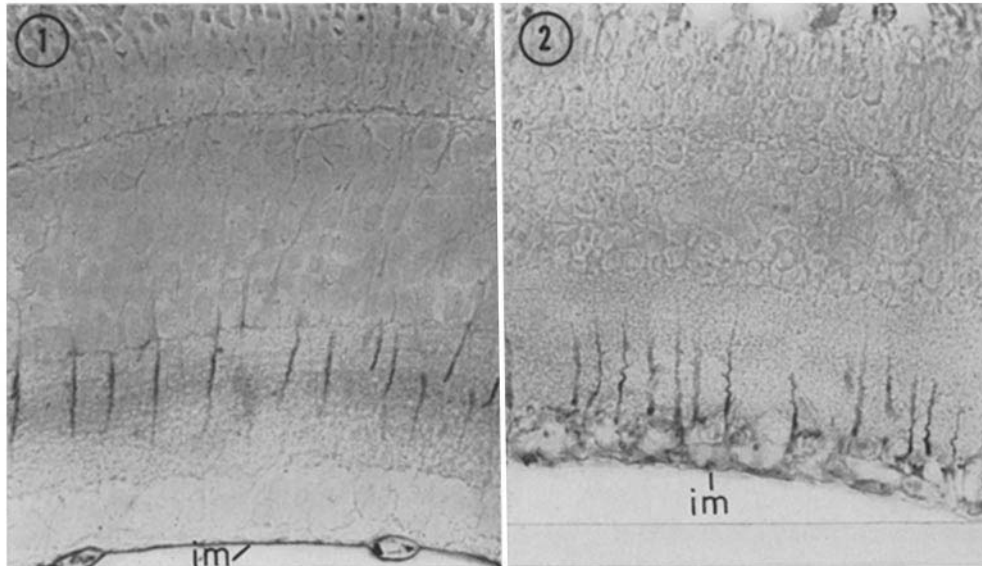
FIGURE 1

Toad retina maintained for 5 minutes in the stronger ferrocyanide medium (see text). The retina was detached from the pigment epithelium prior to immersion. At the end of the experiment the electroretinogram was almost abolished. The vitreal processes of Müller cells are stained by Prussian blue. A broad band of diffuse background staining is also observed at the inner retinal layers. *im*, inner limiting membrane. × 480.

FIGURE 2

Toad retina maintained for 20 minutes in the weaker ferrocyanide medium (see text). The retina was detached from the pigment epithelium prior to immersion. At the end of the experiment the amplitude of the electroretinogram was reduced by about 40 per cent. The inner ends of Müller cells are stained. *im*, inner limiting membrane. × 420.

to the segments of Müller cells located within the inner synaptic layer and inner part of the bipolar cell layer, while in Fig. 2 the inner ends of Müller cells also are stained. The extent to which Müller cells are stained usually varies throughout the retina, but only rarely are Prussian blue deposits seen occupying the full length of the cells. Usually Müller cells are not stained at the retinal layers external to the bipolar cells. Although Prussian blue staining of the Müller cells is the more conspicuous finding, a diffuse and less intense background staining can be noticed also at the level of the inner retinal layers (Fig. 1).

Identical patterns were obtained either by immersing the isolated retina into the ferrocyanide medium or by placing the medium into the ocular cavity. This circumstance, together with the observation that Prussian blue deposits are found predominantly at the inner layers of the retina, indicates that ferrocyanide penetrates only at the vitreal surface.

Lengthening the time during which the retina was maintained in the ferrocyanide medium did not affect the distribution of the stain. After about 5 minutes' immersion, patterns with all the characteristics described were obtained. This is in agreement with studies reporting in the retina an equilibration time of 3 minutes for inulin (2). After prolonged maintenance of the retina in the ferrocyanide medium (45 to 60 minutes), Prussian blue precipitates were present in some ganglion cells.

Immersion of the retina in the weaker ferrocyanide medium (1 part of isotonic sodium ferrocyanide solution to 9 parts of saline solution) during periods of up to 20 minutes did not abolish the electroretinogram, but in the stronger medium (1 part of isotonic ferrocyanide to 3 parts of
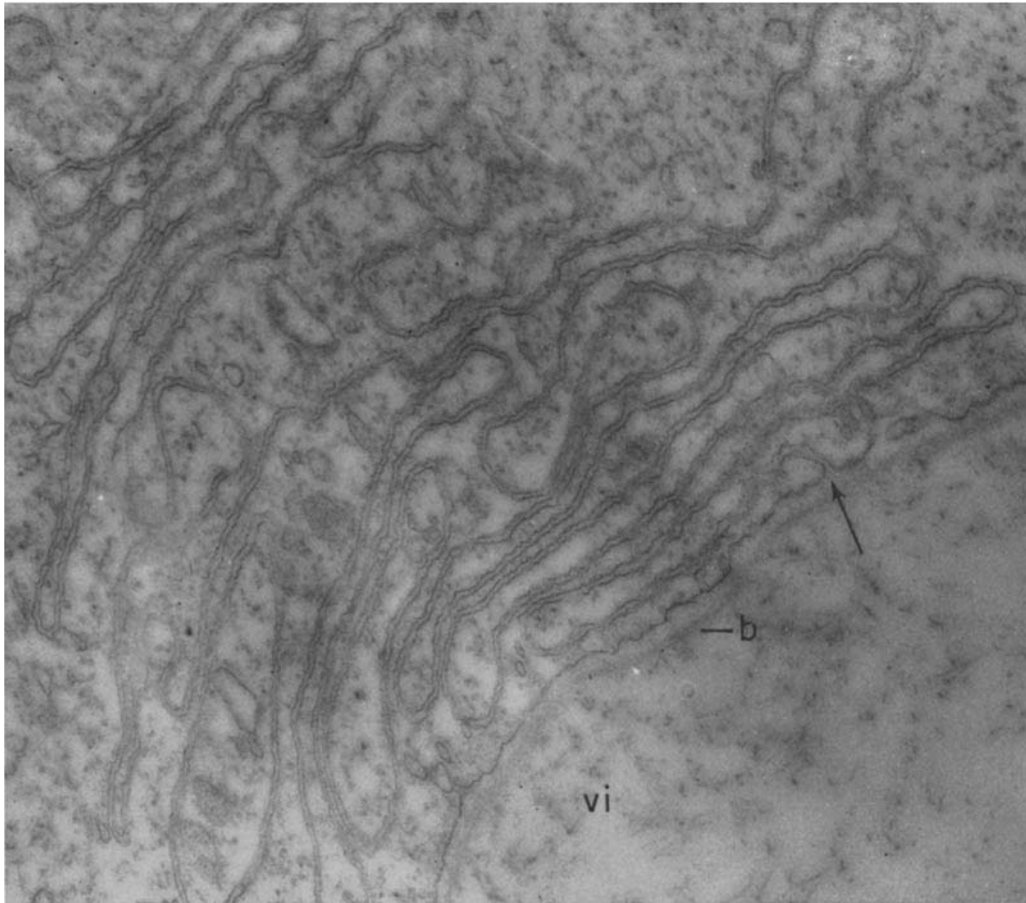
FIGURE 3

Electron micrograph of the vitreal end of a Müller cell of the toad retina. An infolding of the plasma membrane (arrow) is seen forming an extensive system of channels. Within the cytoplasm of the Müller cell numerous small granules (see text) are observed. *b*, basement membrane separating the plasma membrane of the Müller cell from the vitreous humor (*vi*). × 45,000.

saline) the electroretinogram disappeared in a few minutes. When the ferrocyanide medium (with buffered saline) was placed into the ocular cavity, the electroretinogram remained almost unchanged during the 60 minutes that the longest experiments lasted. In any event, no differences in staining pattern were observed in these different conditions, the distribution of Prussian blue remaining unchanged whatever the amplitude of the electroretinographic response.

