ORGANIZATION OF THE PRIMATE RETINA: LIGHT MICROSCOPY

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With an Appendix:

A SECOND TYPE OF MIDGET BIPOLAR CELL IN THE PRIMATE RETINA

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The structure of the human, but mainly of the rhesus monkey, retina as examined by Golgi-staining techniques is described and interpreted on evidence from both light and electron microscopy. One type of rod bipolar cell and two types of cone bipolar cell are recognized. The rod bipolar is exclusively connected to rods. The midget bipolar is postsynaptic to only one cone but each cone is also presynaptic to a diffuse cone (flat) bipolar. Such flat bipolar cells are in synaptic relationship with about seven cones. No other bipolar cell types have been found. The brush bipolar of Polyak is interpreted as probably a distorted rod bipolar, while Polyak's centrifugal bipolar is a misinterpretation of the morphology of diffuse amacrine cells. When presumptive centrifugal bipolars were observed they appeared to be a developmental stage of amacrine cells. In the outer plexiform layer two types of horizontal cell have been defined. Each type of horizontal cell has a single axon and two kinds of horizontal cell axon terminals are recognized. In the inner plexiform layer there are two main classes of amacrine cells: the stratified amacrines and the diffuse amacrines. Each class of amacrine has a wide variety of shapes. Polyak's midget ganglion cell is confirmed and his five other kinds of ganglion cell are classified into diffuse and stratified ganglion cells according to the level at which their dendrites branch within the inner plexiform layer. A fuller summary is given by the diagram and in the legend of figure 98, p. 174. A new type of midget bipolar is described in the Appendix (p. 177).

Introduction

Cajal (1892 and 1937) some 75 years ago appreciated that the vertebrate retina has considerable anatomical and experimental advantages for a correlation of central neural structure and function. It is accessible and its cellular components are precisely orientated. The advent of electron microscopy and modern electrical recording techniques has re-affirmed Cajal's prescience.

In two previous papers we have described the synaptic contacts of the five main cell types of the primate retina as observed with the electron microscope and interpreted these as far as possible in functional terms (Dowling & Boycott 1965, 1966). These papers provided valuable new criteria for the probable sites of synaptic contact of the different retinal elements, but they did not give much information as to the distribution of synapses in three dimensions, or of the spatial relationships of the different types of nerve cells. For these kinds of information, light microscopy is necessary.

The Golgi method of staining and its many variants have provided, until recently, more information about the probable relationships between the cells of the central nervous system than any other simple histological technique. However, the retinae of most mammals, and particularly those of adult mammals, have traditionally been difficult to stain by the Golgi method. This probably accounts for the fact that Cajal's well-known descriptions of mammalian retinae seem to be based mainly on successfully prepared

material from unusual sources such as the ox and the dog. Previous authors frequently discussed the problems imposed by difficulties of fixation and staining of certain retinal elements, such as the very important amacrine cells, and emphasized the need to use young animals. The method we have mainly used has worked well on adults, and this may sometimes account for some of our differences with Polyak.

After initially inadequate results using the Golgi-rapid and the Golgi–Cox methods of staining, we have obtained good results with Colonnier's modification of the Golgi–Kopsch method (Colonnier 1964), on the retinae of rhesus monkeys between 9 months and 5 years old and middle-aged humans. Colonnier's method employs glutaraldehyde as the primary fixative instead of formaldehyde. It regularly gave good impregnation of most types of primate retinal neurons, particularly in the central area of the retina. The present paper describes our findings and attempts to create a picture of primate retinal architecture based on both light and electron microscopy.

Polyak's 1941 treatise is a most detailed description of the retina of the rhesus monkey, chimpanzee and man. That book, together with his 1957 text, provides a thorough summary and bibliography of the subject. A reference to Polyak in the text will mean his 1941 book; reference to his 1957 summary will be explicit. Cajal's first general account of the vertebrate retina was in 1892, and a German translation by Greeff appeared in 1894. The original paper was reprinted in 1933 with some emendations by Cajal and to this we shall refer without date reference when Cajal's name is used. Cajal often referred to the retina in other works of his and specific reference will be made to these.

During the account we shall have frequent reason to qualify statements in Polyak's books. It is salutary to remember that the major errors in those books are mistakes of interpretation that would have continued in the literature had it not been for the advent of electron microscopy. It is also true to say that the present work could not have proceeded so rapidly without Polyak's detailed records of many years of patient observation, nor would it have been worth the effort without the recent advances that have been made in the physiological understanding of the vertebrate retina.

Material and Methods

Most of our observations have been on rhesus monkey; the observations on man have been sufficient only to confirm that his retina is in principle structurally similar. We have made no observations on other *Primates*. Seventeen eyes of ten rhesus macaques (*Macaca mulatta*) were used.* The animals were aged from 9 months to 5 years. The eyes were removed from the animals, hemisected and the posterior pole cut into three or four pieces so that, under saline, the central area of the retina could be gently stroked off the sclera in one piece. The following account is mainly about the central area of the retina, although limited observations have been made in the periphery. That is to say, observations were made within a radius of 4 to 5 mm from the centre of the foveal pit. These dimensions include the fovea, the parafovea and perifovea as defined by Polyak and also

* The animals were either anaesthetized with Nembutal or killed by air embolism. We are grateful to Dr A. Silverstein and his colleagues for provision of some of the adult monkeys and all the foetal material referred to in later pages.

the central edge of Polyak's 'near periphery'. We shall refer to all this as the central area. Peripheral to the central area the connexions of the retina are substantially similar, although the details of the shapes of the cells vary as the ratio of rods to cones changes and the number of ganglion cells decreases (see Polyak). Mention of these differences is made where they can lead to some confusion over the types of cells present.

Three retinae were fixed in an osmium dichromate mixture and 14 were fixed in a glutaraldehyde dichromate mixture. The former method is the well known Golgi-rapid and the latter is the modification of the Kopsch–Golgi method initiated by Colonnier (1964). The rapid Golgi method gave good impregnation of a few cells, but Colonnier's modification stained more cells and has been more generally consistent; of 12 central areas used only two failed to reveal any useful cells. The peripheral parts of the retina stained adequately, but there was a greater incidence of incompletely impregnated nerve cells and more glia cells seemed to be stained. Fixation of pieces of peripheral retina in buffered glutaraldehyde before fixation in the glutaraldehyde-dichromate mixture gave similar results, except perhaps for a tendency for more Müller's (glial) cells to be impregnated. The Golgi–Cox method as used by Sholl (1953) was unsuccessful on the two pieces of retina attempted. Two retinae were stained according to a modification of Stell's (1965*a*) usage of the repeated impregnation method of Cajal. These retinae were prepared by Miss H. Kolb, who kindly made them available to us. One retina produced a few well-stained diffuse amacrine cells and some ganglion cells but little else; the other retina had nothing stained. The procedure consisted of fixation for $1\frac{1}{2}$ h in 2·5% glutaraldehyde in 0·05 M sodium phosphate buffer, followed by 90 min in a mixture of ice-cold 3·3% OsO_4 in 0·1 M potassium dichromate. Then, *A*, 2 days in a mixture of 0·25% OsO_4 in 0·1 M potassium dichromate in the dark; followed by a wash in 1% silver nitrate and, *B*, 2 days in 1% silver nitrate. After *B* the stages *A* and *B* were repeated twice before rapid embedding and sectioning in collodion.

Human material was obtained from patients who had small tumours of the anterior part of the eye but whose vision was normal. The four retinae used were from middle-aged patients (40 to 60 years old). Three were stained by the Colonnier method and one by the rapid Golgi procedure.

All retinae were cut at a thickness of about 75 μm for horizontal sections, and 95 μm for the vertical sections. The mounting medium was Fisher's 'Permount' as introduced by Vaisamurat & Hess (1953) for Golgi material, and thus coverslips could be used. No fading of the impregnation has been seen in two years, but darkening of the unstained background of some of the sections has occurred, quite probably due to exposure to the microscope light. Guillery (1966) has had similar experience. Where high magnifications were necessary the cells were examined with a Carl Zeiss Neofluar 100/1·30 using a condenser of N.A. 1·4. Where resolution was critical (see p. 132) no extra advantage was obtained using a Zeiss apochromat $\times 100$, and for the most part the working distance of this lens was too small for the thickness of sections that had to be used.

Terminology

Because of the peculiarities of the development of the vertebrate retina and its adult arrangement, some definitions of terminology are necessary. The term 'horizontal section' will mean a section taken at right angles to the long axis of the rods and cones. 'Vertical section' will mean a section taken parallel to the long axis of the photoreceptors. Any positions of cells in the retina are given as a direct line from the centre of the foveal pit. Such measurements are approximate because fixation in Golgi fixatives may distort the retina considerably and irregularly and no allowance was made for its natural curvature. For these reasons comparisons of individual kinds of cells are made, wherever possible, with respect to adjacently stained cone pedicles. No special search has been made for possible differences between nasal and temporal fields which, however, do not appear to differ in the details of the relationships of the neural elements. Occasionally it is necessary to refer to the vitreal or the scleral surface of a retinal layer but this is cumbersome. With for example, a bipolar cell, the scleral process will be referred to as 'an apical dendrite' and the vitreal process will be called 'an axon'.

Some of the text-figures are redrawn, with due acknowledgement, from the data of Polyak and Cajal to facilitate comparison of the present observations with their data. As far as possible the figures for the plates have been kept at two magnifications, $\times 800$ and $\times 2000$; this simplifies comparisons of different cells. It is rare for any cell in a photomicrograph to be displayed in its entirety. To facilitate identification by future workers their dimensions as measured under the light microscope, are, where appropriate, given in the figure legends. In order further to lighten the text, some detailed points are discussed in the legends. An index is given to the plates at the heading for each cell type.

A part of the definition of different kinds of cells involves measurements of the extent to which their processes spread within the retina. Such measurements are given here (see, for example, figure 96). They are always given as the maximum lateral extent of the processes irrespective of whether the fields are elliptical or circular or irregular. These data are a formal statement of some of the characteristics of the cell type and give an indication of the relative cell sizes. Such measurements may sometimes have significance for interpretation of the functional organization of the retina, as, for example, in the case of the hypothesis that the diameter of dendritic spread of the ganglion cells corresponds to the physiologically determined centre field (p. 166). However, to take the case of the rod bipolar cells in figure 23, plate 35, it must be that the number and distribution of the rods to which those cells are connected, and the relation of those rods to horizontal cells and to other rod bipolar cells are much more important than the shape or size *per se* of the dendritic fields. In this example, outlining the fields around the actual points of contact of those cells would produce very irregularly shaped fields. Considerable attention has been directed recently to the correlation of the shapes of dendritic fields with the neural basis of visual discrimination in *Octopus* and other animals (Young 1966). But without detailed knowledge of the synapses of cells it is difficult at present to be certain what such interpretations may mean. At least so far as the arrangements of the primate retina are concerned it is the definition of the units and the specification of their connexions that it is immediately important to understand. The actual shapes and sizes of the cells often appear to be more

determined by factors that have nothing directly to do with their function. For example the cells in figures 88 and 89, plate 43, are clearly stratified diffuse ganglion cells. That in figure 89 is from the periphery of the retina where it might be expected to be larger than those of figure 88 from the central area, because the number of bipolars per unit area in the periphery is smaller and ganglion cells therefore tend to be larger (Polyak). Yet the cell in figure 89 is smaller, and the problem arises as to whether this is really a stratified diffuse ganglion cell, or a midget ganglion cell that is this shape simply because the terminals of a midget bipolar in the periphery are often of a different shape corresponding to differences in the rod/cone ratio between the centre and the periphery of the retina. On the whole little of functional importance can be said about the shapes and sizes of cells until factors of this kind can be disentangled. The problem is further discussed on page 173.

Interpretation of Golgi-stained material

The chemical basis of Golgi-staining is almost entirely obscure. Much of the account that follows necessarily discusses the artifactual appearance that can arise with a particular cell type. A few general remarks, however, are appropriate. An important part of the present work has been to discover the relationships of the rods and cones to the neural elements. Any Golgi variant impregnates only a small proportion of the cells in a piece of tissue. That is its advantage, for if impregnation of all cells occurred, no cells could be resolved (Sholl 1956). But the selectivity of the method means that the synaptic relationships of the cells with each other have to be confirmed by different methods. For example, Cajal (1937), in his autobiography, explains that his definition of separate rod and cone bipolars was based on the argument that rods and cones must be separately connected into the neural elements, otherwise there would be no point in having separate photopic and scotopic receptors. He explains that he had no direct anatomical proof of this because the photoreceptors and the neural elements could not be stained in relationship to each other. Most later workers have ignored this very important point. Polyak, adopting only the anatomical criteria then available and without Cajal's intuition, was entitled to claim that rod bipolars were also connected to cones (p. 119). Mere contact between nerve cell and nerve cell, as revealed in a Golgi preparation, is no proof of a synaptic relationship; see, for example, the axo-somatic junction of the rod bipolars (p. 144). Using Golgi methods alone it is possible to suggest, but it is not possible to be certain of, the sites of synaptic contact between cells.

Quantitative procedures are possible using Golgi methods (see, for example, Sholl (1956) and Ramon-Moliner (1961)). However, at present, it is not often possible to make worthwhile statements as to the relative proportions of different kinds of cells in the retina. Furthermore, although we believe, using Polyak's data and our own, that we have now identified most of the types of cells present in the primate retina, it is important to remember that it is difficult to be entirely certain that this has been done. As described on pp. 151 and 155 some kinds of amacrines were only observed in one preparation (made available to us by Miss H. Kolb). One of these amacrines, the bistratified amacrine cell, had never previously been observed in mammals (Cajal 1911). Just as it is difficult, at least in the retina, to be sure that representatives of all the kinds of cells have been stained, so

it is difficult to assert that a kind of cell claimed by another author does not exist. That is why our assertion that Polyak's centrifugal bipolar cell is not present in adult monkeys requires such detailed argument (p. 129).

It is now possible with the electron microscopy of Golgi-stained cells to get direct evidence of the relationship of one cell to another (Stell 1967; Blackstad 1965) and the importance of this is illustrated very well on page 177. But electron microscopy cannot yet be used to trace processes over long distances; for this light microscopy is necessary. Consequently the description and arguments for the connexions of a particular cell have to be somewhat involved and the evidence for a particular connexion may for the present still remain circumstantial.

Divisions and cell types of the primate retina

Figure 1, plate 32, is part of a vertical section about 1·25 mm from the centre of the human fovea, and figures 2 to 7 of plate 33 show higher magnifications of various parts of the central retinal area. Table 1 gives the thicknesses of some of the layers in monkey and man. Figure 1 shows the main divisions of the retina. Except for the central portion of the fovea of man and monkey, which contains only cones, the rest of the retina clearly demonstrates both categories of receptor. In the region illustrated there are numerous rods in between the cones, and they are clearly distinguishable from each other, both at their scleral ends (figures 1 and 2) and at their vitreal terminations where they synapse with the neural elements (figures 2, 3, 4 and 6, plate 33). The clarity of this distinction in primates has recently been questioned, apparently on the basis of a comparative study of vertebrate

TABLE 1. THICKNESS OF SOME OF THE LAYERS OF THE PRIMATE RETINA IN MICRONS (μm)

	monkey		man
	parafovea (0·5 mm from foveal centre)	periphery (over 5 mm from foveal centre)	parafovea (1·25 mm from foveal centre)
inner segments	25	25	25
outer nuclear layer	20	40	30
fibre layer	50	10	50
outer plexiform layer	10	10	10
inner nuclear layer	60	30	60
inner plexiform layer	35	30	35
ganglion cell perikaryon layer	50	15	60
nerve fibre layer	10	30	60

The measurements are taken from tissue fixed for electron microscopy, embedded and sectioned in Araldite (Dowling & Boycott 1966). The thickness of the optic nerve fibre layer varies considerably depending on whether the section is towards the optic nerve head or the other side of the fovea. In the first instance it is thicker and in the latter thinner.

retinae (Pedler 1965). It is true, of course, that the foveal cones are so narrow that when seen by light microscopy they appear rod-like, as was appreciated by Schultze (1866, 1873). Electron microscope observations confirm Schultze's conclusions and show that the fine structure of the foveal cones of primates is like that of other cones in the retina; and that the fine structure of the outer segments of both foveal and peripheral cones is clearly distinct from the fine structure of the outer segments of rods (Dowling 1965; Missotten 1965). There seems no reason, therefore, further to confuse problems of retinal anatomy by rejecting the anatomical evidence for the duplex nature of the primate retina on the evidence from possibly unresolved difficulties in amphibian, reptilian, and avian retinae; especially when the evidence for two classes of receptors is so heavily supported by physiological and psychophysical evidence.

Below the outer limiting membrane (figures 1, 2) are the rod and cone nuclei that form the outer nuclear layer. In the region of these sections the fibres of the receptors pass obliquely away from the fovea. As figures 3 and 4 show, the fibres of the photoreceptors end at the outer plexiform layer in cone pedicles and rod spherules. The differences between the two are clearly recognizable by both light and electron microscopy (Missotten, Appelmans & Michiels 1963). The outer plexiform layer consists of the processes of the retinal nerve cells that synapse with the cone pedicles and rod spherules. The term is here restricted to the zone from the receptor bases to the outer edge of the inner nuclear layer.

The inner nuclear layer contains horizontal cell perikarya along its scleral (outer) edge. The bipolar cell perikarya are mostly in the middle, and the amacrine cell perikarya are along the vitreal (inner) edge (figures 5, 6). There is some displacement of the perikarya between the layers and bipolar cell perikarya may be found at either edge of the inner nuclear layer, while 'displaced' ganglion cells may also be found along the vitreal edge (figures 7, plate 38). Figures 3 and 4 show aggregations of stained material at the inner faces of the rod spherules and cone pedicles, as was noted by Held (1905) for cones. This corresponds to the invaginations formed by the processes of the bipolar and horizontal cells that are inserted into the receptor cell bases. In the same plate, material that electron microscopy shows to be mitochondrial can be seen in the centre of the cone pedicles. On the side of the inner nuclear layer nearest the inner plexiform layer occasional

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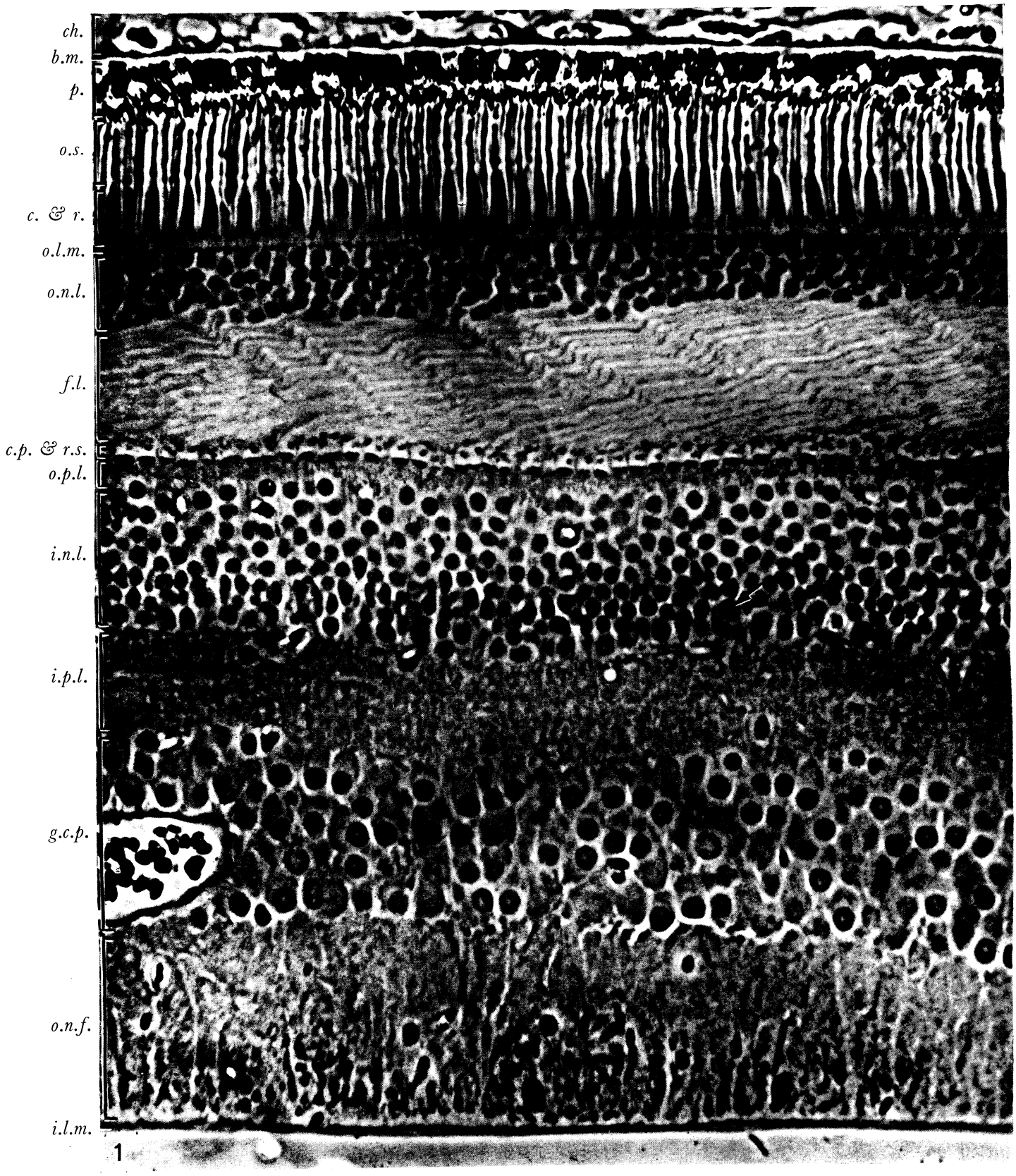
Description of plate 32

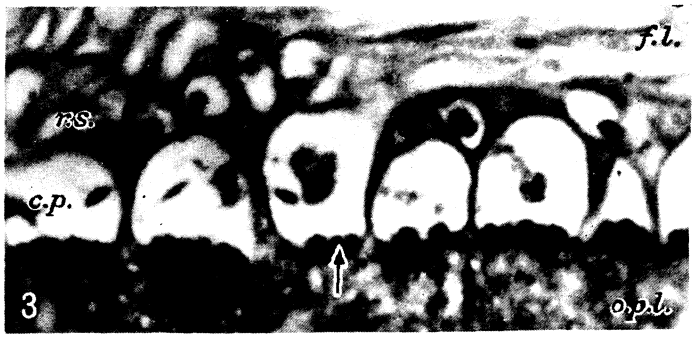
Vertical section through a human retina

Figure 1. Fixed in osmium tetroxide for electron microscopy (Dowling & Boycott 1966) embedded in Araldite and cut about 2 to 3 μm thick. The picture was taken of a piece about 1·25 mm from the centre of the fovea and was photographed by phase-contrast microscopy. From the outer edge of the pigment epithelium to the inner limiting membrane the retina was 400 μm thick. The thickness of the individual layers is given in table 1. The arrow points to a displaced ganglion cell. Magn. × 550. *ch*, choroid; *b.m.*, Bruch's membrane; *p.* pigment epithelium; *o.s.*, outer segments of rods and cones; *c* & *r*, inner segments of cones (*c*) and rods (*r*); *o.l.m.*, outer limiting membrane; *o.n.l.*, outer nuclear layer; *f.l.*, fibre layer; *c.p.*, cone pedicles,; *r.s.*, rod spherules; *o.p.l.*, outer plexiform layer; *i.n.l.*, inner nuclear layer; *i.p.l.*, inner plexiform layer; *g.c.p.*, ganglion cell perikarya; *o.n.f.*, optic nerve fibre layer; *i.l.m.*, inner limiting membrane. A portion of a blood vessel with erythrocytes shows in the ganglion cell perikaryon layer.



