ORGANIZATION OF THE OUTER PLEXIFORM LAYER OF THE PRIMATE RETINA: ELECTRON MICROSCOPY OF GOLGI-IMPREGNATED CELLS

By HELGA KOLB
Wilmer Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland, 21205 U.S.A.

(*Communicated by G. P. Wells, F.R.S.—Received 2 June 1969—Revised 3 November 1969*)

[Plates 51 to 57]

CONTENTS

Golgi-impregnated retinae of rhesus monkeys have been examined by serial section electron microscopy to establish in a quantitative manner the neural connexions in the outer plexiform layer. The results have shown that there are two types of midget bipolar cell, here called the invaginating midget bipolar and the flat midget bipolar. Both types of midget bipolar are exclusive to a single cone. The invaginating midget bipolar has been found to fit a dendritic terminal process into every invagination in the cone pedicle base. The flat midget bipolar has dendritic terminals that make superficial contact on the cone pedicle base. There are twice as many dendritic terminals and points of contact with the cone pedicle on a flat midget bipolar top as compared with an invaginating midget bipolar top. These observations, together with light microscope counts of the numbers of the two types of midget bipolars, suggest that there are two midget bipolars per cone.

The diffuse cone bipolar (the flat bipolar) also makes superficial contacts on the cone pedicle base, and serial sections have shown that a flat bipolar contacts about six cones. Rod bipolars connect exclusively to rods and their dendritic terminals always end as one of the central processes that penetrate the invagination. Horizontal cell dendrites end exclusively in cone pedicles and their axon terminals end in rod spherules. The point of contact with both the types of receptor is as the lateral elements of the invaginations. A single small horizontal cell contacts about seven cones and a large horizontal cell contacts about twelve cones. The numbers of contacts per cone pedicle decrease from the centre to the periphery of the horizontal cell's dendritic field, suggesting there is an overlap of four to six horizontal cells onto a single cone pedicle. The horizontal cell axon terminals are too numerous to assess in absolute numbers but there is only one terminal to a given rod spherule from any particular axon.

Introduction

The two anatomical methods that have been most useful for the study of the neural relationships in the retina are light microscopy of Golgi-impregnated tissue and electron microscopy of thin sections. Light microscopy of Golgi-impregnated retinae (Cajal 1933; Polyak 1941; Boycott & Dowling 1969) has provided information on the shapes and spread of the various nerve cells, but this method is limited to giving only circumstantial evidence of the synaptic connexions between the neurons. Electron microscopy, on the other hand, can provide the detailed pictures of the probable sites of synaptic contact between nerve cells, but it is not possible often to be certain which cells are involved, even with exhaustive and exacting serial sectioning methods (Sjöstrand 1958; Missotten 1965; Pedler & Tilly 1965).

The present paper will describe the neural connexions in the outer plexiform layer of the primate retina as revealed by a combination of these two methods. Golgi-impregnated cells are selected by light microscopy and subsequently examined and studied by electron microscopy (Stell 1965; Blackstad 1965). This method provides a direct test for the claims of authors who used either light microscopy (Boycott & Dowling 1969) or electron microscopy alone (Missotten 1965). The present paper will also provide some quantitative data regarding retinal contacts, as all the cells studied have been analysed by serial sections. In addition, there is a more detailed description of the new type of midget bipolar cell first reported by Kolb, Boycott & Dowling (1969).

In the mammalian retina, Cajal (1933) described two types of bipolar cells and at least two types of horizontal cells (outer and inner horizontal cells) that contact the receptors in the outer plexiform layer. He reasoned that the bipolars and horizontal cells were exclusive to either rods or cones. Polyak (1941) studied the primate retina in detail, and described three types of diffuse bipolars, a 'private' midget bipolar, and one type of horizontal cell in the outer plexiform layer. His large 'mop' bipolar he considered to make contact with both rods and cones. The flat and brush bipolars were considered to be primarily diffuse cone bipolars, but he claimed that they contacted rods as well. Polyak described only one type of horizontal cell, which he believed to be in synaptic contact with a group of cones and linked by the axon to a group of both rods and cones. Polyak's important contribution was his discovery of the midget bipolar and his realization that this bipolar was in exclusive relationship to a single cone. The midget bipolar has only been seen in the bird fovea and chameleon fovea (Cajal 1933) apart from the primate retina.

With the advent of electron microscopy it became evident that some of Polyak's ideas could not be substantiated. For example, electron microscopy showed that there are no synapses on the sides of rod and cone receptor terminals as Polyak believed. Boycott & Dowling (1969) have reassessed the light microscopy of the primate retina, in order to clarify some of Polyak's uncertainties and conclusions. Boycott & Dowling (1969) described five types of cells that contact the receptors in the outer plexiform layer. The midget and flat bipolars were thought to be exclusive to cones, and the rod bipolar (Polyak's mop bipolar) was considered to be exclusive to rods. Polyak's brush bipolar was not found but his horizontal cell was thought to be distinguishable into two types, one of which seemed to be a cone horizontal cell, and the other, on the light microscope criteria, was suggested to be a rod horizontal cell. Boycott & Dowling's distinction of the cell types and suggested mode of contact of these cells with the receptors are based on circumstantial evidence, as they point out in the paper. They have come

to their conclusions by relating the size and level of termination of the terminal dendrites on the cells they are studying by light microscopy, to the known ultrastructure of the rod and cone terminals.

Missotten (1965) has attempted to analyse the neural connexions in the outer plexiform layer of man by serial section electron microscopy. This method is limited by the extreme difficulty of interpreting serial sections; only portions of cells can be reconstructed and these partial outlines compared with the Golgi-impregnated cells of light microscopy (see Boycott & Dowling 1969). However, Missotten's findings are particularly important in having clarified the ultrastructure of the rod spherules and cone pedicles in the primate.

The rod spherules (Sjöstrand 1953, 1958, 1961; de Robertis & Franchi 1956; Ladman 1958; Cohen 1963, 1964; Villegas 1960, 1964; Missotten, Appelmans & Michiels 1963; Missotten 1965; Evans 1966) make synaptic contacts with a cluster of nerve endings penetrating the spherule in a single invagination. Synaptic ribbons, presynaptic vesicles and arciform densities characterize the hilus of the invaginations. The deeply inserted terminal buds are situated in a lateral position and were identified as horizontal cell axon terminals in man (Missotten 1965). In goldfish the laterally placed terminals were identified as horizontal cell dendrites (Stell 1965, 1967). The less deeply inserted terminal buds have been traced to rod bipolars in man (Missotten 1965) and to the large bipolar of the goldfish (Stell 1967).

Cone pedicles of vertebrates have been studied in detail by various authors (de Robertis & Franchi 1956; Cohen 1963, 1964; Pedler & Tansley 1963; Kalberer & Pedler 1963; Pedler & Tilly 1964; Villegas 1960; Stell 1965, 1967; Evans 1966; Dowling 1968; Dowling & Werblin 1969). In the primate cone pedicle many synapses occur in invaginations but there are also superficial contacts on the pedicle base (Missotten 1965; Pedler & Tilly 1965; Dowling 1965; Villegas 1964; Cohen 1961, 1965; Dowling & Boycott 1966). The superficial contacts are simple shallow dents in the cone pedicle base. The number of invaginations per pedicle varies according to the position of the cone pedicle in the retina. For example, there are 12 in the foveal cone pedicle (Dowling 1965), but 23 to 25 in the pedicles 1.5 to 2 mm from the foveal pit. In each invagination there are three processes, the 'triad' (Missotten 1965): a central process measuring about 0.2 μm across and two deeply inserted lateral processes each measuring about 0.5 μm across. A synaptic ribbon, synaptic vesicles and membrane thickenings are associated with these triads. The central members have been identified as midget bipolar dendrites and horizontal cell dendrites (Missotten 1965). The lateral elements are thought to be horizontal cell axon terminals (Missotten 1965) or horizontal cell dendrites (Stell 1967). Superficial contacts are on the base of the cone pedicle and have been traced to a cell that could be a flat bipolar (Missotten 1965).

The electron microscopy of Golgi-impregnated cells, described here, has also revealed some important information concerning the reliability of the Golgi method. Besides being able to show the type and extent of the neural connexions and the specificity of certain cells for the rod or cone systems, the findings also indicate that Golgi artefacts can lead to a misinterpretation of cell types if light microscopy alone is used. Particularly this study shows that arriving at conclusions about cell contacts solely on the basis of the size and shape of the terminals as seen by the light microscope can be misleading.

Material and methods

The eyes of young rhesus monkeys (*Macaca mulatta*) were removed under anaesthesia, the globe opened and the posterior pole trimmed and reduced to one large piece of central retina. Under saline immersion, the vitreous humour was removed, the retina eased off the choroid and sclera, and the the optic nerve severed. The tissue was then prepared in one of the following four ways: (1) The tissue was fixed overnight in 3.5 % glutaraldehyde in 0.1 M sodium phosphate buffer and then stained by the Colonnier modification of the Golgi–Kopsch method (Colonnier 1964). (2) Tissue was fixed overnight in 1.75 % glutaraldehyde in 0.05 M sodium phosphate buffer and the Golgi–Colonnier method then proceeded with as above. (3) Tissue was fixed for 1 h in 2.5 % glutaraldehyde in 0.05 M sodium phosphate buffer and then processed by the Golgi triple impregnation method modified after Cajal (Stell 1965). (4) Some of the material studied by electron microscopy was that used by Boycott & Dowling (1969). Their material was all processed according to the method of Colonnier (1964).

After silver impregnation the tissue was embedded in collodion (Necoloidine B. D. H.), sectioned at 90 μm and mounted in Fisher's Permount on glass slides. Isolated stained cells were selected by light microscopy and the whole section containing these cells re-embedded in Araldite. After trimming away as much excess material as possible the specimens were mounted on Araldite capsules and could be viewed under the light microscope. Serial sections of the chosen cells were cut on a Porter-Blum MT-2 microtome using a glass knife, and the sections mounted on Formvar carbon-coated slot grids. The sections were examined, after staining with lead citrate, in the RCA EMU-3G electron microscope.

All the cells analysed in this electron microscope study are from the central retina within a 5 mm radius of the foveal pit.

Earlier experiments with other concentrations and osmolarities of the fixative were carried out, but unfortunately the quality of the fixation with accompanying Golgi impregnation of the cells has not yet been improved beyond that of the methods described in the previous section. The four methods described gave the best results in terms of numbers of cells stained and the ultrastructural appearance of the tissue. The quality of the fixation is poor by conventional electron microscopy standards, but is adequate to determine unequivocally the course of the dendrites of stained cells to their point of contact on the receptor terminal.

The Golgi–Colonnier method is characterized by poor contrast, owing to the absence of osmium in the fixative, poor cytoplasmic preservation, breakdown of the mitochondrial cristae, and sometimes gross distortion and swelling of whole dendritic processes. The adapted Golgi triple impregnation method gives better contrast and the processes particularly at the synaptic sites, maintain a more normal size, but the cytoplasm and mitochondria and other organelles are less well preserved than the Colonnier method.

Golgi-stained cells are easily recognized in the electron microscope by the presence of the dense stain deposited in the cell cytoplasm; however, the smaller processes, particularly the dendritic terminals, are often only partially stained. Sometimes the fine processes consist of an empty space with a minute speck of stain at the rim, and in such cases only serial sections showing the process joined to a larger stained dendrite is proof of the neural contact. It is also very difficult to find completely isolated cells for electron microscopy and almost inevitably what appears to be a 'clean' area of tissue with an isolated cell does actually contain staining of other cell processes when viewed under the higher magnification of the electron microscope.

All these difficulties emphasize that only serial sectioning will give an adequate and accurately interpretable result.

Observations

Midget bipolars

The midget bipolar, first described by Polyak (1941) in primates, is thought to be monosynaptic to a single cone pedicle. It is typified by the single dendrite passing through the outer plexiform layer to terminate in a top (bouquet) that has a diameter corresponding to the diameter of a cone pedicle (Polyak 1941; Boycott & Dowling 1969). Until quite recently only one kind of midget bipolar was thought to exist in the primate retina. This midget bipolar has projecting finger-like terminal dendrites on the top, that can be clearly distinguished by light microscopy. Missotten (1965) and Boycott & Dowling (1969) have predicted that the terminal dendrites fit into the cone pedicle invaginations as the central element of the triad. This type of midget bipolar is here called the invaginating midget bipolar and has been analysed by serial sections (see below). In addition, a new type of midget bipolar has been discovered by electron microscopy (Kolb *et al.* 1969). This new midget bipolar cell, here called a flat midget bipolar, makes the superficial type of contacts on the cone pedicle base and is never seen to have terminal dendrites projecting into the invaginations. The absence of distinct terminal dendrites on the flat midget bipolar top distinguishes it from the invaginating midget bipolar by light microscopy. Therefore, it is now possible to identify the two types of midget bipolars by the light microscope (Kolb *et al.* 1969). A recent light microscope count of the flat midget bipolars compared with the invaginating midget bipolars throughout the central and some additional peripheral retina, suggests that they are equal in number. It is likely, therefore, that each cone pedicle makes contact with each type of midget bipolar.

Invaginating midget bipolars

Seven Golgi-impregnated invaginating midget bipolars have been examined by electron microscopy. In all cases the dendrites make synaptic contact with a single cone pedicle as the central elements of the triads.

Figure 2, plate 51, is a light micrograph of an invaginating midget bipolar stained by the Golgi–Colonnier method (method 4). The cell body is typically small (8×12 μm) and lies high in the inner nuclear layer just below the horizontal cells. The single dendrite passes up through the outer plexiform layer to form a top 6 μm in diameter under the line of cone pedicles. Individual terminal dendrites measuring 0.2 μm across can be seen as small projections. The axon is long and slender and ends as a bag of processes in the third of the inner plexiform layer nearest the ganglion cells. Figure 1, plate 51, is an invaginating midget bipolar top at a higher magnification. The individual processes on the apical dendrite are clearly visible and some of them (arrowed) are forked, which suggests that they are inserted into separate triads.

Figure 3, plate 51, is a micrograph of an invaginating midget bipolar as it appears by electron microscopy. The plane of section is fortunate in that the cell body, main dendrite and its branch inserting into the cone pedicle are in line. The terminal dendrite forms the central process of the triad. Note a neighbouring triad (arrowed) apparently has no stained central element, but an adjoining section (see below) shows this process also to have stain and to be from the same midget bipolar cell. Sixty serial sections were obtained of this particular

invaginating midget bipolar, and an almost complete reconstruction of its dendritic top and mode of contact with this single cone pedicle was possible. The main dendrite branched into three close below the cone pedicle; further branches were inserted into the cone pedicle as the central element of the triads. Figures 4 to 6, plate 51, show areas of the cone pedicle that were contacted. The central elements of the triads contain a Golgi-impregnated process. Figure 6 shows the mode of branching of the dendrites close to the cone pedicle.

This cone pedicle contained 25 invaginations and the central elements of 24 of them were found to be impregnated processes from the invaginating midget bipolar. A few sections of the series were missing and this probably explains why the 25th triad seemed to lack a stained process. How the loss of a few sections could lead to a misinterpretation of whether a triad receives a stained central element or not is illustrated by figures 7 to 10. These are serial sections through two triads labelled A and B in the figures. Both contain a stained central element in figures 7 and 8. The stained terminals are sub-branches of the same terminal dendrite and obviously correspond to the forked dendrite seen in the light micrograph of figure 1, plate 51. In figure 9, triad B has no staining and in figure 10, neither does triad A. If serial sections had not been studied and either figure 9 or 10 had been picked at random, the interpretation might have been that one or another of these triads did not receive a stained process. Similarly, in figure 3, the triad marked with an arrow appears to be unstained, but the adjoining sections (figures 7 to 10) show that this triad is contacted by the Golgi-impregnated midget bipolar. This illustrates the importance of serial sections for the right quantitative results. The serial section analysis of this invaginating midget bipolar has proved with almost complete certainty that this bipolar is in contact with one cone only and has a terminal dendrite to fit into every triad of this cone pedicle.

Six other invaginating midget bipolars have also been investigated by electron microscopy but not as completely as the one described above. Analysis of the number of stained central elements of the triads seen in comparison with the total number of triads for four of these cells is seen in table 1. The evidence suggests that all the triads of a cone pedicle do receive a dendrite

Description of plate 51

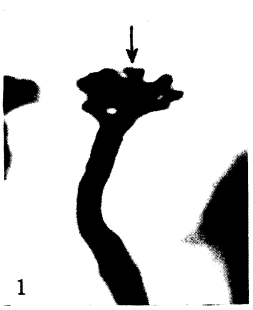
Figure 1. Light micrograph of an invaginating midget bipolar dendritic terminal. Individual terminal dendrites can be distinguished, one of which is branched at the point of insertion into the cone pedicle (arrowed). Golgi rapid triple impregnation (method 3) (× 3000).

Figure 2. Invaginating midget bipolar. Whole cell. Only four terminal dendrites can be seen. Note the cell body lies high in the inner nuclear layer and the axon terminates (out of focus) low in the inner plexiform layer. The cell lies within 1 mm of the foveal pit. Golgi–Colonnier (method 4) (× 1500).

Figure 3. Electron micrograph of an invaginating midget bipolar. Terminal dendrite arising from the main dendrite is inserted into the cone pedicle (CP) as the central element of the triad. Note a neighbouring triad (arrowed) appears unstained in this section. Golgi rapid triple impregnation (method 3) (× 14000).

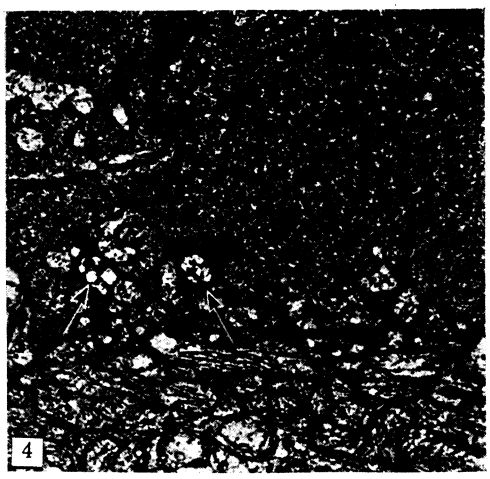
Figures 4 to 6 are sections through different regions of the cone pedicle contacted by the invaginating midget bipolar (figure 3). The central elements of the triads are the stained terminal dendrites (arrowed) of the invaginating midget bipolar. Golgi rapid triple impregnation (method 3) (× 12000).

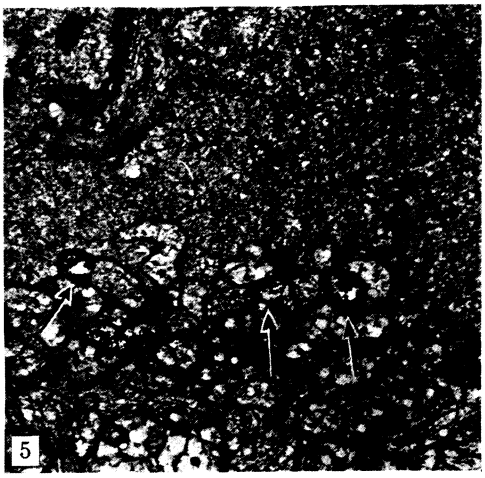
Figures 7 to 10. Four serial sections through two triads (A and B) to illustrate the reason for serial sections. In figure 7 both A and B contain a stained central element (sub-branches of the same terminal dendrite). In figure 8, A contains a clearly stained element, but B has the appearance of a hole, although it can still be interpreted as a stained terminal dendrite. In figure 9, B contains a central element but it now appears unstained while A still has a clearly stained central element. By figure 10, however, A has an apparently unstained central element and B is no longer recognizable as a triad. A in figure 10 is the triad marked by an arrow in figure 3. Golgi rapid triple impregnation (method 3) (× 22000). L, lateral elements of triad; C, central element of triad.

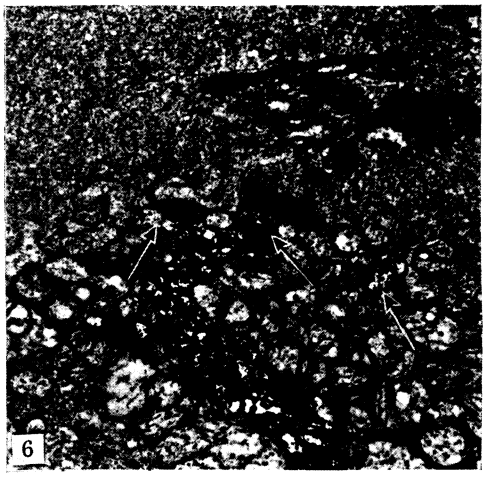

1


2

3


4


5


6


7

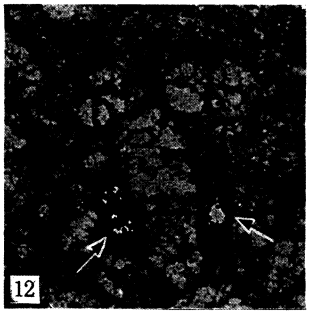

8

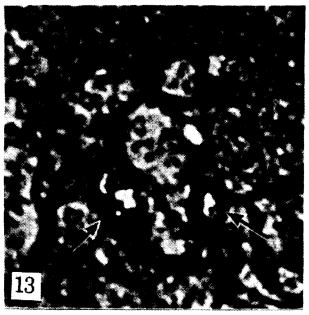

9


10

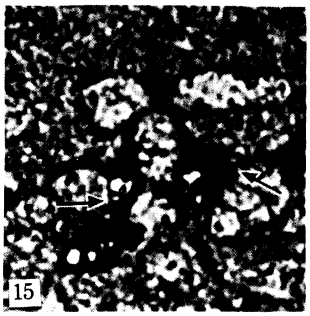
(Facing p. 266)

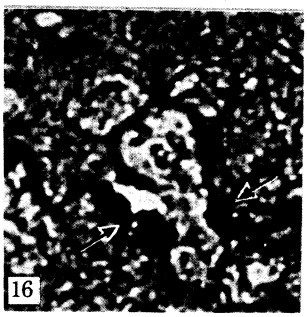

11


12


13


14


15


16


17

18

from the invaginating midget bipolar concerned with that particular cone pedicle. All the cells that have been analysed by electron microscopy were from the central areas of the retina. In the periphery there are branched midget bipolars (Polyak 1941; Boycott & Dowling 1969) and this may mean that they are in contact with two cone pedicles. Also some of the midget bipolars in the periphery have tops that are smaller than the diameter of a cone pedicle base, and the possibility exists that two invaginating midget bipolars are going to one cone (Boycott & Dowling 1969).

TABLE 1. THE NUMBER OF DENDRITIC TERMINAL PROCESSES SEEN ON THE INVAGINATING MIDGET BIPOLAR CELL STUDIED BY ELECTRON MICROSCOPY OF GOLGI-IMPREGNATED MATERIAL

invaginating midget bipolar	number of triads observed	central elements stained
1	25	24
2	19	17
3	14	12
4	12	9

Flat midget bipolars

The flat midget bipolar was first shown to be different from the invaginating midget bipolar by electron microscopy, but it is now possible to distinguish the two by light microscopy also (Kolb *et al.* 1969). The cell body generally lies lower in the inner nuclear layer than that of the invaginating midget bipolar and the axon terminals end at a higher level in the inner plexiform layer, usually just below the inner nuclear layer. The striking difference between the flat and invaginating midget bipolars, overlooked by Polyak and by Boycott & Dowling, is in the appearance of the dendrite top. Figure 11, plate 52, shows the light microscope appearance of the dendritic top of a flat midget bipolar. The main dendrite ends as a flattened or slightly dome-shaped, solid-looking plaque, with a diameter corresponding to the diameter of a cone pedicle. No projecting terminal dendrites are visible, although occasionally small horizontally running

DESCRIPTION OF PLATE 52

FIGURE 11. Light micrograph of a flat midget bipolar dendritic top, lying within a mm of the foveal pit. Note the flattened or slightly convex, solid plaque appearance of the dendritic top. Occasionally a few individual terminal dendrites can be seen but they never appear as finger-like projections. Golgi–Colonnier material (method 4) (× 3000).