Cyanide or iodoacetate, when added to the ferrocyanide medium, did not prevent or increase the penetration of ferrocyanide into the retina. Consequently, the penetration of ferrocyanide into

Müller cells appears not to be an energy-requiring process, but a passive diffusion. This conclusion received additional support from the electron microscope findings.

When the retinae were rinsed in saline medium for 10 minutes prior to fixation, practically no Prussian blue precipitate was observed. This observation indicates that ferrocyanide is not bound to any tissue components within the retina.

Electron Microscope Observations

a) THE FINE STRUCTURE OF MÜLLER CELLS: Electron microscope data on some

aspects of the fine structure of Müller cells in mammalian, reptilian, and amphibian retinae can be found in previous reports by Sjöstrand (34, 35), Wald and De Robertis (39), Lasansky (24), Cohen (4) and Kidd (23). The following description will be concerned in the main with the structural details which are more relevant in relation to the findings on ferrocyanide distribution.

Müller cells are elongated elements extended across the retinal layers in between the inner and outer limiting membranes. Their organization varies considerably according to the level in the retina, and even within the same retinal layer Müller cell processes may not exhibit a similar appearance. Differences in the texture and density of the ground cytoplasm are to a great extent responsible for this changing aspect of the Müller cell. Thus, at the outer nuclear layer the cytoplasmic matrix of Müller cells usually presents a low electron opacity and within it many paired

membranes are seen (Fig. 7). At the inner ends of Müller cells a similar situation occurs (Fig. 3). On the contrary in other processes the cytoplasmic matrix is so dense that it may obscure other cytoplasmic constituents. This aspect is frequently observed in sections of Müller cell processes at the inner synaptic and bipolar cell layers (Fig. 6). Numerous granules 100 to 200 A in diameter are usually found scattered in the cytoplasmic matrix of the Müller cell (Figs. 3 and 5). The nature of these particles is unknown, but they might represent glycogen granules (30). The presence of glycogen within the Müller cells has been reported by Shimizu and Maeda (33).

Among the other cytoplasmic elements the mitochondria are very characteristic (24). In general they have almost no cristae (Figs. 4, 5, 7, and 8) although exceptions to this rule may be observed also (Figs. 7 and 8). At the outer nuclear layer these mitochondria are sometimes attached to the plasma membrane (Fig. 8) and this finding
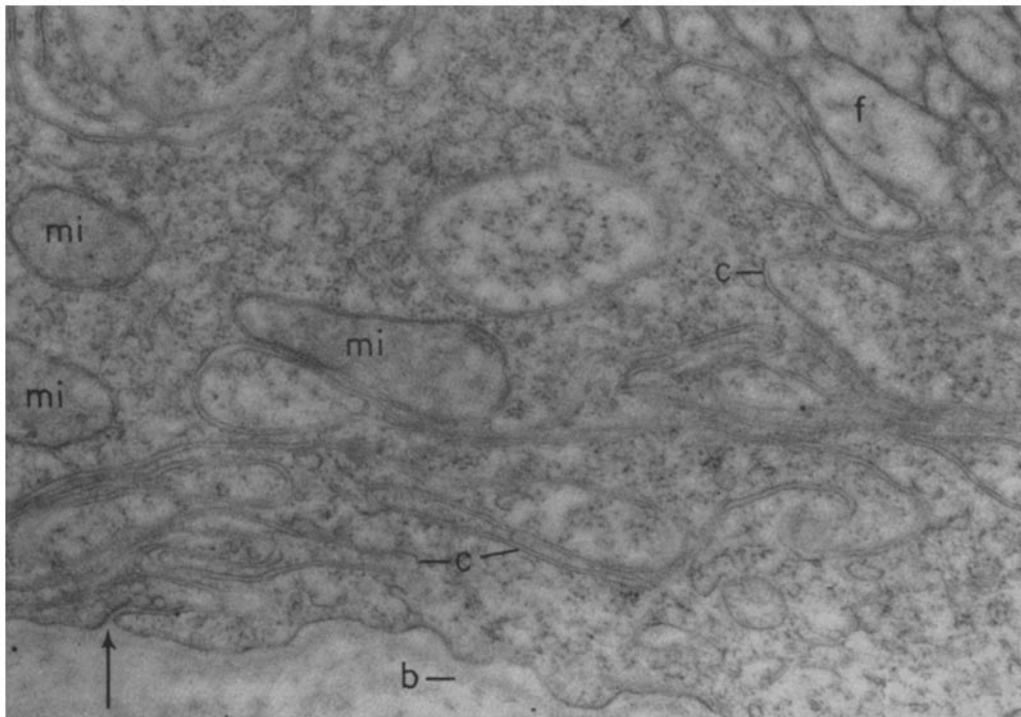

FIGURE 4

Same as Fig. 3. The arrow points to an infolding of the plasma membrane of the Müller cell. Three typical Müller cell mitochondria are observed (mi). c, channels formed by infoldings of the plasma membrane of the Müller cell. f, optic nerve fibers. b, basement membrane. × 45,000.
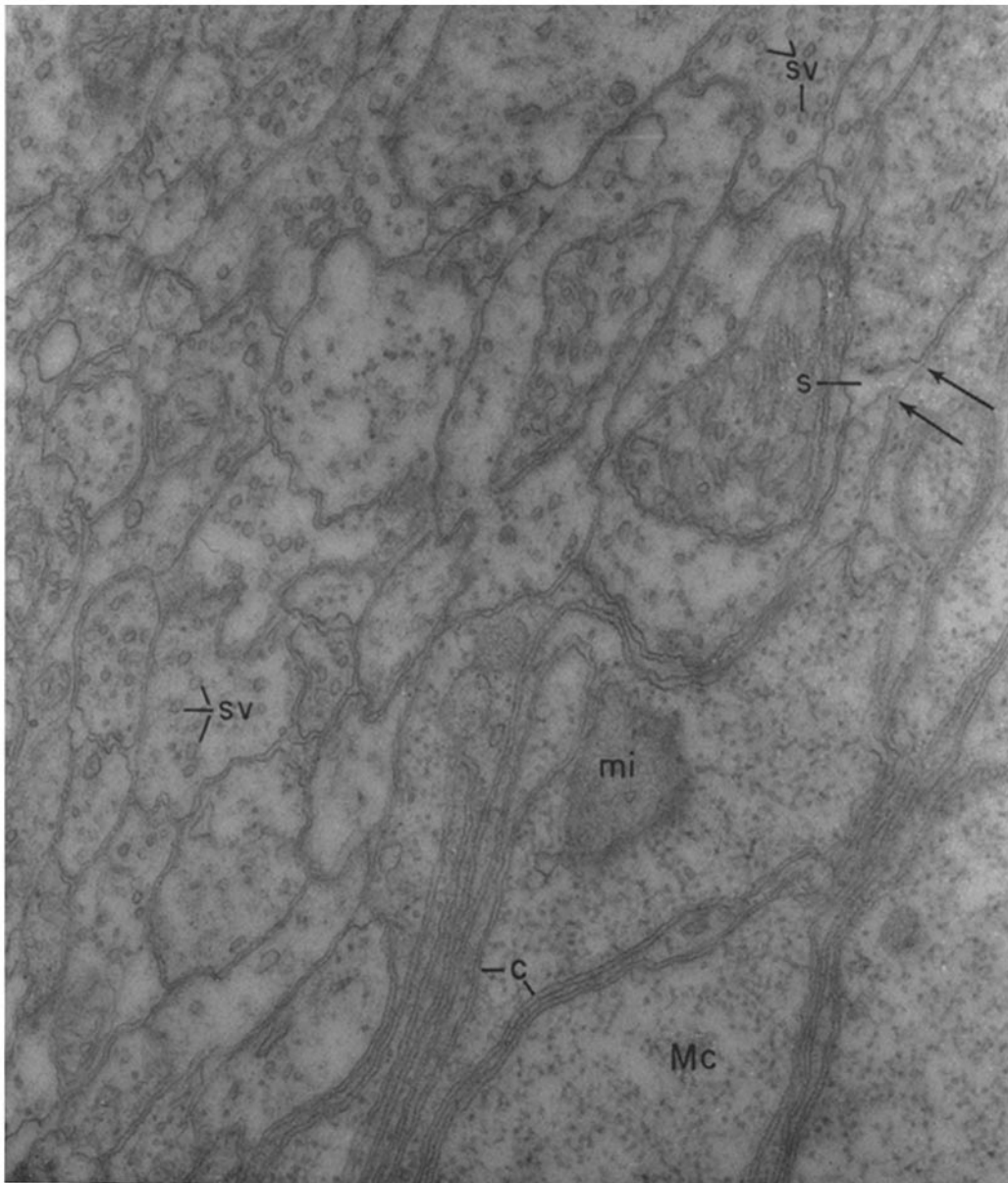
FIGURE 5