(*Facing p.* 116)


b.m.
p.
o.s.
c. & r.
o.l.m.
o.n.l.
f.l.
p. & r.s.
o.p.l.
i.n.l.
2


f.l.
r.s.
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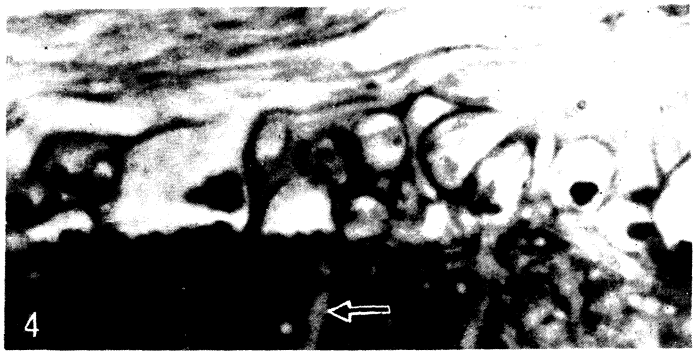
3
o.p.l.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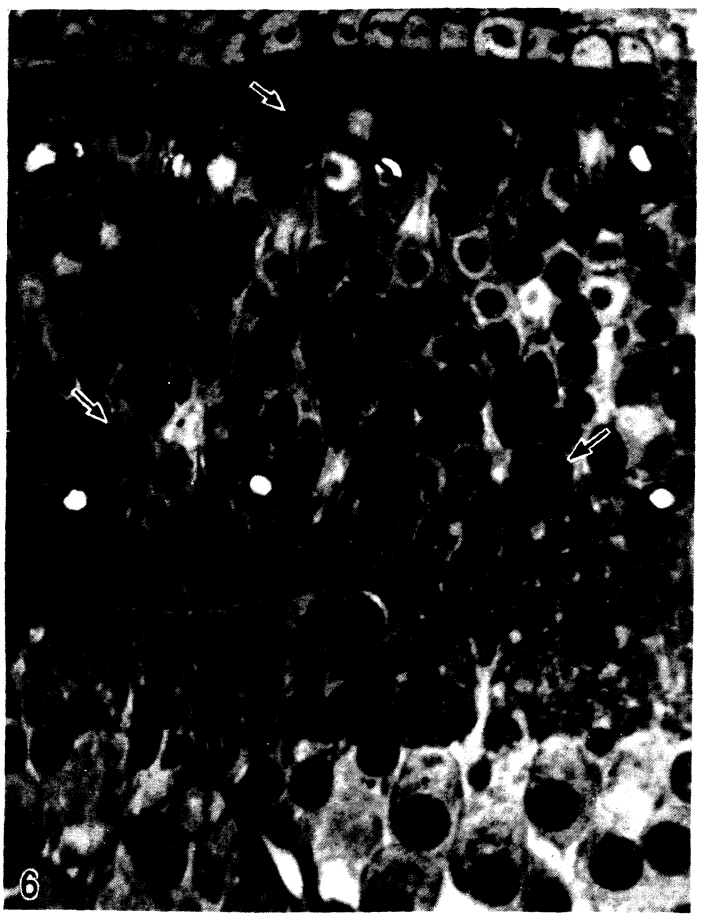
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i.n.l.
7
i.p.l.

Description of plate 33

Vertical sections through a human retina. The material was treated as in figure 1, plate 32, but stained by the method of Richardson *et al.* (1960). Magnifications all approximately $\times 1000$ except for figures 3 and 4 at $\times 2000$.

Figure 2 shows the outer plexiform and photoreceptor layers. The arrow points to a midget bipolar cell, whose apical dendrite can be seen going to a cone pedicle. For abbreviations see figure 1, plate 32.

Figure 3 shows that, where the cone pedicles are adjacent to each other, the rod spherules are stepped back from the outer plexiform layer. The arrow points to a triad of which several can be seen in each cone pedicle. The dark centre in each rod spherule also represents sites where dendrites invaginate into the photoreceptor base. The material in the centre of the cone pedicles is mostly mitochondrial. The diameter of the cone pedicles in this region was 8 μm and of the rod spherules 3 μm.

Figure 4 shows that further towards the periphery of the central area the rod spherules come to lie between the cone pedicles. Some of the rod spherules border the outer plexiform layer but here rods are more numerous and the spherules are piled up on each other (see also figure 28, plate 35). The arrow points to what is probably a portion of rod bipolar dendrite that passes around the cone pedicle towards the rod spherules. In this picture and figure 3 the apparently different sizes of the cone pedicles are, of course, due to the fact that they are not in the same plane of section.

Figure 5 shows that with thin sections it is possible to identify amacrine cells by this procedure. Arrows point to two large amacrine cell perikarya each about 10×15 μm, whose nuclei are lobulate. The small perikarya lining this border of the inner plexiform layer are also lobulate, as can just be seen in the cells indicated by arrows in figures 6 and 7. This is a useful criterion for the identification of amacrine cells by electron microscopy (Dowling & Boycott 1966). In this region of the retina the ganglion cells are stacked two deep. The ganglion cell perikaryon labelled *g*, is probably that of the kind of cell illustrated in figure 88, plate 43.

Figure 6. The field of this section is from the bases of the photoreceptors to the ganglion cell layer, and shows, amongst other things, an interstitial amacrine cell in the inner plexiform layer. The lower arrows indicate amacrine cells with visibly lobulate nuclei. The upper arrow indicates a perikaryon, bordering the outer plexiform layer, which is probably that of a horizontal cell. Whereas at the border of the outer nuclear layer and the inner nuclear layer the perikarya are nearly all those of amacrine cells (see, however, figure 7), and these may be stacked two perikarya thick (see figure 62, plate 40), the border between the former layer and the outer plexiform layer contains a single layer of horizontal cell perikarya. These may, however, be interspersed with bipolar cell perikarya as shown in figure 2. Between the two borders the perikarya are mostly those of bipolar cells and Müller's (glial) cells. Towards and in the periphery, the number of cells per unit area and consequently the thickness of the inner nuclear layer decreases (table 1). The cell in the centre of the inner plexiform layer, on the evidence available from cells previously described in this position (see p. 148), is an interstitial amacrine cell. However, the nucleus has a readily visible nucleolus and does not appear to be lobulate. As can be seen here, it superficially resembles a small ganglion cell perikaryon. There is, therefore, the possibility that such cells have axons and are, in fact, ganglion cells not amacrine cells.

Figure 7 shows a perikaryon with a nucleus to one side of the cell and a large amount of cytoplasm in proportion to the nucleus. Ganglion cell perikarya as seen in the other figures on this plate differ from the perikarya with small nuclei and a thin sheath of cytoplasm, which are those of bipolar cells, and the perikarya with lobulate nuclei, which are those of amacrine cells (arrow). The perikaryon (which is 14×20 μm) illustrated here, therefore, resembles those of the ganglion cells. Because it is in the amacrine cell portion of the inner nuclear layer it is referred to as a 'displaced' ganglion cell (p. 164).

perikarya with indented nuclei are seen (figures 5, 6). This is a common feature of the nuclei of primate amacrine cells. The inner plexiform layer comprises the neuropil between the inner nuclear and ganglion cell layers. The paler streaks seen in figures 5 and 6 are the axons of the bipolar cells, the larger dendrites of the ganglion cells and the larger processes of the amacrine cells. Many of the irregular pale areas represent portions of the larger bipolar terminals. In the regions illustrated the ganglion cells are piled from two to five perikarya deep. They are, therefore, all from the central area. Beyond the ganglion cell perikarya are the axons of the ganglion cells which make up the optic nerve fibre layer (figure 5).

Results

Outer plexiform layer

Introduction

It is at the outer plexiform layer that the rods and cones come into synaptic relationship with the neural elements of the retina. There are horizontal cells that extend laterally between the photoreceptors, and bipolar cells whose axons conduct the effects of the photoreceptors to the inner plexiform layer. Cajal reasoned that the rods and cones should have mutually exclusive pathways to the inner plexiform layer (p. 114). Polyak also recognized this distinction but supposed that the rod bipolars had, in addition, synaptic contact with cones. Cajal found only one main type of cone bipolar in mammals and a less common, 'giant cone bipolar'. Although Cajal was the first to describe in birds and reptiles a cone bipolar that is connected exclusively to a single cone, he did not find such a bipolar in mammals. Polyak found this kind of bipolar, the midget bipolar, in primates. In addition, he described two other main kinds of cone bipolar, the flat and the brush bipolars, as well as a new type of bipolar which he regarded as centrifugal in its direction of conduction. Cajal found two main kinds of horizontal cell in vertebrates which, at least in mammals, he suggested might go separately to rods and to cones; while Polyak, in primates, found only one kind of horizontal cell that he thought connected the cones to rods and perhaps other cones.

With an electron microscope it can be seen that most of the processes from the bipolar and horizontal cells that contact the receptors penetrate into invaginations in the receptor terminals. Above each invagination in the receptor terminal is a synaptic ribbon (De Robertis 1962; Sjöstrand 1953). In rods there is usually one invagination into which from four to seven processes may penetrate (Missotten *et al.* 1963); while in cones there are from 12 to 30 or even more invaginations. Each cone invagination is made up of a group of three processes and such a group has been called a triad by Missotten (1965). Stell (1965*b*) has further shown by the electron microscopy of Golgi-stained material that horizontal cell dendrites terminate in the lateral position in the invaginations of the cone pedicles, while the centrally placed elements are bipolar cell dendrites. In the cone pedicles of primates, it is the midget bipolar dendrites that end as the central element in the triad (Missotten 1965; see appendix, page 177). The diffuse cone bipolar dendrites do not penetrate into the invaginations but end superficially on the base of the cone terminal (Missotten 1965; Kolb, in preparation). In the following account of the outer plexiform layer the classification of the types of bipolar and horizontal cells will be discussed and more evidence given to show how they are most probably arranged in relation to the photoreceptors.

Observations on bipolar cells

Rod bipolar cells (figures 11 to 13, 17, plate 34; figures 23, 29, plate 35).

Polyak's evidence that rod bipolar cells synapse with cones depended on the observation that the processes of the dendrites on the way to the rod spherules pass very close to the sides of the cone pedicles (figure 12, plate 34). From the appearance of the branching of the dendrites of these bipolars he named them 'mop bipolars' (figure 19*a*). Electron microscopy reveals that there are no synaptic contacts between any bipolar cell dendrites and the sides of cone pedicles, or for that matter the sides of rod spherules (Missotten 1965; Dowling & Boycott 1966). Thus, as Cajal emphasized, the rod bipolars are probably connected only to rods.

The apical dendrite of a rod bipolar cell divides many times within the outer plexiform layer to form a number of small processes that enter the rod spherules. Where they enter the spherule the dendritic tips are about 0·2 μm in diameter (figures 23, 29, plate 35). The spread of the branching of the apical dendrites of the rod bipolars is such that it is possible that a single rod spherule receives only one process from any one bipolar. However, adjacent rod bipolars are occasionally stained in a Golgi preparation and the dendritic fields can be observed to overlap by about 10 to 20 % of their diameter (figures 13, 23, plates 34, 35). It is possible, therefore, that any one spherule is innervated by at least two different rod bipolars (p. 138; see also Missotten *et al.* 1963).

The exact distribution and number of the dendritic processes on a rod bipolar in part vary with a rod bipolar's position relative to the centre of the fovea but also with a variation in the innervation ratio of rods to bipolars within the same area. There seems to be a tendency for rod bipolars farther away from the fovea to have more processes than those nearer; this corresponds with increasing rod densities towards the periphery. Compare figures 11 to 13 and 17, plate 34 from which an impression of this can be obtained. In thin sections fixed for electron microscopy careful observation confirms Polyak's conclusion that there are no rod spherules on the foveal slope, either in human or rhesus monkey retinae. At the outer edge of the foveal slope there are a few rods and their terminal spherules. Here the cone pedicles are still close together so that the spherules are displaced away from the outer plexiform layer (figure 3, plate 33). The rod bipolar dendrites go up between the cone pedicles to the rod spherules. Measurements of the diameter of the dendritic spread of rod bipolars in the central area are rather constantly about 20 to 25 μm, although occasionally they are as large as 30 μm or as small as 15 μm in diameter. Because of this rather constant diameter of the dendritic field, the rod bipolars near the foveal slope can only reach a few rods and, therefore, regularly have fewer dendritic processes; usually between 10 and 15 (figure 11, plate 34). Peripheral to this the rod spherules increase in number and are to be found between the cone pedicles as well as piled up on each other (figure 4, plate 33). The dendrites of any one rod bipolar thus terminate at slightly different levels, giving an irregular appearance to the top of the dendritic tree (figure 19*a*, and figures 12, 29, plates 34, 35). In such a region the processes on each bipolar are more numerous and a single rod bipolar cell can have as many as 45 processes and, therefore, could contact as many as 45 rods. However, a simple statement, that all rod bipolar cells more than a certain distance from the fovea always contact more

Description of plate 34

Bipolar cells as observed in vertical sections of the rhesus macaque retina. The magnification for all cells is the same at × 800 and they are all stained by the Golgi–Colonnier procedure. As nearly as possible the outer plexiform layers of the first four pictures have been alined so that the different levels of termination of the apical dendrites can be compared.

Figure 8. A midget bipolar cell on the left and a midget ganglion cell to the right. The apical dendrite of the midget bipolar cell is about 6 μm in diameter. The cells are 1·0 to 1·5 mm from the centre of the foveal pit. [For more details of the bipolar cell see p. 179.]

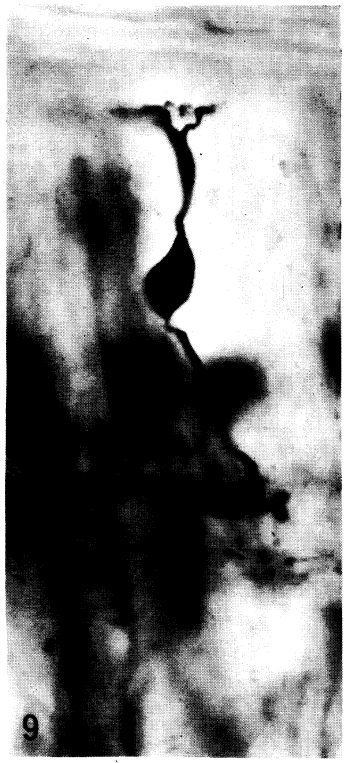
Figure 9. A flat bipolar cell showing two of the branches of the apical dendrite in sharp focus and that these turn sharply to pass parallel to the cone bases. The axon terminals of this cell end in the upper half of the inner plexiform layer. On the average the axon terminals of flat and rod bipolar cells have a lateral extent approximately equal to, or a bit less than, that of the diameter of spread of the apical dendrites. This cell was about 0·5 mm from the centre of the fovea. The dendritic diameter is 20 μm.

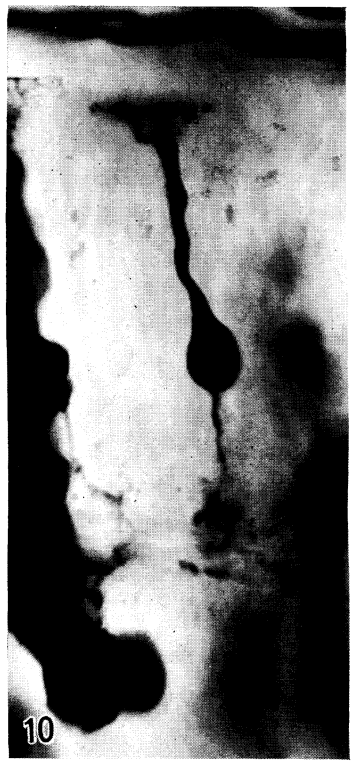
Figure 10. A flat bipolar cell that might be confused with Polyak's brush bipolar unless the apical dendrites are examined sufficiently closely to reveal that they turn parallel to the base of the cone pedicles. The axon terminals of this cell branched in the middle of the inner plexiform layer but only a small portion of them is visible in this illustration. The cell was about 500 μm away from that in figure 8 and 200 μm from that in figure 11; all were on the same section. The dendritic diameter is 22 μm.

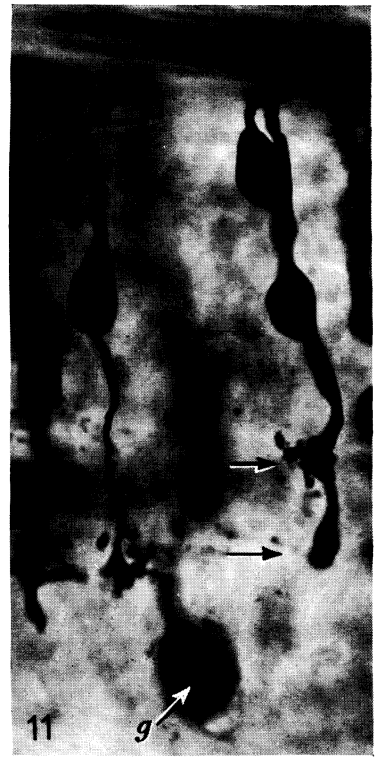
Figure 11 contains three bipolar cells. On the left is a rod bipolar cell and on the right are two midget bipolar cells. Comparison of the top of the midget bipolar dendrites with those of the rod bipolar dendrites shows that the latter extend above the level of the base of the cone pedicles. It can also be seen, as in figures 12 and 13 and in figure 29 plate 35, that the processes of the dendrites of the rods end at different levels. The axon on the rod bipolar swells at its end into two large swellings, each about 5 μm by 5 μm, which bear a number of smaller terminals on fine processes. The largest swelling is sufficiently near to the ganglion cell layer to form an axosomatic junction (contrast its level of termination with that of the midget bipolar cell). The diameter of the dendritic spread is 25 μm. Comparison of this cell and that of the rod bipolar of figure 13 shows that it has fewer dendritic processes. The apical dendrites of the two midget bipolar cells on the right show how the dendritic terminals can be sufficiently close together for the presumption to be that the two dendrites supply only one cone. The diameter of the tops of the two cells together is between 6 and 7 μm, which corresponds well with the 8 μm diameter of the cone pedicles. The arrows in the inner plexiform layer show the level of termination of the axons of each midget bipolar cell. If the cells synapse with midget ganglion cells they must be different midget ganglion cells and this one cone, therefore, could affect two midget ganglion cells. If they synapse with the same ganglion cell then it is likely to be a diffuse ganglion cell. The ganglion cell (*g*) is an incompletely stained diffuse ganglion cell. The cells of figures 8, 10 and 11 were on the same section of the retina within about 500 μm of each other and 1 to 1·5 mm from the foveal pit. [For more details of these cone bipolars see p. 179.]

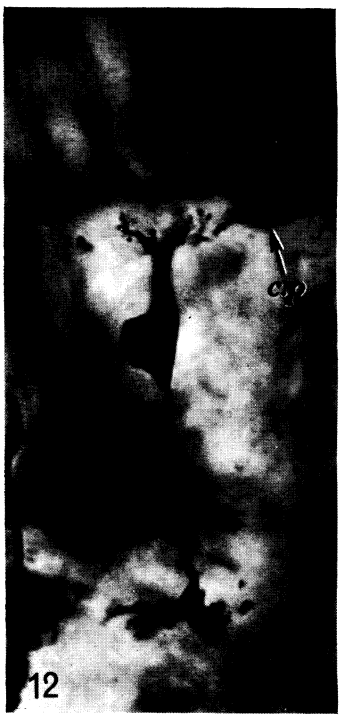
Figures 12, 13. Rod bipolars showing the manner of branching of the apical dendrites and that the dendritic terminals end at different levels corresponding to the levels of the rod spherules (figure 4, plate 33). In figure 12 a process goes close to and apparently touches (arrow) a stained cone pedicle (*c.p.*) but this is not a synaptic contact (p. 119). This illustration also shows how the rod bipolar axon terminal branches near the ganglion cell perikarya. Figure 13 contains a second rod bipolar just out of focus and illustrates how the dendritic fields of nearby rod bipolar cells may overlap. The diameter of the dendritic field of both cells is 30 μm.

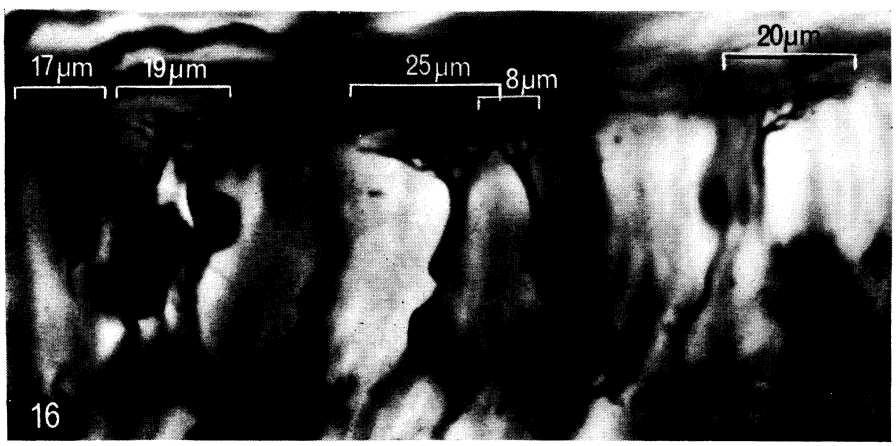
(*Continued on back of plate* 34)











(*Facing p.* 120)

FIGURE 14. A flat bipolar cell near the periphery of the central area. The dendrites of the cell can be clearly seen to turn parallel to the cone pedicle (*c.p.*). By contrast with the bipolar cells in figures 8–13 this and other more peripheral bipolar cells (figures 15, 17 and 18) are shorter. This is due to thinning of the inner nuclear layer (table 1). The diameter of the dendritic spread of the bipolar cell is 30 μm and the cone pedicle diameter is between 9 and 10 μm. The cell was between 2 and 3 mm from the centre of the foveal pit.

FIGURE 15. A midget bipolar from towards the periphery of the central area showing the difference in shape between the axon terminals of such bipolars and those nearer the fovea, for example as shown in figure 8 of this plate. The invaginating processes of the apical dendrite show clearly. The diameter of the dendritic spread is 8 μm and the diameter of spread of the axon terminals is 15 μm.