FIGURES 12 to 16. The electron micrographs are of triads in the cone pedicles contacted by the flat midget bipolar. The terminal dendrites of the flat midget bipolar (arrowed) lie alongside the central element and even push halfway up into the triad (figures 12 and 13). However, they never penetrate right into the invagination but make superficial contacts with the pedicle base. The stained flat midget bipolar terminal dendrites are approximately half the diameter of the invaginating midget bipolar (central element) dendrites. Golgi rapid triple impregnation method (method 3) (× 20000).

FIGURE 17. Light micrograph of two midget bipolars making contact with a single cone pedicle. The cell whose nucleus is shown is probably an invaginating midget bipolar, whereas the other cell is probably a flat midget bipolar. Golgi–Colonnier (method 2) (× 3000).

FIGURE 18. An electron micrograph of a flat midget bipolar cell and the cone pedicle (CP) to which it is related. The terminal dendrites end as superficial contacts on the pedicle base on either side of the central element of the triad of invaginations (arrowed). The cell was from a peripheral area of retina, 5 mm from the foveal pit, and, therefore, the cell body lies higher in the inner nuclear layer than in foveal regions. Golgi triple impregnation method (method 3) (× 12000).

branches can be resolved. The top looks like a badly impregnated invaginating midget bipolar in the light microscope and consequently was ignored by previous authors. Electron microscopy shows that the light microscopic appearance is caused by the fact that the flat midget bipolar top consists of twice the number of processes as are found on the invaginating midget bipolar; and because they lie nearer together than 0.2 μm and never project up into the cone pedicle they cannot be separately resolved by light microscopy.

Four flat midget bipolars have been serially sectioned and examined by electron microscopy. Figure 18, plate 52, shows the electron microscopic appearance of one of these cells. The section has passed through the cell body and part of the main dendrite and the centre of the single cone pedicle with which it is in contact. The cone pedicle is 9 μm in diameter. The terminal dendrites of the impregnated cell do not enter the invaginations but instead make superficial contact with the cone pedicle base. Figures 12 to 16 are a higher magnification of triads of the cone pedicle of figure 18. The impregnated dendrites of the flat midget bipolar make superficial contacts on either side of the process that forms the central element of the triad (i.e. the invaginating midget bipolar). Very often, as illustrated by figures 12 and 13, the two flat midget dendrites even seem to have pushed partially into the invagination. The flat midget bipolar dendrites are finer in diameter (0.1 μm) than the invaginating midget bipolar terminal dendrites (0.2 μm) (see figures 12 and 13, plate 52).

A complete reconstruction of the flat midget bipolar was possible and the course of the dendrites in the dentritic top could be followed. The branches of the single main dendrite were more numerous than for the corresponding invaginating midget bipolar and there were more terminal dendrites twisting to run horizontally and obliquely before coming to their point of contact, two to every triad. The cone pedicle contained 25 triads, and 48 impregnated flat midget bioplar dendrites were seen to make superficial contact. The three other flat midget bipolars analysed by electron microscopy showed similar results. Like the invaginating midget, the flat midget always contacts only one cone pedicle. Table 2 summarizes the results regarding the number of flat midget bipolar contacts on the cone pedicles for the cells studied.

TABLE 2. THE NUMBER OF DENDRITIC TERMINALS SEEN ON THE FLAT MIDGET BIPOLARS STUDIED BY ELECTRON MICROSCOPY OF GOLGI-IMPREGNATED MATERIAL

flat midget bipolar	number of triads observed	number of superficial contacts observed
1	25	48
2	24	45
3	19	34

Occasionally, two midget bipolars with tops that are converging onto a single cone pedicle are stained by the Golgi method (see figure 17, plate 52). The interpretation is that the midget bipolar with the higher placed nucleus (shown) is an invaginating midget bipolar and the other midget bipolar with the lower nucleus (not shown here but see Boycott & Dowling 1969, figure 11, plate 34) is a flat midget bipolar.

Flat bipolars

The flat bipolar is a diffuse bipolar that contacts only cones (Cajal 1933; Boycott & Dowling 1969). Polyak (1941) considered the flat bipolars to be related to cones mainly, but thought they might contact rod spherules that lay at the same level as the cone pedicles. The typical

appearance of a flat bipolar about 2 mm from the foveal pit is illustrated in figure 20, plate 53. The cell body lies in the centre of the inner nuclear layer and the long dendrite extends up into the outer plexiform layer to branch into three or four sub-branches. These branches pass obliquely up to a level just under the line of cone pedicles, where a number of fine terminal dendrites turn sharply to run horizontally under the receptors. No terminal dendrites are seen to project out of the horizontal plane. Horizontal sections of flat bipolars (Boycott & Dowling 1969) show that the dendrites are formed into groups corresponding in size to a cone pedicle and the inference is, therefore, that these dendrites contact cone pedicles. Boycott & Dowling (1969) have estimated by counting the numbers of such groups of dendritic terminals that six or seven cone pedicles are probably contacted by a flat bipolar cell. They also suggest that the flat bipolar is making superficial contacts with the cone pedicle because the terminal dendrites never appear to pass up vertically out of the flat plane, as they would have to if they were to be inserted into the cone pedicles in invaginations. However, Boycott & Dowling point out that the flat bipolars do not stain well at their terminals and, therefore, the light microscope evidence is not yet strong enough to be certain how the flat bipolar makes contact with the cone pedicle or how many cones are contacted. Missotten (1965) has followed some terminals from their point of superficial contact on a cone pedicle base, back to a cell body that may be a flat bipolar.

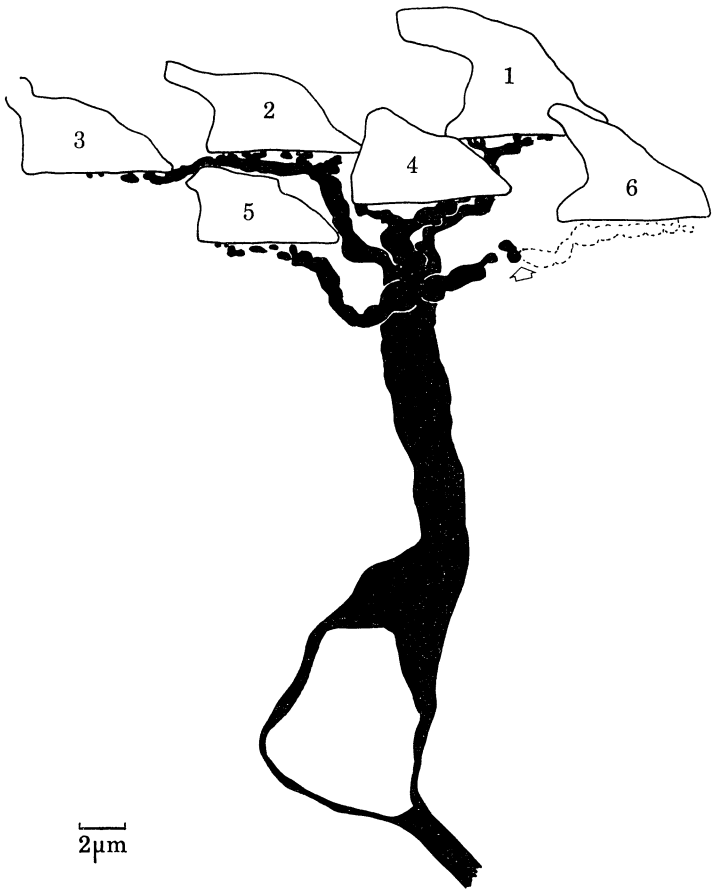


FIGURE 24. Drawing from tracings of serial sections of a flat bipolar cell. The tracings have been staggered to separate the cone pedicles. Six cone pedicles are contacted by the flat bipolar although one branch was not followed completely (arrowed at point of break) and the dotted outline represents its probable course and termination on pedicle 6.

Figure 19, plate 53, shows the appearance of a Golgi-impregnated flat bipolar under the electron microscope. The top of the main dendrite is seen emerging into the outer plexiform layer and a portion of the four primary branches can be seen (arrowed). Immediately above are two cone pedicles that are contacted by this flat bipolar. The horizontal course of two fine dendrites close under the cone pedicles can be seen and some of the terminal dendrites are making contact with the cone pedicles. The higher magnification of figures 21 to 23, plate 53, shows that the impregnated terminal dendrites make superficial contacts on the pedicle base, very often next to the central invaginating midget bipolar process of the triad (ribbon of triad arrowed). Only one flat bipolar dendrite is associated with the triad as compared with the two, on either side of the central process, that is typical of the flat midget bipolar (see previous section).

A serial reconstruction of this Golgi-impregnated flat bipolar was attempted but the dendritic spread of the terminal was between 15 and 20 μm and unfortunately some portions of the series were lost. Figure 24 is a drawing made from superimposed tracings of the electron micrographs, staggered in such a way that the cone pedicle and main dendrites of the cell are obvious. Five cone pedicles were seen to be contacted, and it is assumed that a sixth was also contacted because one branch was not followed to its destination (arrowed at the point where no more sections were available, and interpreted to continue as the dotted line). The main dendrite branched into four in the centre of the outer plexiform layer. One of these branches subdivided shortly above, and another branch passed under one cone pedicle and continued to a neighbouring cone, giving off terminal dendrites to both. All the cone pedicles in the dendritic field were contacted. There was no evidence of this flat bipolar making any contact with a rod spherule. The flat bipolar made about as many contacts per cone pedicle as there were triads in the cone pedicle. This seemingly rather small number of contacts, on the cone base, from this Golgi-impregnated flat bipolar may be a result of poor staining in the fine terminals, as Boycott & Dowling noticed. Alternatively, the finding probably means that

Description of plate 53

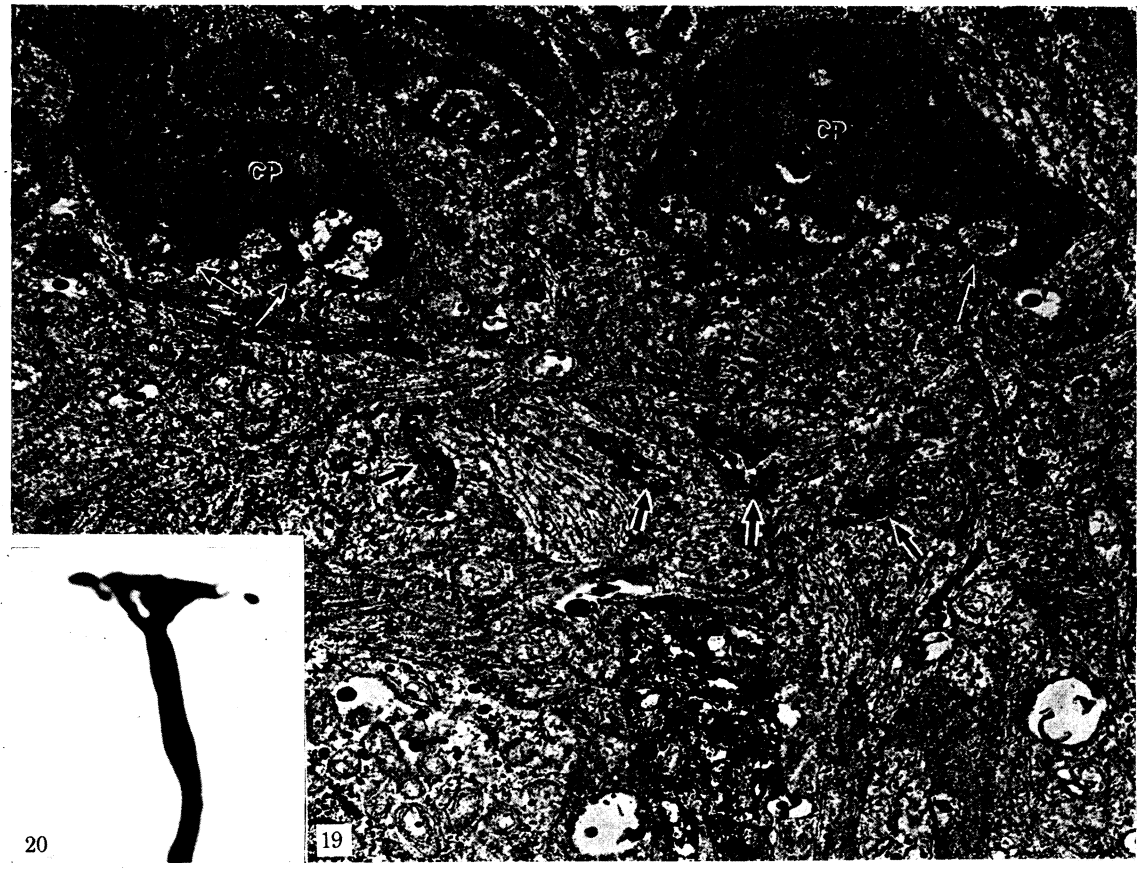
Figure 19. Low-power electron micrograph of a portion of a flat bipolar cell and two of the six cone pedicles it makes contact with. Part of the main dendrite is seen emerging into the outer plexiform layer and portions of its four branches (thick arrows) can be seen in the centre of the outer plexiform layer. Two cone pedicles (CP) are contacted by the dendrites (thin arrows). Note the horizontally running dendrites close beneath the cone pedicles. Golgi–Colonnier (method 2) ($\times$ 12000).

Figure 20. Light micrograph of a flat bipolar cell top. The dendritic spread is 15 to 20 μm. Golgi–Colonnier (method 2) ($\times$ 1400).

Figures 21 to 23. Higher magnifications of the superficial contacts made by the flat bipolar dendrites. The triads are marked with an arrow. The stained terminal dendrites always end as flat superficial contacts on the base of the cone pedicles (thick arrows), usually on either side of the central elements of the triads (figures 22, 23). The horizontally running course of dendrites coming from beyond (the left of) the cone pedicle is well illustrated in figure 23. Golgi–Colonnier (method 2) ($\times$ 22000).

Figure 25. Low power electron micrograph of a portion of a rod bipolar cell. The main dendrite is seen pushing up through the inner nuclear layer and two sub-branches appear in the outer plexiform layer. The fine dendrites (arrowed) pass up between and into the rod spherules (R). A cone pedicle (CP) is avoided. Golgi rapid triple impregnation (method 3) ($\times$ 5000).

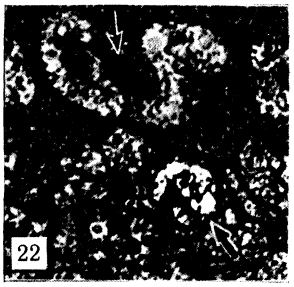
Figures 26, 27. Higher magnifications of the rod bipolar dendrites in the rod spherules. The stained dendrites are always the central (C) element of the invaginated processes. In figure 27 two central elements are seen but only one is from the stained rod bipolar cell. (L) lateral elements. Golgi rapid triple impregnation (method 3) ($\times$ 25000).



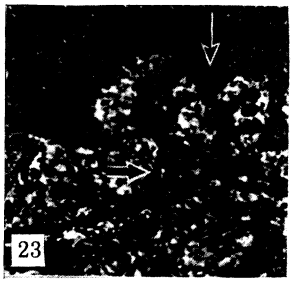
19

20

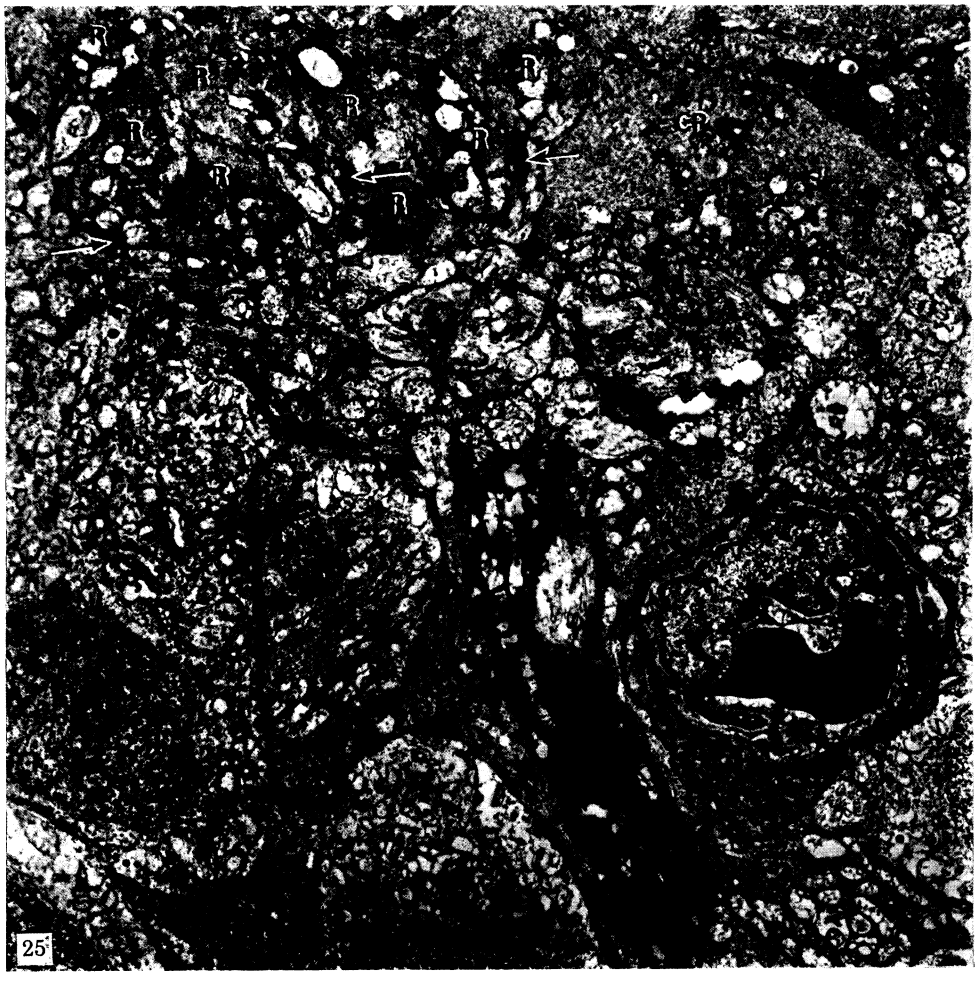
21



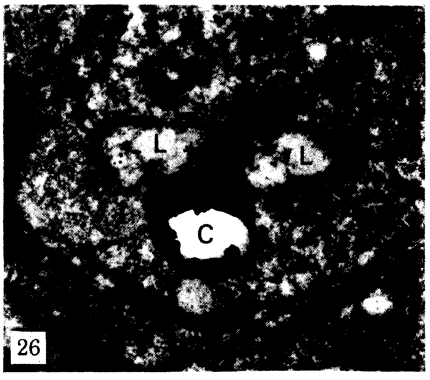
22



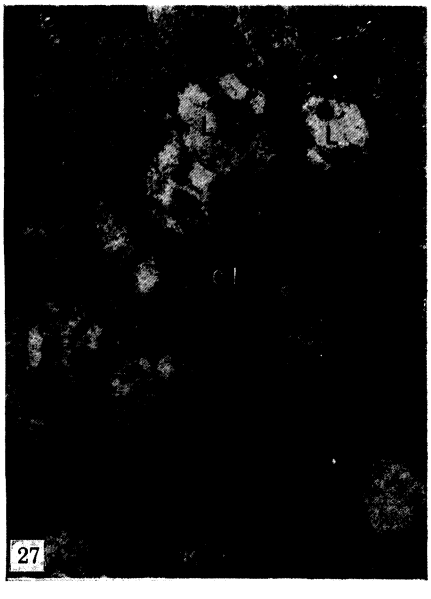
23



25

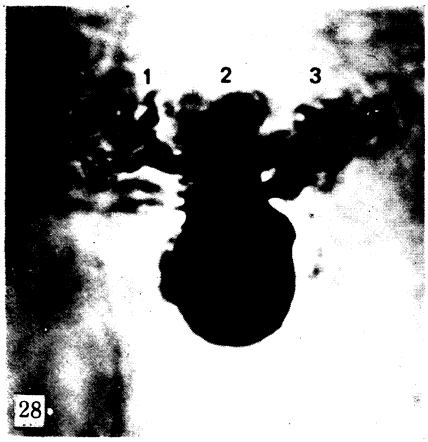


26



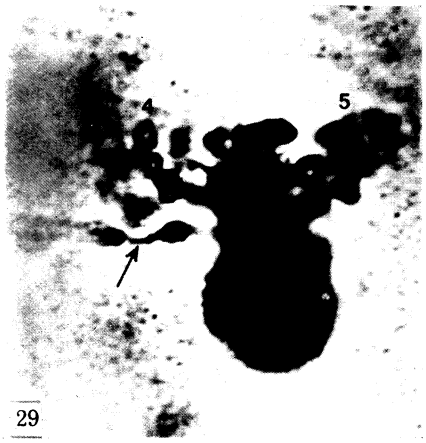
27

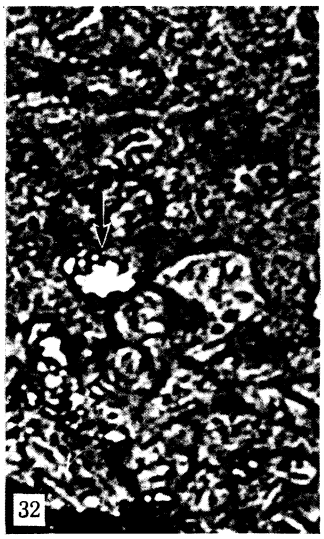
(Facing p. 270)

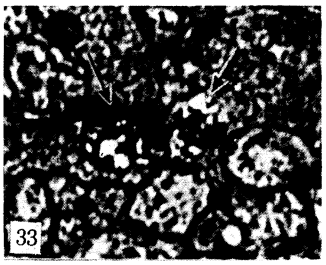

1
2
3
28

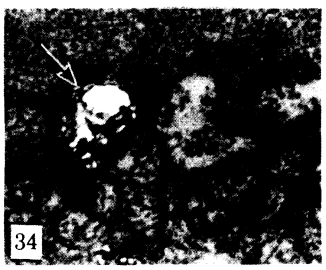

30


31


4
5
29


32

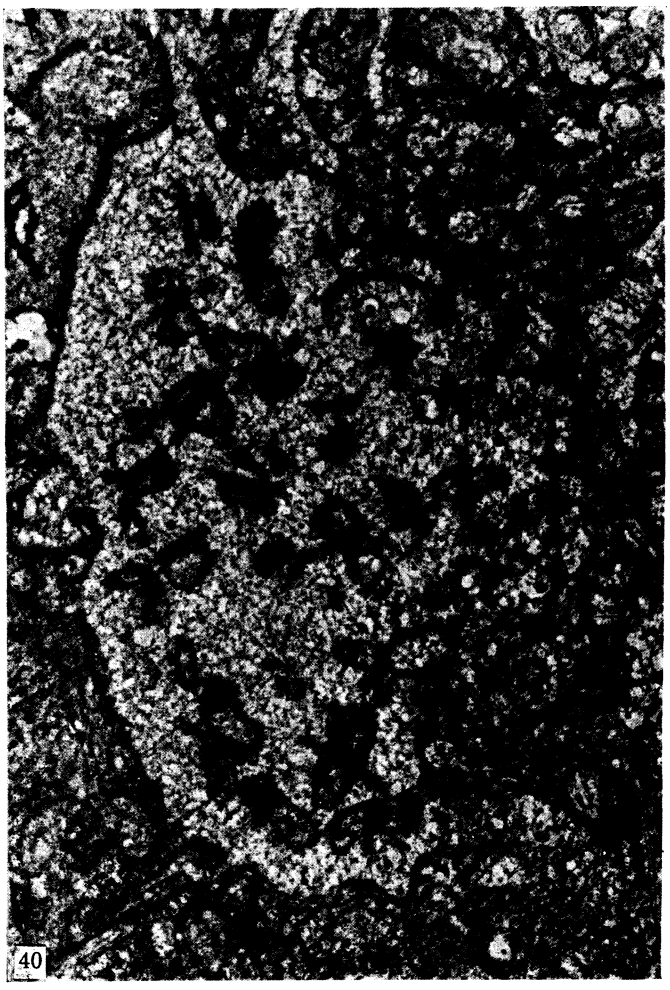

33

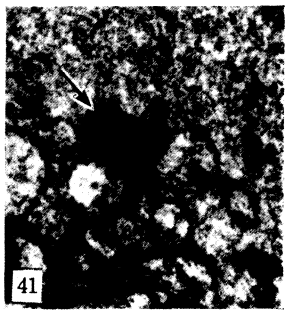

34


35

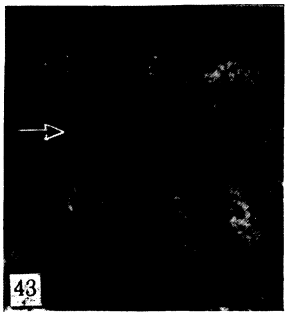

1
2
3
38


1
2
3
39


40


41


42


43

there is an overlap of flat bipolars on to cone pedicles, because certainly nowhere near all the superficial contacts on a cone pedicle can be accounted for by the number observed for the above-described impregnated flat bipolar, plus a flat midget bipolar.