Electron micrograph of a toad retina at the inner plexiform layer. Numerous synaptic endings containing synaptic vesicles (sv) are observed. On the right there is a Müller cell process (Mc) exhibiting several closely packed channels (c). Two channels are open (arrows) into an expanded part of the intercellular space (s). Many small granules are seen within the cytoplasm of the Müller cell. mi, Müller cell mitochondrion. × 60,000.

has been interpreted as suggesting a metabolic relationship between Müller cells and visual cells (24). Elements of the endoplasmic reticulum are also seen in the cytoplasm of Müller cells; they consist of small vesicles 400 to 1200 A in diameter (Fig. 7).

At the inner synaptic layer large vacuoles of unknown nature are often encountered in the Müller cells (Fig. 6). In some places these vacuoles are very closely packed, the membranes of adjacent vacuoles being separated by spaces of about 100 to 150 A (Fig. 12). These narrow spaces appear to be continuous with the lumen of the plasma membrane infoldings since copper ferrocyanide deposits were found also within them (see below).

The most important feature in relation to the study on ferrocyanide penetration is a conspicuous system of paired membranes observed within the processes of Müller cells, virtually extending throughout the entire cytoplasm (Figs. 3 to 5, 7, and 8). Each of these paired membranes has a thickness of about 40 A and the intervening space measures about 100 to 200 A. At the inner limiting membrane it is easily observed that the paired membranes actually are very tortuous infoldings of the plasma membrane of the Müller cell (Figs. 3 and 4). This arrangement is very similar to that found in the basal part of epithelial cells in kidney tubules, ciliary body, choroid plexuses, and submaxillary gland (28). At the inner surface of the retina the lumen of the infoldings is separated from the vitreous humor by a basement membrane (Figs. 3 and 4). Within the retina, the lumen of the infoldings is continuous with the intercellular spaces (Fig. 5). As found in other retinae (38), the intercellular spaces in the toad retina are about 100 A wide. At the inner synaptic layer the width of these spaces is rather irregular, and expanded areas are commonly observed (Fig. 5).

At the outer limit of the neural retina, structures analogous to terminal bars (34, 39) appear to attach the plasma membranes of Müller cells and visual cells (Fig. 8). The presence of these terminal bars may explain why ferrocyanide does not penetrate at this side of the retina (see light microscope observations).

b) THE DISTRIBUTION OF COPPER FER-ROCYANIDE: Preservation of tissue structure in the OsO_4-copper sulphate fixative is quite unsatisfactory, probably as a result of the low pH of the solution. The cytoplasmic matrix is extracted in many places, mitochondria are swollen, and cell
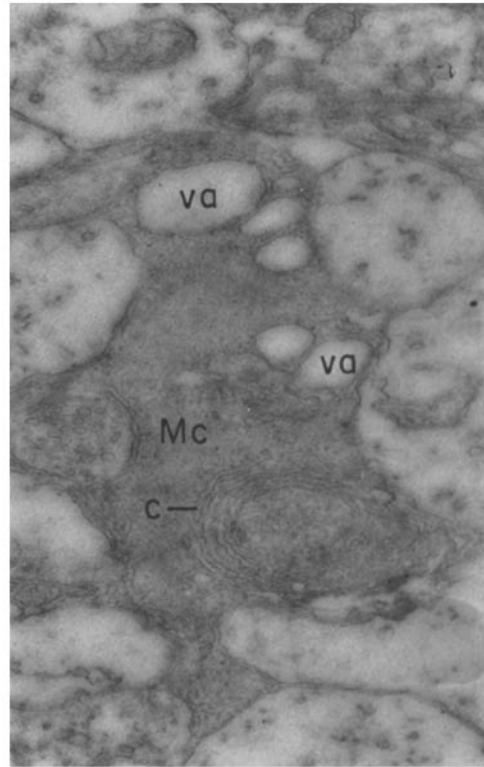

FIGURE 6

Electron micrograph of a toad retina at the inner synaptic layer. Mc, Müller cell process containing a very dense cytoplasmic matrix and several large vacuoles (va). c, Müller cell channels. × 39,000.

membranes have a low electron opacity. Copper acetate gave somewhat better results than sulphate. However, with copper sulphate a better definition of cell membranes was achieved, and a compromise between these inconveniences had to be made in different stages of this study. Besides, copper acetate produced sometimes a copper precipitate, which is easily distinguishable from copper ferrocyanide deposits and is also found in control preparations (retinae fixed without any previous treatment or after immersion in a ferrocyanide-free unbuffered saline solution). This drawback was not experienced when using copper sulphate.