FIGURE 16. Dendrites of flat bipolars on the foveal slope. The diameters of the dendritic fields of those just out of focus have also been inserted. For details see text (p. 127) and table 2.

FIGURE 17. Rod bipolar from the periphery of the retina. The mode of termination of the axon appears different when compared with those of the same kind of cell in the central area. Presumably this may be correlated with a sparser population of ganglion cells. The dendritic diameter is 35 μm.

FIGURE 18. A flat bipolar cell from peripheral retina. The arrow indicates part of a horizontal cell axon. The cell is apparently of a very different shape to that of those flat bipolars in the central area but high-power examination of the apical dendrites showed it to be a flat bipolar. Such an illustration exemplifies the ease with which the numbers of types of cell could be multiplied. Here, at least, it is not the shape of the cell that is important but the connexions and the manner in which they are made.

rods than the rod bipolars nearer the fovea, cannot be made. It is possible to find, within 2 to 300 μm of each other, rod bipolars with 15 or more processes and others with up to 45 processes. It is clear, therefore, that in regions of presumably equivalent rod density the number of rods connected to individual rod bipolars may differ but the area of the retina from which each rod bipolar collects is approximately the same regardless of the rod to rod bipolar innervation ratios. However, those rod bipolar cells with smaller dendritic diameters seem to be nearer the fovea. It is hard to imagine any functional significance for the variations in the innervation ratio of the rods to individual rod bipolars. They are certainly to be related in part to the variations in the distribution of the numbers of rods packed between the cones. However, in other mammalian retinae Cajal has described similar variations in the number of branches on rod bipolar dendrites, suggesting that they connect variously with from 3 or 4 to 15 or 20 rod spherules. There could, therefore, be some as yet unimagined functional significance for these differences between rod bipolars within similar retinal areas.

We have seen no evidence of a Landolt club on rod bipolars in the central area, although such are described by Polyak for rod bipolars in the periphery of the primate retina. Landolt clubs certainly exist in amphibia, reptiles and birds and have been identified by electron microscopy in amphibia (Hendrickson 1966).

Midget or single cone bipolar cells (figures 8, 15 plate 34; figures 24 to 26, plate 35).

(*a*) *Midget bipolar cells on and near the foveal slope.* Polyak was the first to recognize the importance of the midget bipolar and to describe it thoroughly (figure 19*b*). As he pointed out, such a kind of bipolar had been seen before, in primates, by several authors, including Held (1905). It had not, however, been recognized as in direct synaptic contact with only a single cone. Perhaps because a midget bipolar cell type has not been observed in other mammals, and perhaps also because Polyak's anatomical statements have often been summarized unclearly (see, for example, Fulton 1949; von Frisch 1964; Dowling 1965; Berrill 1967), it is often supposed that a cone which synapses with a midget bipolar synapses with that bipolar and none other. If this were not thought to be true for the whole retina, then it was sometimes supposed to be true for the foveal cones (e.g. Morgan 1965). None of this is true, and it is not what Polyak wrote. Polyak stated that a midget bipolar is connected to only one cone, but that 'the same cones that are in contact with the midget bipolars are also related to other varieties of bipolars'. Nonetheless, there is a great deal of confusion and in a summary of retinal structure in 1962 Granit had to write '...that the accepted view is that there are as many foveal cones as bipolars and thus a relation of 1:1'. In view of the quotation from Polyak and, as Granit pointed out, Vilter's (1949) data showing that there are three times as many bipolar cells as cones, it is difficult to see how the idea that primate cones may synapse with only one bipolar got such currency and caused confusion of discussion (see, for example, discussion of Pedler 1965).

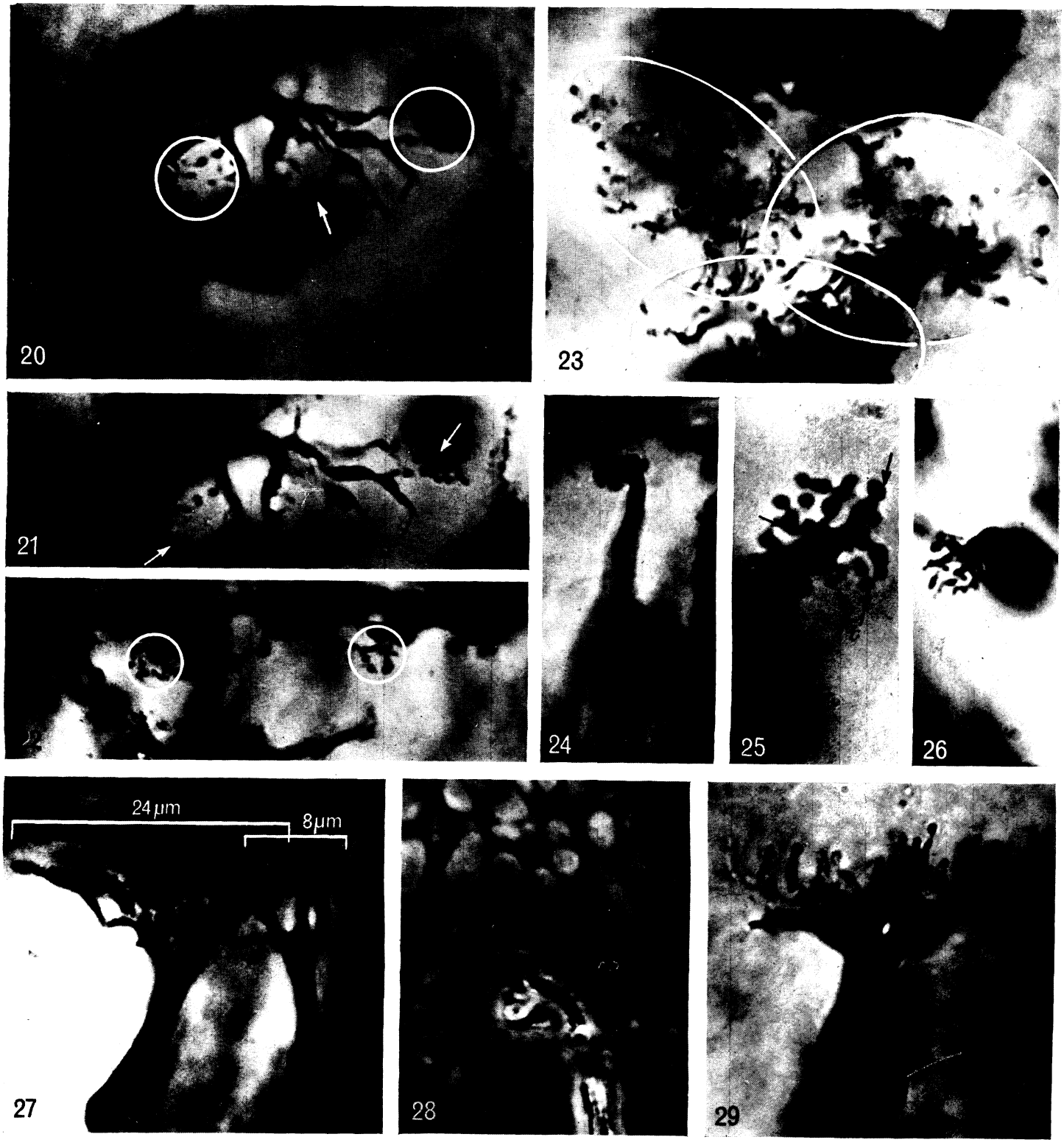
We agree with Polyak's observation that the apical dendrite of each midget bipolar cell can be seen as unbranched in the outer plexiform layer until it reaches the level of the cone pedicles (figure 2, plate 33; figure 8, plate 34), when it is often seen to terminate in a number of small knobs about 0·2 μm in diameter (figures 24 to 26, plate 35). However, his

evidence that they synapse with only one cone had to be based essentially on the correspondence between the diameters of the tops of the apical dendrites and the diameters of the cone pedicles. In view of the limitations imposed by observation of Golgi material (p. 114), this, of itself, does not exclude other possibilities.

The tops of midget bipolars are shown in figures 8 and 24 to 26, plates 34 and 35. The number of dendritic terminals on some of the cells can be counted. Of 20 midget bipolar cells within 150 μm of the centre of the fovea the diameter of their dendritic spreads varied between 4 and 7 μm, their average diameter was about 5 μm. This corresponds to

FIGURE 19. Diagram of three main kinds of bipolar cell found in the central area of the rhesus macaque retina as seen in vertical section. *a*, rod bipolar; *b*, midget bipolar (see p. 177 for the two kinds of midget bipolar); *c*, flat bipolar. No attempt has been made to represent all the branches on the cells. Detailed variations in shape of the different types are well illustrated in Polyak. The scale is given by the diameter of the top of the midget bipolar, which is approximately 6 μm (see also plates 34 and 35).

the diameter of the cone pedicles in that region (Dowling 1965). The apex of the midget bipolar dendrite and its branches could, therefore, fit comfortably into a cone base. However, because the cone pedicles are packed tightly together in the region near the foveal pit, it might be argued either that the apical dendrite of a midget bipolar sits in the middle between two cones and sends half of its processes to each or that it is sited between four cones and sends a quarter of its processes to each. There could not be a relationship with more than four cones because the diameter of spread of the midget bipolar dendrite is insufficiently great. However, if either of these conditions were to occur, the branches of the dendrites would have to be gathered into two or four groups to the side of the meridian of the apical dendrite. This would give an arrangement similar to the groupings of the dendrites of a single type A horizontal cell, which innervates seven different cones (p. 134). The processes of the midget bipolars are, in fact, evenly distributed (figures 24 to 26, plate 35), and thus in the region of the fovea they clearly contact one cone pedicle each.



Bipolar cells of rhesus macaque retina as observed in vertical and horizontal sections. All are stained by the Golgi–Colonnier procedure and the magnification is × 2000 except for figure 25 at × 4000.

FIGURE 20. Horizontal section showing a flat bipolar cell with the dendrites branching in a plane and terminating in fine processes that are distributed so that they can be thought of as corresponding to the diameter of the base of a cone pedicle. The circles represent bases of cone pedicles 7 to 8 μm in diameter. This picture does not show the whole of the top of the flat bipolar but its dendritic spread is 35 μm. We have never regularly stained all the fine branches of

(*Facing p.* 123)

the dendrites of a flat bipolar. The arrow points to an area where we suppose that there are such fine processes that they have not been impregnated. It is certain that these bipolars, at least outside the foveal slope, synapse with three and four cones. The estimate that they synapse with seven cones is based on counting in spaces such as that indicated by an arrow and assuming that if the impregnation were complete an appearance such as in those circled would be obtained seven times on each cell.

FIGURE 21. Same cell as figure 20 at a different focus. The arrow on the left indicates the nest of terminals in focus in the previous picture. The arrow on the right indicates part of a midget bipolar top with its terminals involved with the terminals of the flat bipolar. They are, therefore, presumably both postsynaptic to the same cone.

FIGURE 22. Horizontal section showing a flat bipolar as in figures 20 and 21. Whereas that cell was about 2 mm from the foveal centre this one is from the foveal slope, and the area covered by the terminal processes is correspondingly smaller because the cone pedicle diameters on the foveal slope are between 4 and 5 μm (see circles). This flat bipolar has a dendritic diameter of 25 μm and has *seven* aggregates of fine terminals.

FIGURE 23. Two rod bipolar cells and a portion of a third as seen in horizontal section to illustrate the appearance of their terminal dendrites. The approximate fields of each cell are shown by circles and their degree of overlap indicated. Fifty-four terminals were counted on the upper left cell and 42 on the cell on the right. Many of the terminals of the lower cell were obscured by glia.

FIGURE 24. Midget bipolar cell from the middle of the foveal slope observed in vertical section but with the tip viewed obliquely showing the small terminals that are inserted into the centre of the triads. The cell was adjacent to those of figure 27. The diameter of the tip of the dendrite is 5 μm and the estimated number of terminal knobs was 11.

FIGURE 25. Top of a midget bipolar about 2 mm from the foveal centre at a magnification of $\times$ 4000. The larger swellings, two of which are indicated by arrows, are probably due to mitochondria. The estimated number of terminals (*t*) was 16 to 19 and the diameter of dendritic spread is 6 μm; a cone pedicle 25 μm away measured 7·5 μm in diameter.

FIGURE 26. View of the terminals of a midget bipolar cell as seen in horizontal section. This cell was about 1·5 mm from the foveal centre. The diameter of the top was 5 μm and the number of dendritic terminals was 14. A nearby stained cone had a diameter of 6 μm.

FIGURE 27. Two of the flat bipolar cells illustrated in figure 16, plate 34, at a magnification of $\times$ 2000 to show how, when the dendrites of these cells turn parallel to the cone base, they can appear, because of the perspective, to have irregular swellings and clumps on the dendrites.

FIGURE 28. Vertical section of one of the branches of a stained flat bipolar cell that had been embedded in Araldite and cut about 3 μm thick. It shows how the finer branches of the terminals are arranged along the cone pedicle (*c.p.*) base. The picture was taken by phase-contrast microscopy. This cell is from the near periphery so that numerous rod spherules (*r.s.*) are to be observed beside the cone and stacked upon each other (see also figure 4, plate 33). The diameter of the cone pedicle is 8 μm.

FIGURE 29. A higher magnification of the rod bipolar cell in figure 13, plate 34, showing, as far as possible in a photograph, that the rod bipolar dendritic terminals end at different levels. The diameter of rod spherules is about 3 μm and dendrites invaginate into the centre of the spherule, so from the spacing of the terminals in this figure and in figure 23 above, it is possible to estimate that any one rod bipolar cell sends only one dendrite to any one rod spherule in its field. Where rod bipolar dendritic fields overlap, an individual rod spherule could receive processes from two separate rod bipolars. It must be remembered however, that, as Missotten *et al.* (1963) showed, the number of processes in an individual rod spherule differs for different spherules within the same area, so that to discover the exact connexions of the rod spherules will be more difficult than for cones.

Foveal cones in rhesus monkey have a cone pedicle about 4 to 5 μm in diameter which contains on average, 12 invaginations and 12 synaptic ribbons. Each invagination is made up of three processes which gives a total of 36 processes invaginated into each cone base (Dowling 1965). The counts of the number of apical processes on the same 20 midget bipolar cells measured above varied between 7 and 14 for an average of between 11 and 12. Therefore, the most likely arrangement is that one process from a midget bipolar goes to each invagination in a cone pedicle. This would agree with the electron microscopic identification of midget bipolar dendrites as the central element in the triads. The two lateral elements in the invaginations, on the other hand, have been shown to be horizontal cell processes (p. 135).

There remain some observations at variance with the above interpretation and, as described below, there are some further variations in the midget bipolars towards the periphery. Polyak, in his description of midget bipolar cells, said that the number of processes on their single apical dendrite varied from 1 to 36. There are, however, no cone pedicles with fewer than about 10 triads although there are peripheral cones with 36 and more. We have found no unequivocal instances of midget bipolar cells with fewer than seven processes on the apical dendrites and there are usually not less than 10. It is likely, therefore, that Polyak's cells with fewer than seven or eight processes were incompletely impregnated. If Polyak's observation is correct such cells must end in processes much larger than the usual 0·2 μm diameter of the midget bipolar terminals. Electron microscopy shows that none of the central processes of the triads in any cone pedicle is larger than about 0·2 to 0·3 μm in diameter (Dowling and Boycott 1966). However, as the succeeding paper (p. 177) shows there are midget bipolar cells on which the top of the apical dendrite appears by light microscopy as a single mass as many microns in diameter as the adjacent cone pedicles. These were also observed by Polyak.

(*b*) *Midget bipolar cells in the para- and perifoveal regions.* Cone pedicle diameters increase with increasing distance from the fovea at least up to 4 or 5 mm from the foveal pit centre. Correspondingly, the number of triads, and therefore the number of processes invaginated into the cone bases, are also found to increase. If the preceding arguments for fitting a single midget bipolar into a single cone base are correct the tops of the midget bipolars should show a corresponding increase in diameter as the cone pedicle diameters increase. The number of processes on these bipolars (since there are more triads) should also increase. Because Golgi procedures stain only a very few cells in any given population it was uncommon to find instances where a cone pedicle is stained nearby a stained midget bipolar cell, thus permitting directly comparable measurements to be made in a given area. Such was possible in 10 instances where cone base diameters of 7 to 8 μm were found within 200 μm of midget bipolar cells that had a top diameter of the same order as the cone base (figure 30*a*, figure 15, plate 34). The processes on the tops of such bipolars are, like those for the foveal cones, about 0·2 μm in diameter, but they are often also more numerous, ranging from 19 to 21 in number (figure 25, plate 35). This corresponds very well with the number of triads estimated from electron microscopy to be in cone bases of that size. However, in regions where the cone pedicle diameters are large, it is still possible to find midget bipolar tops that are as small as about 5 μm in diameter and with only about 12 terminal processes (figure 15, plate 34). From such arguments it follows, either

that in some cones the centres of the triads are filled by processes other than those of the midget bipolars, or that away from the fovea there can be some different relationships between the midget bipolar cells and the cones. Proof is obviously difficult but the circumstantial evidence of three observations suggests the latter interpretation to be the more likely.*

1. There are only two kinds of cone bipolar cell; of these, only the midget bipolar cell has processes ascending towards the cone base, the processes of the flat bipolar run parallel to the cone base.

2. Midget bipolar cells have been stained so that two apical processes of about 5 μm lie close enough together to suggest that they enter a single cone (figure 30*c* and figure 11, plate 34).

3. Midget bipolar cells with the apical dendrite split into two (a kind described by Polyak as abnormal) are found. Where this is so the total diameter of the tops of the two branches of the dendrite often corresponds to the diameter of the cone base (figure 30*b*).

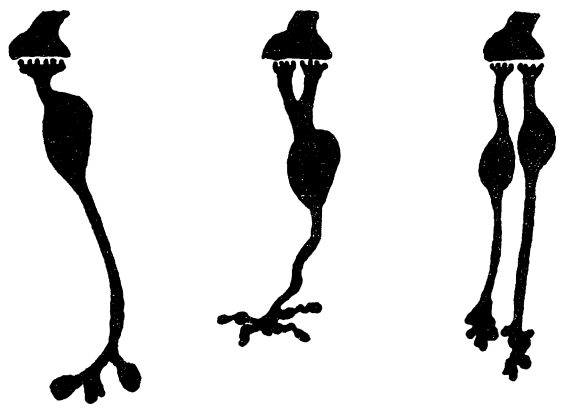


FIGURE 30. Diagram of midget bipolar cells in vertical section to show some of the arrangements of the synapses that may exist between them and the cones towards the edge of the central area and in the periphery of the retina. Note also the differences in the axon terminals of *a* and *b* when compared with the midget bipolars (figure 19*b*) nearer the fovea. The cells are shorter than those nearer the fovea because the inner nuclear layer is thinner (Table 1). The diameter of the top of cell *a* is approximately 8 μm. Figure 99 p. 178 shows the arrangement for flat and invaginating midget bipolars.

Midget bipolars are occasionally found with a divided apical dendrite so spread out that each part must synapse with two separate cones. Such cells have been seen by Held (1905), Polyak and ourselves. Yet, in general, just as in the immediate foveal region, each cone is connected to only one midget bipolar cell; if not, it is connected to two midget bipolars that are very close together. We have no means of estimating the frequency of this arrangement. However, when two cells stain as closely together as those in figure 11,

* Since this account was written a midget bipolar cell with contacts on the cone pedicle base has been found (see p. 177). The arguments here are referring to the problem of filling the centres of triads with invaginating midget bipolar processes and are none the less valid.

plate 34, it can be seen that they may not necessarily contact the same midget ganglion cell, although they could contact the same diffuse ganglion cell (p. 157). The instances of midget bipolar cells going to two cones probably need no explanation because the cones could well be of the same colour-coding and so make no important functional difference amongst a large population of cells. Such cells are most usually found towards the periphery of the central area and outwards.

Diffuse cone bipolar cells (figures 9, 10, 14, 16 and 18, plate 34; figures 20 to 22, 27 and 28, plate 35).

(*a*) *Brush and flat bipolar cells.* In Polyak's introduction to his definition of separate brush and flat bipolar cells he suggests that they may not really be so different from each other. His feeling of uncertainty is repeated in the 1957 book, and perhaps because of this his exact distinctions between the two are difficult to follow. The basic distinction of the flat bipolar cell from all other bipolars is that the apical dendrite branches as it passes through the outer plexiform layer, but at the line of the receptor cell bases the processes turn sharply to follow a course parallel to those bases (figure 19*c*, and figures 9, 10 and 14, plate 34). We agree with this description. On the other hand, according to Polyak, a brush bipolar cell branches more frequently in its passage through the outer plexiform layer and when the dendrites have turned parallel to the receptor cell bases there are fine processes that go directly upwards towards the receptors. In this way, as Polyak himself points out, they resemble the branching of the rod bipolar dendrites going to the rod spherules. The sort of contrast Polyak describes is illustrated by a comparison of the cells in figures 9 to 14, plate 34.

In general we have found that the observational difficulty is not to distinguish between flat and brush bipolars but between brush and rod bipolars. Distortion of the retina during the Golgi fixation can make rod bipolar cells resemble Polyak's description of a brush bipolar. It is hard to give reliable justification, using Golgi techniques, for the complete non-existence of a structure claimed by another worker. In our material we have several times seen stained rod and cone terminals in proximity to a bipolar cell resembling Polyak's brush bipolar. Such a bipolar might appear to have its dendrites entering a stained cone, but careful examination of the dendrites showed that the processes went around the sides of the cone and on towards the unstained rod spherules. Where this was to be observed it was clear that the bipolar being examined was a rod bipolar and that it differed from other rod bipolars only in having its dendrites compressed. It is likely that Polyak's identification of a brush bipolar is a misinterpretation of rod bipolars distorted during fixation and staining of the retina. Also Polyak apparently did not appreciate that rod bipolars vary in their exact appearance. Those rod bipolars, near the foveal slope and elsewhere, that have fewer processes (see p. 119) can resemble Polyak's description of a brush bipolar. In addition, as figures 17 and 18, plate 34 indicate, a peripheral flat bipolar can superficially appear like Polyak's description of a brush bipolar. We have, therefore, concluded that there is only one kind of diffuse* cone bipolar in the primate retina—Polyak's flat bipolar.

* Here the term diffuse is as used by Polyak to mean connecting to more than one cone, in contrast to the single-cone or midget bipolar (see footnote p. 147).

An important observation to support this conclusion is that on the foveal slope, which is free of rod spherules, we have frequently stained flat bipolars but have never stained a cell that could be regarded as a brush bipolar. Furthermore, since as best we can tell at present all the elements contributing to the invaginations in the cone pedicles can be accounted for, there is no room for the processes of another cone bipolar. If there were another kind of cone bipolar it would have to have the relationships to the cones of a flat bipolar. In that case it would be, by definition, a flat bipolar.