Rod bipolars

A bipolar exclusive to rods was first described for vertebrates by Cajal (1933). Polyak (1941) considered this type of bipolar to contact both rods and cones in the primate retina and he named it the mop bipolar. He claimed that the mop bipolar dendrites 'adhere to the side of cone pedicles and, besides, touch the adjoining rod spherules'. Electron microscopy (Missotten 1965) shows that there are no synapses on the sides of cone pedicles or rod spherules, and fingers of glia lie between any dendrites passing up to the rods and the sides of cones. The recent light microscope observations of Boycott & Dowling suggest that the size of the dendritic terminals and varying levels at which they end above the line of cone pedicle bases is circumstantial evidence that the rod bipolar dendrites are contacting rods spherules only. Between 15 and 45 stained terminals on a rod bipolar have been counted by Boycott & Dowling (1969).

The rod bipolar is easily recognized by light microscopy. The large perikaryon lies in the inner nuclear layer and sends a thick main dendrite up to the outer plexiform layer, just within which it divides into three or more branches. Side branches of these pass vertically or obliquely through the outer plexiform layer giving off fine dendrites that terminate at different levels. The whole tuft of dendrites usually has a diameter of between 15 and 30 μm. The axon passes down into the inner plexiform layer to end as widespread terminals close to the ganglion cell perikarya.

A single small rod bipolar has been serially sectioned for electron microscopy. The dendritic spread of this cell was 15 μm. Figure 25, plate 53, shows the main dendrite and two branches

---

Description of plate 54

Figures 28, 29. Light micrographs of a type *A* horizontal cell in two planes of focus. Five groups of dendritic terminals are apparent and each group of terminals appears as an arrangement of optical sections of spheres in the same plane. The axon is arrowed. Golgi rapid triple impregnation (method 3) (× 2000).

Figures 30, 31. Electron micrographs of triads of a cone pedicle contacted by a type *A* horizontal cell. The dendritic terminals are the lateral elements of the triads. The lateral elements appear rounded and densely stained (figure 31) or slightly distorted with a clear centre (arrowed in figure 30). Golgi–Colonnier (method 1) (× 14000).

Figures 32 to 35. Higher magnification electron micrographs of triads receiving a stained dendrite from a type *A* horizontal cell. One triad has both lateral elements stained (figure 33) but usually only the one is stained (arrowed). The lateral elements appear to retain their normal size and shape. Occasionally the stained lateral element appears as a rim of stain around a hole (figures 32, 34). Golgi rapid triple impregnation (method 3) (× 22000).

Figures 38, 39. Light micrographs of a type *B* horizontal cell in two different focuses. Three groups of dendritic terminals can be seen. The terminals appear in a branched arrangement and are of fine diameter ending at different levels. Golgi–Colonnier (method 4) (× 2000).

Figure 40. Electron micrograph of a cone pedicle contacted by a type *B* horizontal cell, cut in flat section. Seventeen of the lateral elements contain fine dust-like stain. Twenty one ribbons and portions of twenty five triads can be seen. Golgi–Colonnier (method 4) (× 13000).

Figures 41 to 43. Triads with stained lateral elements (arrowed) from type *B* horizontal cells. In figure 41 the lateral element is of normal diameter and filled with a dust-like stain deposit. Figures 42 and 43 are lateral elements with the 'exploded' type of stain deposit. The lateral elements are grossly distorted and enlarged and the stain unevenly deposited. Note figures 42 and 43 are the same magnification as figure 41. Golgi–Colonnier (method 4) (× 15000).

in the outer plexiform layer. Dendritic terminals are seen squeezing between rod spherules to reach other rod spherules stacked at a higher level. A constant finding throughout the whole series of sections was that the rod bipolar always contacted rod spherules only and the cone pedicles were completely avoided. Another important finding was the position of the dendritic contact with the rod spherules. Figures 26 and 27, plate 53, show the typical appearance of the rod bipolar dendrite penetrating into the invagination of the rod spherule as one of the central elements. The larger lateral elements were never stained. This cell contacted 33 rod spherules, and never more than one dendrite penetrated a rod spherule. As there are usually two or more central elements in each rod spherule (Missotten 1965) this finding must mean that the remaining unstained central elements come from rod bipolars with overlapping fields.

Horizontal cells and horizontal cell dendrites

Cajal (1933) described at least two types of horizontal cell in the vertebrate retina and suggested that they were probably exclusive to either rods or cones. Polyak (1941) described only one type, with dendrites in contact with cones but having an axon linking a group of both rods and cones some distance away in the retina. Boycott & Dowling (1969) distinguish two types of horizontal cell on the basis of light microscope differences in appearance of the dendritic terminals. Type *A* horizontal cells have large terminals, measuring 0.5 to 1.0 μm in diameter, which are arranged in groups in one plane of focus. The large dendritic terminals usually appear as optical sections of spheres in flat section, and each group of terminals is the size of a cone pedicle. For these among other reasons type *A* horizontal cells are thought to be cone horizontal cells by Boycott & Dowling (1969). These type *A* horizontal cells appear to be connected to six to nine cones, because usually only this number of groups of terminals are observed (Boycott & Dowling 1969). Type *B* horizontal cells are primarily distinguished from type *A* by the shape of the dendritic terminals. The terminals are smaller in diameter than those of type *A* and appear to end at different levels above the line of cone pedicles. The terminals appeared to be arranged in 10 to 12 different groupings. For these reasons type *B* horizontal cells were thought by Boycott & Dowling to be possible rod horizontal cells.

Figures 28 and 29, plate 54, are views of a horizontal cell at two different focuses, 2 mm from the foveal pit, and viewed from the side. This cell fits the light microscope criteria for a type *A* horizontal cell of Boycott & Dowling. The cell body lies in the outer row of the inner nuclear layer, and the tuft of dendrites arises from the short neck of the cell. The axon (arrowed) arises from the junction of the dendrites with the perikaryon. The dendritic terminals are large (0.5 to 1.0 μm) spherical objects and the span of each group is 7 μm, which is approximately the diameter of a cone pedicle base in this region of the retina.

Electron microscopy of such a type *A* cell is shown in figures 30 to 35, plate 54. The stained dendrites from the Golgi-impregnated cell enter the cone pedicles only, and always occupy the lateral position when present in the triads. The purpose of including figures 30 & 31 is to demonstrate the different appearance of the staining from that of figures 32 to 35 (see figure legends) owing to the two different Golgi techniques used. The diameters of the stained lateral elements in the Golgi rapid triple impregnated series (figures 32 to 35) were closely comparable to those of well-fixed primate material (Missotten 1965; Dowling & Boycott 1966) in being approximately 0.5 μm in diameter; but the Golgi–Colonnier method (figures 30 and 31) often produced a slight swelling of the lateral elements and a suggestion of contortion. However, in every instance the stained terminals of type *A* horizontal cells enter the triad as the lateral

element. Usually only one of the pair of lateral elements is stained but occasionally (figure 33) both of the pair are stained. The staining of both of the lateral elements of a triad occurs most frequently in the cone pedicle that lies in the centre of the dendritic field of the impregnated horizontal cell.

A type *A* horizontal cell was serially sectioned for electron microscopy and a quantitative assessment of both the number of cone pedicles contacted and number of dendrites inserted per cone pedicle was possible. Figure 36 is a direct reconstruction from tracings of electron micrographs staggered to show all the dendrites and cone pedicles clearly. This horizontal cell

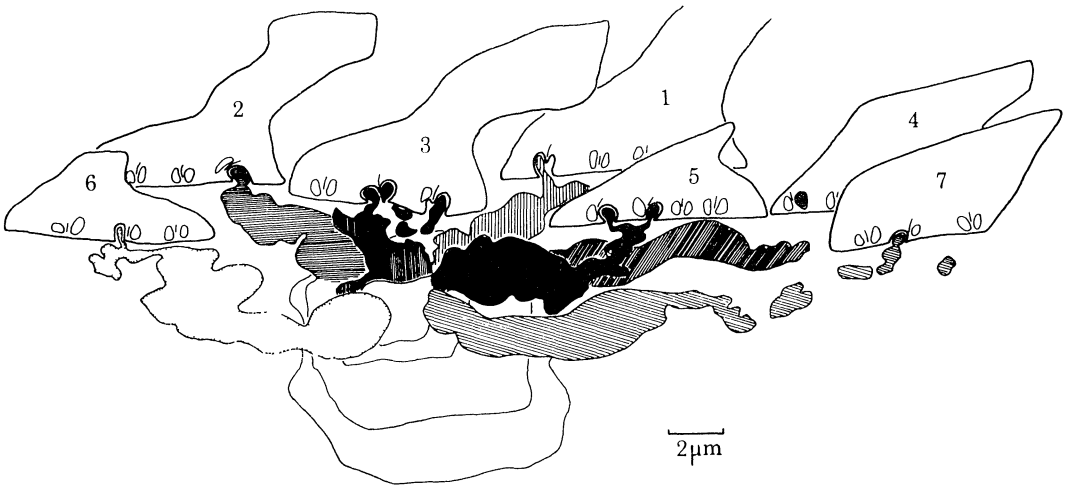


FIGURE 36. Drawing from tracings of serial section of a type *A* horizontal cell. The tracings were staggered. The seven arms of the horizontal cell are represented by different shadings. The corresponding cone pedicles contacted are numbered.

contacted seven cone pedicles. There was no evidence of any dendrites passing to rods. All the cone pedicles in the dendritic field were contacted. There is some evidence from light microscope data (Boycott & Dowling 1969) that cone pedicles in the centre of a dendritic field are occasionally avoided but this has not been substantiated in this electron microscope study. In figure 37 the actual number of stained dendrites contacting each pedicle is represented for this type *A* horizontal cell. Pedicles 2 and 3 were most centrally located in the dendritic field and receive the greatest number of contacts. Pedicle 3 contained a triad with both lateral elements stained. The number of dendrites received per cone pedicle from the horizontal cell decreases as the cone pedicles come to lie further peripheral in the horizontal cell's dendritic field. Table 3 summarizes the counts of dendritic contacts per cone for this horizontal cell. The triads in the cone pedicles that have one or both lateral elements unstained must be receiving dendrites from other horizontal cells. There is no cell process other than horizontal cell dendrites (see later section on horizontal cell axon terminals) that provides the lateral elements of the triads of the cone pedicles, and therefore, there must be overlap of other horizontal cells onto each cone pedicle.

Figures 38 and 39, plate 54, are light micrographs of a horizontal cell that fits the light microscope criteria for a type *B* horizontal cell (Boycott & Dowling 1969). Twelve groups of dendritic terminals were present, although only three are in focus in the micrographs. The cell lies in an oblique plane. The branching characteristic of the groups is identical to that

described for type *B* horizontal cells by Boycott & Dowling, and the terminals are smaller than type *A* terminals and end at different levels. None have the appearance of optical sections of spheres.†

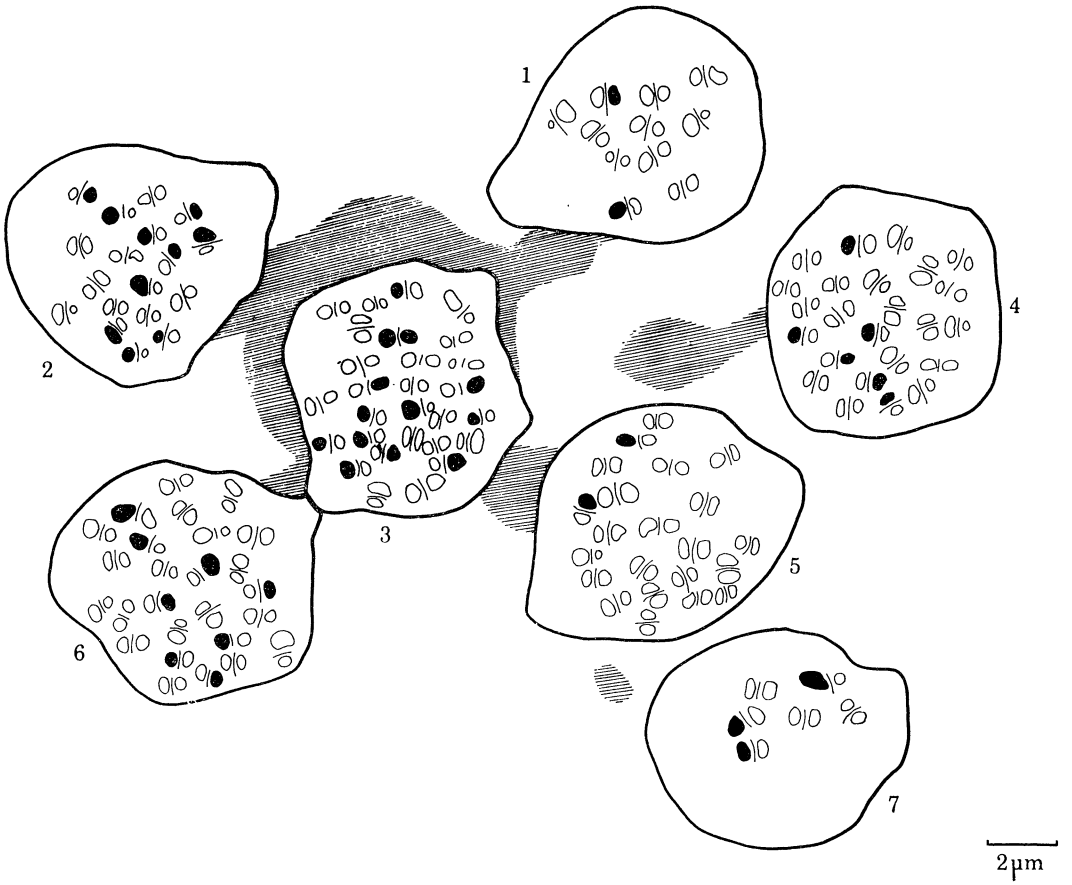


FIGURE 37. Drawing from the vertical tracings that have been converted to a flat section of the type *A* horizontal cell of figure 36. The cell body lies below pedicles 2 and 3 and the main dendrites are indicated by the shaded areas. The triads that were observed in serial section for each cone pedicle are indicated by the ribbon and two lateral elements only. The stained lateral elements received from the horizontal cell are black. Pedicles 3 and 6 were seen to touch each other.

TABLE 3. THE NUMBER OF DENDRITIC TERMINAL PROCESSES SEEN ON THE SMALL-FIELD HORIZONTAL CELL ILLUSTRATED IN FIGURES 36 AND 37

cone pedicle number	triads seen	stained lateral elements	approximate percentage
1	11	2	10
2	18	10	25
3	28	13	25
4	25	6	12
5	22	2	5
6	25	8	15
7*	6	3	25

* This cell and its data were incomplete.

† This horizontal cell and most of the other type *B* horizontal cells described here were selected by B. B. Boycott as type specimens identical to those illustrated by Boycott & Dowling (1969). The cells were from their material and are, therefore, all stained by the Golgi–Colonnier method.

Electron microscopy of such a type *B* horizontal cell shows that, contrary to the Boycott & Dowling prediction, it also is exclusive to cones and the dendritic terminals invaginate into the cone pedicles as the lateral elements of triads (figures 40 to 43, plate 54). The connexions of type *B* cells are no different from type *A* horizontal cells. The reason that type *B* horizontal cells appear so different by light microscopy is evident when the way in which the stain has been deposited in the terminals is analysed by electron microscopy.

Figures 42 and 43 show the appearance of these type *B* horizontal cell dendritic terminals as the lateral elements of the triad. The stain appears to have exploded up into the cytoplasm of the cone pedicle. The stain is not deposited heavily enough to make the dendritic terminals appear grossly enlarged by light microscopy. Rather the appearance is that of fine processes penetrating up to terminate at different levels. Because of the irregularity of shape and deposit of stain throughout the exploded terminal, this would never look like an optical section of a sphere, as do many of the rounded and usually slightly swollen, well-impregnated terminals of the type *A* horizontal cell seen by light microscopy. This particular horizontal cell was found to contact seven cones.

Three type *B* cells have been extensively analysed by serial section electron microscopy. In two of them the staining showed another variation compared with the 'exploded' appearance. Figures 40 and 41 illustrate the appearance of these type *B* terminals. The lateral elements are quite normal in size, i.e. closely comparable to good electron microscope standards (Missotten 1965). The stain either fills the lateral elements entirely as in figure 41 or, more often the stain appears as a sparse fine dust-like deposit (figure 40). In figure 40 a cone has been cut in flat section such that most of the triads are cut in one plane. Twenty-one ribbons are visible and portions of 25 triads. Staining is present in 17 of the lateral elements, and 3 of the triads have both lateral elements stained.

Two of these horizontal cells contacted 12 cones and the other, 10 cones. In no case was there any evidence of cone selection, i.e. the horizontal cell made contact with all the cone pedicles in its dendritic field. Figure 44 is a drawing from tracings of electron micrographs of a type *B* horizontal cell cut in flat section. The shaded area is the outline of the cell body and some of the main dendrites. The perikaryon lies below pedicle 5. Twelve cone pedicles are contacted and the number of stained lateral elements per cone is represented by the black symbols. Table 4 summarizes the numbers of contacts per cone for the horizontal cell of figure 44.

Thus, a total of eleven horizontal cells have been studied here by electron microscopy. Three were selected as typical of Boycott & Dowling's type *A* cell. One of these (figures 28, 29 and figures 32 to 35) was stained by the Golgi rapid triple impregnation method and there was no sign of artefact. The other two were stained by the Golgi–Colonnier method (figures 30 and 31) and were characterized by well impregnated but slightly swollen, rounded terminals. All three contacted the small number of cone pedicles (usually 7). Eight horizontal cells were selected as being type *B* horizontal cells. All were thought to have the small terminals, ending at different levels and arranged in approximately 12 groups as described for type *B* cells by Boycott & Dowling. All were stained by the Golgi–Colonnier method. They showed the following two variations in staining artefact when viewed by electron microscopy: (1) exploded, unevenly stained terminals; and (2) normal size but understained terminals with the fine-dust appearance of the stain. Three of these type *B* cells made contact with the larger number of cones, i.e. 10 to 12 (figure 44). The other five contacted the smaller number, about seven cones.

In other words, cells with the type *B* appearance by light microscopy were not consistently wired up to the larger number of cones.

These findings have revealed two important points about horizontal cells. First, the findings have revealed that horizontal cells, although basically of one type only, can be divided into a

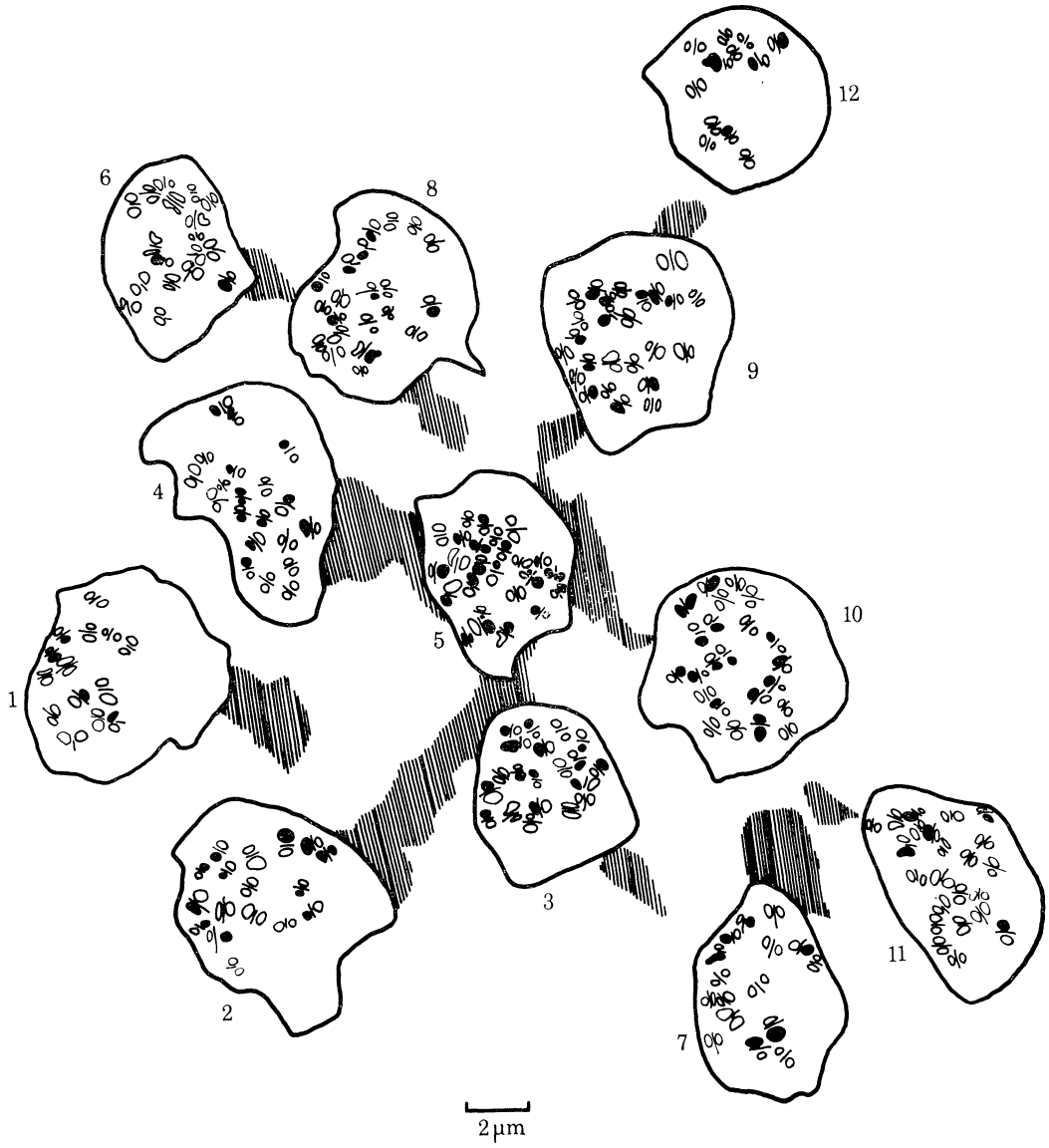
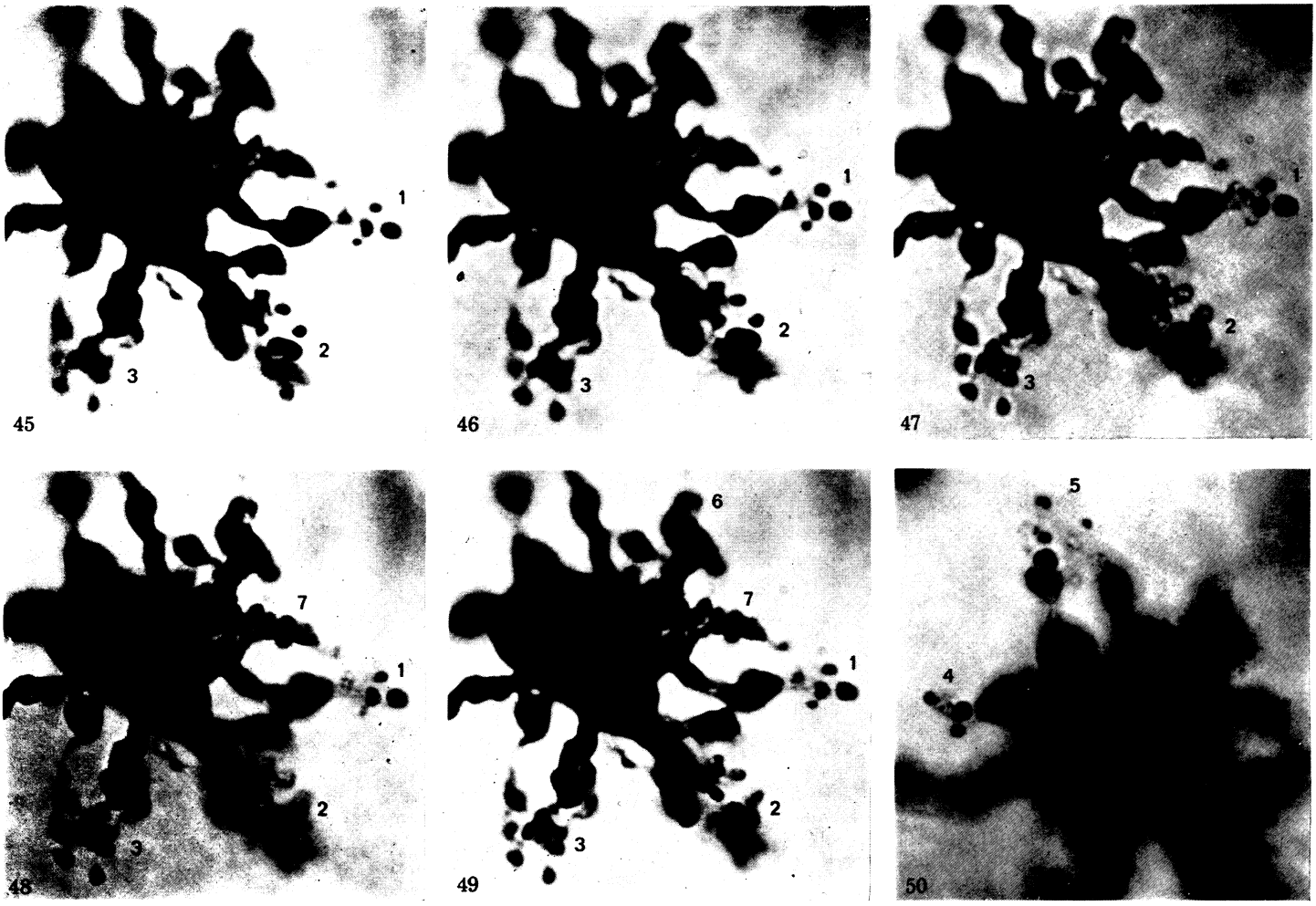