Copper ferrocyanide deposits appeared both as dense dots and as an amorphous dense material filling the diffusion spaces. The dotted appearance was the only one seen when fixation was accomplished at pH 5.5 with copper acetate (Figs. 9 and 12). The distribution of copper ferrocyanide deposits is observed in Figs. 9 to 15. At the layers
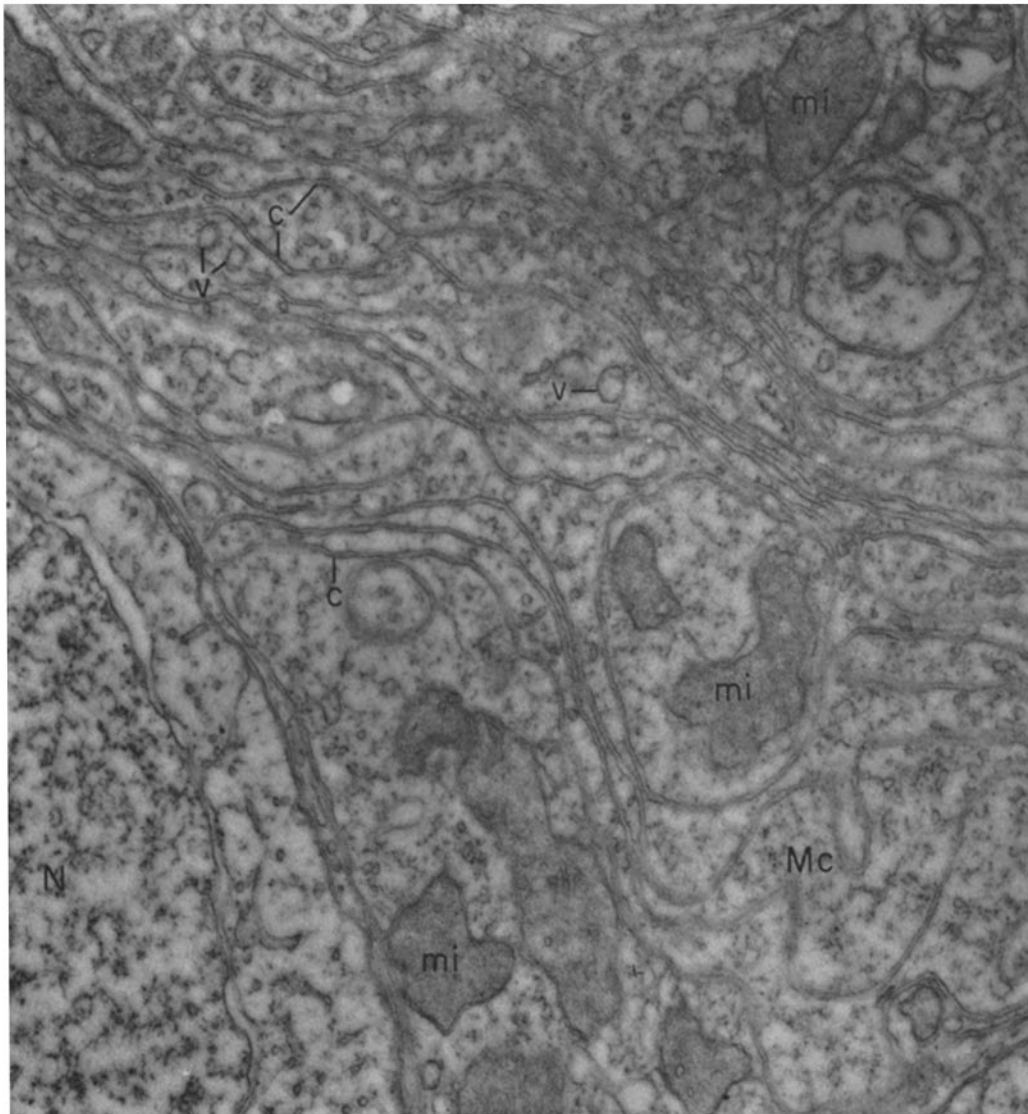
FIGURE 7

Electron micrograph of a toad retina at the outer nuclear layer showing a Müller cell process (*Mc*) containing many of the long and tortuous paired membranes (*c*) that constitute the system of channels. *mi*, Müller cell mitochondrion; *v*, endoplasmic reticulum vesicles; *N*, nucleus of a visual cell. × 30,000.

of ganglion cells and nerve fibers, copper ferrocyanide occupies the spaces within the infoldings of the plasma membrane of the Müller cell (Figs. 9 to 11). At the inner synaptic and bipolar cell layers, the deposits are found also within these infoldings (Figs. 12 and 13), in the spaces between the vacuoles of the Müller cell (Fig. 12) and within the intercellular spaces (Figs. 12 to 15). In agree-

ment with the light microscope observations no copper ferrocyanide deposits were found beyond the bipolar cell layer.

DISCUSSION

The distribution of Prussian blue deposits as seen under the light microscope, with a predominant localization in the Müller cells and a less
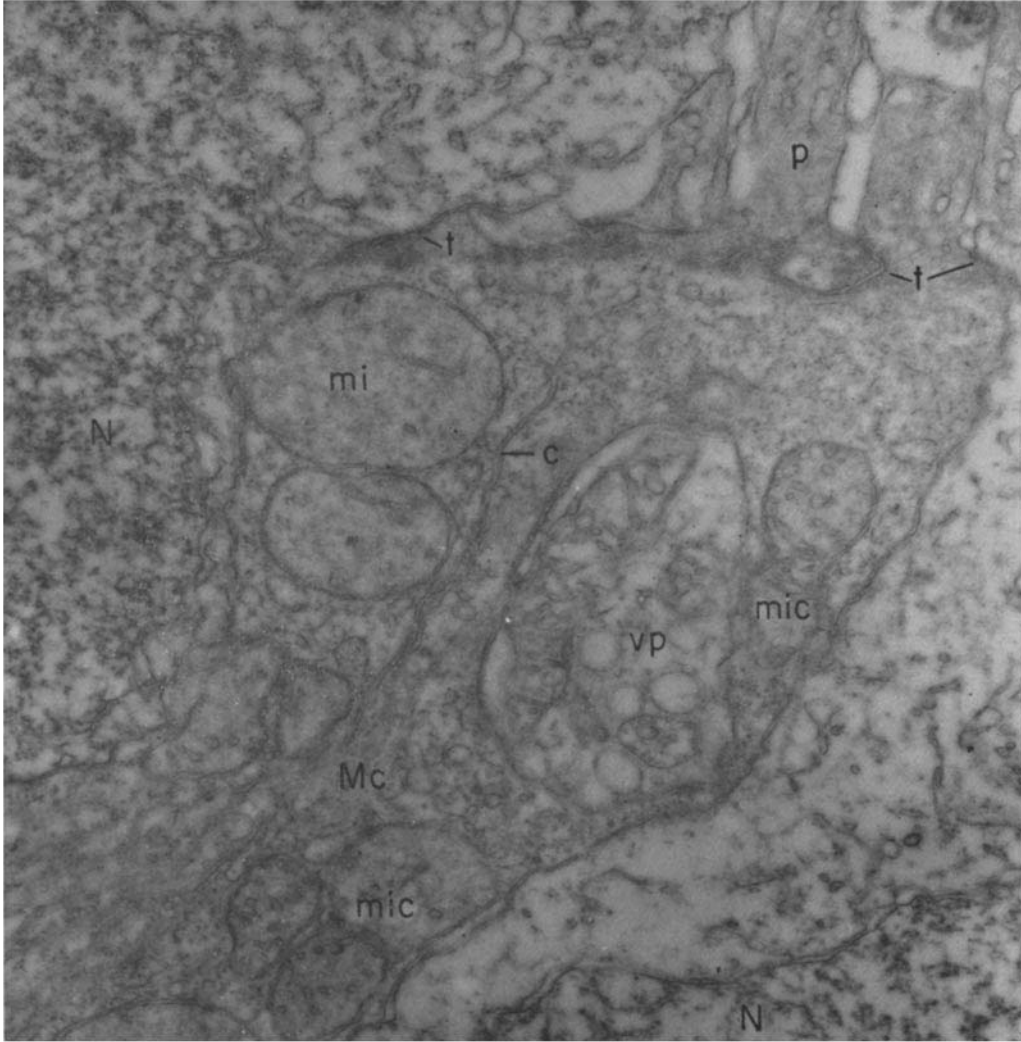
intense diffuse staining, indicates the two retinal compartments into which ferrocyanide penetrates.