(*b*) *Flat or diffuse cone bipolar cells.* We agree with Polyak's description of the branching of the flat bipolar dendrites in the outer plexiform layer and that the penultimate branches turn parallel to the bases of the receptor cells when they reach that level (figures 9, 10 and 14, plate 34). Viewed from above (figures 20 to 22, 27 and 28, plate 35) at the level of the receptor terminals it can be seen that the dendrites divide from the apical dendrite and radiate horizontally to form at their tips, or from side branches, aggregates of very fine processes. These fine branches are in a plane and cover an area that corresponds approximately to the area of a cone base. This correspondence of the area over which the terminals are distributed to the cone pedicle base diameters is illustrated by a comparison of flat bipolar terminals on the foveal slope with those more peripheral (figures 20 to 22, plate 35).

Careful focusing on the terminals of flat bipolar cells shows that the dendrites do not send processes out of the plane in which they lie. This agrees with Polyak's conclusion that these cells are cone bipolars, for in order to enter rods they would have to have processes ascending from their plane of branching to reach up to the rod spherules. The appearance of flat bipolars also agrees with the conclusions that the processes that form the cone triads come from horizontal cells and midget bipolar cells (pp. 137 and 124). It follows that the synapses of flat bipolar cells are with the base of the cones and not as invaginations into the cones. Missotten (1965) showed by electron microscopy of serial sections of a flat bipolar that its processes ran along the outer plexiform layer aspect of a cone pedicle. However, typical synaptic specializations between the cones and these bipolar cells have not been observed in primates (Missotten 1965; Dowling & Boycott 1966). In other retinae membrane specializations at such superficial contacts have been described (Dowling 1968) and in *Necturus* retina synaptic ribbons are associated with the superficial contacts on the receptor terminals (J. E. Dowling, in preparation).

While it is very likely that flat bipolar cells have synaptic relations with the photoreceptors as deduced above, it is not easy to decide: (1) how many cones synapse with a single flat bipolar cell; (2) how many flat bipolars synapse with a single cone. Table 2 shows the diameter of the spread of the dendrites of 17 flat bipolar cells as measured from vertical sections of retinae from two different eyes, and their approximate distance from the foveal centre (figure 16, plate 34). These cells have their apical dendrites on or very near the foveal slope, which is where the cone pedicle diameters are between 4 and 5 μm and lie packed close together. Assuming that the dendrites parallel to the cone pedicles make contacts with them along the whole of their length, then a flat bipolar with a dendritic diameter of 20 μm could contact as many as 10 cones. One with a diameter of 30 μm could contact about 30 cones. However, it is unlikely that this is the arrangement. As pointed out above and illustrated in figures 20 to 22, plate 35, the tips of the dendrites branch to form

an aggregate of very fine processes. The processes are so fine that quite frequently they are not impregnated or are impregnated unevenly (for details see figure legends 20 to 22, plate 35). When these flat bipolar terminals are impregnated they assume an arrangement that gives the impression that they are spreading over an area equivalent to that of a cone base and interdigitating between the processes of the midget bipolar and horizontal cells (figure 21, plate 35). Figure 28, which is a thin vertical section of a Golgi-impregnated flat bipolar cell observed by phase-contrast microscopy, shows the fine processes as dotted along the base of a cone pedicle. We have concluded from such appearances that these structures are the

TABLE 2. DENDRITIC DIAMETERS OF FLAT BIPOLAR CELLS ON THE FOVEAL SLOPE AS MEASURED IN VERTICAL SECTION

distance from foveal centre (μm)	diameter of dendritic spread (μm)	distance from foveal centre (μm)	diameter of dendritic spread (μm)
100 to 200	25	50 to 100	10
	30		15
	20		12
	17		15
	25		15
	19	200 to 300	28
	8		20
directly on edge of foveal pit	29		22
			21

Because of the manner in which the dendrites of flat bipolar cells spread out it is difficult to be certain of the accuracy of the measurements. However, it does seem clear that there are flat bipolars with dendritic diameters of 20 to 30 μm and those with smaller diameters down to 10 μm. In an area like the foveal slope where the cone pedicles are packed closely together, quite small variations in dendritic diameter could produce quite large variations in the number of cones contacted unless, as we think, the cones are only contacted at the tips of the dendrites. In which case the spatial distribution of the seven cones would vary for different flat bipolars. It is hard to imagine what would be the significance of such an arrangement.

points of contact with the cones. Whatever the diameter of dendritic spread of a flat bipolar cell, there appear to be six or seven terminal aggregates of processes on each cell. If these are the only sites of contact of these bipolars with the cones, then it is probable that each flat bipolar is in synaptic relationship with only that number of cones.

Figures 16 and 27, plates 34 and 35, show that at least on the foveal slope, the dendrites of the flat bipolars overlap but we have no direct evidence that any one cone contacts more than one flat bipolar. If Vilter's (1949) data suggesting there are three times as many bipolar cells as there are cones in the foveal region are more or less correct, (see page 121 and Granit 1962) then, since there is one midget bipolar cell to each cone, it is possible that, near the fovea, any one cone is in contact with more than one flat bipolar cell.*

Polyak stated a similar figure for the number of cones contacting a single flat bipolar cell. He also observed, as we have done, that outside the immediate region of the fovea the dendritic diameter of the flat bipolar cells is more regular at about 20 to 30 μm. However, such cells, outside the foveal slope, on the same arguments as above, appear to be connected

* Since it is now known there is more than one midget bipolar to some if not all cones (p. 177) these kinds of figures and arguments are difficult to assess.

to about six or seven cones but we have not been able to observe whether their fields overlap (see Discussion, p. 164).

Centrifugal bipolar cells (figure 68, plate 40)

Although Polyak was diffident about centrifugal fibres, that is to say fibres conducting from the brain to the retina, he described and insisted upon a centrifugal bipolar cell. This cell had dendrites in the inner plexiform layer and an axon to the outer plexiform layer, where it was supposed to contact only cones (figures 31*a* and 31*b*). We have not observed, in its entirety in an adult, such a cell as Polyak described. His photomicrograph 61 B comes from a young chimpanzee and most of his line drawings are from that animal. Yet he insisted on the presence of centrifugal bipolar cells in all three of the primates he studied.

Polyak based his case for certain bipolars conducting centrifugally on the nature of the branching and other appearances of the processes of the cells. The scleral process was supposed to have 'the appearance of the axon' and the vitreal process 'the appearance of dendrites'. He did not give, and could not then have given, other evidence that these bipolars are centrifugal. Thus, even if it be granted that structures with the morphology he described exist, there was, and is, no reason to suppose that they conduct towards the photoreceptors. Since synapses other than those with the receptor terminals have not been observed in the outer plexiform layer of primates (Dowling & Boycott 1966), such cells would have to make presynaptic contact with the receptor terminals.

Polyak admitted he saw only a very few centrifugal bipolar cells intact. Yet he believed they were very common, because he often observed cells with their perikarya on the vitreal side of the inner nuclear layer with no processes to the outer plexiform layer, but with several processes descending from the perikaryon and branching frequently in the inner plexiform layer. Polyak admitted that the branching of the processes in the inner plexiform layer agreed with Cajal's description of the shape of the diffuse amacrine cells of other mammalian retinae. We, too, have often observed such cells and, like Cajal, believe them to be amacrine cells (p. 148). Polyak, however, decided that such cells were not diffuse amacrine cells and insisted that diffuse amacrines were centrifugal bipolar cells whose scleral processes had only rarely stained. As Missotten (1965), also, has noted, this is the only occasion when Polyak claims an extensive and regular failure of the Golgi impregnation of a cell type. Such occurs, but when a cell only partially impregnates it is usually possible to see some evidence of this in the form of interruption of the cell outline, or a faint ghost of a badly impregnated piece as it leaves the well-stained portion. This we have not seen on the scleral side of the many amacrines stained; nor in amacrines studied by electron microscopy has there ever been the slightest sign of a scleral process. Thus we think that the majority of the vitreal processes described by Polyak as dendrites of centrifugal bipolar cells are truly diffuse amacrine cells (p. 148). Polyak made the hypothesis that the dendrites of the centrifugal bipolars were postsynaptic to the somata of ganglion cells. We have shown (Dowling & Boycott 1966) that such axosomatic contacts of the amacrines are always presynaptic to the ganglion cell somata.

It remains, however, to identify the kind of cell that Polyak showed in his photo-

micrograph 61B and in his diagrams of intact centrifugal bipolar cells. That we have probably done in retinae from foetal rhesus monkey of 108 days gestation. Figure 68, plate 40, shows a cell with processes in the inner plexiform layer resembling those of an amacrine cell. At its opposite pole there is a clear process going up to the outer plexiform layer, where it ends. As Cajal (1960) has explained, many, if not all, amacrine cells go through a bipolar phase in the course of their development. The present observation represents confirmation in rhesus monkey of his description, from mouse embryos. It also suggests that Polyak's cells, where they were intact, may have been cells that had

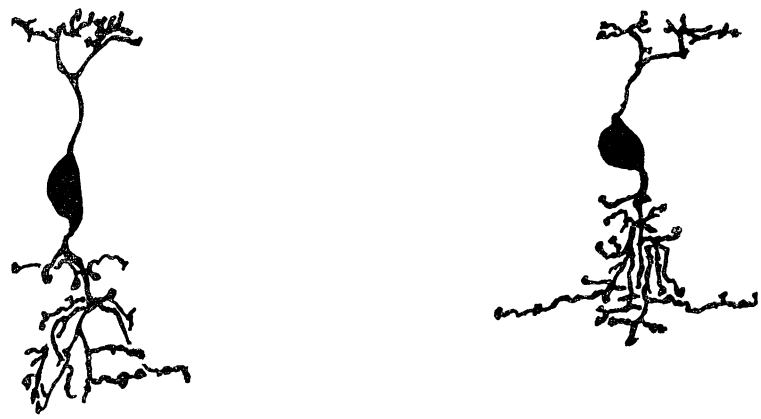


FIGURE 31. Centrifugal bipolars from chimpanzee retina as observed in vertical section by Polyak in the extra-areal region and the periphery of the central area. (Redrawn from Polyak; 31*a* from his figure 66*E*; 31*b* from his figure 66*B*; see also figure 68, plate 40.)

persisted into the young animal. The age of Polyak's animals is unknown, but in his Methods chapter he recommends the use of young animals. It is also noteworthy that his drawings of intact centrifugal bipolars (figure 31) are taken from the extra-areal regions of the retina which lag behind the central area during differentiation (B. B. Boycott & J. E. Dowling, unpublished observations). Cajal (1960) pointed out that 'bipolar' amacrine cells in newborn mice are more commonly found towards the ora serrata. One of us (B.B.B.) has been able to confirm Cajal's observations in this respect in mice. Possibly increasing the likelihood that 'centrifugal bipolars' would more easily be found in chimpanzees (see above) is their longer postnatal development when compared with that of rhesus monkeys. Cajal also described a cell, reproduced here in figure 52*a*, that resembles Polyak's description of a centrifugal bipolar cell. Cajal did not refer to it as such and, indeed, comments that he only found two cells of this kind in all of his vertebrate material.

In conclusion, there are strong reasons to believe that a bipolar cell conducting from the inner plexiform layer to the outer plexiform layer does not exist in the primate retina, that the majority of cells Polyak described as centrifugal bipolars are truly diffuse amacrine cells, and those few cells that have a process to the outer plexiform layer are really a developmental stage of an amacrine cell that had persisted in the young animal.

Centrifugal fibres and association ganglion cells

We have nothing except comment to contribute to the vexed question of the presence or absence of fibres originating in the brain and ending in the mammalian retina. Recently, Dowling & Cowan (1966) identified by electron microscopy the normal and degenerating terminals of such fibres in pigeons. The terminals they identified ended on the somata of amacrine cells as expected from the descriptions of Cajal. We have seen no evidence either by light or electron microscopy of such endings in mammals. Both Cajal and Polyak were somewhat noncommittal as to the presence of centrifugal fibres in mammals. Their descriptions of possible centrifugal fibres were based on the occasional presence in Golgi-stained material of fine fibres that came from the optic nerve fibre layer and branched in the inner plexiform layer. We have occasionally observed such structures but they have always appeared poorly stained and we could never find their origin or their place of termination. Recently, Gallego & Cruz (1965) have raised the possibility that in some mammals, including man, there may be 'association ganglion cells', that is to say ganglion cells whose axons leave the inner plexiform layer, join the optic nerve fibre layer, but then re-enter the inner plexiform layer and branch there. Because of their presumed length, in some cases as much as 5 mm, the axons of such cells would be unlikely to be observed attached to a perikaryon in sections of Golgi preparations. If stained in Golgi material they might provide isolated fibres of the appearance described above.

Three recent papers summarize the centrifugal fibre literature on mammals. That of Brindley & Hamasaki (1966) concluded from a light microscope degeneration study that the cat retina does not receive centrifugal fibres. However, earlier, using similar methods, Cragg (1962) had evidence of their existence in rats but emphasized that they were rather sparse. Brooke, Downer & Powell (1965) identified material they presumed to be degenerating by electron microscopy after cutting the optic tracts of cats and monkeys. These authors did not identify the terminal features of supposedly degenerating axons so that for the present it remains to be proved anatomically whether or not there are centrifugal fibres that go to the neural elements of mammalian retinae. It is also presumably possible that different species of mammals differ with respect to the presence or absence of centrifugal fibres.

Horizontal cells

Introduction

Cajal described two main types of horizontal cell in mammals. Small flattened cells with their perikarya within the outer plexiform layer he called 'outer horizontal cells'. 'Inner horizontal cells' were defined as larger cells with their perikarya in the scleral edge of the inner nuclear layer. Each kind of cell had one axon that took a horizontal course within the outer plexiform layer. In addition, he described a larger variety of the outer horizontal cells, as well as an infrequent subvariety of the inner horizontal cells that was characterized by one or two stout processes descending to the inner plexiform layer. Polyak found only one kind of horizontal cell in the primate retina, which was smaller when situated near the fovea and larger towards the periphery. Its perikaryon was to be found in the scleral edge of the inner nuclear layer. He concluded that the dendrites of the horizontal cells of primates were primarily postsynaptic to the cones and that their

axons terminated in synaptic relationship with rods and cones. He supposed that this single variety was equivalent to Cajal's inner horizontal cells.

Cajal and Polyak had no doubt that horizontal cells are truly nerve cells. Recently Stell (1965*b*) and Missotten (1965) have found that processes of horizontal cells contribute to the invaginations in the cone pedicles of primates. Stell (1965*a*, 1967) has identified horizontal cell dendritic processes in the invaginations of the rod spherules and cone pedicles of goldfishes and *Centropomus*. In some of the horizontal cells of cat and rabbit retinae synaptic vesicles have been found. These vesicles are aggregated at sites of membrane specialization typical of synaptic contacts. The synapses are between horizontal cell processes, and between horizontal cell processes and bipolar cell processes (Dowling, Brown & Major 1966). These horizontal cells are probably also in postsynaptic relationship to the photoreceptors as the horizontal cells of primates are known to be. No synapses of any kind have been found in the outer plexiform layer of primates (Dowling & Boycott 1966 and p. 172).

In agreement with Polyak we have been unable to classify the horizontal cells of primates into 'inner' and 'outer' categories as Cajal was able to do for other mammals. We have, however, found two distinct types of horizontal cell which may be distinguished by differences in their dendritic and axonal terminals. These have been termed type A (figure 32*A*) and type B (figure 33) horizontal cells. These two types of horizontal cell appear very similar. Both apparently have their perikarya at the same level in the scleral edge of the inner nuclear layer. For those two reasons, and the fact that it is not known which kind of Cajal's various types of horizontal cells go into which photoreceptors, we have not attempted comparisons between the A and B type horizontal cells to be described here and the categories defined by Cajal. Recently Gallego (1965) and Dowling *et al.* (1966) have raised the possibility that the 'external' horizontal cells in the cat and the rabbit may have no axon. It is certain that each kind of horizontal cell identified in the present paper does have a processs that may be defined as an axon (p. 139).

Observations on horizontal cells

Critical observation of horizontal cells of primates in vertical section is difficult, and very important information concerning their processes can only be obtained from horizontal sections that are orientated so that the dendritic terminals of the cells can be observed on their ascent towards the photoreceptors. In this regard, the illustration, figure 34, plate 36, has a pleasing and suggestive appearance, but it is not certain evidence that such a cell is partly or exclusively a cone horizontal cell. Viewed in horizontal section either kind of primate horizontal cell can be seen to have dendrites radiating fairly symmetrically from the perikaryon. In a region about 2 or 3 mm from the foveal centre the diameter of the horizontal cell dendritic spread is symmetrical at about 35 μm. Comparison of figures 35 to 39, plate 36, shows that there may be some slight differences in the appearance of the main dendrites. The most marked difference and it is, at present, the only index for discriminating the two kinds of horizontal cell, is in the details of the appearance of the dendritic terminals, and the levels at which the terminals appear to end.

Type A horizontal cells (figures 34 to 37, plate 36; figures 41 to 43 and 46, plate 37).

The following account discusses four interdependent problems: (1) the characterization of a particular horizontal cell as type A; (2) its identification as perhaps a 'cone' horizontal cell; (3) the number of synapses a single cone pedicle has with a single horizontal cell; (4) the number of cones that synapse with a single horizontal cell.

The terminal dendritic knobs on the horizontal cells that we believe, owing to the following arguments and observations, send dendritic processes only into cones, are usually under 1·0 μm in diameter. For the most part they appear to be about 0·5 to 0·75 μm in diameter.* In the illustrations many of them have a clear centre; this is because, when sharply in focus, they appear as optical sections of spheres (figure 36, plate 36; figures 41, 43, plate 37). They cannot be ending in the outer plexiform layer because there are no unidentified structures, synaptic or otherwise, of such a size. By exclusion, therefore, horizontal cells with dendritic terminals of this size are likely to be in relationship with cones. Structures corresponding to the size of these terminal knobs can be identified by comparison of the electron microscopy of the invaginations into the cone pedicles and the light microscopy of the terminals. The dimension of each lateral element of a triad at its greatest diameter in the cone base of a monkey is about 0·4 to 0·5 μm. The diameter of the central element in a triad (the midget bipolar terminal, p. 122) is about 0·2 to 0·3 μm. As figures 24 to 26, plate 35, illustrate, light microscopy of the dendritic terminals of the midget bipolar cells shows them to be smaller than those of the horizontal cells under discussion. Seen by electron microscopy the diameter of a whole triad in the base of a cone about 1·0 mm from the foveal centre is a little over 1·0 μm. Since the space between each of the three elements of a triad is less than 200 Å the parts of a triad, if all three were stained, could not be separately resolved by light microscopy. If only the two lateral elements were stained for light microscopy the triad would appear as approximately a 1·0 μm structure, or slightly larger. Examination of the type A horizontal cells in plates 36 and 37 and figure 32*A* shows that most of their terminals are about half that diameter. It can, therefore, be concluded that the horizontal cell terminals at present under discussion could correspond to one lateral element of a triad.

However as figure 32*A* and figure 41, plate 37, show there are occasional terminals on this kind of horizontal cell that are about 1·0 to 1·5 μm in diameter. Figure 41, plate 37, shows groups of terminals that have been cut off from a horizontal cell. They can, therefore, be more clearly resolved. Some of the terminals appear as optical sections of spheres

* The dimensions given to these structures are approximate, not only because of their small size, but because they are measured on sections 75 to 100 μm thick. It must also be remembered, particularly when comparing light and electron microscope data, that we do not yet know to what extent the Golgi procedures misrepresent and distort the sizes of the processes. For example, it is almost certain that the dendritic terminals of the horizontal cells described in this part are slightly swollen when judged by electron microscope criteria. A major part of the case for recognition of type A and type B horizontal cells depends on *relative* differences in the sizes and appearances of the terminals of the two kinds of cell. Although several retinae have been used in this study we have been careful to check that the two classes of cell we are claiming show the differences and dimensions described in one and the same preparation; and, wherever possible, in the same section.

with a dark centre to them, others appear as if with a line across the sphere. Those terminals with a dark dot in the centre present the kind of appearance that would be obtained if the two lateral elements of a single triad were lightly stained but the terminal of the midget bipolar cell was unstained. The lateral elements in the triads surround the central element. Consequently at the sides they are so close together that they are not separately resolved under the light microscope. Yet in the middle between them it is known that there is a process that keeps them 0·2 to 0·3 μm apart. This unstained central element appears as a small refractile dot. In this instance, therefore, such a horizontal cell terminal is probably

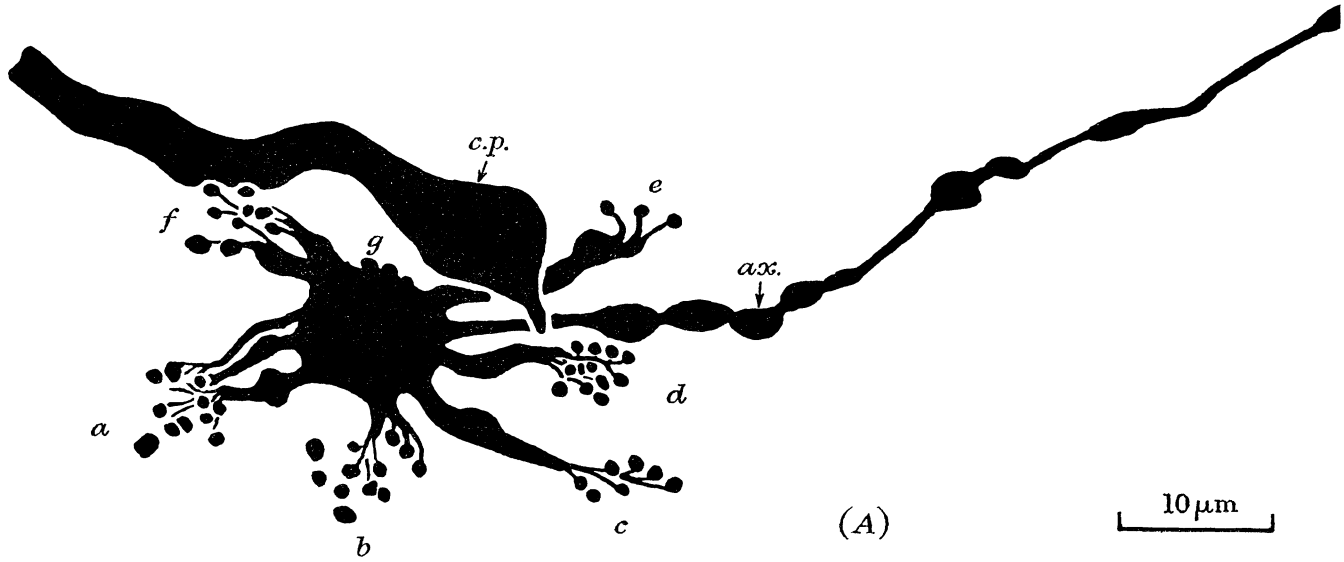


FIGURE 32 (*A*). Type A horizontal cell of rhesus monkey as seen in a horizontal section. Diagram of the cell shown in figures 35 and 36, plate 36. The cone pedicle (*c.p.*) does not in fact synapse with this horizontal cell. Analysis of the terminals on the horizontal cell is given in the text and table 3. Some of the terminals are represented as not joined onto the cell. This is because the processes leading to a terminal are often very fine, and are not always impregnated. They are considered to belong to this cell because it was well isolated from all other cells in the section. The axon (*ax*) could be followed for 200 μm, near the perikaryon it was 2 μm in diameter, the cone pedicle diameter was 7 μm.

not filling just one lateral process of a single triad, but both. In the case of those terminals that appear to have a dark line across them, or where a group of the smaller terminals appear confluent, the most likely interpretation is that single lateral elements in adjacent triads are being innervated by the same horizontal cell. In such cases the adjacent triads are sufficiently close together for the lateral elements, as seen by light microscopy, to appear to touch. Because of the nature of the material it is difficult accurately to observe the terminals with a dark dot in the centre. On all type A horizontal cells the 0·5 to 0·75 μm terminals appear to be much more common than the larger terminals (table 3). It therefore follows that any one such horizontal cell probably most often contributes only one process to a proportion of the lateral elements of the triads of the cone it innervates. This is an important conclusion because it then follows that the remaining lateral elements

of the triads must come from other sources.* These are in fact from adjacent type A horizontal cells and perhaps from horizontal cell axons (p. 139).