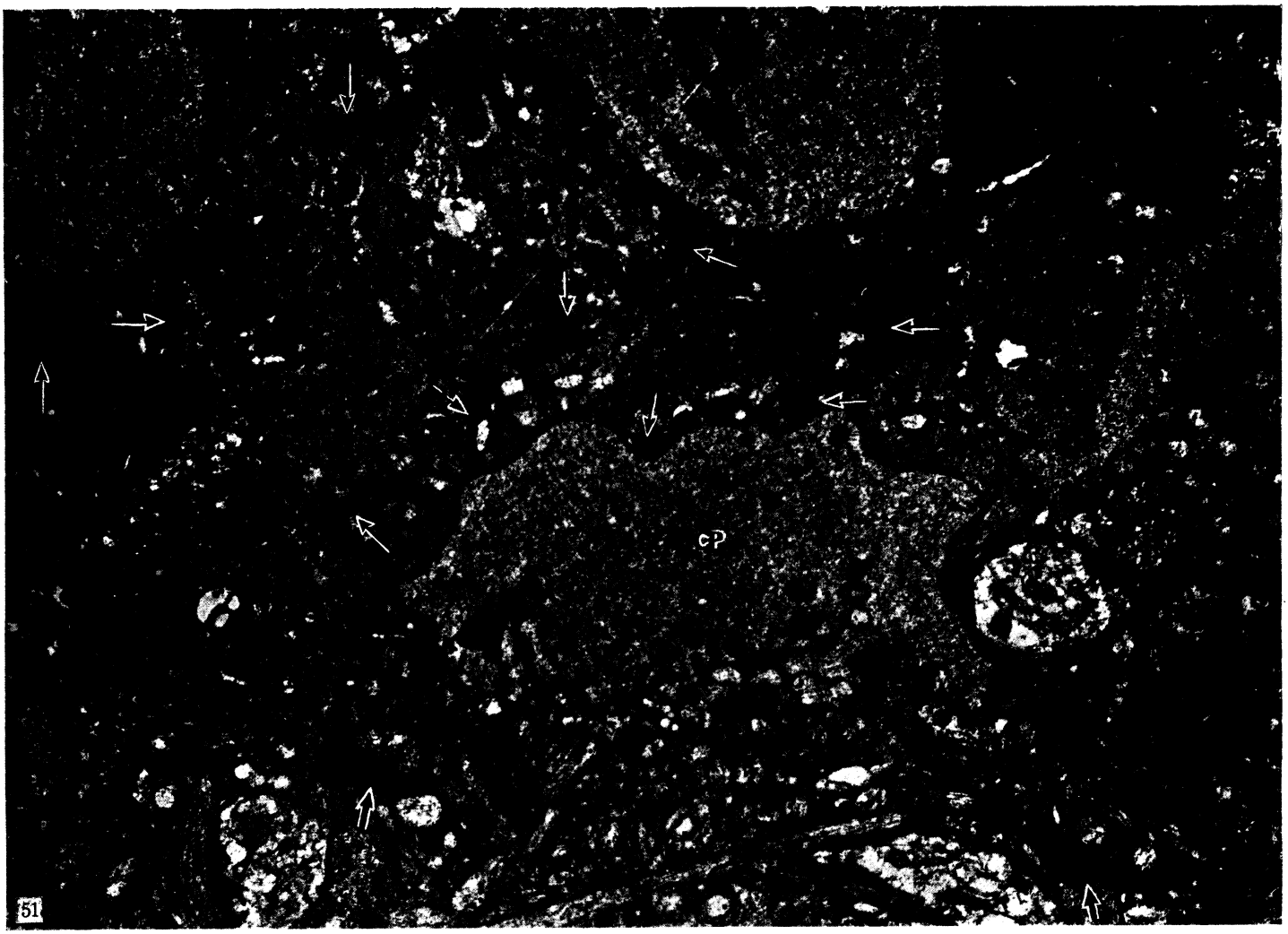
FIGURE 44. Tracing from electron micrographs of a type *B* horizontal cell in flat section. The cell body lies below pedicle 5, and some of the main dendrites are represented by the shaded areas. The number of triads seen for each pedicle are indicated by the ribbon and two lateral elements, and the contribution of dendritic terminals from the horizontal cell are indicated by the black lateral elements.

small field and a large field type. Some horizontal cells contact about seven cones, whereas others contact about twelve cones. The overlap and relationship of the large-field and small-field horizontal cells are yet to be established exactly. However, it seems likely from the data that there is room for at least four small-field horizontal cells and possibly six large-field horizontal cells to overlap onto a single cone pedicle.



FIGURES 45 to 50. Through focus series of a horizontal cell (kindly photographed by Miss E. M. Crawley and donated by B. B. Boycott) that shows terminals of varying size, that could be characteristic of both type *A* and type *B* horizontal cells. In group 1 the largest terminal has an optical section of a sphere appearance (Type *A*) and measures about 1 μm across; the smallest terminals are typical type *B* horizontal cell terminals. Group 2 consists of two large type *A* terminals (1.0 μm), eight or nine medium sized terminals (0.5 μm) and four small type *B* terminals. The span of the group is 8 μm. Group 3 consists of nine terminals of intermediate size, and the diameter of the group is 6 to 7 μm. Group 4 consists of one medium and two small terminals. Group 5 has seven terminals all of which are small except two that seem to have coalesced to form a blob. Group 6 is not clearly resolvable and group 7 has three typical type *A* terminals, which appear as optical sections of spheres. Golgi–Colonnier (method 4) ($\times$ 2000).

(*Facing p.* 276)



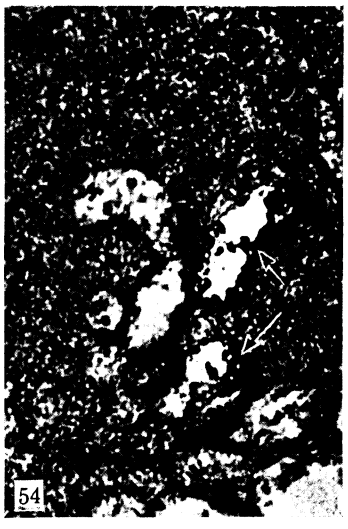


FIGURE 51. Low-power electron micrograph of horizontal cell axon terminals cut in flat section. The stained terminals always occupy the position of the lateral elements in the rod spherules (thin arrows). The cone pedicles are avoided. Branches of the axon are seen running through the outer plexiform layer (thick arrows). Golgi-Colonnier (method 4) (× 9000).

FIGURES 52, 53. Higher magnification of rod spherules cut in flat section. The lateral elements are grossly swollen and fill almost the entire diameter of the rod spherules. The stain (arrowed) is deposited unevenly usually leaving a clear centre. These are type *A* axon terminals by light microscope criteria. Golgi–Colonnier (method 4) (× 20000).

FIGURES 54, 55. Rod spherules cut in oblique section. The stained lateral elements retain their normal size and lobed appearance (arrowed). The stain is deposited evenly throughout the dendritic terminal. Note the magnification is the same as for figures 52 and 53. These are typical type *B* terminals by light microscope criteria. Golgi rapid triple impregnation (method 3) (× 20000).

Secondly it has become apparent that the Golgi–Colonnier method can give an artefactual appearance to the dendritic terminals of horizontal cells; and the size, shape and configuration of these terminals have been important criteria for distinguishing two types of horizontal cells by light microscopy. A misinterpretation of cell connexion and function could easily result if light microscopy alone were relied on. It is tempting, for example, to suggest that type *B*

TABLE 4. THE NUMBER OF DENDRITIC TERMINAL PROCESSES SEEN ON THE LARGE-FIELD HORIZONTAL CELL ILLUSTRATED IN FIGURE 44

cone pedicle number	triads seen	stained lateral elements	approximate percentage
1	14	4	10
2	17	12	33
3	20	13	25
4	25	12	25
5	25	18	33
6	18	2	5
7	16	6	12
8	21	7	10
9	25	11	25
10	23	16	33
11	23	5	10
12	12	4	5

horizontal cells are rod horizontal cells, because they so exactly fit the predictions of what a rod horizontal cell should look like by light microscopy (Boycott & Dowling 1969). It seems in this respect that the Golgi–Colonnier method is less reliable than the Golgi rapid triple impregnation method for staining horizontal cells. Type *A* and *B* are not distinguishable by the latter method; for this reason type *B* cells could not be selected from Golgi-rapid material and therefore all the data for type *B* cells are from Golgi–Colonnier material.

Knowing that horizontal cells stained by the Golgi–Colonnier method can look like either type *A* or type *B*, it is possible to find examples of horizontal cells which clearly possess the characteristics of both by light microscopy. Figures 45 to 50, plate 55, show a through focus series of such a cell. In figure 44 the group 1 terminals are in sharpest focus. The largest terminal is clearly an optical section of a sphere (type *A*; 1 μm) but the smallest is of the size typical of a type *B* horizontal cell terminal. Figures 46, 47 and 48 show the second group of terminals and it is seen to consist of two large terminals (type *A*; 1 μm), eight or nine medium-sized terminals (0.5 μm) and three or four small terminals (type *B*). The span of the group is 8 to 9 μm, i.e. the size of a large cone pedicle. Figures 47, 48 and 49 show group 3 in focus, and the eight or nine terminals composing it are all intermediate in size and type. The span of the group is 6 μm, again the size of a cone pedicle. Groups 4 to 7 are seen in figures 48 to 50 but are less well defined. In group 7, three of the terminals are large and appear as optical sections of spheres. Group 4 consists of one medium and two small terminals, whereas group 5 shows two medium-sized terminals lying alongside each other (so that they look like a large blob) and five other small type *B* terminals. This horizontal cell, therefore, clearly has type *A* and type *B* terminals. Probably there are as many as 10 groups of dendritic terminals, of which seven are clearly visible but three may be obscured by the tilted perikaryon of the cell.

Horizontal cell axon terminals

Electron microscopy of horizontal cell axon terminals provides further evidence that staining artefacts can cause misinterpretation of cell types seen by light microscopy. What appear to be clearly divisible types of axon terminals by light microscopy (Boycott & Dowling 1969) prove to be only staining variations of the same type by electron microscopy. All the axon terminals examined in this study go to rod spherules and occupy the position of the lateral elements of the invaginations (figures 51 to 55, plate 56). Three sets of terminals were examined because they were considered to be type *A* and two sets were thought to be type *B*. In no cases were the cone pedicles involved, although occasionally an axon was seen touching the side of the cone pedicle as it passed up to the rod spherules. There was no evidence of synaptic contact.

Type *A* axon terminals (Boycott & Dowling 1969) are distinguished in the light microscope by their large size (0.5 to 1.0 μm) and appearance as optical sections of spheres. Electron microscopy shows type *A* axon terminals to be the swollen and rounded lateral elements of the invaginations of the rod spherules as illustrated in figures 52 and 53. The stain is often deposited in a uneven ring, leaving a clear centre. These are examples from Golgi–Colonnier stained material where the lateral elements are particularly swollen, and are as much as 1.5 μm in diameter. In normal tissue (Missotten 1965) the lateral elements of the rod spherules rarely exceed 0.5 μm at their largest part and they usually have a complicated shape with several lobes connected by thin stalks.

Figures 51, 54 and 55 are of type *B* axon terminals as determined by the light microscope criteria of Boycott & Dowling and are much more typical of the normal appearance of lateral elements of rod spherules. Figures 54 and 55 are from Golgi rapid triple impregnated material (note that the magnification for these is the same as for figures 52 and 53), whereas figure 51 is Golgi–Colonnier material. Therefore, the reason for two different appearances of horizontal cell axon terminals by light microscopy is explained by the electron microscope findings. The spherical appearance of type *A* terminals is the result of swelling, rounding and patchy stain deposition in the rod lateral elements, whereas the small cluster appearance of type *B* terminals is a normal cross-section of the lobes of the lateral elements. In all cases, only one of the lateral elements is stained. This presumably means that a single rod spherule receives input from two horizontal cells.

Discussion

Figure 56 is a summary diagram of the cells and their connexions in the outer plexiform layer of the rhesus monkey as revealed by this study. The bipolar pathways from receptors to ganglion cells are exclusive either to rods or to cones. The bipolar pathways may be diffuse, collecting information from several receptors; or private, related to a single receptor. The rods are connected only to a diffuse pathway, via the rod bipolar. The cones, on the other hand, are connected to diffuse pathways via the flat bipolar and, in addition, have private pathways via the midget bipolar systems. There are two types of midget bipolar, the invaginating and the flat midget bipolar distinguished primarily by their mode of contact with the cone pedicle. Probably every cone is in contact with both an invaginating midget bipolar and a flat midget bipolar. In addition to the two types of midget bipolar, every cone pedicle is in contact with one or more diffuse (flat) bipolars. A single flat bipolar appears to be connected to six or seven cones. Each rod spherule is in synaptic contact with one to four rod bipolars. Each rod bipolar

has only a single dendrite contacting a particular rod spherule. The dendrites of horizontal cells contact a group of about seven or twelve cones, while the horizontal cell axon terminals contact a group of rod spherules. Each cone is probably in synaptic contact with at least four horizontal cells, whereas each rod is in contact with two horizontal cell axons. Aside from possible inter-receptor contacts (Dowling & Boycott 1966), the horizontal cells appear to be the only neurons in the outer plexiform layer that may be mixing information from rods and cones.

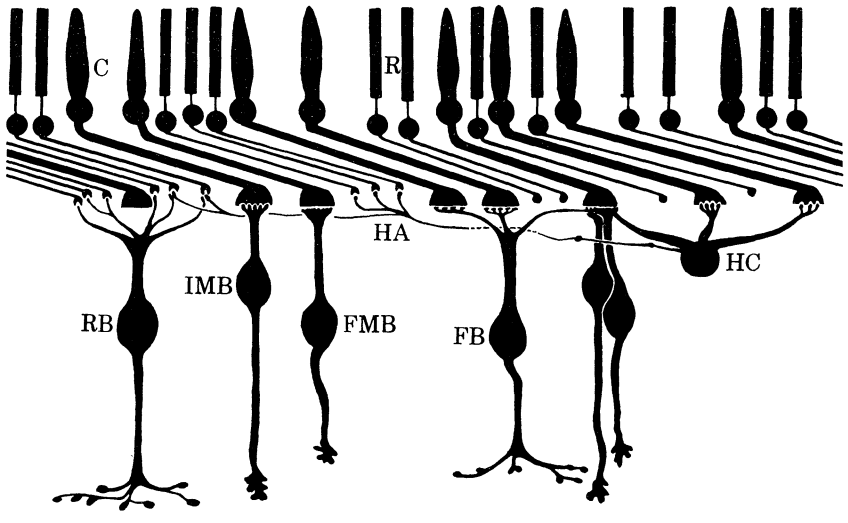


FIGURE 56. Summary diagram of the different nerve cells and the types of connexions that they make with the receptors in the outer plexiform layer of the primate retina. For clarity the diagram is drawn as a vertical section of central retina, close to the fovea. The cells are represented as they would appear in light microscope preparations of Golgi stained retinae. The cells are drawn diagrammatically and do not, except for the midget bipolar cells, show the full extent of their dendritic spread. In general the drawing does not show the overlap of cell types onto their respective receptors, the exception being the third cone from the right, where all the cell types connecting with a single cone pedicle are shown.

The cones (C) have terminals (pedicles) that end on a plane on the border of the outer plexiform layer. The rods (R) terminate in spherules that are stepped up between the cone pedicles. The rod bipolar (RB) makes contact with rod spherules only. Two or three rod bipolars contact an individual rod, but a single rod bipolar only makes one dendritic contact per rod. The dendritic field of a rod bipolar in this area of retina covers thirty to fifty rods. Horizontal cell axon terminals (HA) end in rod spherules only. Probably only two axons contribute a terminal to an individual rod. The axon is represented as a dotted line because the direct evidence for axon terminals being connected to the horizontal cell is not yet available.

The invaginating midget bipolar (IMB) is connected to a single cone. The flat midget bipolar (FMB) is also exclusive to a single cone pedicle. The flat bipolar (FB) is a diffuse cone bipolar and contacts six to seven cones. Probably six or seven flat bipolars overlap onto a single cone pedicle. The horizontal cell (HC) has dendrites ending in cone pedicles only. Some horizontal cells contact about seven cones, while others contact about twelve cones. Approximately four horizontal cells provide dendrites for a single cone pedicle.

A rod spherule is connected to rod bipolars and horizontal cell axon terminals. A single cone pedicle is connected to two midget bipolars, the flat midget and invaginating midget bipolars and in addition to diffuse cone bipolars (flat bipolars) and horizontal cells. The manner in which the dendrites of these nerve cells make contact with the receptors is depicted in the electron microscope drawings of figures 59 and 60.

Previously, only a single type of midget bipolar was thought to exist (Polyak 1941; Boycott & Dowling 1969). The light microscope conclusions of Boycott & Dowling (1969) concerning the mode of contact of midget bipolars with the cone pedicles was based on the appearance of the invaginating midget bipolars alone. The midget bipolars that the present study has shown to be the flat midget bipolars were ignored by these and previous authors because these cells were thought to be poorly stained examples of the invaginating midget bipolar. The prediction that the invaginating midget bipolar makes contact with the pedicle by inserting a dendrite into

the invaginations (Missotten 1965; Boycott & Dowling 1969) has been confirmed by this study. In addition, the study has revealed with certainty that the invaginating midget bipolar processes provide the central elements of all the triads of the cone pedicle, to which it is exclusively connected. There is no evidence of horizontal cell dendrites also occupying the position of the central element (Missotten 1965), and no other cell processes apart from the invaginating midget bipolar terminals have been seen to form the central element of the triads. Therefore, it seems reasonable to assume that every cone pedicle is in contact with an invaginating midget bipolar cell.

With this electron microscope evidence showing that there are two types of midget bipolars it has been possible to return to the Golgi material for the light microscope and find distinguishing features in addition to the shape of the dendritic top, such as the levels of the axon termination and the perikaryon position, that make the flat midget bipolar recognizable throughout the retina. Although Polyak (1941) noticed the difference between midget bipolars that have high or low axon terminals, he did not interpret this as meaning the cells made different types of contact with the cone pedicle. The realization that the flat midget bipolar makes such a different type of synaptic contact with the cone pedicle leads to the conclusion that a cone pedicle is connected to both an invaginating midget bipolar and at least one flat midget bipolar. This conclusion is first corroborated by the light microscope counts in Golgi stained retinae showing that the flat midgets are as numerous as invaginating midgets. Secondly, the cluster of dendrites around the central elements of the triads is a constant feature of all cone pedicles studied by routine electron microscopy.

The difference in level at which the flat midget bipolar and invaginating midget bipolar axon terminals end in the inner plexiform layer may be significant. Probably this means that they are in synaptic contact with different ganglion cells and are influenced by different amacrine cell systems. Polyak (1941) distinguished short and long midget ganglion cells as counterparts to short and long midget bipolars. The function of the flat midget bipolar can only be guessed at, but presumably both of the midget bipolar types are colour coded.

This study has proved that the flat bipolar is a diffuse cone bipolar and has established that the dendrites make superficial contact with the cone pedicle base as predicted by Missotten (1965) and Boycott & Dowling (1969). In addition, the electron microscope findings have shown that the flat bipolar contacts between five and seven cones, all of which lie within the bipolar's dendritic field. The overlap could be as many as six flat bipolars to a single cone pedicle. This may well mean that the flat bipolar is not colour coded. The axon of the flat bipolar, at least in the central area, terminates in the centre of the inner plexiform layer (Boycott & Dowling 1969) and could be considered presynaptic to a diffuse type of ganglion cell. Consequently the flat bipolar may be innervating a non-colour-coded ganglion cell.

It has been clearly established that rod bipolars are exclusive to rods as predicted from both light microscopy (Cajal 1933; Boycott & Dowling 1969) and electron microscopy (Missotten 1965). No evidence has been found in this study of the rod 'mop' bipolar touching cones as suggested by Polyak (1941). The finding that only one dendrite is contributed to the rod spherule from a particular rod bipolar, suggests overlap of rod bipolars on to a single rod spherule. It appears that not every rod spherule in a rod bipolars dendritic field is contacted. Figures 57 and 59, plate 57, show a drawing of a rod spherule with an accompanying electron micrograph for comparison. The figures summarize the Golgi electron microscopy findings of which cell processes end in the invagination of the rod spherule. All the synaptic contacts take place in

the invagination and there is no evidence of any superficial types of contact, other than the cone-to-rod, receptor-to-receptor contacts (Missotten 1965; Dowling & Boycott 1966).

Cones on the other hand, receive some dendrites that make contact with the cone pedicle base but do not enter the invaginations; these are the superficial contacts. Figures 58 and 60 plate 57 show a drawing and an electron micrograph of a triad of a cone pedicle, and give an interpretation of the processes contributing to the triad. The processes of flat bipolars and flat midget bipolars end on the cone base, usually in close association with the triads. The impression from the data is that the flat midget bipolar dendrites often push halfway up into the triad. This same position alongside the invaginating midget bipolar dendrite is also frequently occupied by flat bipolar dendrites. This leads to the conclusion that the arrangement of the triad is not as simple as at first thought, and should be pictured in terms of a complex of dendrites. Thus, the complex is seen to consist of the three deeply penetrating dendrites of the horizontal cells and the invaginating midget bipolar, and a cluster of six to eight dendrites around the central invaginating midget process, some of which extend part way into the invagination to get close to the synaptic ribbon and associated synaptic structures. Some dendrites of flat bipolars make superficial contacts between the triads, away from the complex.