The diffuse background staining is shown by electron microscopy to be due to ferrocyanide penetration into the narrow intercellular spaces, since copper ferrocyanide deposits are found within these 100 A gaps. As stated introductorily, intercellular gaps of a similar magnitude in brain had been assumed not to constitute true extracellular spaces on the basis of experimental evidence indicating that their width is not increased in case of cerebral edema (18, 26, 36). A similar situation was also postulated for the retina (39). The present findings, however, indicate that gaps of about 100 A between the plasma membranes of adjacent cells are sufficient to permit free diffusion of solutes (see 31) and therefore constitute true spaces, which is in agreement with the interpreta-
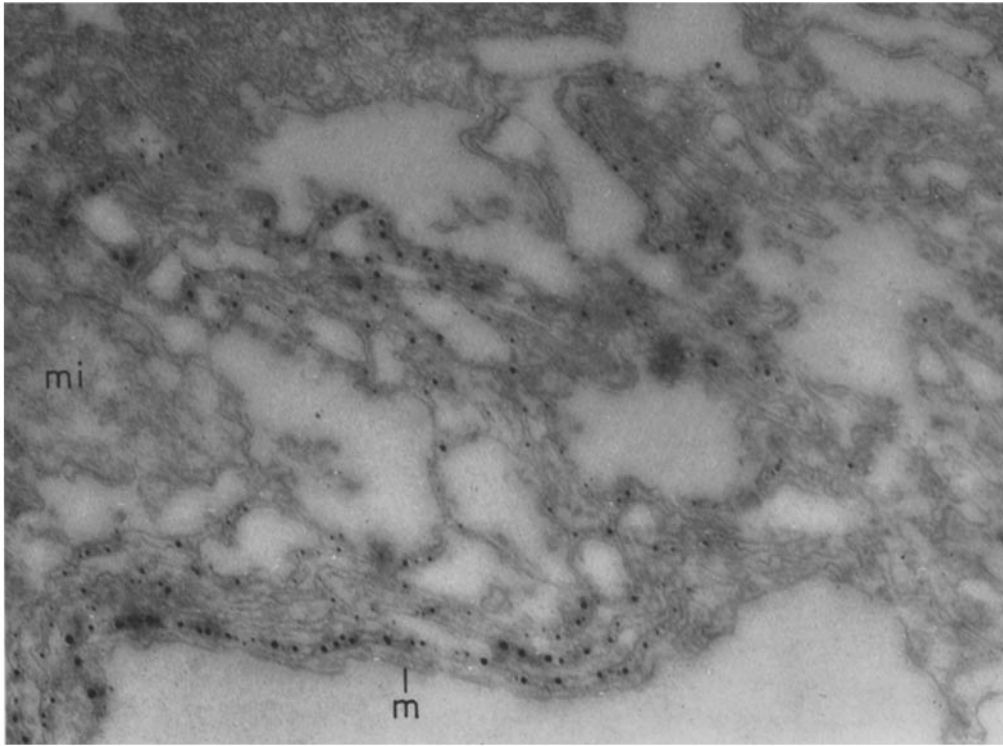
FIGURE 9

Electron micrograph of a toad retina showing copper ferrocyanide deposits (dense dots) within the lumen of the Müller cell channels near the inner surface of the retina. The stronger ferrocyanide medium (with unbuffered saline solution) was placed within the cavity of the excised eye. The experiment lasted 30 minutes, after which the amplitude of the electroretinogram was diminished by about 70 per cent. Fixation was accomplished in a solution containing osmium tetroxide and copper acetate. *mi*, Müller cell mitochondrion; *m*, plasma membrane of the Müller cell at the vitreal surface. × 60,000.

tion advanced by Horstmann and Meves (22). This conclusion facilitates the interpretation of electrical processes in the retina, and probably also in the brain, on the basis of the ionic theory on the origin of the nerve impulse (20, 21) and synaptic potentials (11, 12, 14, 15), since it becomes unnecessary to postulate that the plasma membrane of glial cells is a component of the bioelectrically active membrane (34). Nevertheless, the

possibility still remains that electric currents generated by nerve cells may partially flow through the membrane of glial cells, as recently postulated by Hild and Tasaki (19).

The other localization of Prussian blue deposits, the Müller cells, constituted a more unexpected finding. This light microscope evidence suggested in the early stages of this study that the plasma membrane of Müller cells is endowed with unique