Such arguments suggest that horizontal cells with these sizes of terminals are cone type horizontal cells and provide some information as to the way in which the cones may be related to horizontal cells. Ancillary evidence that these horizontal cells are cells attached

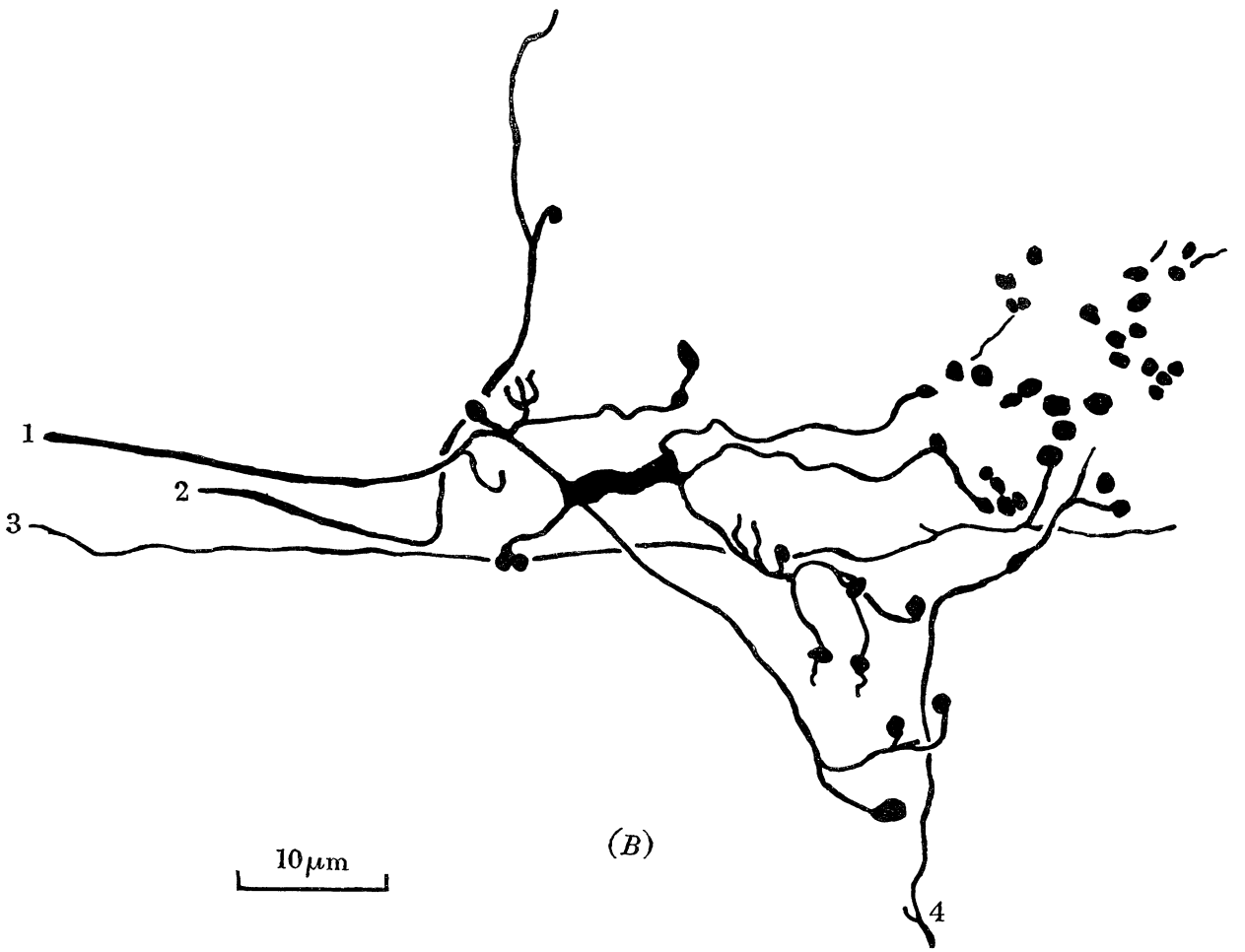


FIGURE 32 (*B*). Axon terminals presumptively of type A horizontal cells of rhesus monkey as seen in horizontal section. Large terminals presumably providing both the lateral elements of an invagination and smaller terminals supplying only one lateral element, are shown. The diagram is of the structures visible in the field of figure 48, plate 38, and it illustrates very well the present difficulty of giving precise information about the anatomy of the axonal terminations of horizontal cells. Axons 1, 2 and 3 could all be branches of one axon from one horizontal cell or three axons from three separate cells. Axon 4 is almost certainly from another horizontal cell and shows how any one region of the outer plexiform layer may be innervated by different horizontal cells from different parts of the retina. The diagram also illustrates how impregnation of the axon may stop abruptly, leaving isolated twigs with no terminals. Axon terminals of this kind on primate horizontal cell axons have also been described by Polyak (see his figure 94).

* Miss H. Kolb, using the electron microscopy of Golgi-impregnated material, has already confirmed this conclusion.

only to cones is provided by the fact that the terminal knobs are in groups that correspond in diameter approximately to the cone base diameters (figure 42, plate 37). Of itself this is not good evidence because the packing of the rods in between the cones might produce similar aggregations of terminals covering a similar area. Important evidence to support the above discussion is that the parts of any given aggregate of terminals of a type A horizontal cell end on a plane; and this corresponds to the fact that the bases of cone pedicles are also flat (figures 3 and 4, plate 33). Examination of the figures on plates 36 and 37

TABLE 3. THE NUMBER OF DENDRITIC TERMINAL PROCESSES ON THE CELL DRAWN AS IN FIGURE 32*A* AND ILLUSTRATED IN FIGURES 35 AND 36, PLATE 36

aggregation	terminals representing one lateral element of one triad	terminals representing two lateral elements of a triad	total number of lateral processes supplied to six cones
a	10	1	12
b	10	2	14
c	7	0	7
d	12	0	12
e	3	0	3
f	7	2	11*
g	The terminals in the aggregation above the perikaryon were uncountable.		
total	49	5	59

* Difficult to resolve details.

shows that for each horizontal cell in the central area there are generally about seven separate groupings of the dendritic terminals. The grouping immediately on the scleral side of the perikaryon is difficult to observe, but by careful focusing it can be seen to be present on most cells (figure 32*A*). Figure 41, plate 37, shows a central group more clearly because the perikaryon has been cut away. If the above conclusions are correct then that illustration and the others of plates 36 and 37 show that a type A horizontal cell does not contribute an equal number of processes to the seven cones with which it is in contact. The cone pedicle immediately above a horizontal cell perikaryon probably receives more processes from that cell than do the other six cones which the cell innervates (figure 41, plate 37). Counts of the number of terminals in the aggregations for the cell drawn in figure 32*A* and illustrated in figures 35 and 36, plate 36, are summarized in table 3.

When these results are matched against the number of triads in a cone base it is clear, as concluded above, that not all the lateral elements of any one cone come from any one horizontal cell. The cell in figure 32*A* was in a region where the cone base diameters were about 7 to 8 μm. As determined by electron microscopy such a cone has about 20 to 25 triads. This means that each grouping of the knobs of a type A horizontal cell, if it filled all the lateral elements of the triads of one 8 μm cone pedicle, would have to have 40 to 50 dendritic terminals of about 0·5 μm in diameter. The greatest number of processes we have been able to estimate as going into one cone from one type A horizontal cell was between 20 and 25. With such a number of processes in a small area the counting errors under light microscopy are considerable. Also some of the variation in number of terminals

any one type A horizontal cell might send to each of seven cones could be due to failure of impregnation of some of the terminals. However, our impression is that this is not so and figure 43, plate 37, shows more direct evidence that at least two horizontal cells could synapse with one cone. Figure 42, plate 37, gives some idea of the density of interlacing of three type A horizontal cells. It would seem certain that at least two individual horizontal cells contribute processes to the triads of each cone and it is possible that more than two do so; conceivably up to as many as four (figure 97). That an exact estimate would be difficult is implicit in the above discussion but it is particularly difficult because an unknown proportion of the contribution to the lateral elements of the triads could come from horizontal cell axon terminals (p. 139). It is also possible that the number of horizontal cells synapsing with a single cone may be different for different cones. However, it is very unlikely that any one cone is connected to only one horizontal cell. From the appearance of stained groups of horizontal cells, such as those in figure 42, plate 37, it is clear that dendrites of different horizontal cells overlap; that is to say, that a single type A horizontal cell would not synapse with all the cones in its dendritic field. A direct impression of this can be obtained from figure 35, plate 36; and figure 32*A* where the stained cone does not synapse with the horizontal cell in whose field it lies.

Type B horizontal cells (figures 38, 39, plate 36; figures 40, 44, 45, plate 37)

After such a necessarily detailed definition of the type A horizontal cells, description and definition of the type B horizontal cells are simpler. Figure 38, plate 36, shows a type B horizontal cell that was within 300 μm of the type A horizontal cell in figures 35 and 36, plate 36, i.e. within the same region of the retina and the same section as some of the cells described above (see footnote p. 133). It is symmetrical and about the same size as the type A horizontal cell. The terminals on this cell (figure 33) about correspond in size to the terminal dendrites of rod bipolar cells (figures 23, plate 35) and the sizes of the processes in a rod spherule when observed by electron microscopy. Figure 39, plate 36, and figures 40, 44 and 45, plate 37, show similar terminals on several type B horizontal cells. Figure 40, plate 37, is an oblique view of one dendrite of a type B horizontal cell with its six terminal branches clearly spaced out and ending at different levels. (It may be compared with the type A horizontal cell process in figure 36, plate 36). Since the terminals of the type B horizontal cells (perhaps corresponding to the layering of the rod spherules (see page 119)) appear to end on different levels, and since they are of the dimensions of the processes within the rod spherule, when seen by electron microscopy, it is reasonable to suggest that these type B cells are postsynaptic to rods. There is considerable variation in the number of rod spherules across the central area of the retina and the ways in which they are stepped back from the cones (figures 3 and 4, plate 33). Consequently there is a good deal of variation in appearance of the type B horizontal cell dendritic terminals. Figure 47, plate 37, shows an appearance of groups of 2 to 4 processes that look at first glance like a terminal knob on a type A horizontal cell when the silver has precipitated. Figure 46, plate 37, shows, however, a type A horizontal cell that could at first be mistaken for a type B horizontal cell but on varying the focus it is more likely to be a type A cell (for details see figure legends). There are about 10 or 12 separate groupings of terminals on any type B horizontal cell

but there is much variation, perhaps corresponding to the variation in the number of rod spherules between the cone pedicles. We have not observed type B horizontal cells near the outer edge of the foveal slope. Whereas a single type A horizontal cell, if the above arguments are accepted, synapses several times with each of about seven cones, it is difficult to estimate how many rods might come into synaptic relationship with a single type B horizontal cell and how many processes there could be to a single receptor terminal. As

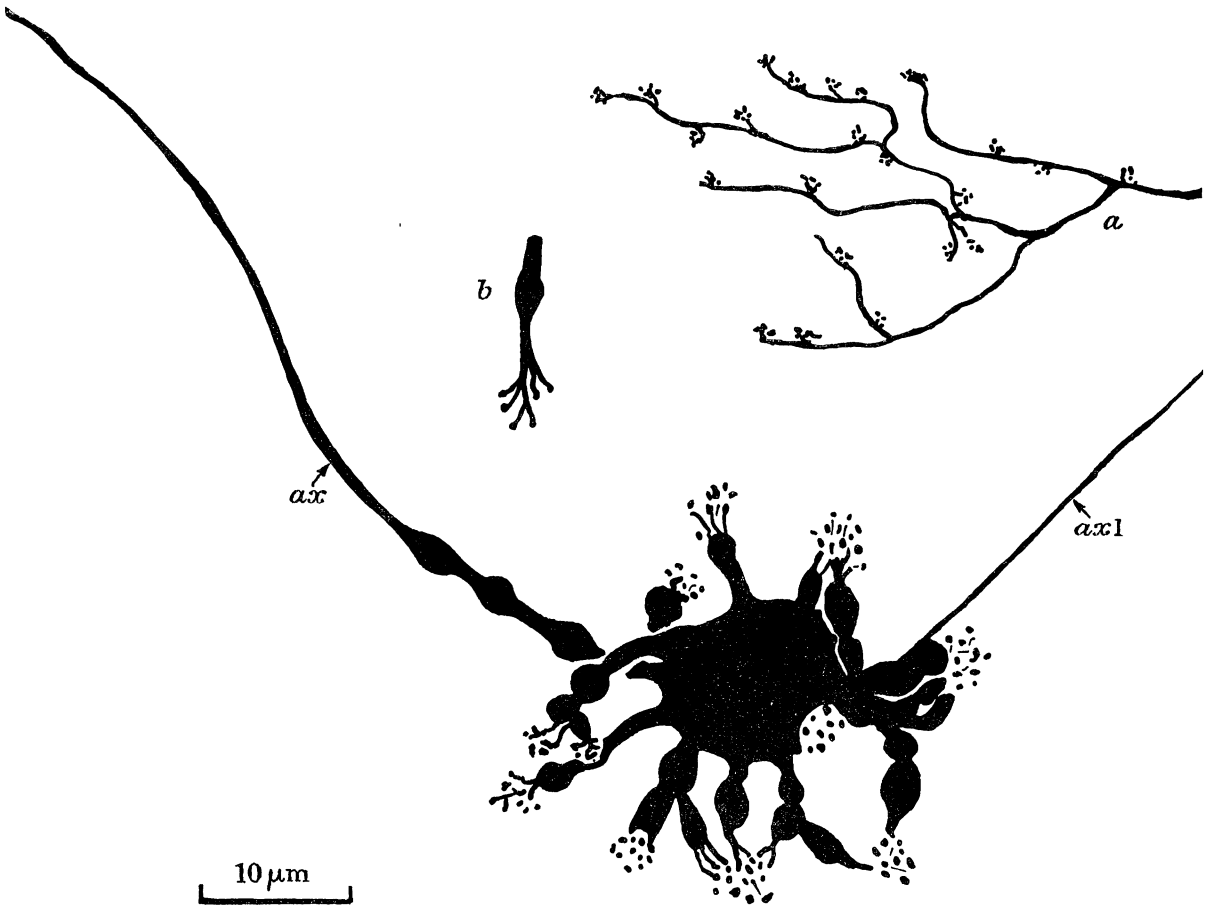


FIGURE 33. Type B horizontal cell of rhesus monkey as seen in horizontal section. Diagram of the cell shown in figure 38, plate 36. The inset *a* is a diagram of the branching of the corresponding type of horizontal cell axon terminals such as those shown in figures 50 and 51, plate 38. Inset *b* shows the branching of a type B horizontal cell dendrite (see figure 40, plate 37). The diagram of the axon is a sketch and not as accurate as that in figure 32*B* because it is difficult at present to resolve the small aggregates of terminals. The axon (*ax*) is 2 μm in diameter near the perikaryon and could be followed for 200 μm. The axon (*ax*1) was followed for 60 μm but could not be resolved as being attached to the cell.

Missotten *et al.* (1963) have shown, the number of processes entering the rod spherules in man varies from spherule to spherule (figure 98). This may well be true for monkey but we have not been able to analyse the innervation of the rod spherules by light microscopy in as much detail as has been possible for the cone pedicles. However, when a group of type B horizontal cells stain, the manner in which they pack together suggests that their receptive fields overlap to an extent similar to that of the type A cells (figure 44, plate 37).

We have been able to identify only two kinds of horizontal cell, of which the type A cells are more easily identifiable and definable. Occasionally what appears to be a kind of horizontal cell intermediate between the two is observed; that is to say a cell apparently with both type A and type B terminals. When such cells are carefully examined, however, the observations can often be explained on the assumption of only two types of horizontal cell. For example, a type A horizontal cell may sometimes be stained along with an overlapping midget bipolar cell. This can give the horizontal cell the appearance of both large and small terminals.

Foveal horizontal cells (figure 37, plate 36)

Only in one preparation have adequately stained horizontal cells been discovered on the foveal slope. They were all type A horizontal cells as described above. Figure 37, plate 36 illustrates one such cell. These cells showed some difference of detail, but none of principle when compared with type A cells peripheral to the foveal slope. Since the foveal slope has only cone pedicles (p. 119) this correspondence is evidence that type A cells are cone horizontal cells.

The dendrites of foveal horizontal cells tend to be elongated circumferentially so that the diameter of the long axis of the cell is about 35 μm, but the short axis about half that. The diameter of the aggregates of the dendritic terminals are smaller corresponding to the smaller size of the foveal cone pedicles (about 4 or 5 μm). Therefore, as in the para- and perifoveae, each foveal cone is innervated by more than one horizontal cell. The groupings of terminals suggest that each foveal horizontal cell could innervate between six and nine cones. A precise estimate was not possible with such limited material. Since there are only cone pedicles on the foveal slope it can be seen from the dimensions of cells such as that in figure 37, plate 36, that any one foveal horizontal cell does not synapse with all the cone pedicles in its immediate vicinity. There must, therefore, be overlap of the dendritic fields of these horizontal cells just as there seems to be in the peri- and parafoveal regions of the retina. Each horizontal cell observed on the foveal slope had a single axon. No horizontal cell axon terminals were stained in the foveal slope region.

Horizontal cell axons (figures 48 to 51, plate 38).

The term axon is here used to refer to a structure that arises either at the side of the perikaryon of a horizontal cell, or from the base of one of its dendrites, and then passes horizontally in the outer plexiform layer. This process does not branch within 200 to 300 μm of the parent cell. Details of the axons of horizontal cells have been difficult to discover for two reasons: (1) The axons leaving either class of horizontal cell are often seen to taper from a diameter of about 1 or 2 μm down to about 0·5 μm or less. They then either peter out or end sharply, often in the middle of a section. It is clear that such axons are incompletely impregnated; (2) Horizontal cell axons can be observed to pass for a distance of 200 to 300 μm from the parent cell, and must go further (see below). They have, therefore, a good chance of being cut even in thick sections and are often observed so to be.

Incomplete impregnation of axons of horizontal cells was typical of Cajal's experience in all vertebrates. As he himself and, recently, Missotten (1965) pointed out, he only stained

two horizontal cells completely. To judge from Polyak's illustrations he stained only one. There are, therefore, no exact data anywhere as to the lengths of horizontal cell axons. Polyak and ourselves are agreed, however, that every horizontal cell in primates has at least one axon and that there seems to be no preferred direction for these to pass across the retina. No axon-like process has been seen to cross the foveal pit.

Because, like others, we have not stained horizontal cell axon terminals so that they can be seen joined to the parent cell body, it is a matter of inference, when such terminals are stained, that they are joined to horizontal cells. The inference is founded on the observation that each horizontal cell has a process as described above and that in the outer plexiform layer horizontally running fibres unattached to a perikaryon or dendrite can be seen to branch and end in specialized terminals. These latter fibres are never observed orientated so that they could be supposed to be originating from anywhere other than from structures adjacent to the outer plexiform layer. Horizontally running fibres ending in terminals have been followed in our material for distances of 300 and 400 μm. Simple addition of this length to the length of an axonal process observed attached to a horizontal cell suggests that axons of horizontal cells are at least as long as 700 μm. Clearly they could be longer and the length could vary for individual cells. Cajal and Polyak suggested axon lengths of as much as a millimetre.

Figure 42, plate 37, shows a very commonly observed aggregate of stained cells in the retina in which it seems that the group of horizontal cells has more axons radiating from it than there are cells. Such appearances raise the question of whether an individual

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Description of plate 36

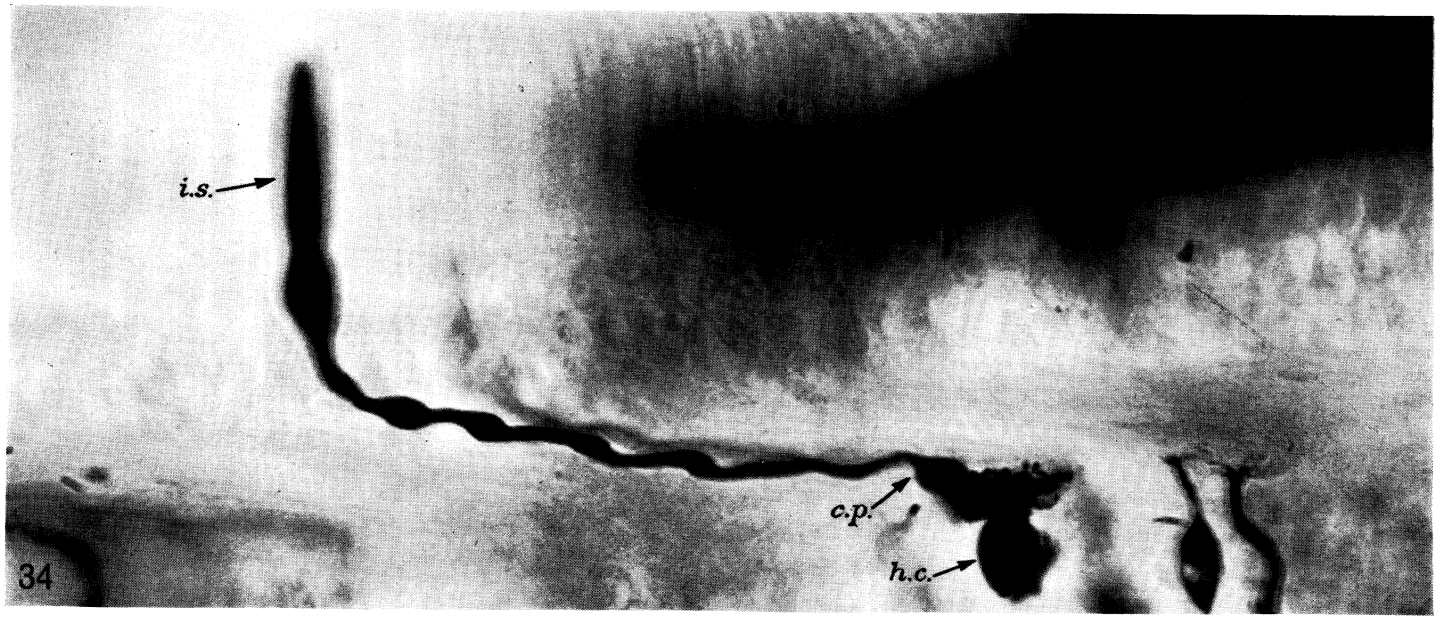
Type A and type B horizontal cells of rhesus macaque retina seen in horizontal section looking down onto the terminals of the cells, with the exception of figure 34 which is a vertical section. Procedure, Golgi–Colonnier. The magnification of figure 34 is $\times$ 800, the others are at $\times$ 2000.