This electron microscope study suggests that only one basic type of connexion is made by horizontal cells in the primate retina. The horizontal cell is in dendritic contact with a group of cones. The axon terminates in a group of rods. There is some evidence from other animals that separate horizontal cells exist for rods and cones. Stell (1966) and Cajal (1933) supposed that the two receptor systems had separate horizontal cells. In the primate, however, there seems to be only the one type of horizontal cell that may link rods and cones. However, it is puzzling that the primate outer plexiform layer is organized into separate rod and cone systems through exclusive rod and cone bipolars, only to be mixed by horizontal cells. If a rod horizontal cell is present in the primate retina it has not yet been identified by the presently available staining techniques. It is still possible that only the dendritic end of a cone horizontal cell and the axonal end of a rod horizontal cell are being demonstrated by the present Golgi technique, but this seems unlikely. Until direct evidence is available that the rod axon terminals are linked to the perikaryon of a cone horizontal cell, this question remains unanswered. Axon terminals ending

---

Description of plate 57

Figure 57. Electron micrograph of a rod spherule of the monkey retina for comparison with the summary diagram of figure 59 (× 58000).

Figure 58. Electron micrograph of a triad from a cone pedicle for comparison with the summary diagram of figure 60 (× 42000).

Figure 59. Summary diagram of a rod spherule to show the arrangement of the processes making synaptic contact. The lobed lateral elements are horizontal cell axon terminals, each from a different horizontal cell (HA1 and HA2). The central elements are rod bipolar dendrites from different rod bipolars (RB1 and RB2). Figure 57 is a normal electron micrograph from which the drawing has been made and slightly adapted to indicate the elements contributing to the invagination.

Figure 60. Summary diagram of the configuration of a cone pedicle triad. The horizontal cell dendrites, usually from different horizontal cells (HC1 and HC2) form the lateral elements of the invagination. The invaginating midget bipolar (IMB) pushes up into the invagination to lie between the two lateral elements. The synaptic ribbon and synaptic vesicles point into the junction of the three processes. The flat midget bipolar (FMB) dendrites lie alongside the invaginating midget bipolar dendrite and push part way up into the triad, but are in contact with the cone pedicle base. The flat bipolar (FB) terminals are also clustered around the invaginating bipolar dendrite and make superficial contact on the cone pedicle base. The drawing is an adapted tracing of the normal electron micrograph of figure 58.

as the lateral elements of the triads in cone pedicles as suggested by Missotten (1965) have not been found. The lateral elements of cone triads always appear to be dendritic, whereas the lateral elements of rod spherules are the axonal endings of horizontal cells. Although all the horizontal cells make the same dendritic connexions, there is a suggestion from the data that there may be two types of horizontal cells on the basis of number of cones contacted. There appear to be small field and large field horizontal cells.

Horizontal cell dendrites from the same cell rarely occupy both lateral elements of a triad except occasionally in the cone pedicles in the centre of a horizontal cell's dendritic field. This means that usually a triad consists of dendrites from different horizontal cells. The significance of this remains obscure. The quantitative results have indicated that there is probably an overlap of at least four and possibly as many as six horizontal cells onto a single cone pedicle.

I am indebted to J. E. Dowling and B. B. Boycott for extremely helpful discussion and critical reading of the manuscript. B. B. Boycott provided valuable advice and donated some of the light microscope material. This work was supported in part by a USPHS grant (NB 05336) to J. E. Dowling and a travel grant from the Royal Society to B. B. Boycott. Publication no. 23 from the Augustus C. Long Laboratories of the Alan C. Woods Building, Wilmer Institute.

References

Blackstad, T. W. 1965 Mapping of experimental degeneration by electron microscopy of Golgi preparations *Z. Zellforsch.* **67**, 819–834.

Boycott, B. B. & Dowling, J. E. 1969 Organization of the primate retina: light microscopy. *Phil. Trans. Roy. Soc. Lond.* B **255**, 109–184.

Cajal, S. R. 1933 La rétine des vertébrés. *Trabajos* (*Travaux*). *Lab. Invest. Biol. Madrid.* **28** Appendix, 1–141.

Cohen, A. I. 1960 The ultrastructure of the rods of the mouse retina. *Am. J. Anat.* **107**, 23–48.

Cohen, A. I. 1961 The fine structure of the extrafoveal receptors of the rhesus monkey. *Expl. Eye Res.* **1**, 128–136.

Cohen, A. I. 1963 The fine structure of the visual receptors of the pigeon. *Expl. Eye Res.* **2**, 88–97.

Cohen, A. I. 1964 Some observations on the fine structure of the retinal receptors of the American gray squirrel. *Invest. Ophthalm.* **3**, 198–216.

Cohen, A. I. 1965 Some electron microscope observations on interreceptor contacts in the human and macaque retinae. *J. Anat., Lond.* **99**, 595–610.

Colonnier, M. 1964 The tangential organization of the visual cortex. *J. Anat., Lond.* **98**, 327–344.

de Robertis, E. & Franchi, C. M. 1956 Electron microscopic observations on synaptic vesicles and synapses of the retinal rods and cones. *J. biophys. biochem. Cytol.* **2**. 307–318.

Dowling, J. E. 1965 Foveal receptors of the monkey retina: fine structure. *Science, N.Y.* **147**, 57–59.

Dowling, J. E. & Boycott, B. B. 1966 Organization of the primate retina: electron microscopy. *Proc. Roy. Soc. Lond.* B **160**, 80–111.

Dowling, J. E., Brown, J. E. & Major, D. 1966 Synapses of horizontal cells in rabbit and cat retinas. *Science N.Y.* **153**, 1639–1941.

Dowling, J. E. 1968 Synaptic organization of the frog retina: an electron microscope analysis comparing the retinas of frogs and primates. *Proc. Roy. Soc. Lond.* B **170**, 205–228.

Dowling, J. E. & Werblin, F. 1969 Organization of the retina of the mudpuppy, *Necturus maculosus*: I. Synaptic structure. *J. Neurophysiol.* **32**, 315–338.

Evans, E. M. 1966 On the ultrastructure of the synaptic region of the visual receptors in certain vertebrates. *Z. Zellforsch.* **71**, 499–516.

Kalberer, M. & Pedler, C. 1963 The visual cells of the alligator: an electron microscope study. *Vision Res.* **3**, 323–329.

Kolb, H., Boycott, B. B. & Dowling, J. E. 1969 A second type of midget bipolar cell in the primate retina. Appendix. *Phil. Trans. Roy. Soc. Lond.* B **255**, 177–184.

Ladman, A. J. 1958 The fine structure of the rod-bipolar cell synapse in the retina of the albino rat. *J. biophys. biochem. Cytol.* **4**, 459–466.

Missotten, L., Applemans, M. & Michiels, J. 1963 L'ultrastructure des synapses des cellules visuelles de la retine humaine. *Bull. Mém. Soc. fr. Opthal.* **76**, 59–82.

Missotten, L. 1965 *The ultrastructure of the human retina.* Brussels: Arscia Uitgaven N.V.

Pedler, C. & Tansley, K. 1963 The fine structure of the cone of a diurnal gecko (*Phelsuma inunguis*). *Expl. Eye Res.* **2**, 39–47.

Pedler, C. 1953 The fine structure of the radial fibres in the reptile retina. *Expl. Eye Res.* **2**, 296–303.

Pedler, C. & Tilly, R. 1964 The nature of the gecko visual cell. A light and electron microscope study. *Vision Res.* **4**, 499–510.

Pedler, C. M. H. & Tilly, R. 1965 The serial reconstruction of a complex receptor synapse. *Wiesbaden Symposium, The structure of the eye* 1965 (ed. J. W. Rohen). Stuttgart: F. K. Schattauer-Verlag.

Polyak, S. L. 1941 *The retina.* Chicago: University of Chicago Press.

Sjöstrand, F. S. 1953 The ultrastructure of the inner segments of the retinal rods of the guinea-pig eye as revealed by electron microscopy. *J. Cell. Comp. Physiol.* **42**, 45–70.

Sjöstrand, F. S. 1958 Ultrastructure of retinal rod synapses of the guinea-pig eye as revealed by three-dimensional reconstructions from serial section. *J. Ultrastruct. Res.* **2**, 122–170.

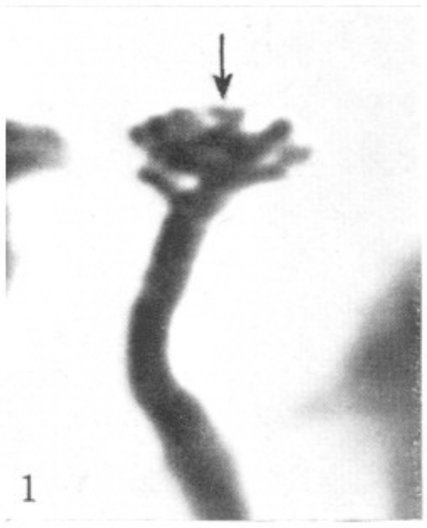
Sjöstrand, F. S. 1961 Electron microscopy of the retina. In *The structure of the eye* (ed. G. K. Smelser), pp. 1–28. New York: Academic Press.

Stell, W. K. 1965 Correlation of retinal cytoarchitecture and ultrastructure in Golgi preparations. *Anat. Rec.* **153**, 389–398.

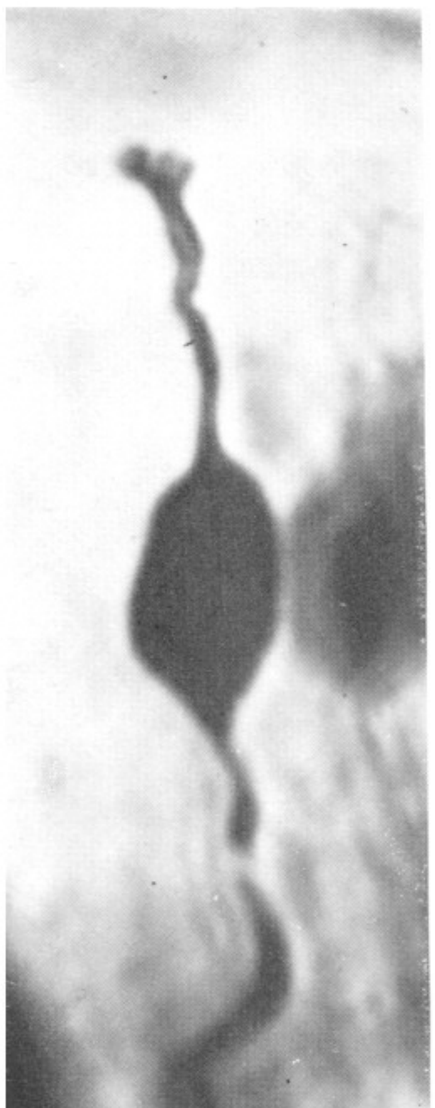
Stell, W. K. 1967 The structure and relationship of horizontal cells and photoreceptor bipolar synaptic complexes in goldfish retina. *Am. J. Anat.* **121**, 401–424.

Villegas, G. M. 1960 Electron microscopic study of the vertebrate retina. *J. gen. Physiol.* **43** (Suppl.), 15–43.

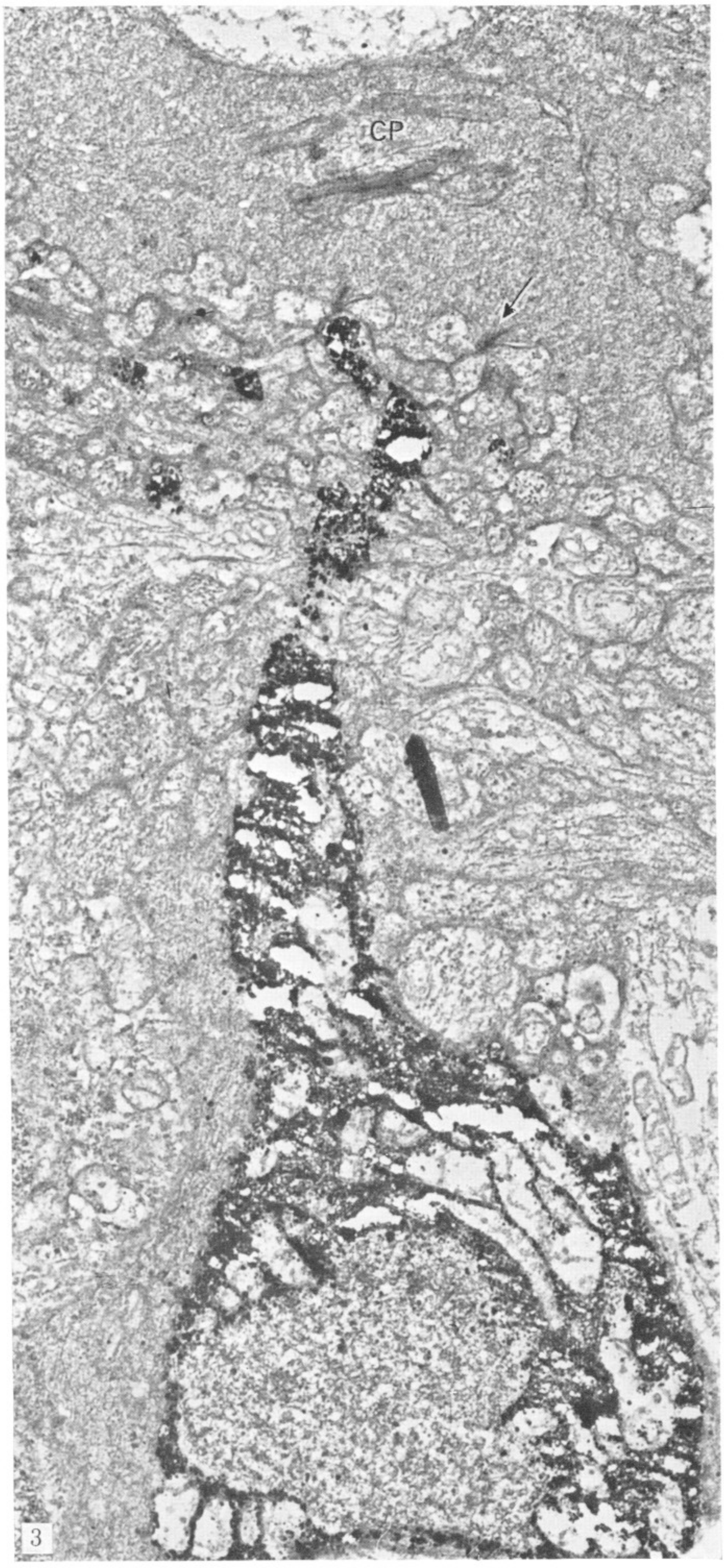
Villegas, G. M. 1964 Ultrastructure of the human retina. *J. Anat., Lond.* **98**, 501–514.



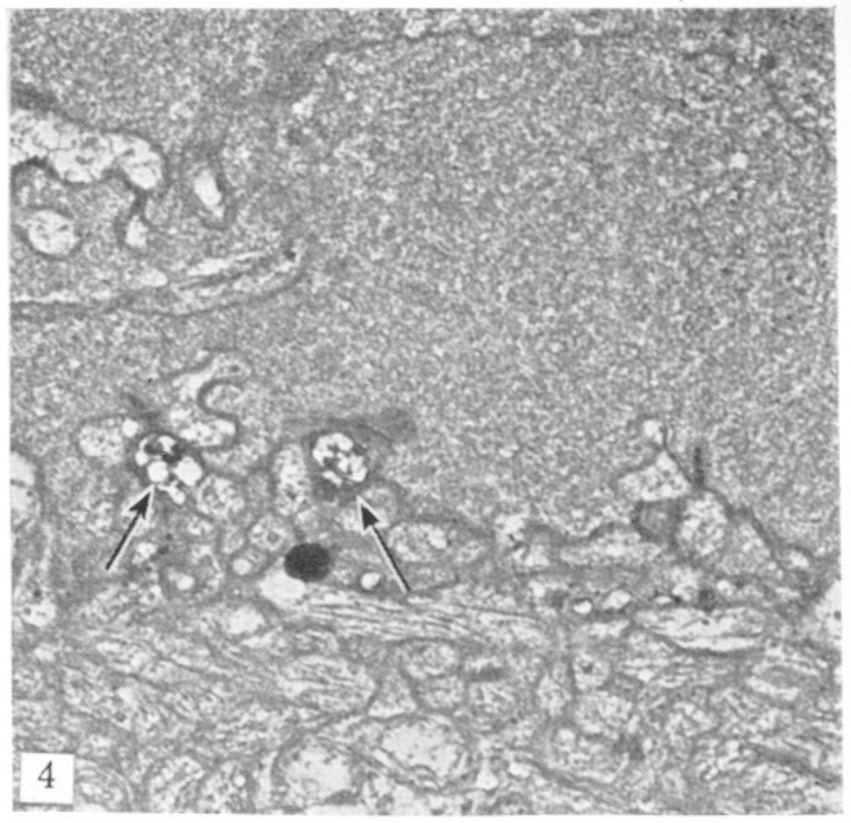
1



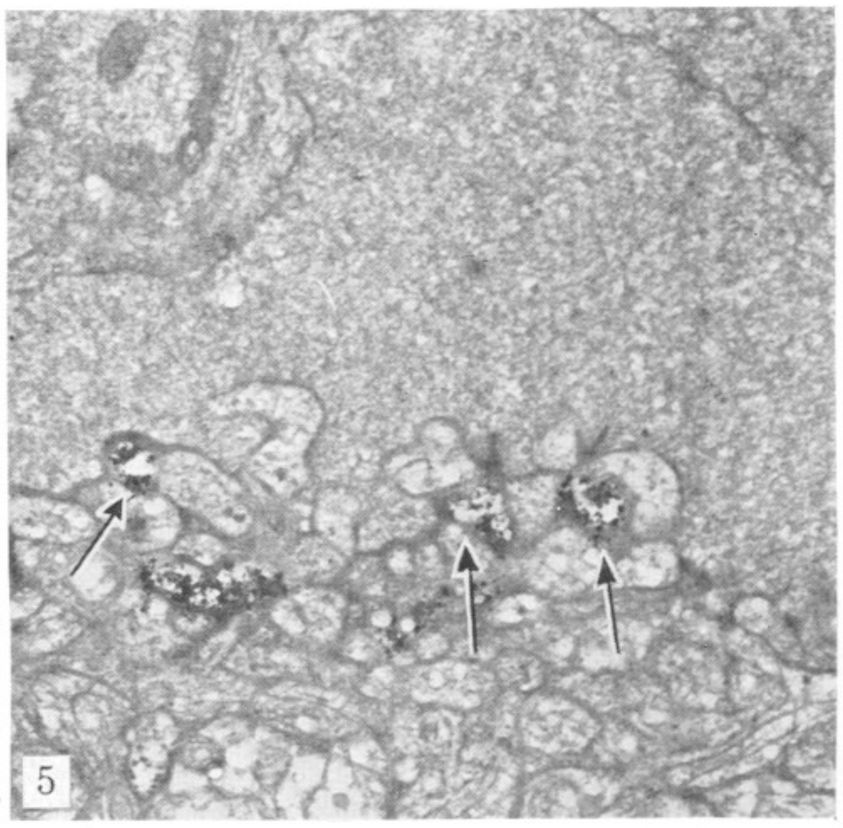
2



3

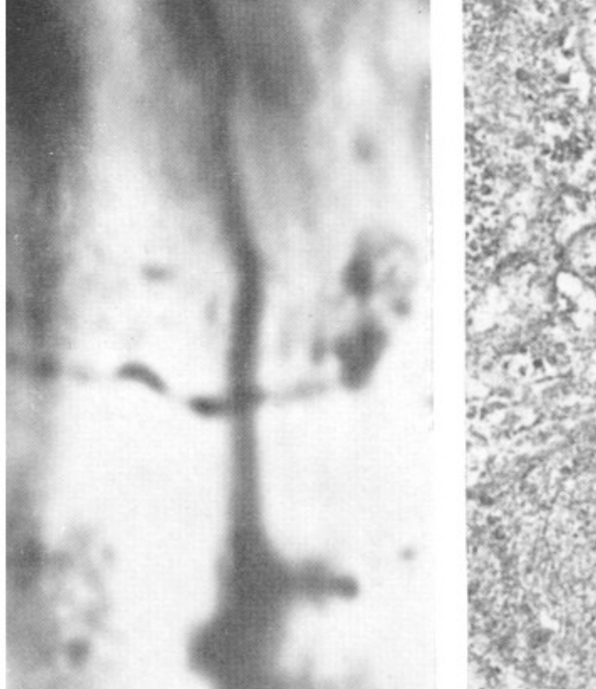


4



5

6



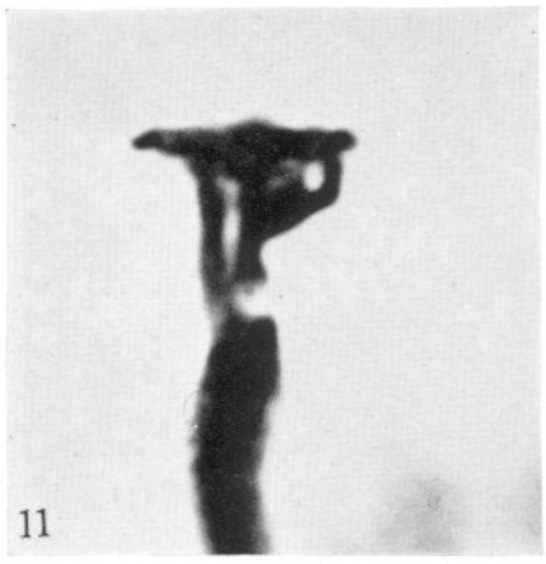
7

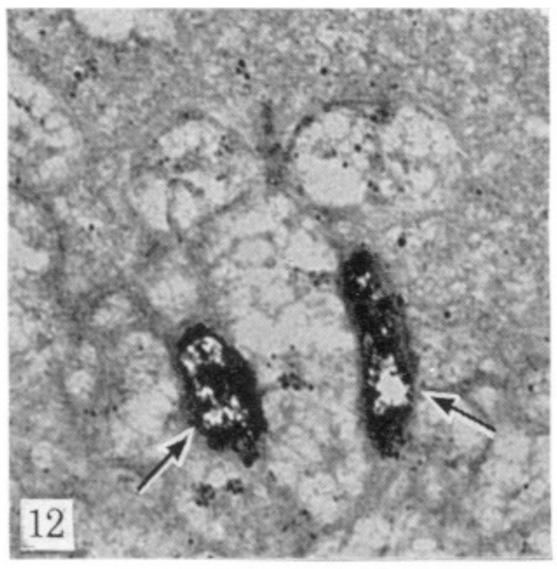
8

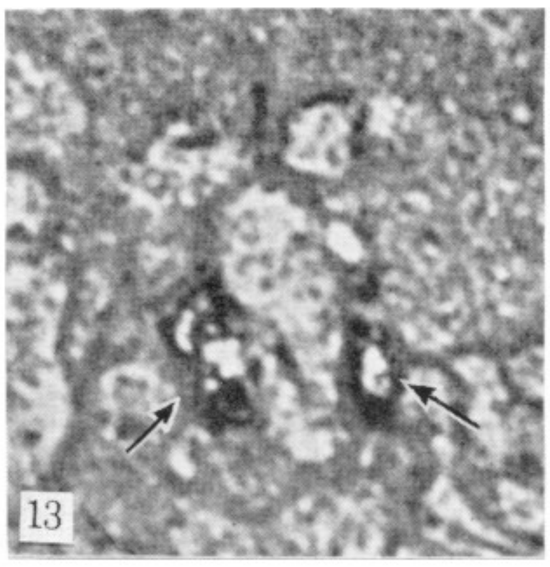
9

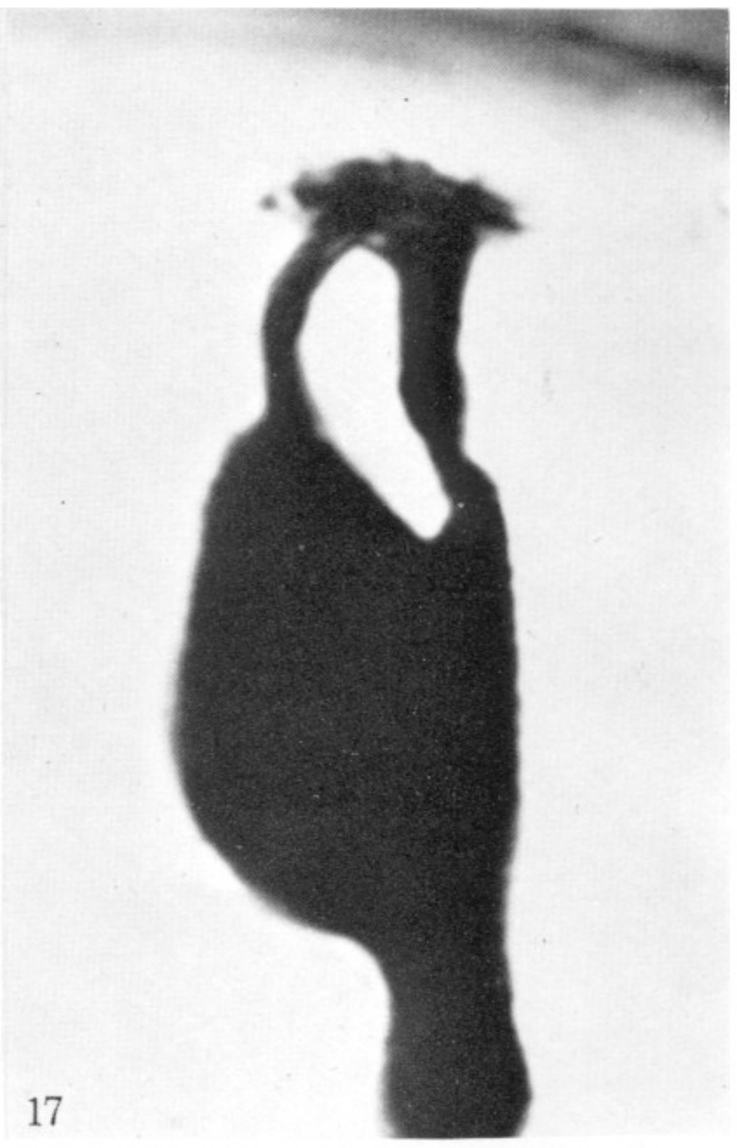
10

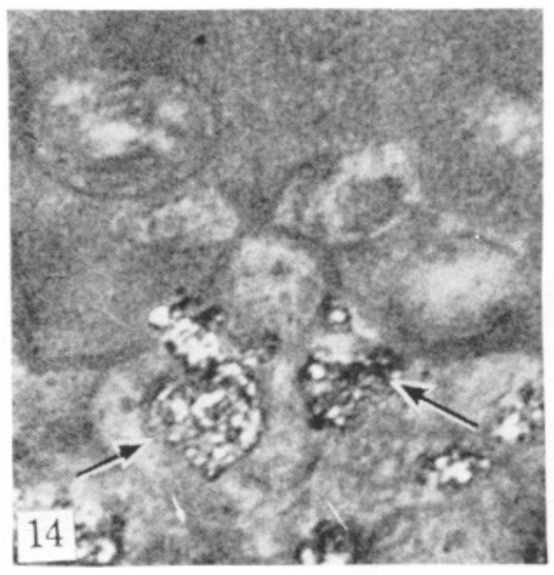
(*Facing p.* 266)