FIGURE 10

Same as Fig. 9. but fixed in an osmium tetroxide–copper sulphate solution. The experiment lasted 40 minutes, after which the electroretinogram was reduced by about 90 per cent. Copper ferrocyanide deposits are also seen within remnants of vitreous humor (*vi*) attached to the retina. × 45,000.
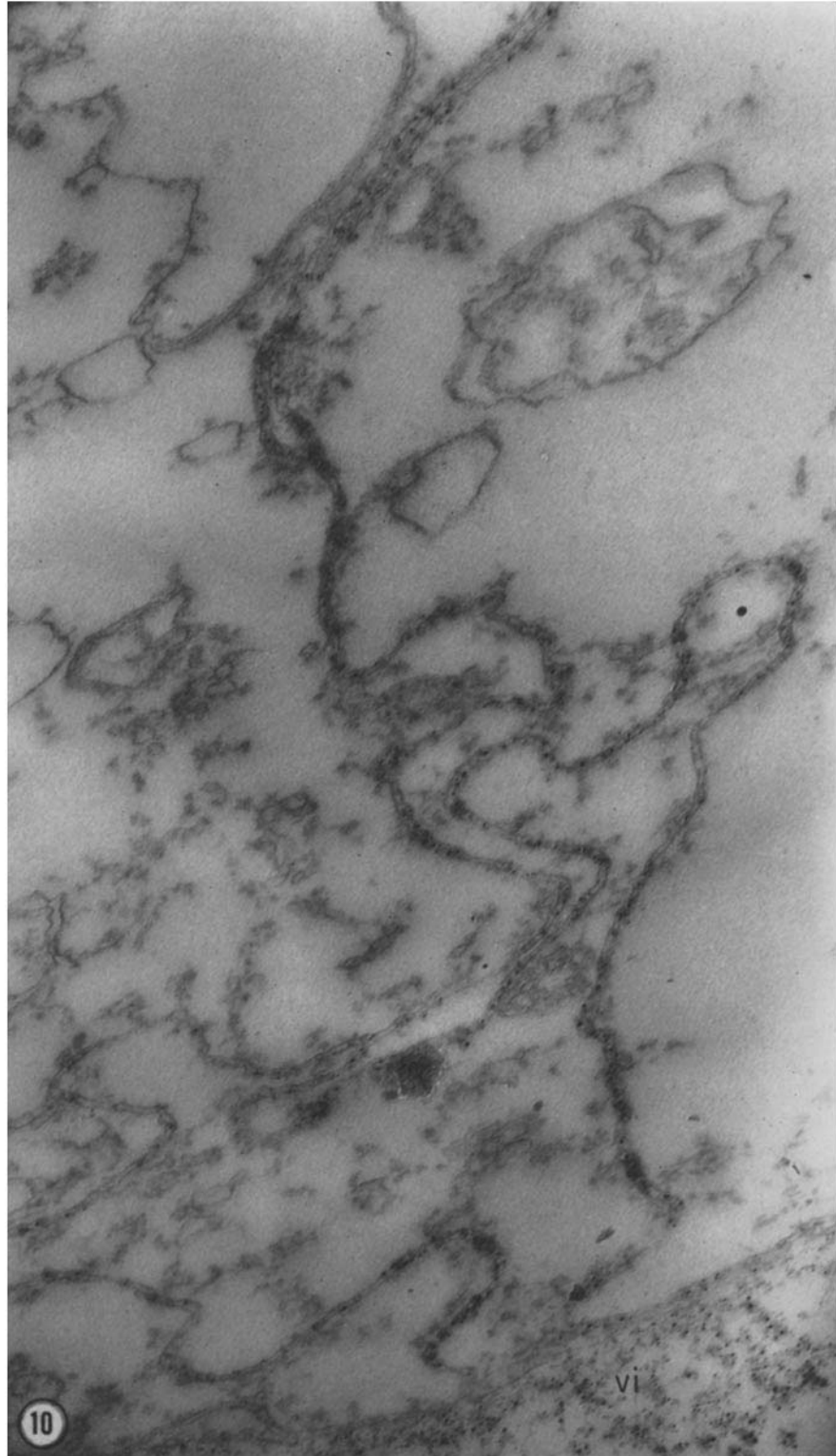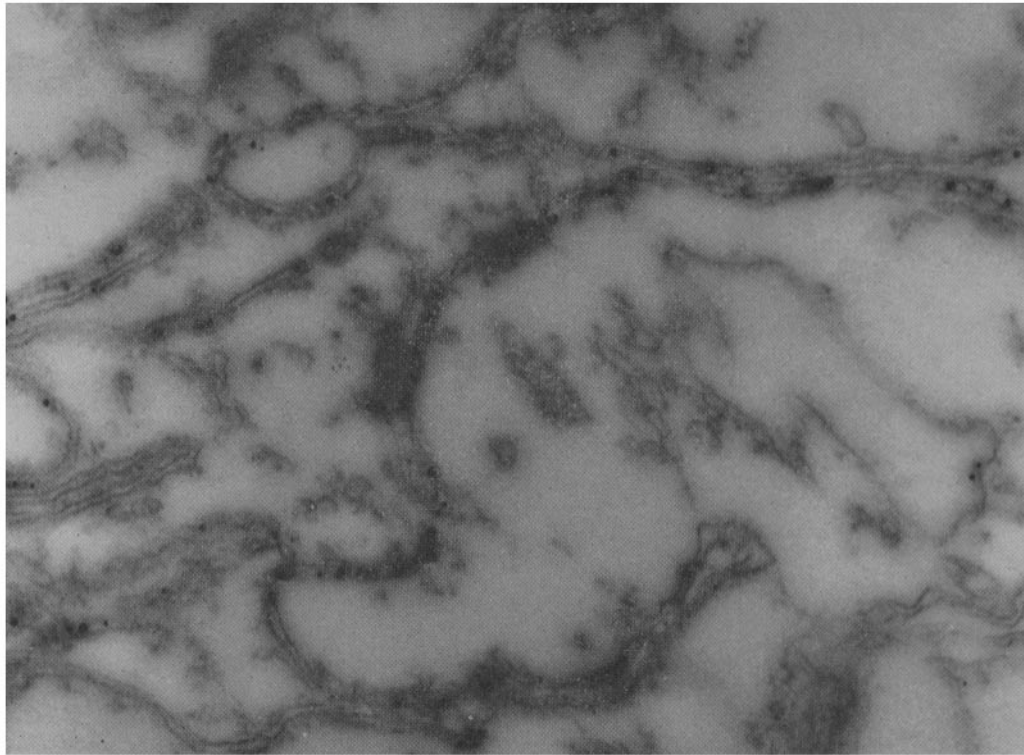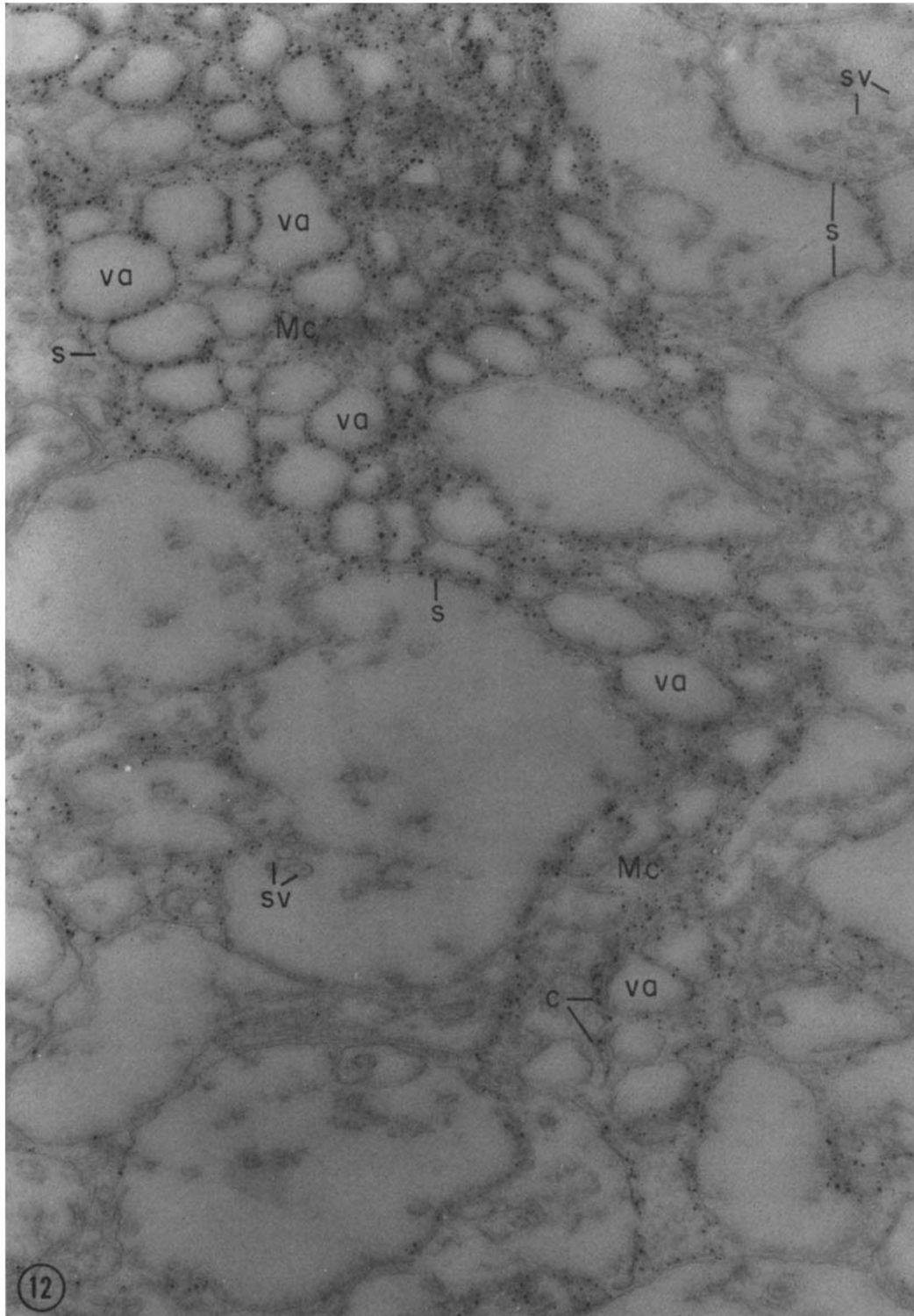
FIGURE 11