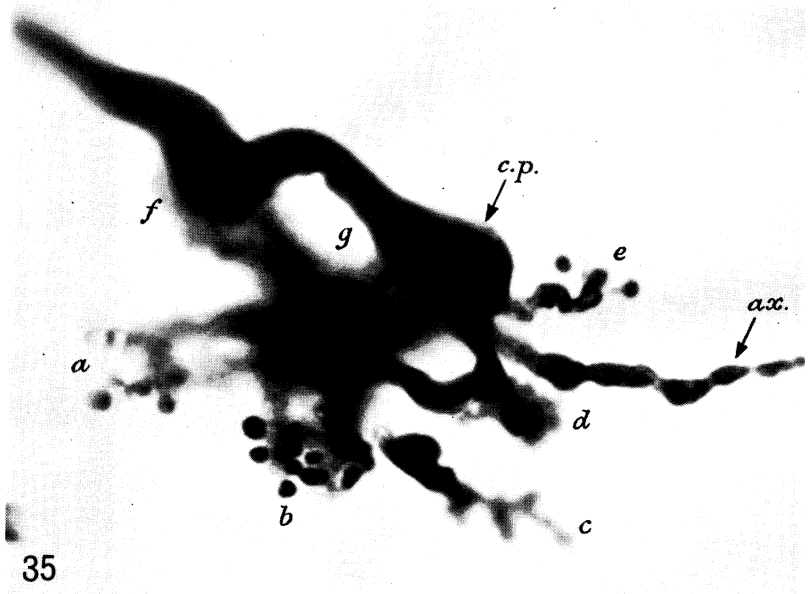
Figure 34. Cone inner segment (*i.s.*) and pedicle (*c.p.*) stained showing the relationship of one cone to one type A horizontal cell. The outer segments of either rods or cones are only rarely stained by Golgi procedures. A few rounded dendritic terminals are just visible on the horizontal cell (*h.c.*).

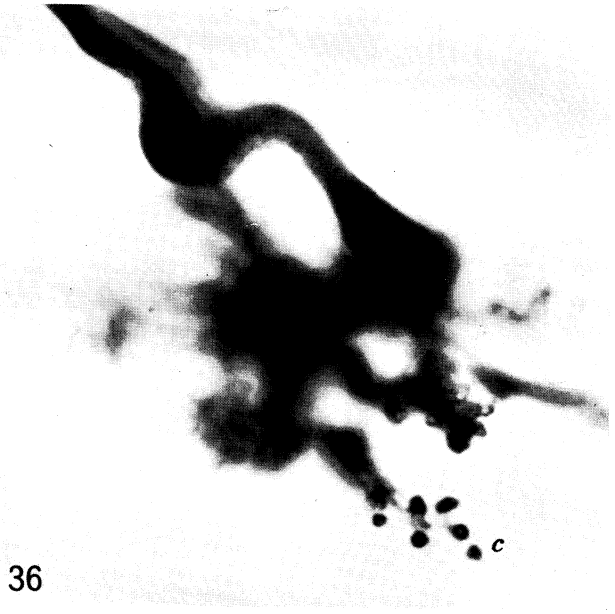
Figures 35, 36. Type A horizontal cell showing aggregations of dendritic terminals and the single axon (*ax*) on the cell. A stained cone pedicle shows within the dendritic spread of the cell but does not synapse with it. Figure 36 is a different focus of the same cell as that of figure 35 and shows the aggregate of terminals (*c*), in sharper focus. The cell is drawn in detail in figure 32*A* and the letters *a* to *g* here identify the terminals in that figure and in table 3. Each aggregation of terminals corresponds with a cone pedicle base. The axon could be followed for 200 μm.

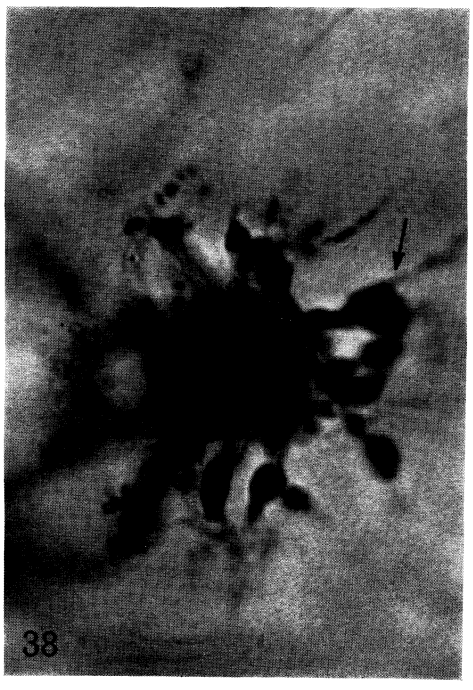
Figure 37. This is a horizontal cell from the rod free slope of the fovea. The perikaryon of the cell is slightly smaller than outside the fovea. Only two aggregations of terminals are in sharp focus (see arrows). They form a smaller mass than peripherally because the cone base diameters are smaller (see legends to figures 20 and 22, plate 35). There were between six and nine aggregates of terminals.

Figures 38, 39. Type B horizontal cells to show the difference in general appearance from the Type A horizontal cells. That in figure 39 is drawn in detail in figure 33 and there the certain axon of the cell is drawn in. Here another axon is in focus (indicated by an arrow) to illustrate the difficulty of decision as to whether or not some processes are axons passing the cell (which we suppose to be true here) or attached to the cell.









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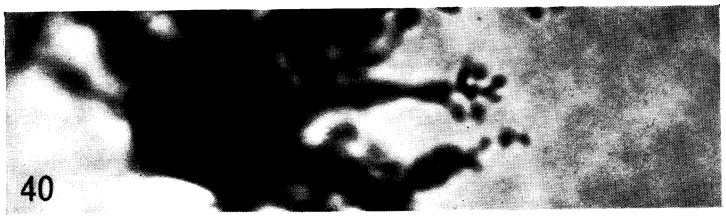
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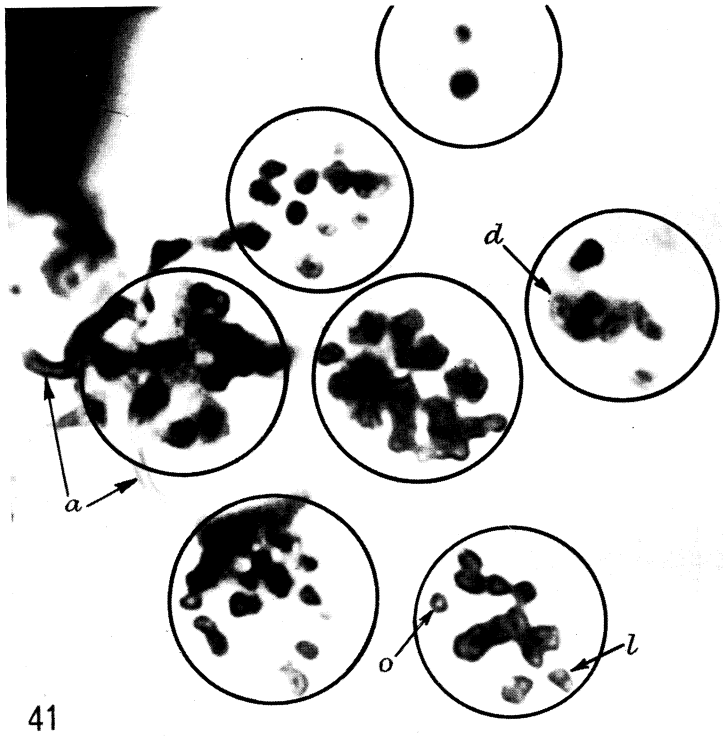
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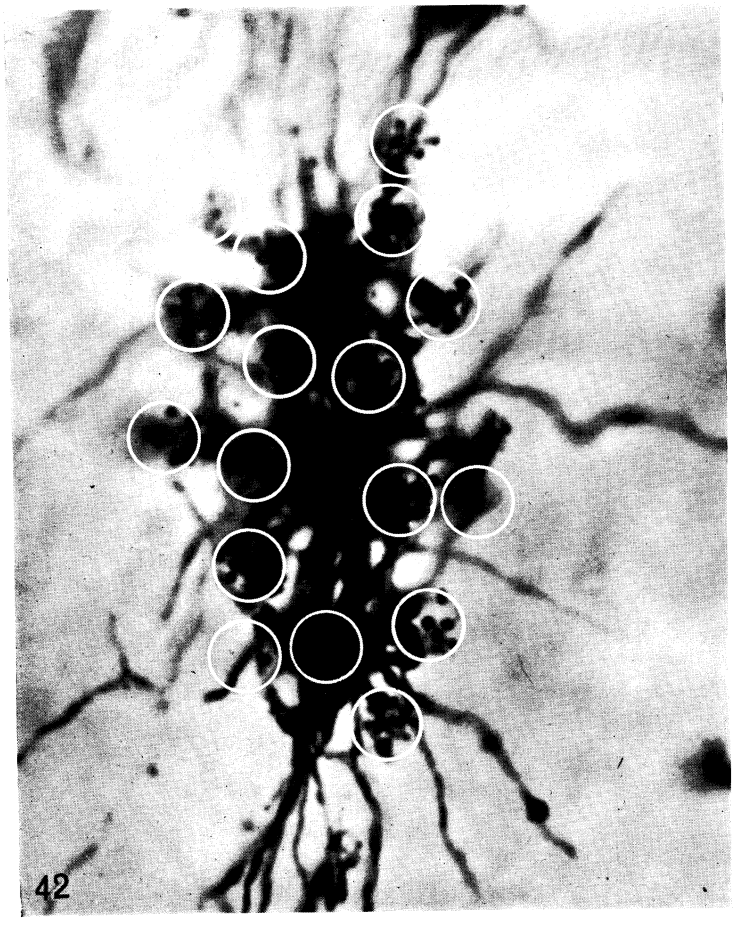
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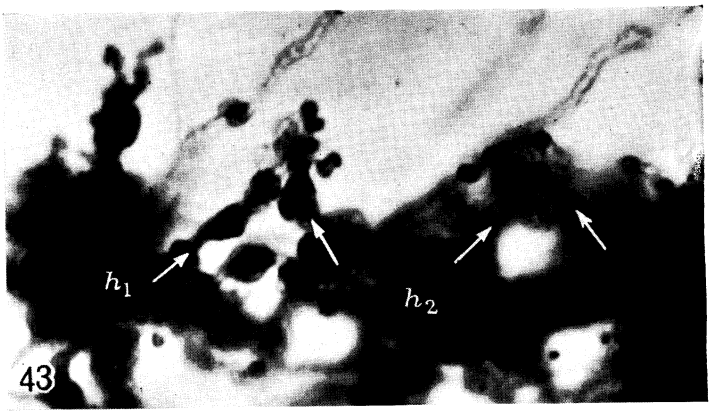
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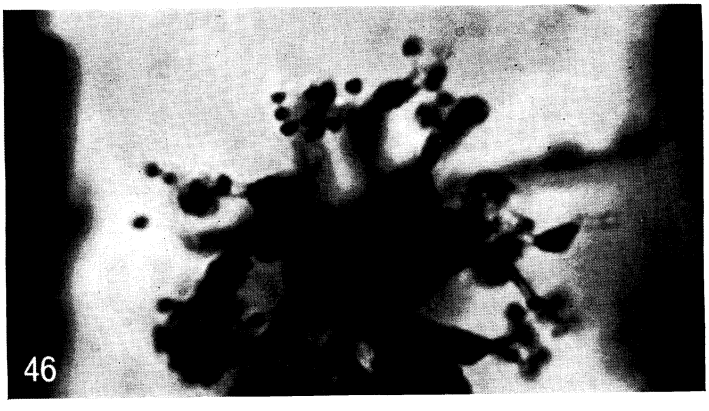
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Description of plate 37

Horizontal sections of rhesus macaque retina illustrating various details of the type A and type B horizontal cell dendritic terminals. All magnifications the same at ×2000 except for figure 41 which is about ×3300, and figure 42 which is ×1500.

Figure 40. Type B horizontal cell dendrite observed obliquely to show the manner of branching and appearance of some of the dendrites of B type horizontal cells.

Figure 41. Type A horizontal cell terminals; the section has passed through the cell so that the terminals are isolated from it. The central group is that which would be immediately above the perikaryon and, therefore, not usually visible. This grouping appears to supply more terminals to a receptor than the other groupings to their receptors and this may be the general rule. It can be seen that many of the terminals appear as optical sections of spheres (*o*), i.e. they have a dark periphery and a clearer centre. Their sizes are such that they correspond to one lateral element of a triad. A few of these are so close together that they appear confluent with only a dark line between them (*l*). These presumably correspond to the lateral elements in two separate but adjacent triads. Others of the terminals appear as optical sections of spheres but with a dark dot in the centre (*d*). This is presumed to correspond to the position of the midget bipolar dendrites in the centre of the triad so that in these instances the terminal represents the two lateral elements of one triad that are not separately resolvable. However, the pieces labelled (*a*) may represent an axon and some of these large terminals could be axon terminals and not dendrites of the horizontal cell (see figure 32*B*). It is, therefore, not possible to give data comparable to those for the cell summarized in table 3. Like that cell, however, the number of terminals differs in each of the seven aggregates. The group at the top possibly has artificially few terminals because this was near the edge of the knife-cut through the section. Nearby cone base diameters were between 8 and 9 μm. The diameter of the central aggregation of terminals is 8 μm.

Figure 42. Group of three type A horizontal cells. The groupings of the terminals so that they correspond to the cone bases are indicated by circles around each group. It must be imagined that between these cone pedicles there are rod spherules and processes going to them as well as to cones not represented on the figure. The diameter of cone bases in this region was between 7 and 8 μm. Aggregations of terminals are not clearly seen from the side (figure 34, plate 36) and from above some aggregations are difficult to observe because of the impregnation of the perikaryon. This group of cells could supply 17 cones. A large number of axons can be seen in the field but there would only be one for each horizontal cell.

Figure 43. Two type A horizontal cells h_1 and h_2 to show that contributions could be made to one cone by two separate horizontal cells. The two sets of arrows point to the dendrites going to an aggregation of terminals which it is supposed are going to one cone.

Figure 44. Two separate type B horizontal cells h_1 and h_2 to show that they could each contribute to the same receptors. The arrows indicate the separate contributions from the different cells.

Figures 45 to 47 are three cells from the same retina to attempt to illustrate some of the difficulties there may be in observation of differences between the dendritic terminals of type A and type B horizontal cells. Figure 45 is clearly a type B horizontal cell; but figure 46, a type A horizontal cell, is near the fovea and the terminals are smaller than in figure 35, plate 36. Because the cell is in the middle of the section, resolution is poorer than in figures 35 to 37, plate 36. The cell, therefore, resembles a type B horizontal cell and careful assessment is needed to avoid confusion. Figure 47 is a type B horizontal cell. Since the angle at which the terminals are viewed affects their separate resolution, a cell such as that illustrated here may look at first as though the terminals are optical sections of spheres, rather than groups of terminals of the size of the type B cells. Type A terminals, where the impregnation has precipitated discontinuously, may also superficially resemble such a cell as that here illustrated.

horizontal cell may have more than one axon. Some of these axons are very fine and do run very close to the horizontal cell dendrites. So close that it is impossible to be certain that they are not part of the cell as in the type B horizontal cell of figure 38, plate 36, but it is equally impossible to be certain that they are.

We have concluded for the time being that all primate horizontal cells have only one axon because isolated, clearly stained cells have only one. The appearance of the cells in figure 42, plate 37 gives, therefore, an impression of the complexity of criss-crossing arrangements of the axons and is not evidence one way or the other that either type of horizontal cell has more than one axon. The observational problem is, however, difficult (see legend to figure 38, plate 36).

There are two kinds of axon terminals. Because of their similarities to the dendritic terminations of the horizontal cells they can be referred to as type A and type B axon terminals. We assume that those axonal terminals that are 'A type' come from type A horizontal cells and those that are 'B type' come from type B horizontal cells. Missotten (1965) by electron microscopy concluded that horizontal cell axons go to the lateral positions in the cone triads and the rod spherules, although he did not identify horizontal cell dendrites in the rod spherules.

The distinctions between the two kinds of horizontal cell axon endings can be seen in plate 38 and figures 32*B* and 33. The axons which end with larger terminals clearly resemble type A horizontal cell dendritic terminals. Polyak has described similar large terminals on axons. Some of the terminal spheres we have been able to observe are in the range of 1 to 1·5 μm. They are, therefore, more like the larger dendritic terminals of the type A horizontal cells and it is possible that the axon more usually contributes the two lateral elements of a single triad. Some of the terminals in figure 49, plate 38, look as if the lateral element of one side of the triad may be continuous around the midget bipolar terminal with the lateral element of the other side. However, none of these type A axon terminals has been observed with a refractile dot in the centre as have some of the cone horizontal cell dendrites. The possibility is, therefore, that they may form superficial contacts on the cone pedicle base. However, the rather large number of larger terminals in figure 41, plate 37, may be due to the fact that some of the spheres with dark dots in the centre are axon terminals mixed in with dendritic terminals, but because of the plane of the section were not identifiable as belonging to a horizontal cell axon. There were some pieces of axon near this aggregate of terminals.

The endings of the axons of the type B cells (figures 50 and 51, plate 38) are difficult to observe. As the figures show the type B axons branch and send up short processes that end in small terminals. These terminals look as if they are in groups of from one to four and presumably represent branches of the fine process on which they are borne. As the figures show, a group of terminals may be about the right diameter to fit into the 3 μm diameter rod spherules. Because of the size of these structures and their closeness it is difficult to resolve them in such a way that the number of processes contributing to a receptor terminal can be stated, but it does seem that it could be as few as one, or as many as four. In serial electron microscope sections Missotten *et al.* (1963) and Missotten (1965) have shown that the horizontal cell processes contributing to a rod may be irregular in shape. Thus what here appears as a group of type B terminals under the light microscope may only reflect the

irregularity of a single terminal within a spherule. The structures are too small for light microscopy alone to resolve this problem.

It has not been possible even to begin to make meaningful estimates of the possible numbers of receptors contacted by a single horizontal cell axon, because we have never been convinced that all the branches of a single axon have been stained. Following a portion of either an 'A type' or a 'B type' axon to its terminals the impregnation can be seen to be sharp up to and including the terminals, but branches from the well-impregnated part of the axon before it forms terminals can be observed to peter out or to stop sharply (figure 48, plate 38, figure 32*B*). One thing seems certain and that is that axons of the same or either kind of terminal that arise in different parts of the retina may branch and overlap with each other.

Introduction

Inner plexiform layer

The inner plexiform layer consists of the axons and terminals of the bipolar cells, the processes of the amacrine cells and the dendrites of the ganglion cells. Using the electron microscope we have described seven types of synaptic contact (Dowling & Boycott 1965, 1966). The bipolar cells are presynaptic to processes from amacrine cells and ganglion cell dendrites. Amacrine cell processes are also presynaptic to the bipolar terminals and to ganglion cell dendrites and somata; they also synapse with each other. Some rod bipolar cell terminals make special axosomatic junctions with ganglion cell perikarya.

Bipolar cell axons and terminals (plates 34 and 42).

The axons of the bipolar cells extend from the vitreal side of the bipolar cell perikaryon to the inner plexiform layer. They are about 1 to 2 μm in diameter and quite smooth. Electron microscopy shows that they have no synapses along their length and that they are usually sheathed by a glia cell process. Bipolar cell axons terminate at all levels in the inner plexiform layer. The general morphology of the terminals differs according to the type of bipolar cell (see Polyak, and figures 19, 30 and 98), but all the types of bipolar axon end in one or more terminal swellings or varicosities and their ultrastructural features are all similar. The terminal swellings vary in size from a micron or so in diameter to elongated swellings of irregular outline as much as 5 μm long and 3 μm wide (see plate 34). Under the electron microscope the terminal swellings are found to contain numerous evenly distributed synaptic vesicles. At the presumed sites of presynaptic contact there are synaptic ribbons in the cytoplasm which point between a pair of postsynaptic processes (figure 98). We have called this arrangement a dyad (Dowling & Boycott 1965, 1966). In the central area of the primate retina one process of the pair is usually from an amacrine cell and the other from a ganglion cell dendrite. The amacrine member of the dyad is often observed to have, and quite probably always has, a reciprocal junction back onto the bipolar terminal within about 0·5 to 1·0 μm of the synaptic ribbon (figure 98). These dyads and reciprocal junctions are packed tightly around each bipolar axon terminal.

As seen by light microscopy the terminal varicosities of the midget bipolar cells are large and in the central area of the retina form a single lobulated structure which is closely embraced by the dendrites of the midget ganglion cells (figures 77 and 78, plate 42). Towards the edge of the central area, and in the periphery, the midget bipolar axon terminal

may branch (figure 15, plate 34) and even resemble that of a flat bipolar (figures 19*c* and 30*b*). Polyak gives a detailed discussion of these points and the varied morphology of other bipolar terminals. Such variations are doubtless related to the decrease in the number of ganglion cells from the centre of the retina towards the periphery. The axon of the flat bipolar cells usually branches into two to four processes which bear several terminal swellings as large as 2 or 3 μm in diameter. In the central area of the retina the diameter of spread of the branching of the axon of a flat bipolar cell is usually about equal to the diameter of spread of branching of the apical dendrites and this may also be true for such cells in the periphery (plate 34). The terminal swellings of the axons of rod bipolars may resemble those of the flat bipolars in appearance, especially in the periphery (figures 17, 18, plate 34). However, in addition to small swellings, sometimes on a fine side branch, they generally have, at least in the central area, a large swelling bigger than any ever found on a flat bipolar cell (see plate 34 and figure 19*a*).