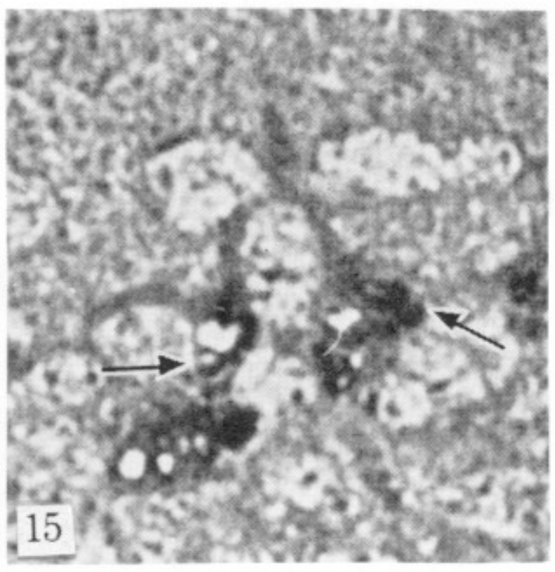

11

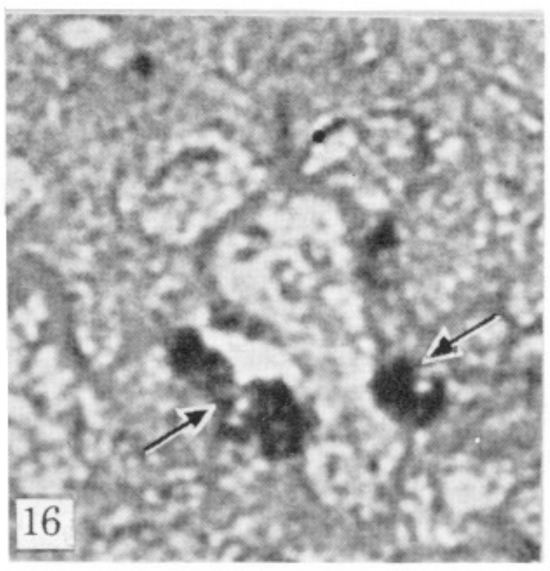

12

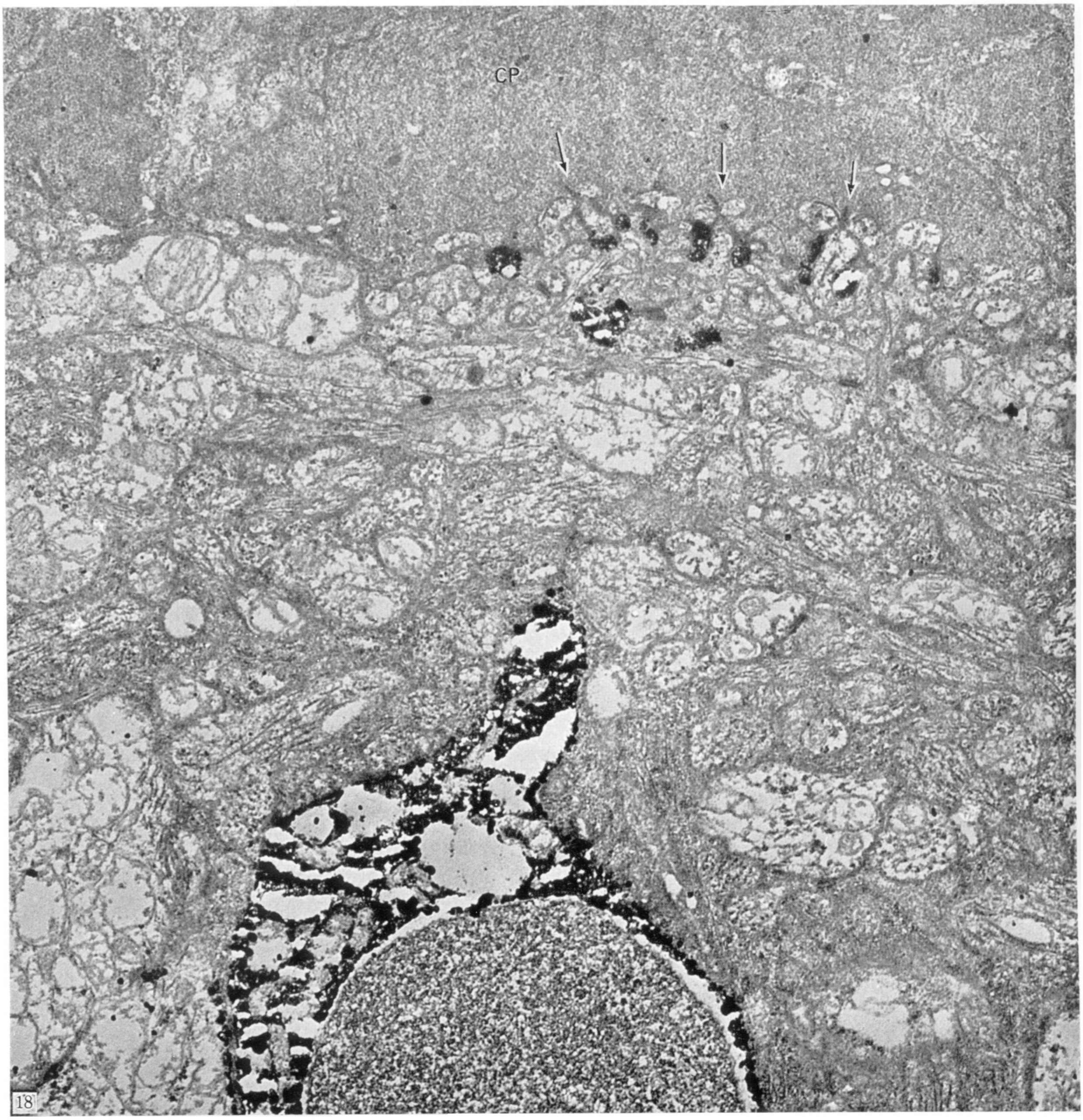

13


17

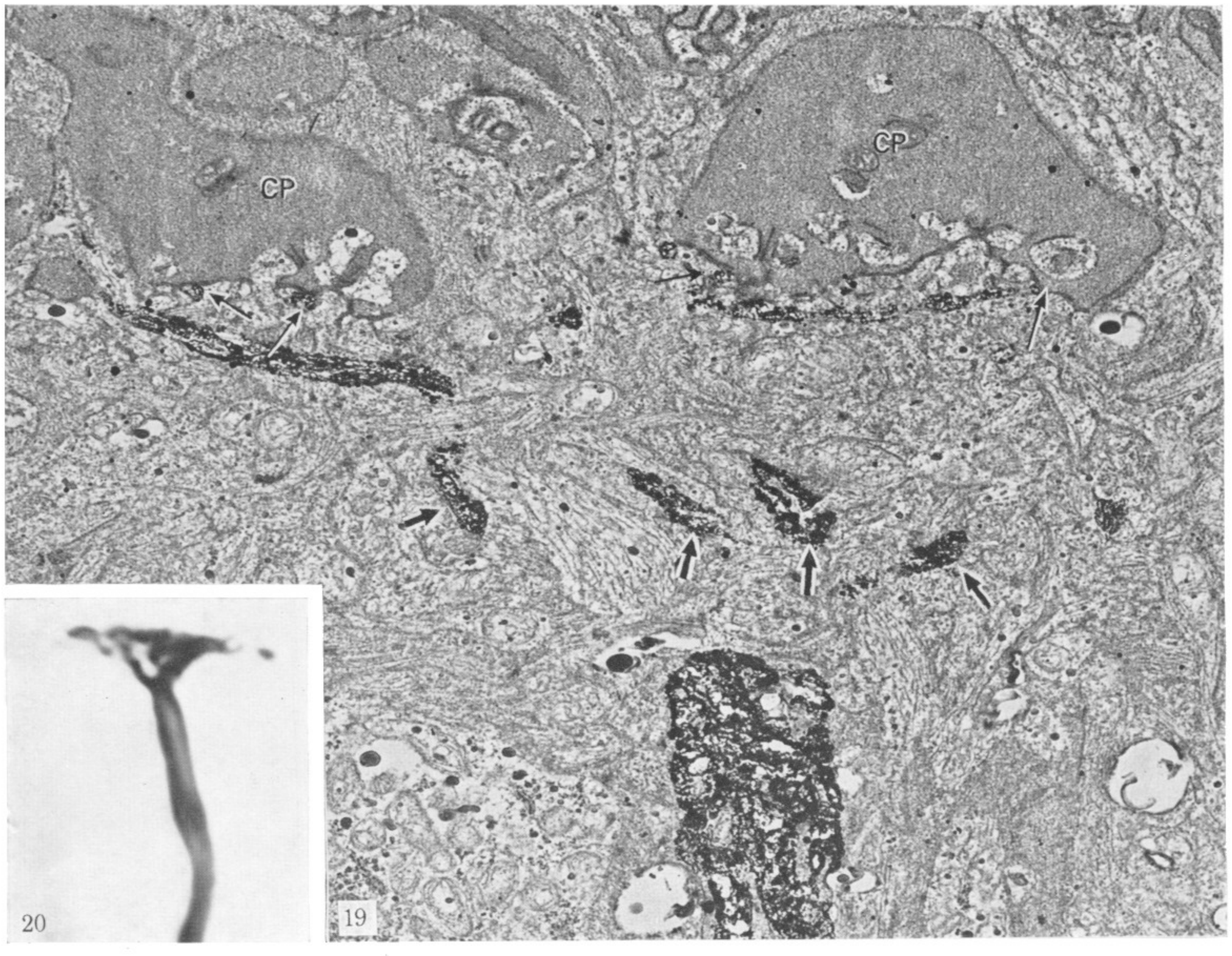

14


15


16

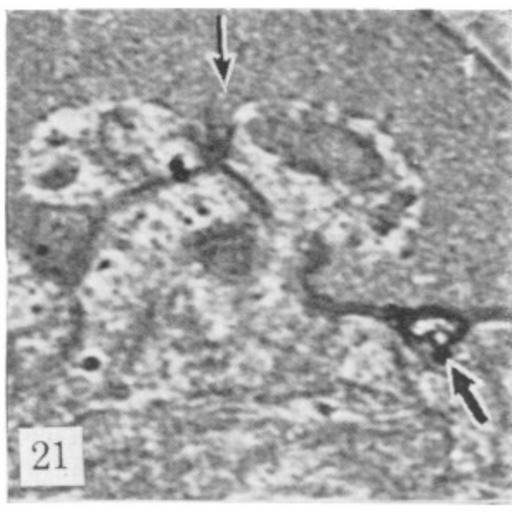


18

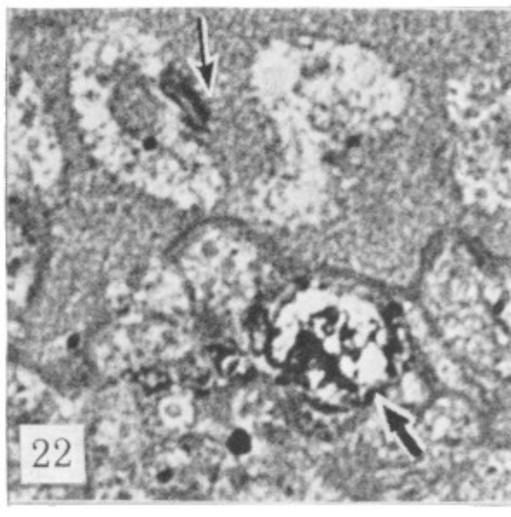


19

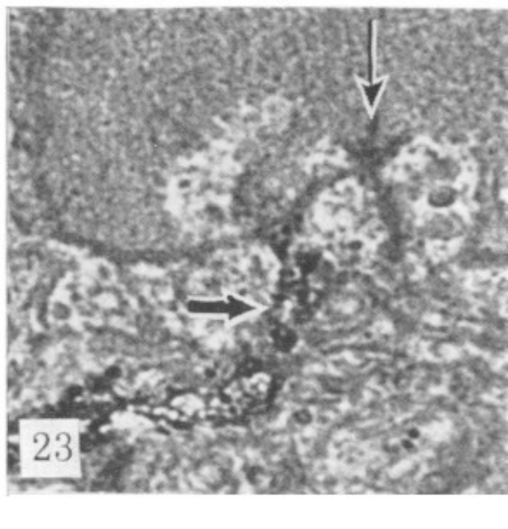
20



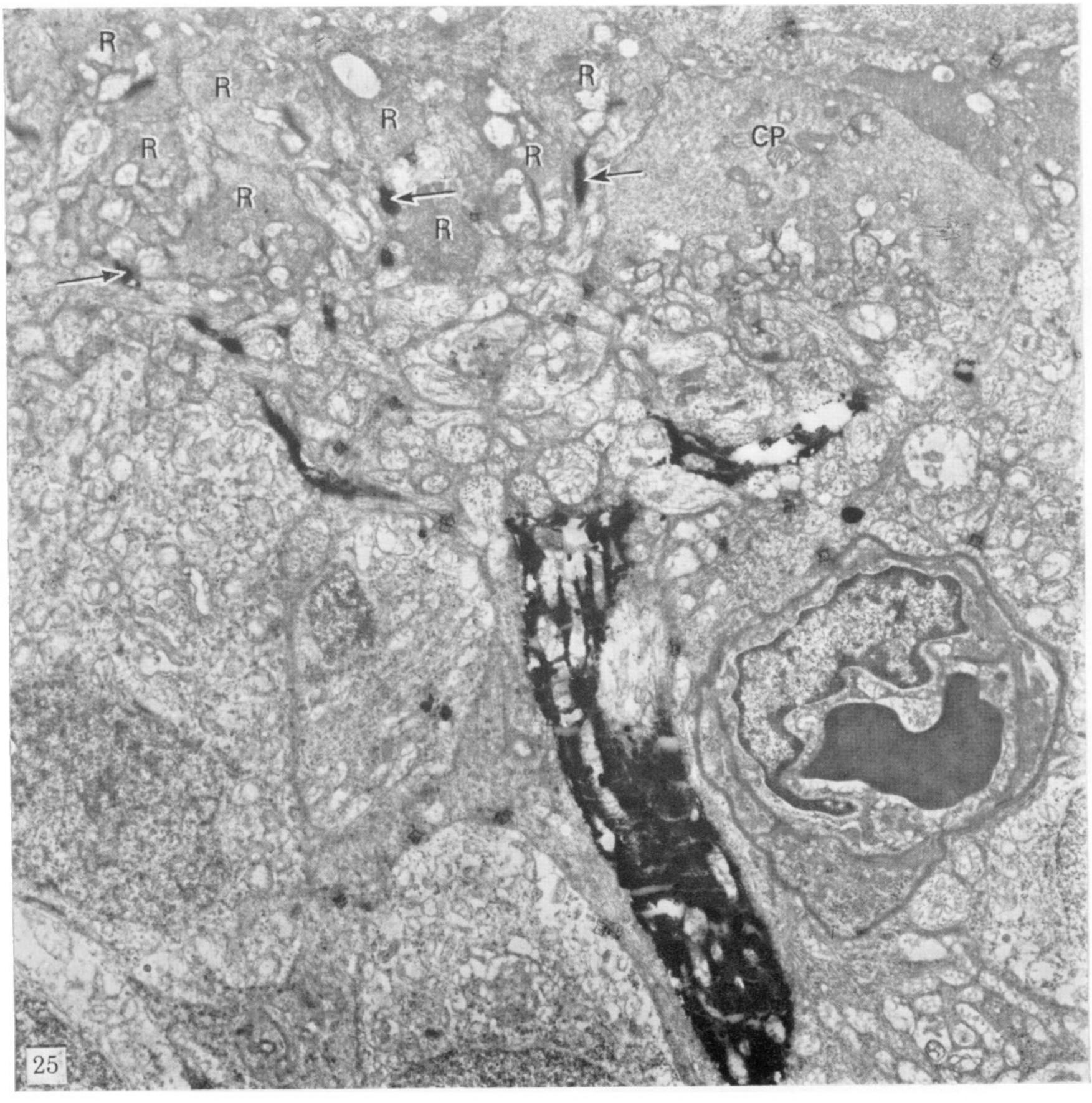
21



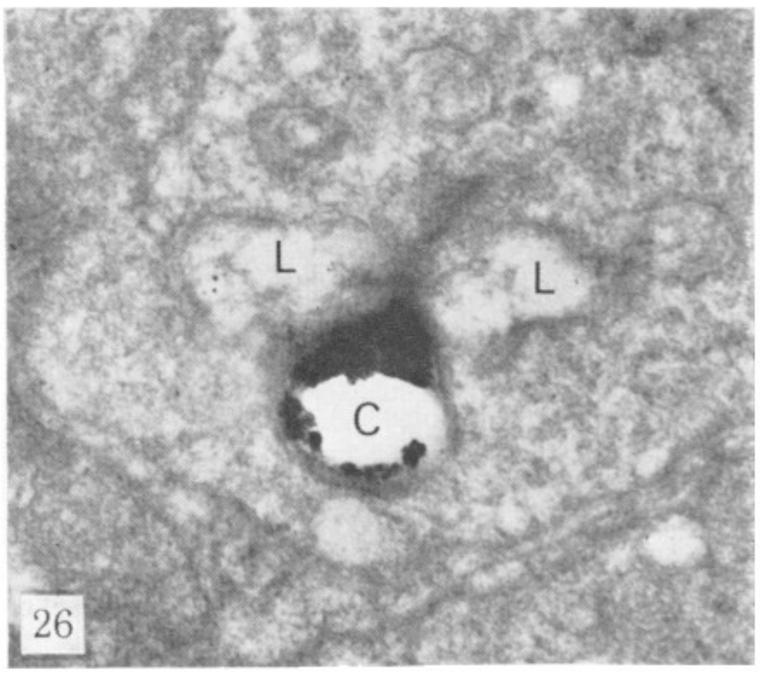
22



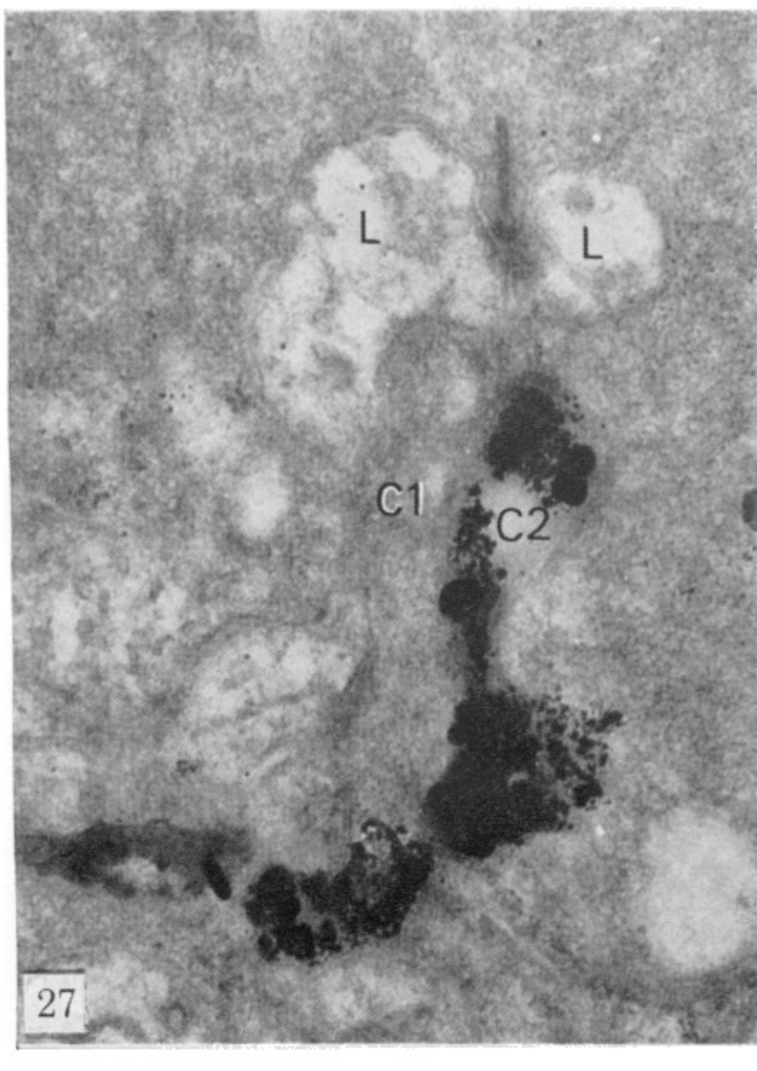
23



25

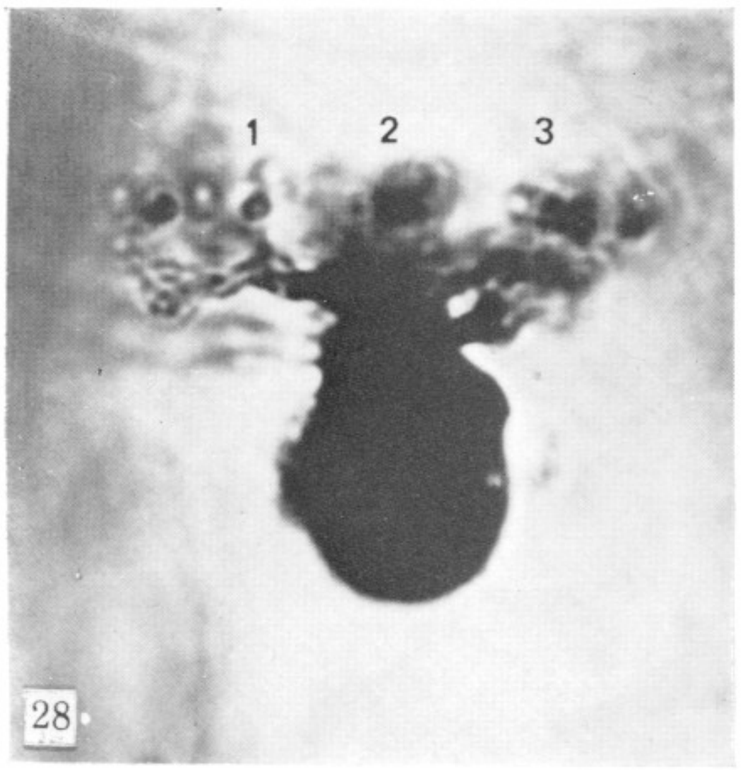


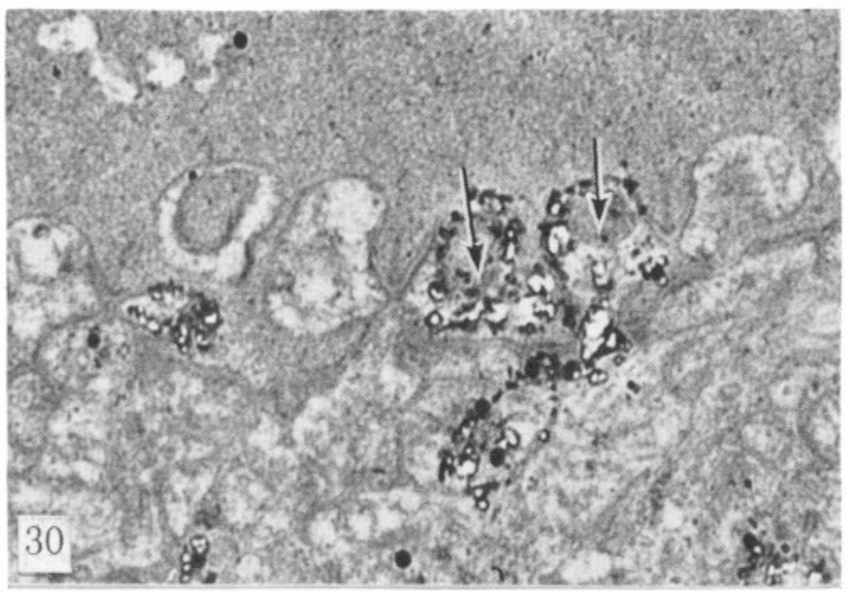
26

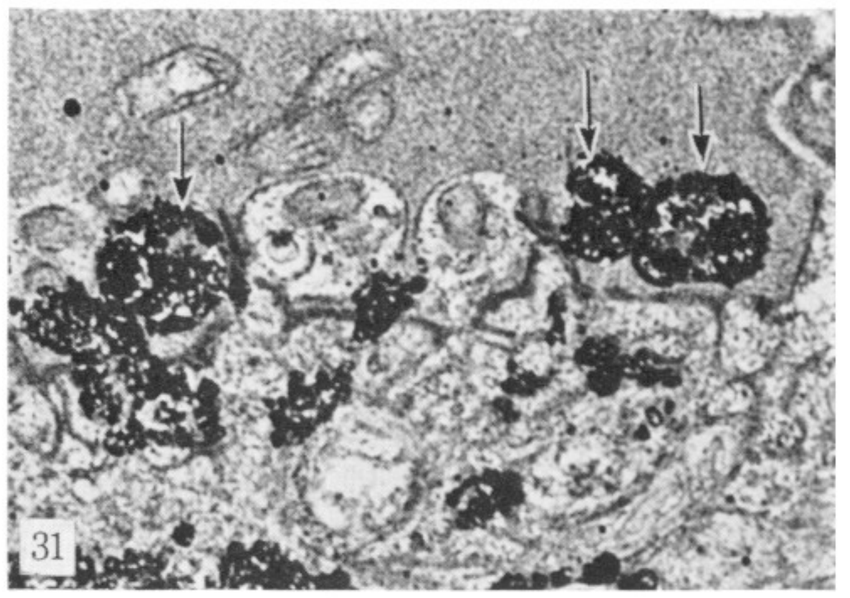


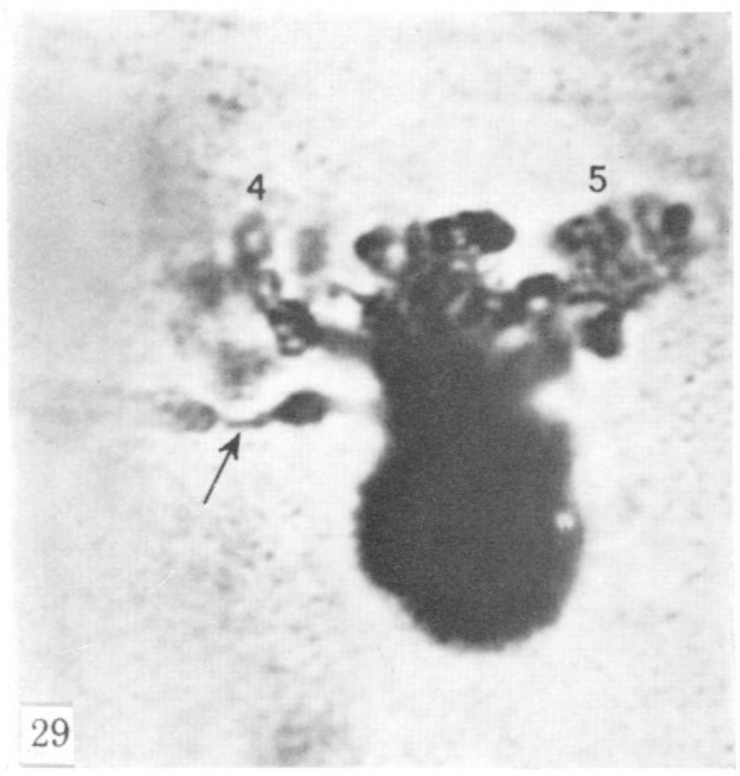
27

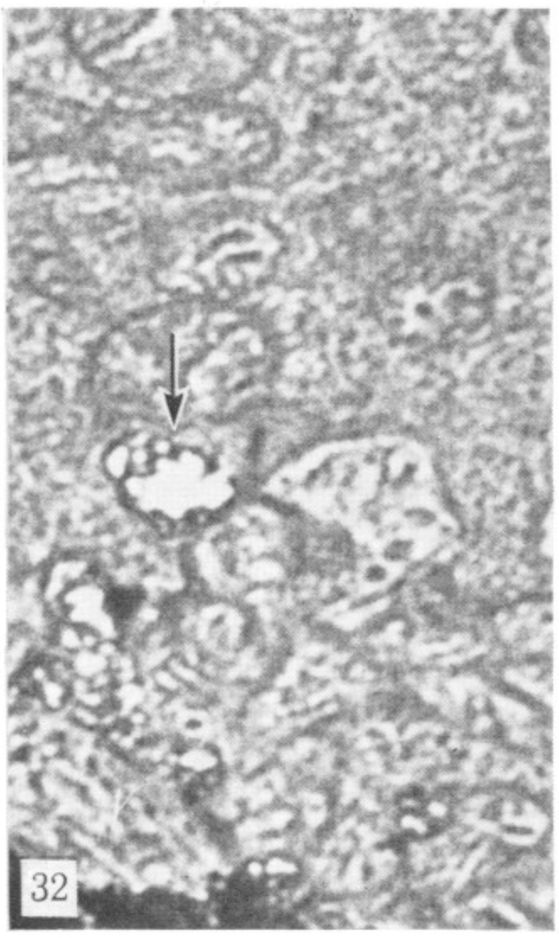
(*Facing p.* 270)