Copper ferrocyanide deposits within the infoldings of the plasma membrane of the Müller cell at the level of the ganglion cell layer. The isolated retina was immersed for 20 minutes in the weaker ferrocyanide medium. This treatment reduced the amplitude of the electroretinogram by about 50 per cent. Fixation in an osmium tetroxide–copper sulphate solution. × 60,000.

permeability properties. Electron microscopy proved this not to be true since ferrocyanide is actually segregated from the Müller cell cytoplasm, being within channels which are a component of the extracellular space. As stated before, these numerous channels are seen to be formed by infoldings of the plasma membrane of the Müller cell throughout the entire length of the cell, although this arrangement is more evident at the inner limiting membrane. It was not possible to establish whether these infoldings have a blind end or course uninterruptedly from the inner surface of the retina to the intercellular spaces. In the latter case, the infoldings would represent a diffusion pathway of communication between the intercellular spaces of the retina and the vitreous humor and in a sense they would be elaborated analogues of the channels that in satellite cells of giant fibers place the periaxonal space in communication with the connective tissue spaces (16). Although this is a very likely possibility, it is difficult to think that the only purpose served by the channels in the Müller cell is that of providing a diffusion route. This bizarre membranous

FIGURE 12

Müller cell process (Mc) at the inner synaptic layer with copper ferrocyanide deposits in the spaces in between vacuoles (va) and within channels (c). Copper ferrocyanide is observed also within the intercellular spaces (s). Synaptic vesicles, sv. Technical details as in Fig. 9. × 60,000.
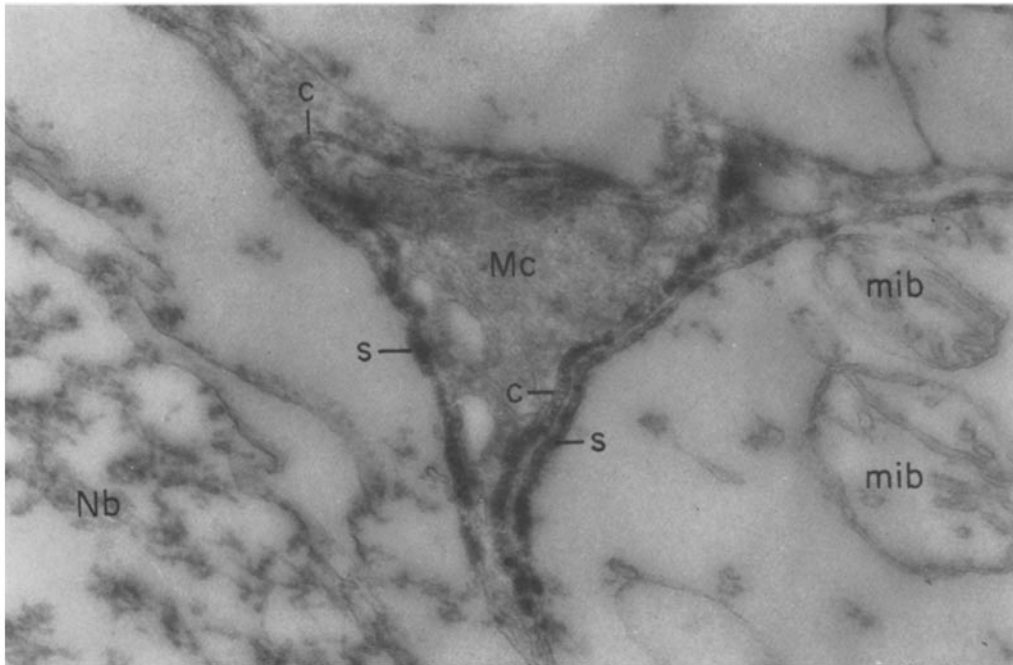
FIGURE 13

Müller cell process (*Mc*) at the inner part of the bipolar cell layer. The copper ferrocyanide deposits appear as an amorphous dense material filling the intercellular spaces (*s*) and the Müller cell channels (*c*). *Nb*, nucleus of a bipolar cell; *mib*, mitochondria of a bipolar cell. Technical details as in Fig. 10. × 60,000.

system enormously multiplies the surface of the Müller cell exposed to the extracellular fluid. Therefore, its existence could be taken as an indication that Müller cells are engaged in very active water and ion transport, by extrapolating the observation of similar membrane infoldings in epithelia of kidney tubules, ciliary body, choroid plexuses, and submaxillary gland (28). An important role of glia cells in the water-ion metabolism of the central nervous system has been already postulated by Gerschenfeld *et al.* (18).

The present observations do not include any quantitative data and therefore it is difficult to determine whether they are consistent with observations on the physiological measurements of the size of the extracellular space in retina and brain. The values for extracellular space in brain of 14 to 22 per cent (1, 5) and in retina of 29 per cent (2), obtained by measuring uptake of "extracellular substances," seemed much too high even when the intercellular gaps are considered as diffusion spaces. In the toad retina, values as high

as those mentioned would not be too unexpected since in this case the extracellular space includes an intercellular and a glial compartment, the latter being represented by the plasma membrane infoldings of the Müller cells. However, similar infoldings have not been found so far in the Müller cells of other retinae (4, 23, 34, 35, 39) and, therefore, general statements cannot be made at the present time. The same can be said with respect to the brain, because early reports describing the presence of plasma membrane infoldings in glial cells (6, 25) have not been confirmed posteriorly (10, 13).

REFERENCES

1. ALLEN, J. N., Extracellular space in the central nervous system, *Arch. Neurol. Psychiat.*, 1955, **73**, 241.
2. AMES, A., III, and HASTINGS, A. B., Studies in water and electrolytes in nervous tissue. I. Rabbit retina: Methods and interpretation of data, *J. Neurophysiol.*, 1956, **19**, 201.
3. BOYLE, P. J., and CONWAY, E. J., Potassium accumulation in muscle and associated changes, *J. Physiol.*, 1941, **100**, 1.
4. COHEN, A. I., Electron microscope observations of the internal limiting membrane and optic fiber layer of the retina of Rhesus monkey, *Am. J. Anat.*, 1961, **108**, 179.
5. DAVSON, H., and SPAZIANI, E., The blood-brain barrier and the extracellular space of the brain, *J. Physiol.*, 1959, **149**, 135.
6. DEMPSEY, E. W., and LUSE, S., Fine structure of the neuropile in relation to neuroglia cells, *in*