The rod bipolar terminals also differ from the terminals of other kinds of bipolars in that they are usually found only in the inner third of the inner plexiform layer nearest to the ganglion cell perikarya; whereas those of other bipolar terminals may end at all levels of that layer. Both Cajal and Polyak describe these terminals as making axosomatic contact with perikarya of the ganglion cells that are adjacent to the inner plexiform layer. However, electron microscopical observation shows that these terminals only occasionally make axosomatic contact with the ganglion cell perikarya. In general, their synaptic relationships are axodendritic, through dyads, just like other bipolar terminals. When rod bipolar terminals form an axosomatic junction it is often peculiar in that it consists of a tight junction, or nexus, between the membrane of the ganglion cell perikaryon and the bipolar terminal. This junction could be, therefore, a site of electrical transmission (Dowling & Boycott 1966; Bennett *et al.* 1967). The significance of the arrangement is obscure, especially since such axosomatic contacts are found only on those ganglion cells whose perikarya are

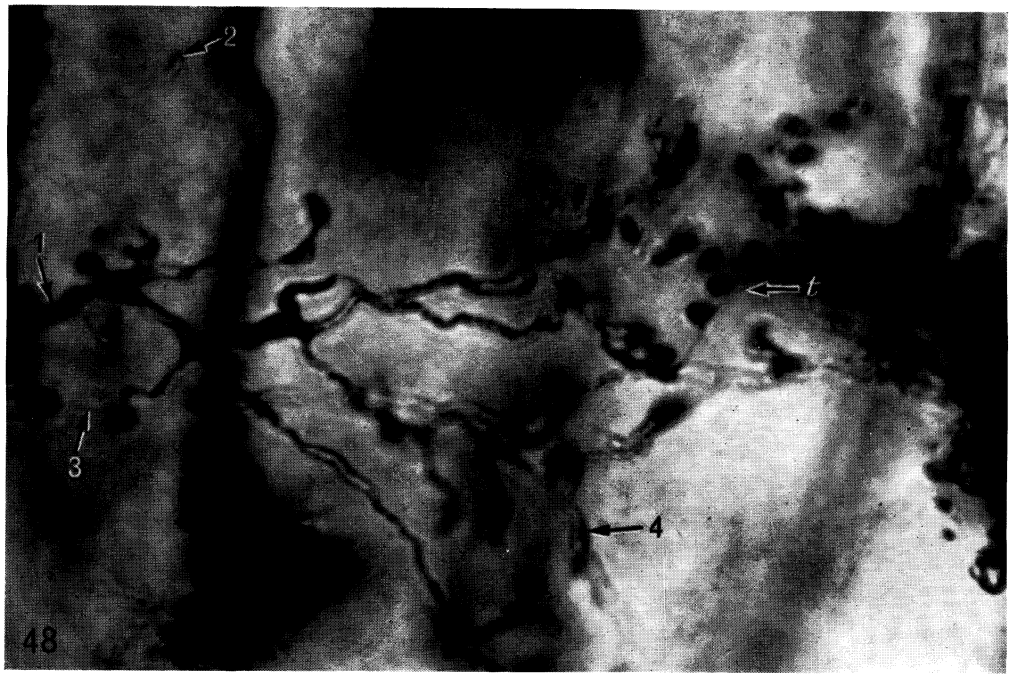
Description of plate 38

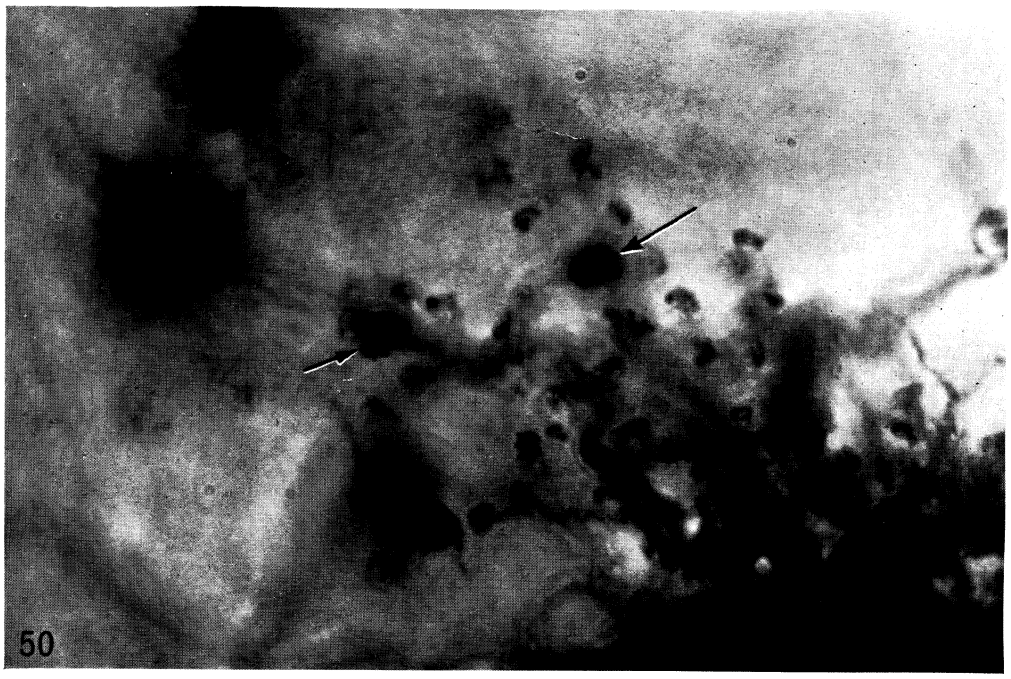
Type A and type B horizontal cell axon terminals. Rhesus macaque retina, all Golgi–Colonnier procedure, viewed in horizontal sections looking down on the terminals at × 2000.

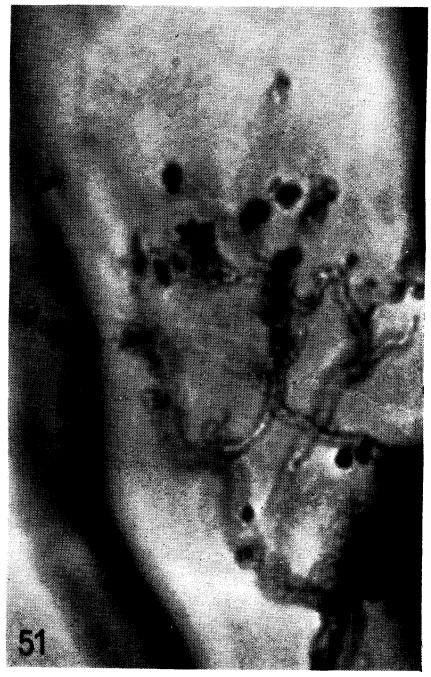
Figure 48 gives an idea of the branching of an axon that ends in large terminals, which is, therefore, a type A horizontal cell axon. The picture is taken within the depth of the section so that the terminals knobs (*t*) are not well resolved. The axon (1) and others out of focus in the field are drawn in detail in figure 32*B*, p. 135. In that figure a rather larger field is drawn. The numbers on this picture indicate places where the axons drawn in the diagram are just visible on the plate; 1 and 3 are the ones most visible in this picture.

Figure 49 shows type A axon terminals. Two of these are unusual in that they appear as a bent oval (see arrows). They are also rather large.

Figures 50, 51. Axons in the outer plexiform layer which end in terminals of small diameter. They are grouped together in bunches with varying numbers of processes. Because of their small size and resemblance to the terminals on the type B horizontal cell dendrites they are presumed to be axons of those cells. In figure 50 the two round structures just out of focus and designated by arrows are rod spherules of 3 μm in diameter. A schematic representation of the termination of a type B axon is in figure 33*a*, p. 138.







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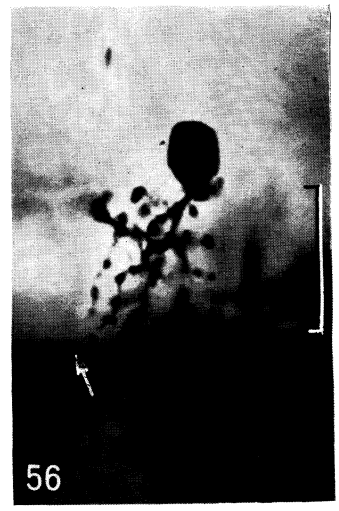
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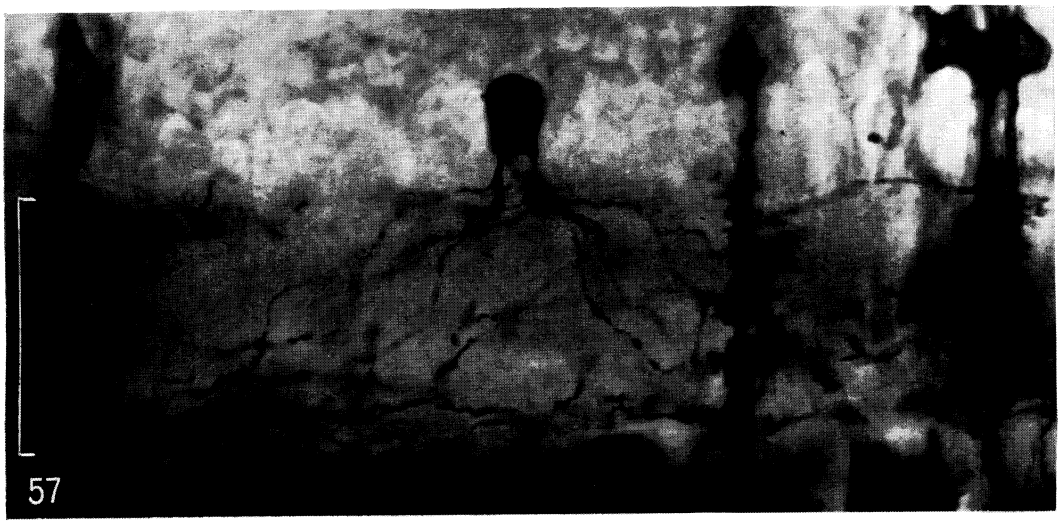
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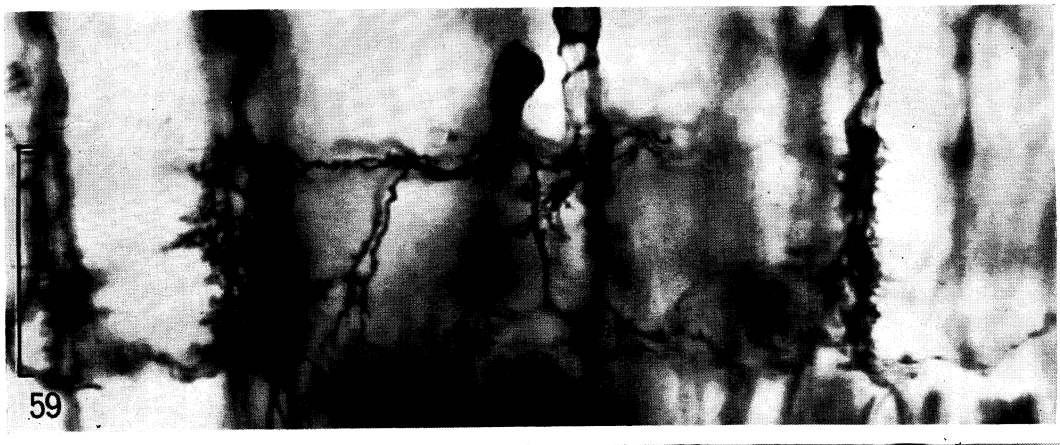
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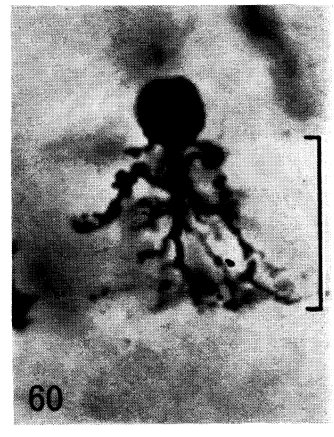
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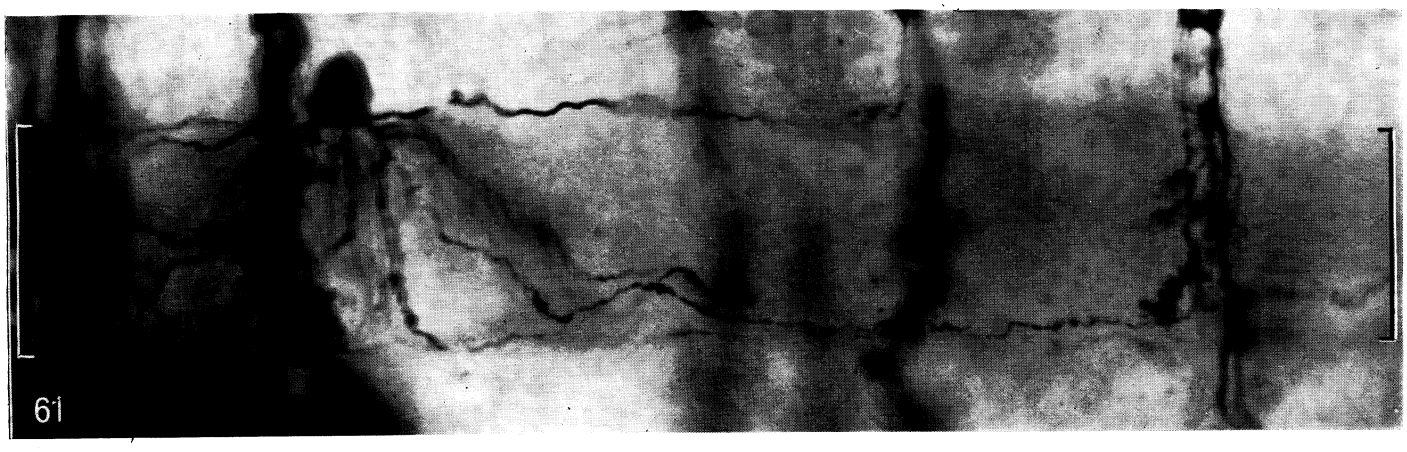
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Description of plate 39

Amacrine cells as seen in vertical section of rhesus macaque retina. Figures 55, 57, 59 and 61 were stained by Miss H. Kolb; figure 56, stained by the Golgi-rapid technique, is an amacrine cell from human retina; figures 58 and 60 are stained by the Golgi–Colonnier method. All cells are at the same magnification, × 800. The braces at the side indicate the thickness of the inner plexiform layer which varies slightly according to the position of the cell relative to the fovea and the angle of the section.

Figure 55. On the left a wide-field diffuse amacrine cell whose processes overlap with those of a stratified diffuse amacrine cell. On the right, out of focus, is a midget ganglion cell. The stratified diffuse amacrine cell resembles that drawn from Cajal in figure 53*c*. Its perikaryon is $9 \times 10\,\mu$m and the diameter of spread of its processes is $80\,\mu$m. The wide-field diffuse amacrine has a perikaryon of $10 \times 12\,\mu$m and the diameter of spread of its processes is $70\,\mu$m. This, however, may be an incompletely impregnated cell (see figure 61).

Figure 56. Narrow-field or small diffuse amacrine cell from human retina. The processes of the cell seem a little finer than in the monkey (figure 58) but both show the characteristic varicosities that are found on these cells. The arrow points to a presumed axosomatic contact with a ganglion cell perikaryon. Such contacts have been seen by electron microscopy (Dowling & Boycott 1966).

Figure 57. Wide-field diffuse amacrine cell. Dimensions: perikaryon, $8 \times 12\,\mu$m; central core of process $80\,\mu$m extending to $100\,\mu$m and more near the ganglion cell perikarya. Total lateral extent of the processes about $300\,\mu$m.

Figure 58. Narrow-field diffuse amacrine cell, the arrow indicates a possible axosomatic contact. The peculiar shape of the top of the perikaryon is due to a partially stained perikaryon just behind the cell. Dimensions: perikaryon, $8 \times 10\,\mu$m; diameter of lateral spread of processes about $25\,\mu$m.

Figure 59. Bistratified amacrine cell. Its processes branch in the inner plexiform layer near the ganglion cell somata and immediately under the inner nuclear layer. An occasional spine can be seen on the processes between the two layers but they otherwise seem quite smooth. Dimensions: perikaryon $8 \times 15\,\mu$m, both layers of processes have a diameter of spread of about $100\,\mu$m.

Figure 60. Narrow-field diffuse amacrine cell from peripheral retina. Its dimensions are about the same as those cells in figures 56 and 58 that are within a millimetre of the foveal pit.

Figure 61. A wide-field diffuse amacrine cell showing a process passing immediately under the inner nuclear layer and another process passing along near the ganglion cell somata. Many of the processes passing through the inner plexiform layer can be seen as just out of focus. Those processes just under the inner nuclear layer eventually join the processes near the ganglion cell perikarya just off the picture on the right. Dimensions: perikaryon $9 \times 11\,\mu$m; on the left the processes could only be followed for about $200\,\mu$m before they were lost in stained glia; on the right they could be followed for $400\,\mu$m. The diameter of the central conical zone of processes through the inner plexiform layer is between 70 and $80\,\mu$m. The total measured lateral extent of the processes of this cell is, therefore, $600\,\mu$m. At the end of the processes the cell in fact appeared understained so that it could well have had a larger spread, say up to 800 or even $1000\,\mu$m. We have seen portions of amacrine cells resembling this, with processes extending horizontally for as much as 1·0 mm in perch retina. Vrabec (1965 and 1966) has also described rather large-field amacrines in a marine fish. The cell here resembles those in figures 55 and 57. However, a notable difference, that may or may not be important, between these cells is that the processes of the cell in figure 57 clearly branch loosely at the level of the ganglion cell perikarya. The processes of this cell apparently did not so branch. Although this cell in figure 61 has a field amongst the largest we have observed in the retina it is in that part of the retina where the ganglion cell perikarya are stacked 4 or 5 deep i.e. very near the fovea. It is in fact nearer to the fovea than all the other cells on this plate except that of figure 59 to which it is adjacent and with whose processes it overlaps.

adjacent to the inner plexiform layer. Axosomatic contacts have never been observed on cells deeper in the ganglion cell layer.

Cajal described axosomatic junctions for rod bipolars in many different mammals and fishes. However, typical synaptic specializations between those terminals and the ganglion cell perikarya have not been found by electron microscopy in primates or elsewhere (see Cohen (1967) and Raviola & Raviola (1967)). It is an irony that Cajal (1954) should have regarded the rod bipolar/ganglion cell somata junctions as the best and most typical example of an axosomatic junction. It does, however, give further point to a present-day discussion of the pitfalls of claiming synapses from the study of Golgi material alone (p. 114).

Classification of amacrine and ganglion cells

The succeeding account describes the sizes and shapes of the interneurons of the inner plexiform layer and the ganglion cells that are the final common pathways to the brain. There is little variety in the shapes of cells in the outer plexiform layer, but the appearance of those in the inner plexiform layer is very varied. In a previous paper we argued that the horizontal dimensions of the dendritic field of any ganglion cell corresponded to the centre of the receptive field of a physiologically determined unit. The peripheral, opponent field, was supposed to be mediated by the amacrine cells through synapses between amacrines; and between amacrine processes and ganglion cell dendrites (Dowling & Boycott 1966). That hypothesis gave a structural explanation for the physiology of the simple concentric receptive fields of the retina. It did not require a knowledge of, or provide a framework for explanation of, the different shapes of the cells in the inner plexiform layer. The varieties of shapes of cells to be described in this section have, therefore, to be classified with their kinds of synapses known but with no clear hypothesis as to the importance of any given shape to the mode of functioning of the retina (see pp. 113, 167). Consequently the classification used may well be misleading from the functional point of view. We have, however, had in mind the present need to simplify and to generalize cell types as much as possible.

Polyak recognized two kinds of amacrine cells, but divided the ganglion cells into two classes, one of which was subdivided to give a total of six varieties of ganglion cells. 'Monosynaptic ganglion cells' contained only the midget ganglion cell variety; 'diffuse ganglion cells' included five further kinds (p. 156). However, as Polyak himself pointed out, there were many intermediate and therefore confusing varieties. This makes his classification awkward to use. Cajal's classification of ganglion and amacrine cells was with regard to the position of the branching of their dendrites in the inner plexiform layer. Although inevitably imperfect it still seems to us to be more immediately useful because it is more easily memorable and is, in principle, the same as his classification of the amacrine cells. There is the additional advantage that, for the present, comparisons with the structure of the inner plexiform layer of other vertebrates are more easily made using Cajal's system.

It is interesting that Cajal (1891) at first listed the different kinds of mammalian ganglion cells by their different shapes and sizes. A year later (Cajal 1892), and still in his 1911 summary, he classified them and the amacrine cells according to the position of the branching and their processes in the inner plexiform layer. He recognized three classes of amacrine cells and four classes of ganglion cells in the mammalian retina, and classified

them according to whether the processes or dendrites predominantly took a horizontal or a vertical course (see figures 52 and 53).

There are, therefore:

(1) *Diffuse amacrine and ganglion cells** which are cells whose processes and dendrites branch at all levels in the inner plexiform layer and the horizontal extent of whose branching is generally less than that of the stratified amacrines. Often such cells have a greater frequency of branching per unit area than the cells whose processes are stratified (figures 52*b*, *h*, 53).

(2) *Unistratified amacrine cells and ganglion cells*, which are those cells whose branches are confined to a single horizontal stratum in the inner plexiform layer (figure 52*c* to *g*).

(3) *Bistratified amacrine and ganglion cells*, which are those cells the branching of whose processes occurs on two distinct horizontal strata in the inner plexiform layer (figure 59, plate 39).

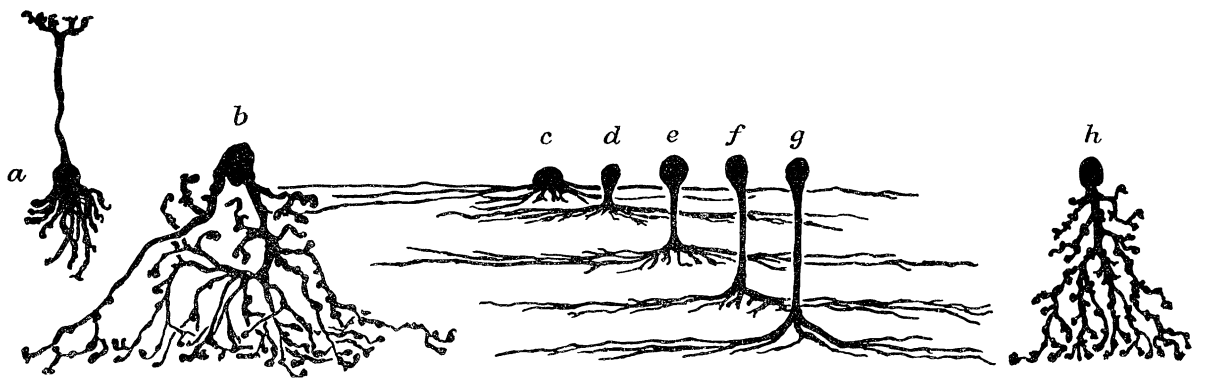


FIGURE 52. Summary of the most commonly stained kinds of amacrine cells as described by Cajal for mammals. (Redrawn from Cajal (1911) *a* and *b*, from figure 191*g* and *h*; the rest from figure 192. Probably the majority of the cells are from ox and dog retinae.) *b* and *h* are diffuse amacrines; *c* to *g* are unistratified amacrines; *a* represents a cell with a process into the outer plexiform layer and other processes in the inner plexiform layer. Cajal only observed this cell twice (see p. 130). Amacrine cells are illustrated in plates 39 to 42.