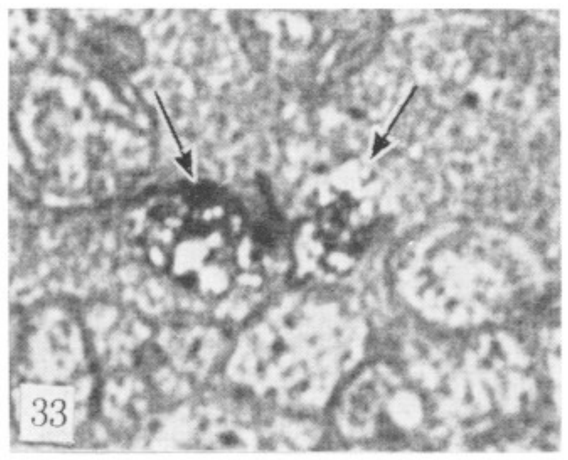

1
2
3
28

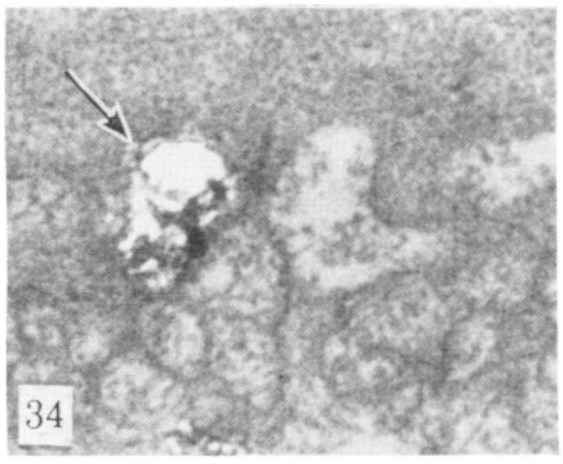

30

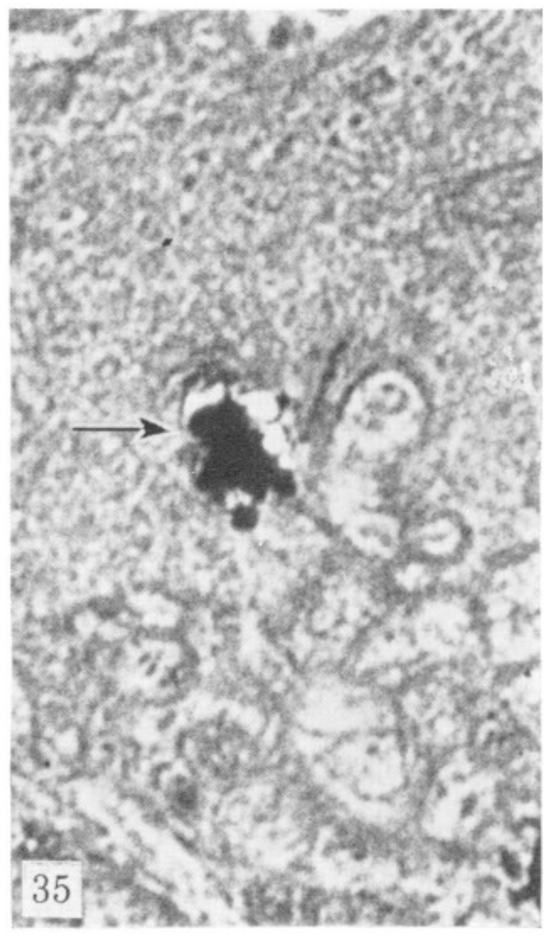

31

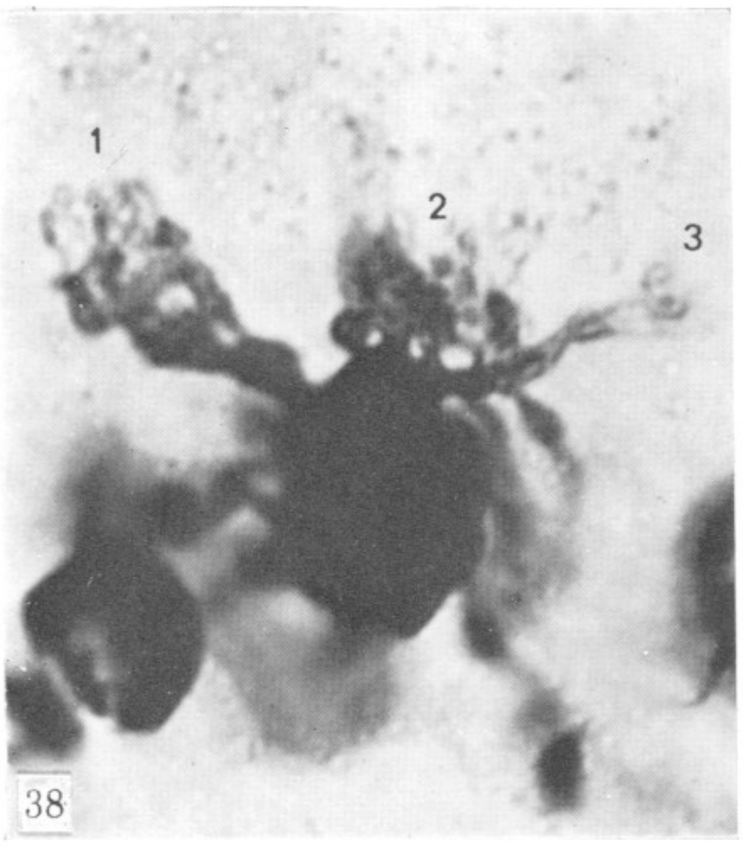

4
5
29

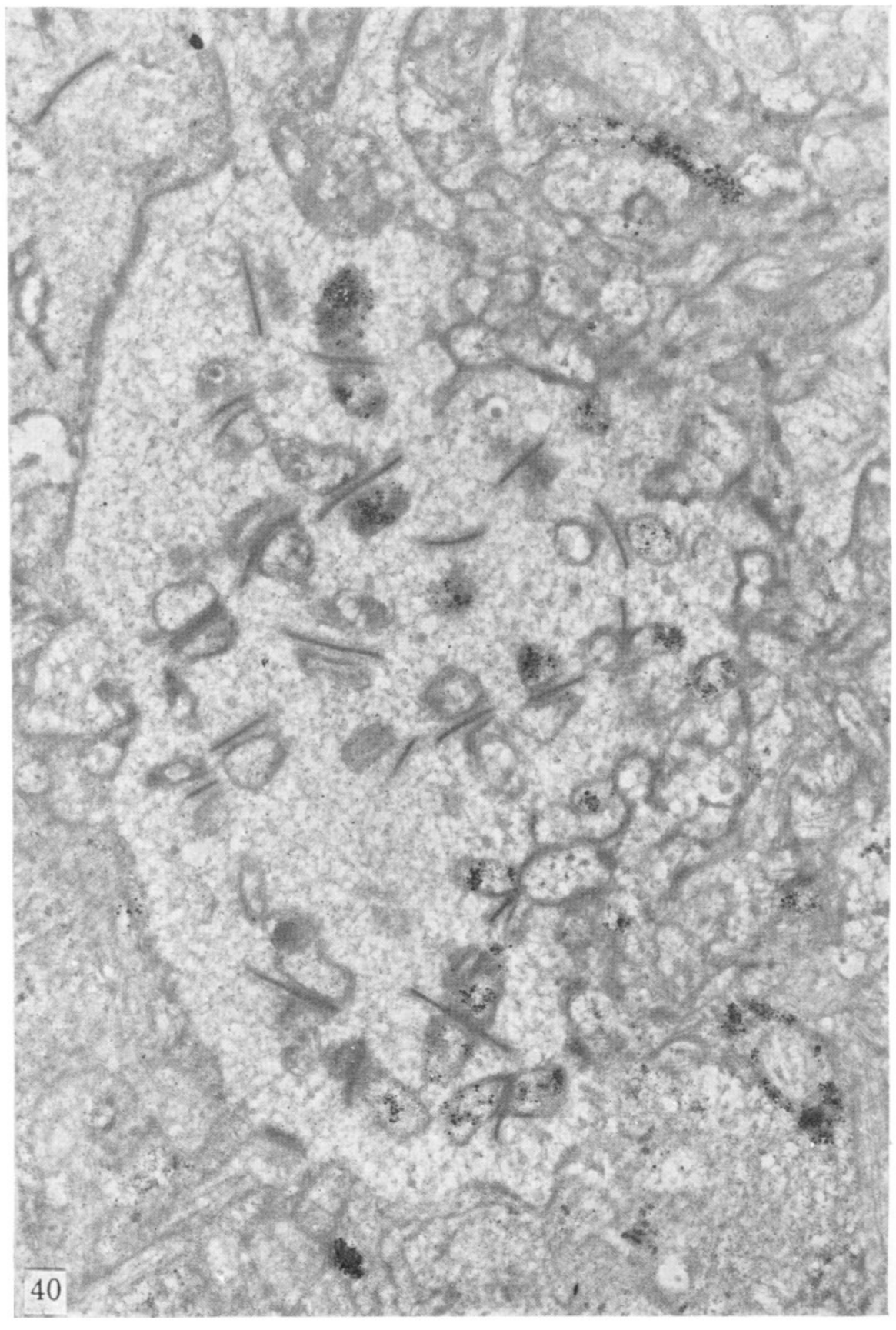

32

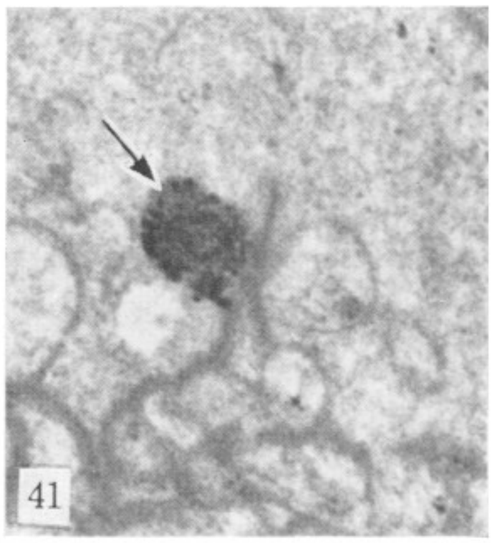

33

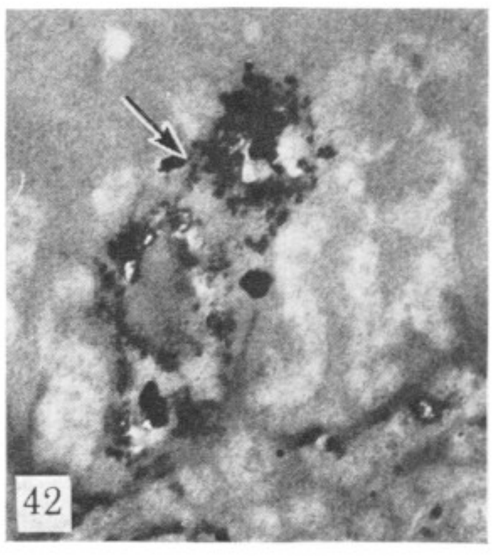

34

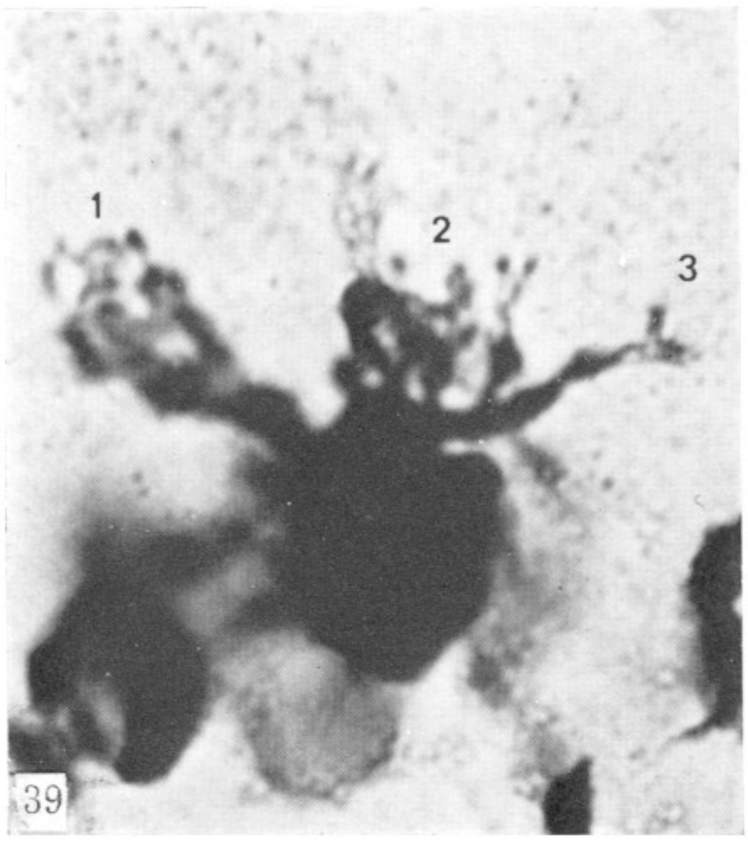

35

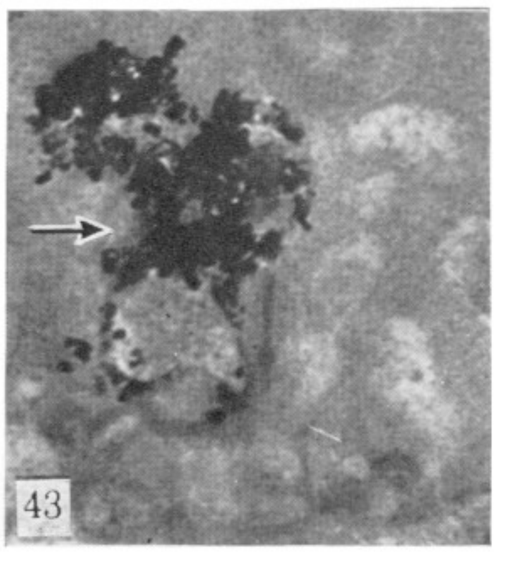

1
2
3
38

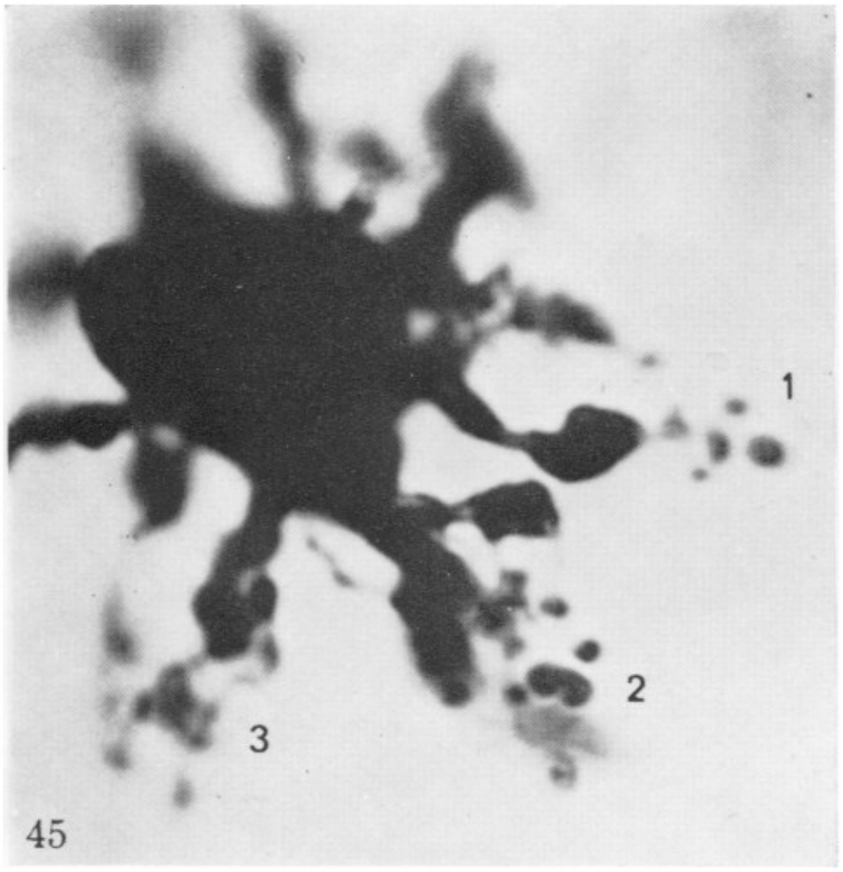

40

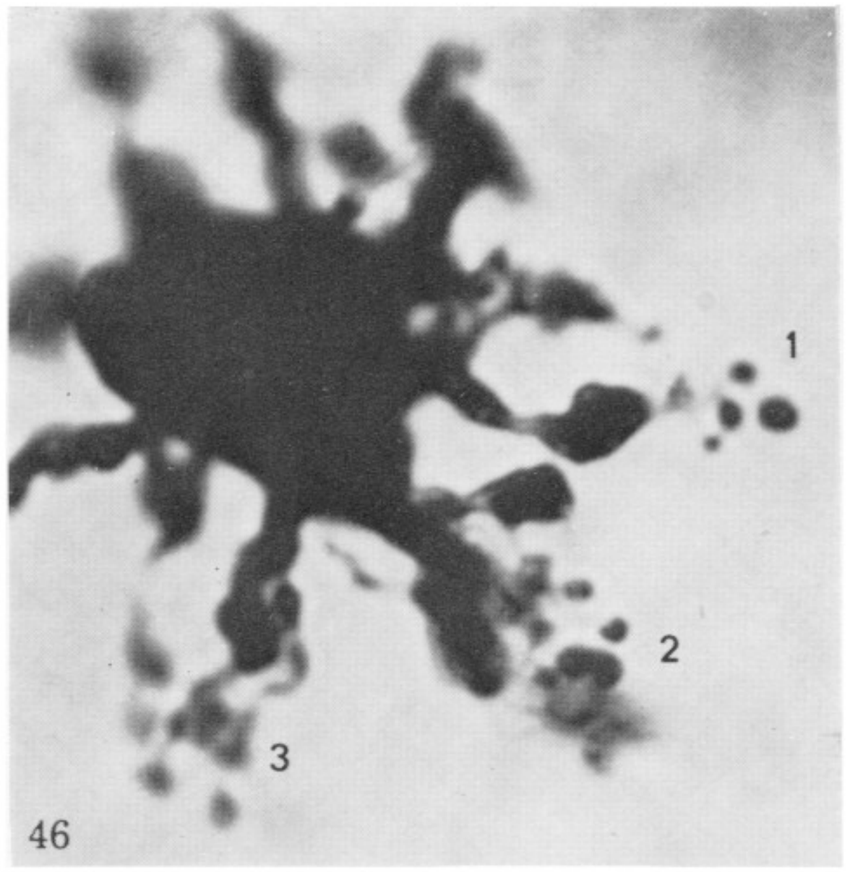

41

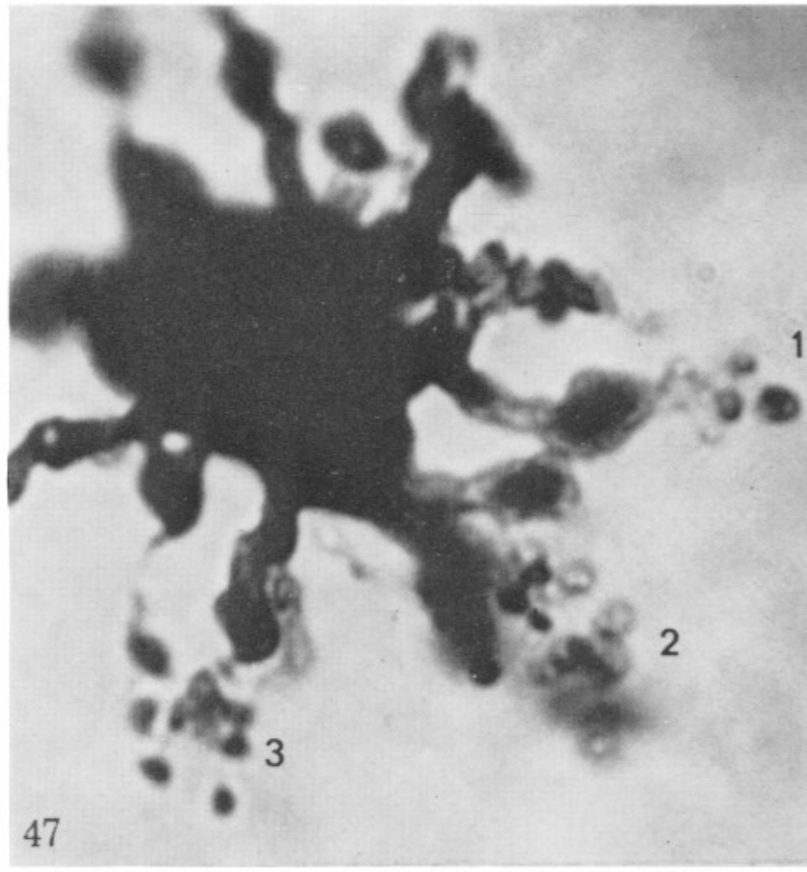

42

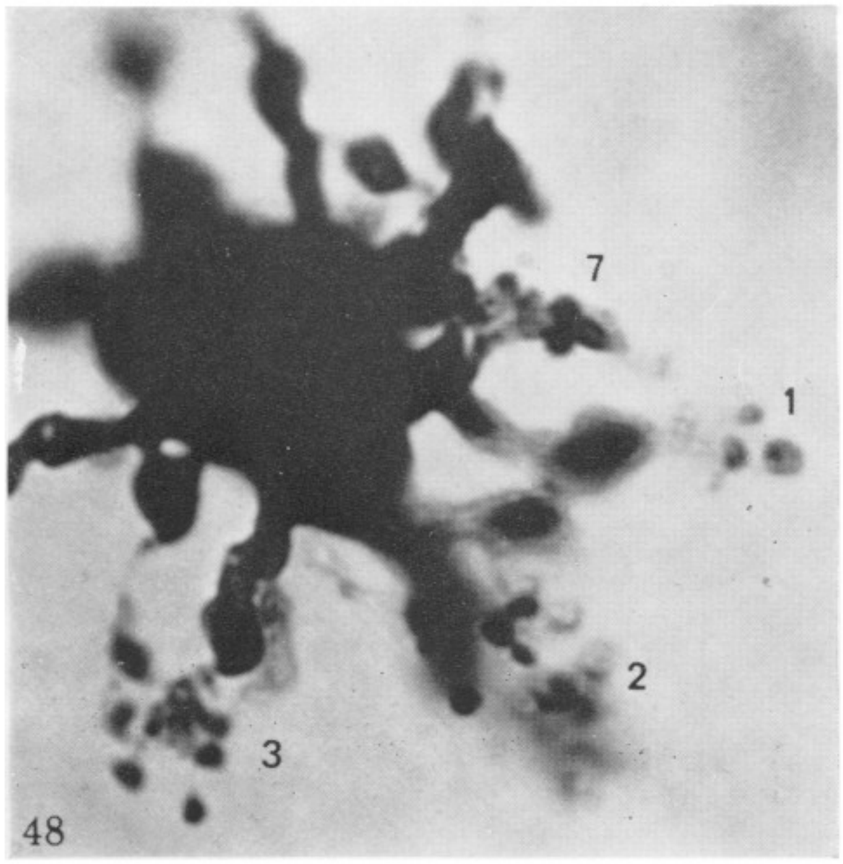

1
2
3
39

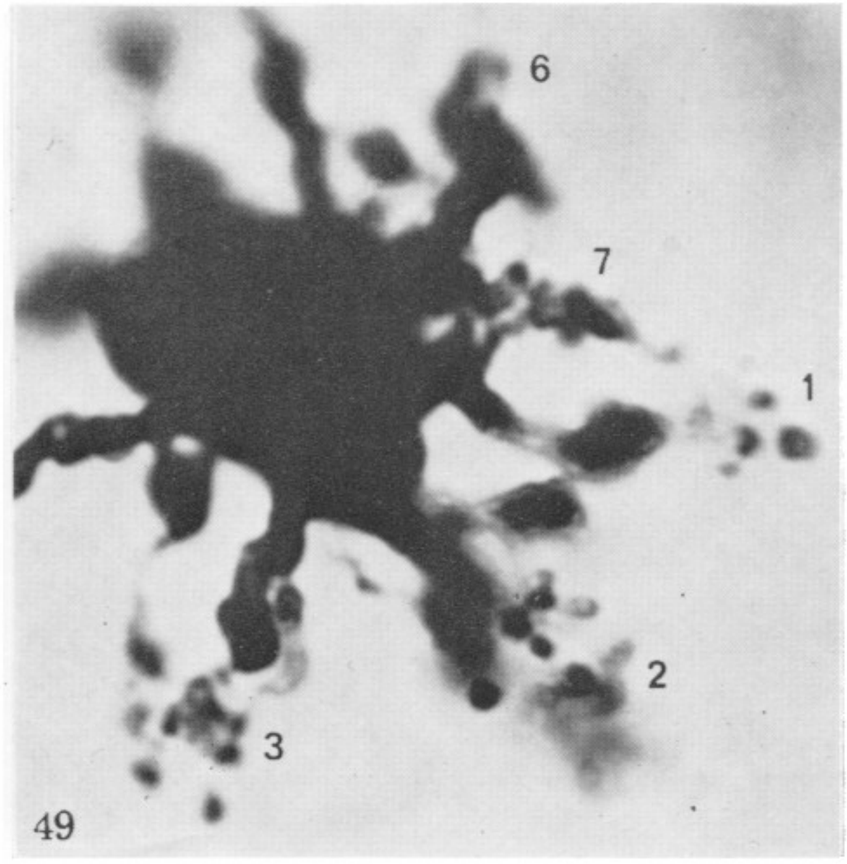

43

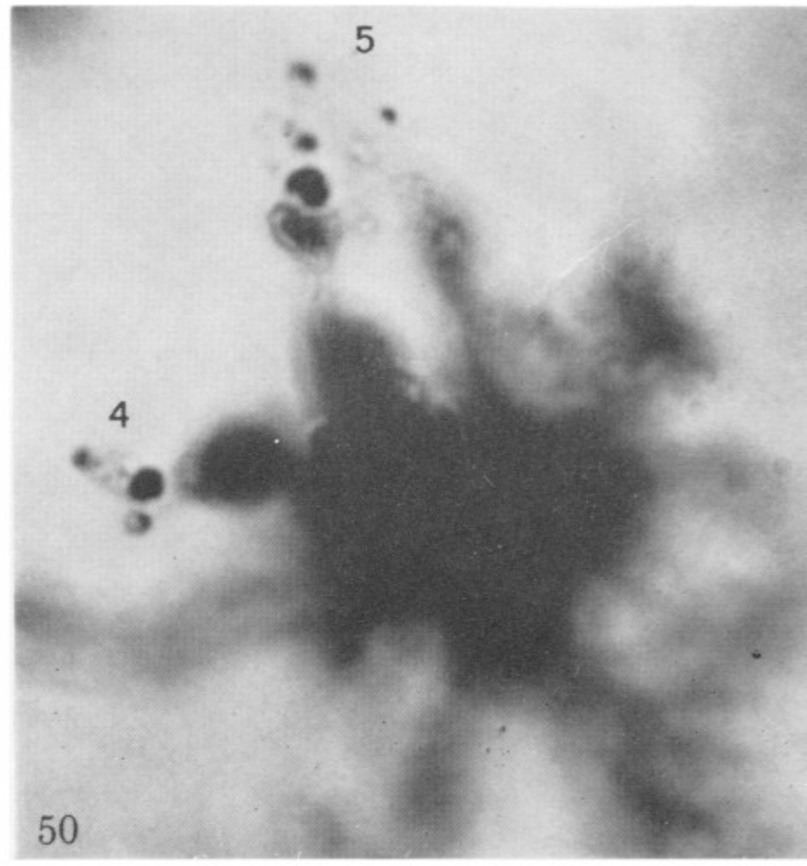




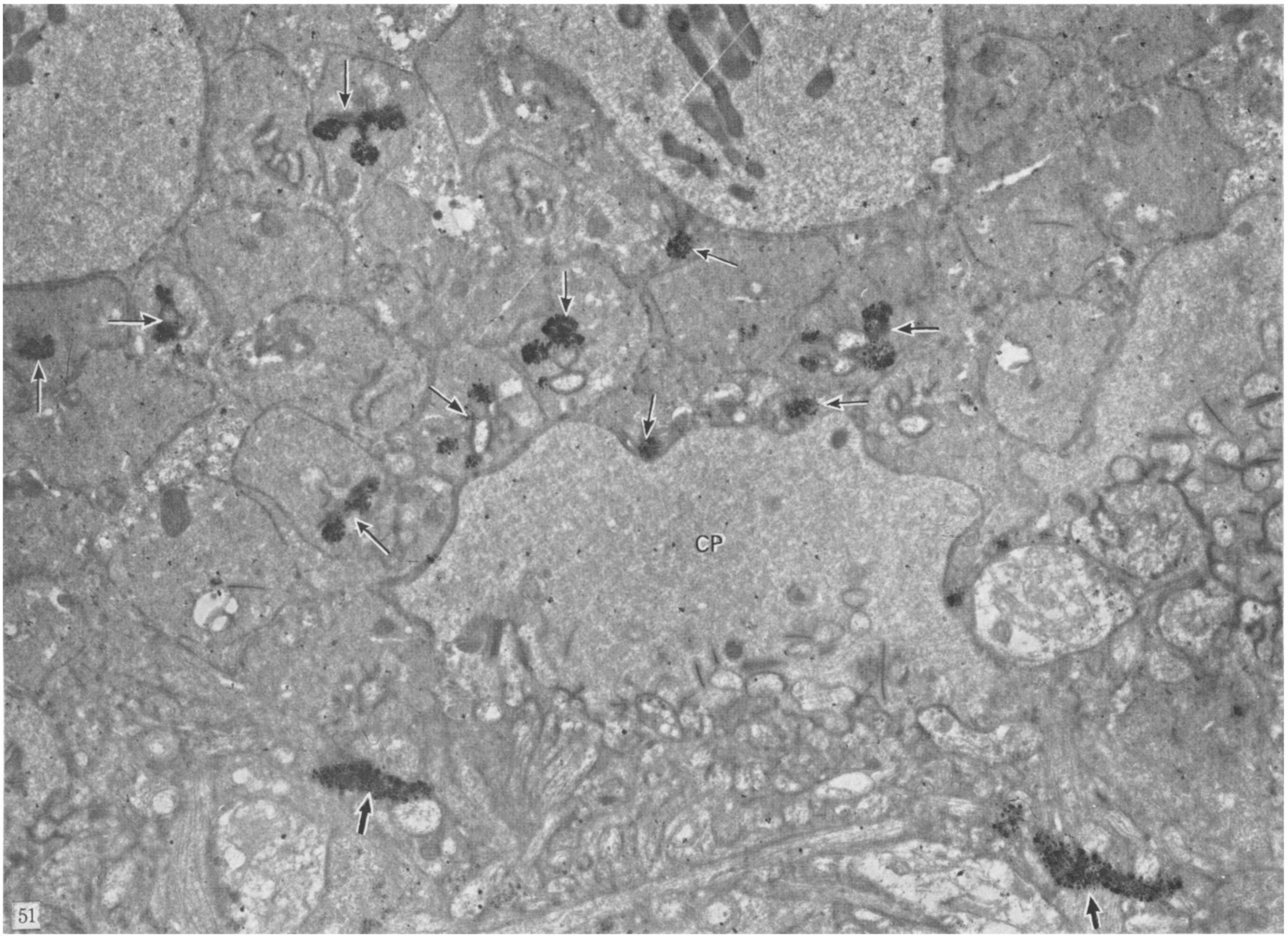


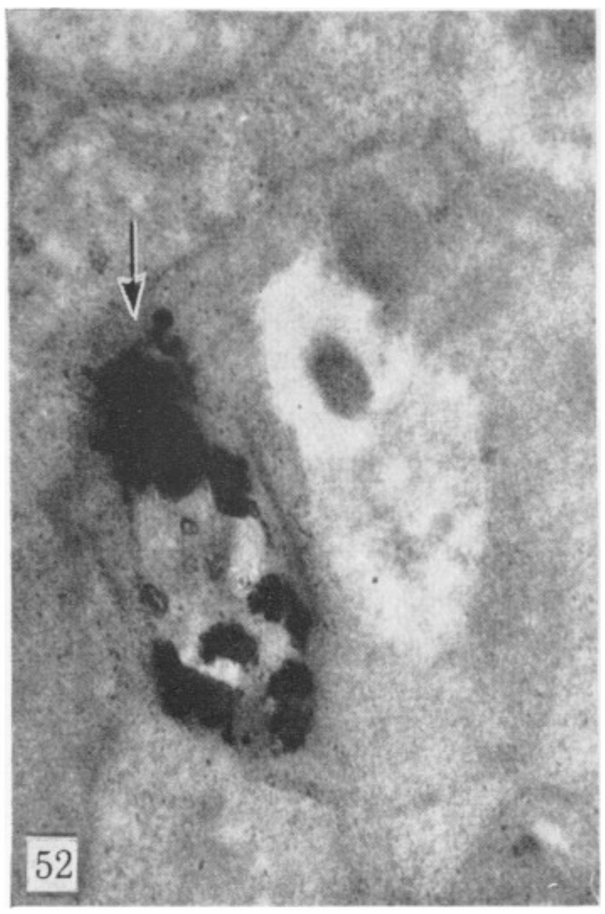


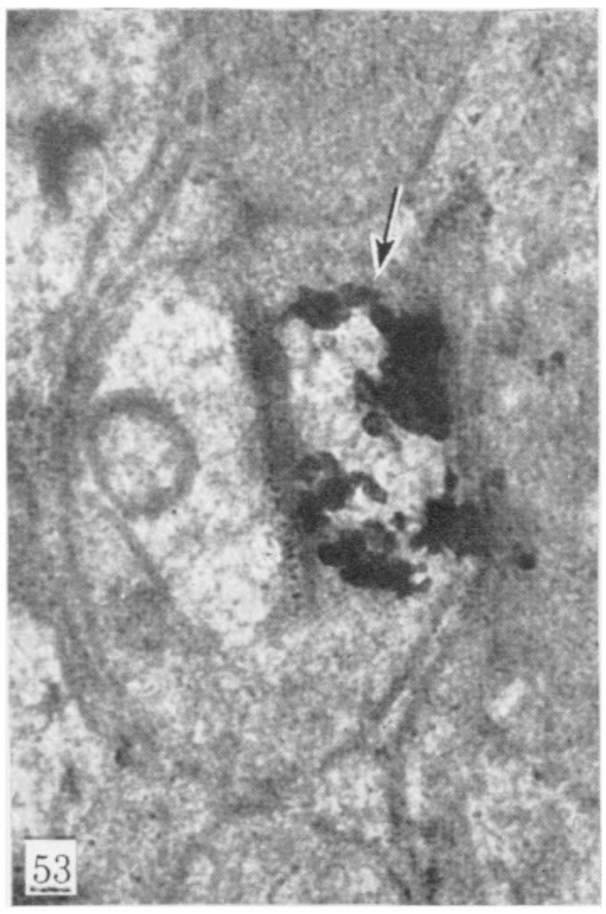


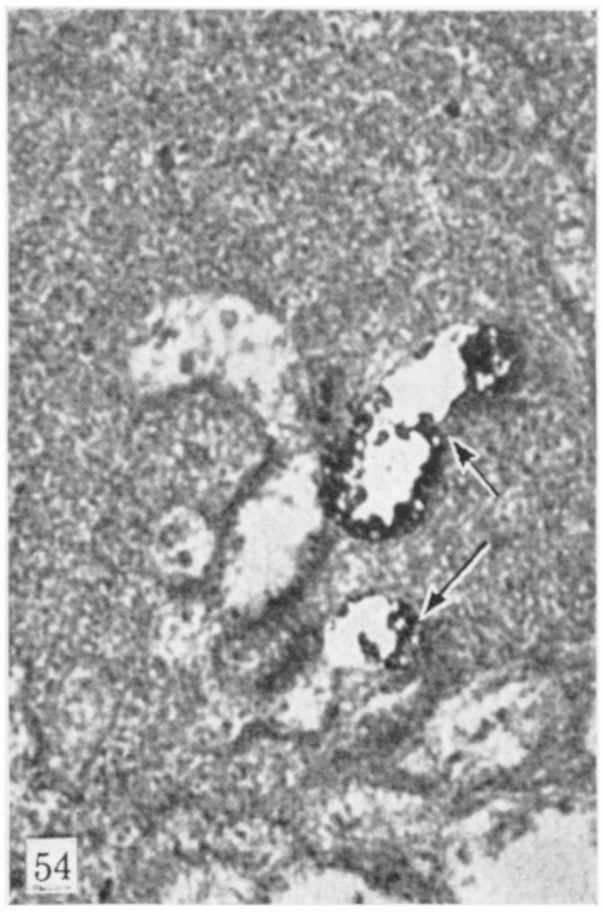


FIGURES 45 to 50. Through focus series of a horizontal cell (kindly photographed by Miss E. M. Crawley and donated by B. B. Boycott) that shows terminals of varying size, that could be characteristic of both type *A* and type *B* horizontal cells. In group 1 the largest terminal has an optical section of a sphere appearance (Type *A*) and measures about 1 μm across; the smallest terminals are typical type *B* horizontal cell terminals. Group 2 consists of two large type *A* terminals (1.0 μm), eight or nine medium sized terminals (0.5 μm) and four small type *B* terminals. The span of the group is 8 μm. Group 3 consists of nine terminals of intermediate size, and