Biology of Neuroglia, (W. F. Windle, editor), Springfield, Illinois, Charles C. Thomas, 1958, 99.
7. DEMPSEY, E. W., and WISLOCKI, G. B., An electron microscopic study of the blood-brain barrier in the rat employing silver nitrate as a vital stain, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 245.
8. DE ROBERTIS, E. D. P., Submicroscopic organization of some synaptic regions, *Acta Neurol. Latinoamer.*, 1955, **1**, 3.
9. DE ROBERTIS, E. D. P., and FRANCHI, C., Electron microscope observations on synaptic vesicles in synapses of the retinal rods and cones, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 307.
10. DE ROBERTIS, E. D. P., and GERSCHENFELD, H. M., Submicroscopic morphology and
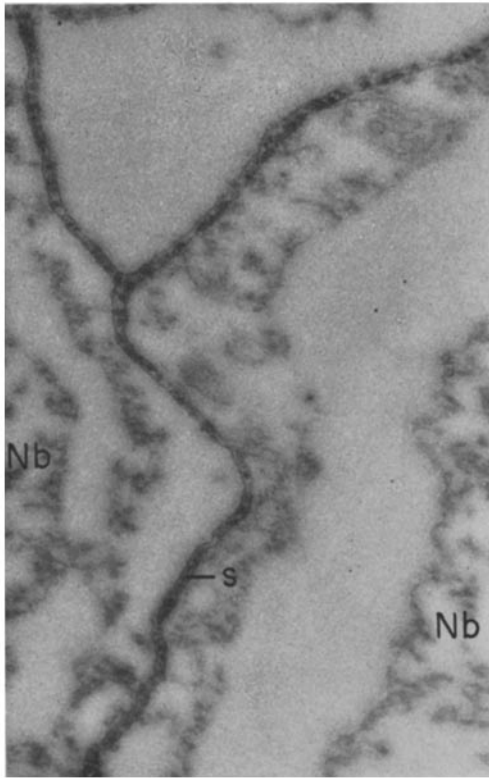
FIGURE 15

Copper ferrocyanide deposits within the spaces in between bipolar cells. *Nb*, bipolar cell nuclei; intercellular space, *s*. Technical details as in Fig. 11. × 68,000.

function of glial cells, *Internat. Rev. Neurobiol.*, 1961, **3**, 1.

11. ECCLES, J. C., The Physiology of Nerve Cells, Baltimore, Johns Hopkins University Press, 1957.
12. ECCLES, J. C., The mechanisms of synaptic transmission, *Ergebn. Physiol.*, 1961, **51**, 299.
13. FARQUHAR, M. G., and HARTMANN, J. F., Neuroglial structure and relationships as revealed by electron microscopy, *J. Neuropathol. and Exp. Neurol.*, 1957, **16**, 18.
14. FATT, P., Biophysics of junctional transmission, *Physiol. Rev.*, 1954, **34**, 674.
15. FATT, P., and KATZ, B., An analysis of the end plate potential recorded with an intracellular electrode, *J. Physiol.*, 1951, **115**, 320.
16. FRANKENHAUSER, B., and HODGKIN, A. L., After effects of impulses in the giant nerve fibers of Loligo, *J. Physiol.*, 1956, **131**, 341.
17. FURUKAWA, T., and HANAWA, J., Effects of some common cations on electroretinogram of the toad, *Japan. J. Physiol.*, 1955, **5**, 289.
18. GERSCHENFELD, H. M., WALD, F., ZADUNAISKY, J. A., and DE ROBERTIS, E. D. P., Function of astroglia in the water-ion metabolism of the central nervous system, *Neurology*, 1959, **9**, 412.
19. HILD, W., and TASAKI, I., Morphological and physiological properties of neurons and glial cells in tissue culture, *J. Neurophysiol.*, 1962, **25**, 277.
20. HODGKIN, A. L., Ionic basis of electrical activity in nerve and muscle, *Biol. Rev.*, 1951, **26**, 339.
21. HODGKIN, A. L., Ionic movements and electrical activity in giant nerve fibers, *Proc. Roy. Soc. London, Series B*, 1958, **148**, 1.
22. HORSTMANN, E., and MEVES, H., Die Feinstruktur des molekulären Rindengraues und ihre physiologische Bedeutung., *Z. Zellforsch.*, 1959, **49**, 569.
23. KIDD, M., Electron microscopy of the inner plexiform layer of the retina in the cat and the pigeon, *J. Anat.*, 1962, **2**, 179.
24. LASANSKY, A., Morphological bases for a nursing role of glia in the toad retina. Electron microscope observations, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 237.
25. LUSE, S. A., Electron microscopic observations of the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 531.
26. LUSE, S. A., and HARRIS, M. D., Electron microscopy of the brain in experimental edema, *J. Neurosurgery*, 1960, **17**, 439.
27. MAYNARD, E. A., SCHULTZ, R. L., AND PEASE, D. C., Electron microscopy of the vascular bed of rat cerebral cortex, *Am. J. Anat.*, 1957, **100**, 409.
28. PEASE, D. C., Infolded basal plasma membranes found in epithelia noted for their water transport, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 203.
29. RALL, D. P., and PATLAK, C. S., The extracellular space in brain, *Fed. Proc.*, 1962, **21**, 324.
30. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron-micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
31. SCHMITT, F. O., Ultrastructure of nerve myelin and its bearing on fundamental concepts of the structure and function of nerve fibers, *in* The Biology of Myelin, (S. Korey, editor), New York, P. B. Hoeber, 1959, 1.
32. SCHULTZ, R. L., MAYNARD, E. A., and PEASE, D. C., Electron microscopy of neurons and neuroglia of cerebral cortex and corpus callosum, *Am. J. Anat.*, 1957, **100**, 369.
33. SHIMIZU, N., and MAEDA, S., Histochemical

studies on glycogen of the retina, *Anat. Rec.*, 1953, **116**, 427.

34. Sjöstrand, F. S., Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstruction from serial sections, *J. Ultrastruct. Research*, 1958, **2**, 122.

35. Sjöstrand, F. S., Electron microscopy of the retina, *in* The Structure of the Eye, (G. R. Smelser, editor), New York, Academic Press, Inc., 1961, 1.

36. Torak, R. M., Terry, R. D., and Zimmerman, H. M., Fine structure of cerebral fluid accumulation. I. Swelling secondary to cold injury, *Am. J. Pathol.*, 1959, **35**, 1135.

37. Trujillo-Cenoz, O., Electron microscopic study of rat gustatory buds, *Z. Zellforsch.*, 1957, **46**, 272.

38. Villegas, G. M., Electron microscopic study of the vertebrate retina, *J. Gen. Physiol.*, 1960, **43**, suppl., 15.

39. Wald, F., and De Robertis, E. D. P., The action of glutamate and the problem of the extracellular space in the retina, *Z. Zellforsch.*, 1961, **55**, 649.

40. Weed, L. H., The absorption of cerebrospinal fluid into the venous system, *Am. J. Anat.*, 1923, **31**, 191.

41. Woodbury, D. M., Timiras, P. S., Koch, A., and Ballard, A., Distribution of radiochloride, radiosulphate, and inulin in brain of rats, *Fed. Proc.*, 1956, **15**, 501.

42. Wyckoff, R. W., and Young, J. Z., The nerve cell surface, *J. Anat.*, 1954, **88**, 568.

43. Wyckoff, R. W., and Young, J. Z., The motoneuron surface, *Proc. Roy. Soc. London, Series B*, 1956, **144**, 440.