the diameter of the group is 6 to 7 μm. Group 4 consists of one medium and two small terminals. Group 5 has seven terminals all of which are small except two that seem to have coalesced to form a blob. Group 6 is not clearly resolvable and group 7 has three typical type *A* terminals, which appear as optical sections of spheres. Golgi–Colonnier (method 4) ($\times$ 2000).









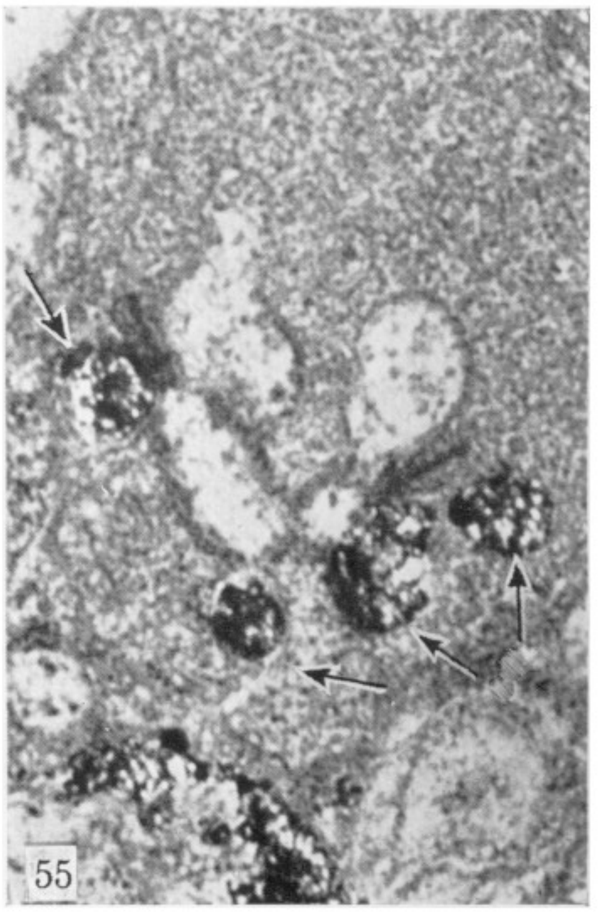


FIGURE 51. Low-power electron micrograph of horizontal cell axon terminals cut in flat section. The stained terminals always occupy the position of the lateral elements in the rod spherules (thin arrows). The cone pedicles are avoided. Branches of the axon are seen running through the outer plexiform layer (thick arrows). Golgi-Colonnier (method 4) ($\times 9000$).

FIGURES 52, 53. Higher magnification of rod spherules cut in flat section. The lateral elements are grossly swollen and fill almost the entire diameter of the rod spherules. The stain (arrowed) is deposited unevenly usually leaving a clear centre. These are type *A* axon terminals by light microscope criteria. Golgi–Colonnier (method 4) ($\times 20000$).

FIGURES 54, 55. Rod spherules cut in oblique section. The stained lateral elements retain their normal size and lobed appearance (arrowed). The stain is deposited evenly throughout the dendritic terminal. Note the magnification is the same as for figures 52 and 53. These are typical type *B* terminals by light microscope criteria. Golgi rapid triple impregnation (method 3) ($\times 20000$).