(4) *Multistratified ganglion cells* are those ganglion cells whose dendrites branch on more than two levels. Cajal described no amacrines of this kind in mammals. He had a fourth category of amacrine cell and a fifth category of ganglion cell. These were the *displaced amacrine and displaced ganglion cells*.

Displaced amacrine cells have their perikarya in the ganglion cell layer instead of the vitreal border of the inner nuclear layer. Their processes are usually disposed in a single stratum. Correspondingly, displaced ganglion cells have their perikarya in the vitreal side of the inner nuclear layer and their processes are likewise stratified (figures 94, 95, plate 44). Both kinds of cell are apparently more common and highly organized in amphibian, reptilian and avian retinae than in mammalian. Often included as displaced

* There is one confusing point of terminology between Polyak and Cajal. Polyak used the term 'diffuse ganglion cell' to mean a ganglion cell with a greater dendritic spread, either horizontal or vertical; and, he presumed, more synapses than the midget or monosynaptic ganglion cell. We shall use the term in Cajal's sense of a cell whose processes spread vertically through the inner plexiform layer.

amacrine cells are the *interstitial amacrines* whose perikarya are actually in the inner plexiform layer. Except for Cajal's descriptions little is known about them. We have occasionally seen what may be such perikarya in the primate retina (figure 7, plate 33) but have only partially stained one such cell with Golgi procedures.

Observations on amacrine cells

Introduction

The term 'amacrine cell' was given by Cajal to those nerve cells of the retina and other parts of the brain that have no axon. He thus distinguished them from other structures, such as spongioblasts and glia with which they had been confused. The apparently exclusively 'dendritic' nature of amacrine cells has been a puzzle to both anatomists and physiologists. The difficulty of imagining how a nerve cell without an axon could work was a major reason which led Polyak to conclude that many of the amacrines in the primate retina were centrifugal bipolars with incompletely impregnated axons (p. 129). Cajal, however, had regarded them as important interneurons of the inner plexiform layer. Electron microscopy has revealed that amacrine cell processes contain synaptic vesicles and that one and the same process can stand in pre- and postsynaptic relationship to other nerve cell processes. Thus some of these difficulties have for the most part been resolved and amacrine cells are established as important retinal interneurons (Dowling & Boycott 1965, 1966; Rall *et al.* 1966).

Polyak, however, did describe two kinds of amacrine cell: the 'knotty amacrine', which he regarded as equivalent to a stratified amacrine of Cajal (figure 54*a* and *c*); and the 'tasselled amacrine' (figure 54*b*), which he thought to be equivalent to Cajal's 'giant amacrine'. As the figure legends and text indicate, these comparisons were probably mistaken.

Diffuse amacrine cells (figures 55 to 58, 60 and 61, plate 39; figure 67, plate 40)

Two kinds of diffuse amacrine cell were described by Cajal and these are readily discriminated from stratified and other amacrines. They are the ones which Polyak regarded as the dendrites of centrifugal bipolar cells. The kind shown in figure 52*b* and 52*h* we shall refer to as a narrow-field diffuse amacrine cell. The second kind we shall call a wide-field diffuse amacrine (figure 53*a*, *d*). In the rhesus monkey the perikarya of the former are about 8 to 10×8 to 10 μm in diameter, while those of the latter are sometimes just slightly larger, perhaps reaching as much as 12 μm in their largest dimension. The perikarya of both kinds of diffuse amacrine appear on average to be smaller than those of many of the stratified amacrine cells, which are as big as 10 to 12×15 to 18 μm (see, for example, figures 5 to 7, plate 33). Careful measurements have not yet been made of the range of sizes of amacrine cell perikarya because the presently most important features of the cells are in the branching and distribution of their processes within the inner plexiform layer.

(*a*) *Narrow-field diffuse amacrine cells* (figures 56, 58 and 60, plate 39). From the perikaryon of a narrow-field diffuse amacrine cell one, or sometimes two, processes enter and immediately branch within the inner plexiform layer. The successive branches extend to the ganglion cell layer. The processes branch dichotomously and come to fill a field of

between 10 and 50 μm in diameter. Generally the diameter of the fields of such cells is around 25 μm. Probably the fields are, on average, narrower on the foveal slope but, within the central area, there is apparently no simple progression from narrower towards wider fields in the cells that are found at increasing distances from the foveal pit. Cells with both narrower and wider fields have been stained close to each other in many regions of the central area. The cell in figure 60, plate 39, is from the periphery of the retina and is very little different in diameter from the others illustrated on that plate. It could as well be from the central area as the periphery. As illustrated by comparison of the two cells (figure 52*b*, *h*) from Cajal (1911), the processes of a narrow-field diffuse amacrine cell may be fine with occasional swellings (figure 52*b*; figure 56, plate 39, figure 74, plate 42)

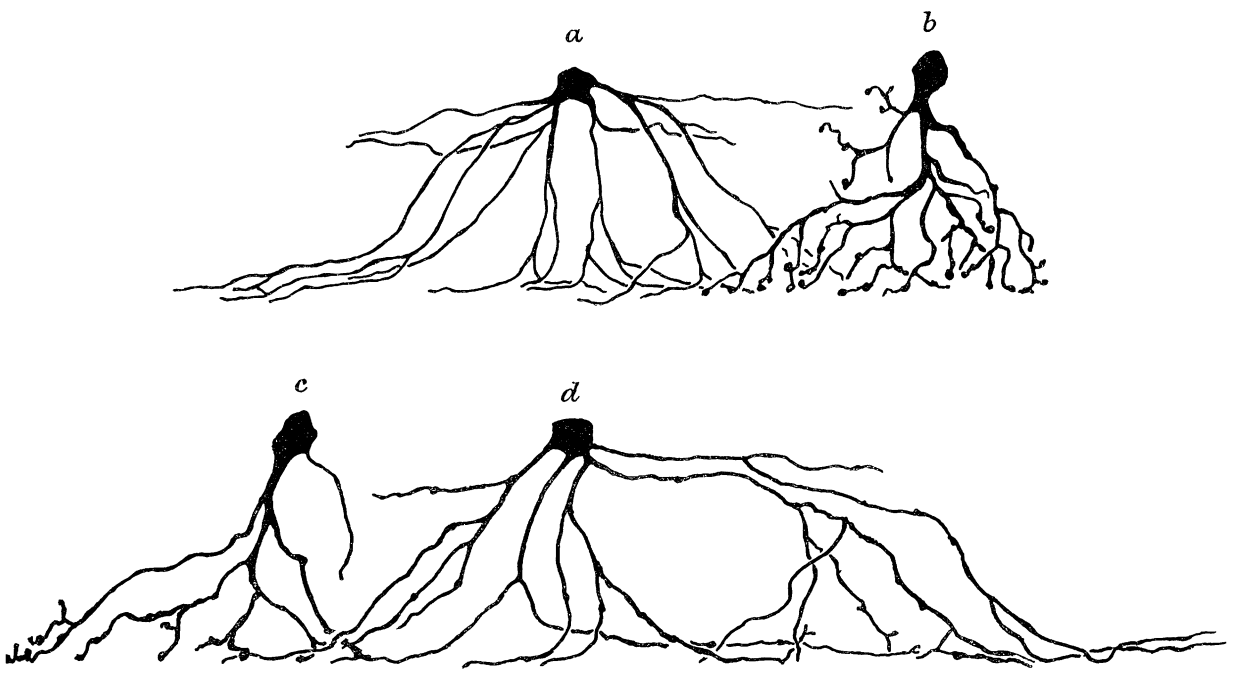


FIGURE 53. Amacrine cells from ox retina stained by a methylene-blue method (redrawn from Greeff (1894) translation of Cajal (1892, figure 8, plate 7), and Cajal (1911, figures 197 and 192*m*)). *b* and *c*, diffuse amacrine cells; *a* and *d* were originally referred to by Cajal (1892) as spongioblasts. *a* and *d* are here regarded as wide-field diffuse amacrines. Although Cajal apparently never explicitly changed from calling these particular cells spongioblasts (i.e. developing nerve cells) to calling them amacrines he treated them as such. Cajal also remarks that the cells in this diagram stain more readily with a methylene blue method than with the Golgi method. He is also explicit that they are quite common cells. Our experience is that they have stained intact only in one Golgi preparation (see p. 151). Such cells are illustrated, plate 39. We have not attempted methylene blue.

or thicker and more varicose (figure 52*h*; and figure 58, plate 39). It is not clear whether there is anything of significance in these differences, although the density of branching of those with the finer processes may perhaps be greater (figure 74, plate 42).

The branches of the narrow-field diffuse amacrines reach the ganglion cell layer where they could form axosomatic contacts such as Cajal described on the ganglion cell somata. Figures 56 and 58, plate 39, show a process with a terminal swelling of this kind. We have

identified amacrine axosomatic junctions by electron microscopy (Dowling & Boycott 1966), but it is not clear whether this kind of diffuse amacrine cell, or the wide-field diffuse amacrine, or both are involved in such a junction.

(*b*) *Wide-field diffuse amacrine cells* (figures 55, 57, 61, plate 39). The general shape of the perikaryon of the wide-field diffuse amacrine cell may be somewhat rectangular due to the fact that several branches leave at either side of its vitreal surface. Cells resembling these were described by Cajal (see figure 53*a*, *d*). The processes in monkey are thin (about 1·0 μm or less in diameter) and rather smooth with only occasional small swellings. The branches near the perikaryon form a cone through the inner plexiform layer enclosing a

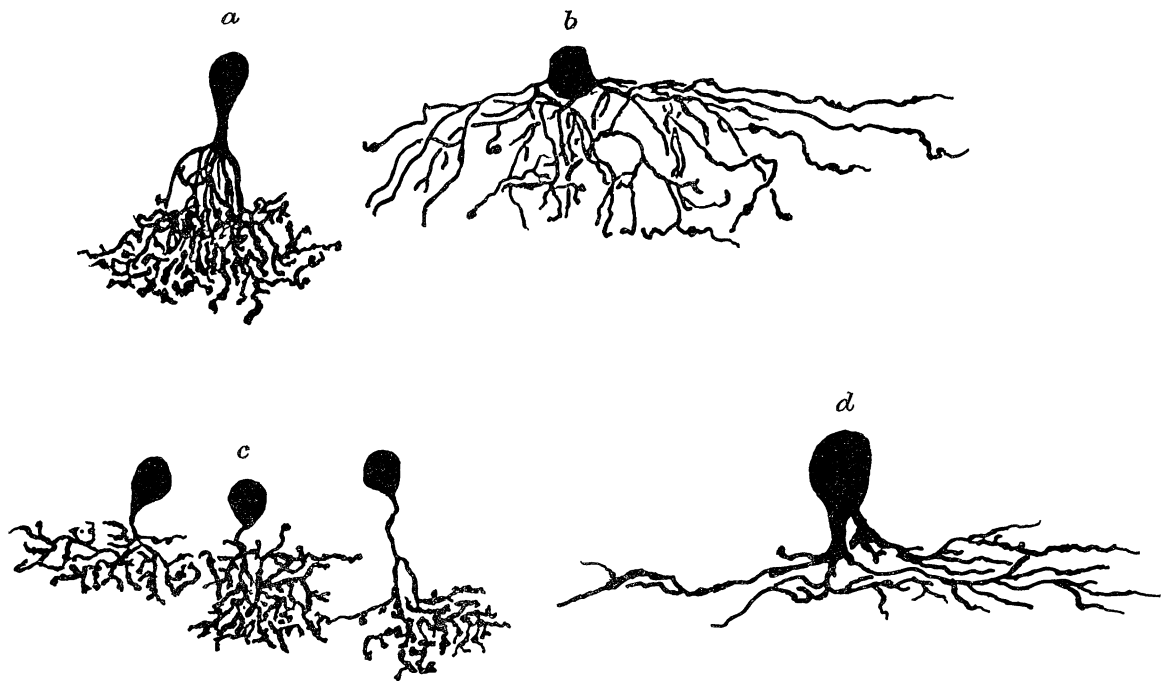


FIGURE 54. Amacrine cells from the central area of the rhesus macaque retina (redrawn from Polyak; *a*, figure 67A; *b*, figure 67B; *c*, figure 67D; *d*, figure 67E). *a* and *c* are probably stratified diffuse amacrine cells (see p. 151); *b* is probably a wide-field diffuse amacrine cell (see p. 150); *d* is probably an understained cell as illustrated in figure 65*m*, plate 40 (see also p. 155). See also plates 39 and 42.

field of about 30 to 50 μm in diameter. A few of the branches at first run horizontally just under the inner nuclear layer (figure 61, plate 39) but usually all pass through the inner plexiform layer towards the ganglion cell perikarya. They do not terminate there and instead turn to continue more or less horizontally for as much as 250 μm, sometimes looping into the centre of the inner plexiform layer and down again to the ganglion cell perikarya. Figures 55, 57 and 61, plate 39, illustrate this and Cajal's cells show a similar form (figure 53*a*, *d*). In total the diameter of spread of the processes of such cells at the level of the ganglion cell perikarya can be as much as 600 μm (see legend figure 61, plate 39). The branches near the ganglion cell somata are in the region of the inner plexiform layer where the rod bipolar terminals are most common (p. 144). It is, therefore, an obvious suggestion that the wide-field diffuse amacrine cell is particularly likely to be in synaptic

relationship with rod bipolar terminals. The processes of this kind of amacrine cell are sufficiently smooth for it to be unlikely that they form axosomatic contacts with ganglion cell perikarya (p. 150).

Wide-field diffuse amacrine cells present difficult problems of assessment. We have recognized only some 10 of them in the material provided by Miss Helga Kolb (p. 112). However, once observed in that material, partially stained cells resembling them were identified in our own preparations. Polyak's cell illustrated in figure 54*b* resembles one of these. The apparently greater density of branching in his figure is probably due to the fact that the cell was drawn flat. Cajal said that these cells stained more readily with methylene blue than with Golgi procedures. It seems likely, therefore, that they are not as uncommon as their infrequency of impregnation in the present investigation suggests.

(*c*) *Stratified diffuse amacrine cells* (figure 55, plate 39; figures 65 and 67, plate 40). As defined by Cajal cells were termed 'diffuse' when their processes branched at all levels of the inner plexiform layer (p. 146), but another feature of most diffuse amacrine and ganglion cells is that their processes branch more frequently per unit area than the stratified cells. It is common to observe amacrine cells whose pattern of branching is very like that of the narrow-field diffuse amacrines but whose maximum branching occurs at one level of the inner plexiform layer. These were observed by Polyak (figure 54*a*, *c*) and are illustrated in figure 55, plate 39; figures 65, 67, plate 40. The density of the branching may be very great (figure 67, plate 40) or rather less so (figure 55, plate 39). The diameter of spread of the branching of the cells is between about 20 and 50 μm and is usually within about a third to a half of the inner plexiform layer. The cells illustrated are all ones with branching near the ganglion cell perikaryon layer. However, some have been observed branching in the middle of the inner plexiform layer and in the half of that layer near the inner nuclear layer. Figure 54*c* from Polyak gives some indication of the three levels of branching. Though quite frequently stained these cells have been difficult to observe in detail in our material since they were often obscured by the branching of the dendrites of stained ganglion cells and the processes of glia cells. The cells in figures 73 and 74, plate 42, show such amacrines in relation to a ganglion cell and suggest that these amacrines could be making frequent, though probably not exclusive contacts with a particular ganglion cell that has its dendrites at a particular level (see p. 164). They were not formally described by Cajal but the cells in figure 53*b* and *c* perhaps represent stratified diffuse amacrine cells as defined here.

Stratified amacrine cells (figure 59, plate 39; figures 62 to 66, plate 40; plate 41).

Stratified amacrine cells are those amacrines whose branches lie along a plane in the inner plexiform layer. From Cajal it is apparent that some mammals (figure 52) and certainly birds (see Oliveira Castro 1966) have stratified amacrines with one or more processes that go to a particular level in the inner plexiform layer and then branch symmetrically within that layer. Oliveira Castro's (1966) paper shows this particularly well for the chicken. And figure 72, plate 41; figure 75, plate 42, illustrate the stratification of amacrine cell processes in a reduced silver preparation of a pigeon. Something of the stratification of the processes of amacrine cells and of the dendrites of ganglion cells in the inner plexiform layers of various vertebrates can be observed by phase contrast microscopy.

Description of plate 40

Amacrine cells as seen in vertical section of rhesus macaque retina. All at × 800 except figure 62 which is × 500. The extent of the inner plexiform layer is indicated by the braces. Figures 62 and 65 to 67 are from Miss H. Kolb's preparation, the remainder are Golgi–Colonnier material.

Figure 62. Unistratified amacrine cell with processes extending immediately under the inner nuclear layer. Out of focus to the right is a midget ganglion cell. There is a second stratified amacrine cell behind that in the foreground, none of its processes are in focus in this picture. The processes of that cell in the foreground extend to give a total diameter of 500 μm. An unusual density of the background of the section shows up the amacrine perikaryon layer (*a*). The perikaryon of this cell is $9 \times 15\,\mu$m.

Figure 63. A commonly observed stratified amacrine cell. The diameter of spread of the processes is 150 μm and the perikaryon is $10 \times 16\,\mu$m.

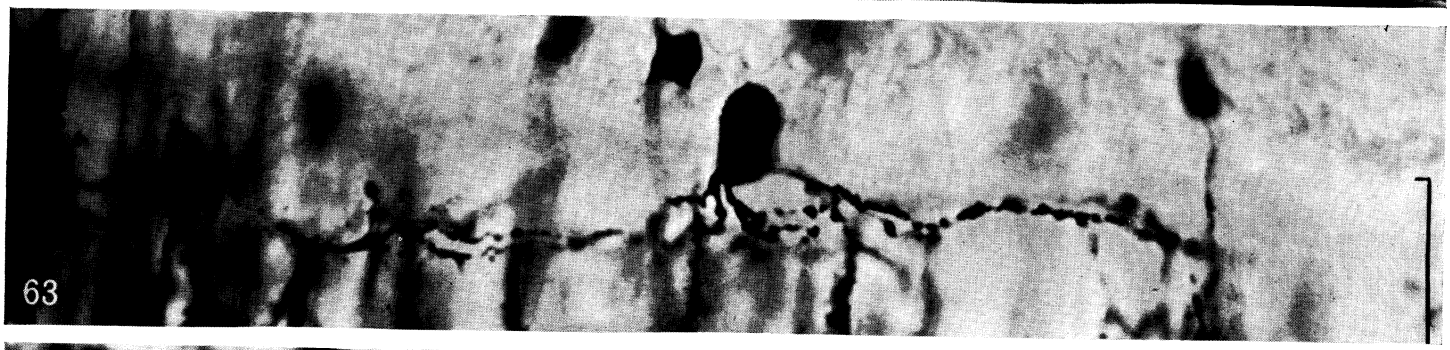
Figure 64. Two cells, like that of figure 63, showing clearly that their processes branch on the same level in the inner plexiform layer. Glia obscures the fact that their processes overlap. (See also figure 71, plate 41.) The perikaryal diameters are 10×10 μm.

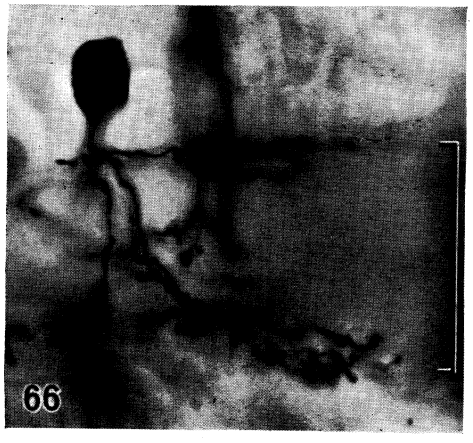
Figure 65. Multistratified amacrine cell (*m*) in the foreground, with a stratified narrow-field diffuse amacrine (*s*) just behind. The processes of the multistratified amacrine mostly take a sinuous course through the centre of the inner plexiform layer. They spread in either direction to give a total diameter of between 400 and 600 μm; the perikaryon is 12×16 μm. The dimensions of the stratified diffuse amacrine are: perikaryon 8×10 μm; diameter of spread of processes 50 μm (see figure 54*d*, p. 150).

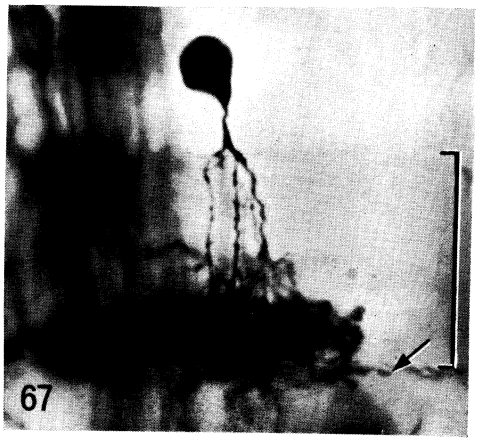
Figure 66. Part of a bistratified amacrine cell showing how some of the processes run down and branch very near the ganglion cell somata (see also figure 59, plate 39).

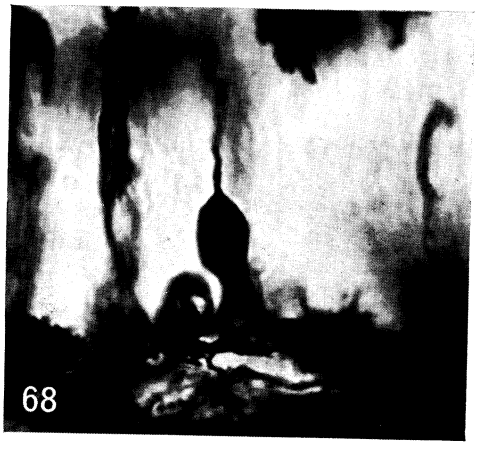
Figure 67. Stratified narrow-field diffuse amacrine with smooth processes that branch very frequently and densely in the third of the inner plexiform layer near the ganglion cell somata. The cell was sufficiently isolated for it to be certain that the processes are all from this one amacrine cell, although the fibre marked with an arrow is from an adjacent amacrine cell like that illustrated in figure 61, plate 39. A similar stratified cell is seen in figure 65 (*s*). The frequency of branching of these two stratified cells is very much greater than that of the stratified diffuse amacrine cells in figure 55 plate 39, but here the diameter of spread of the processes is 40 μm, half the spread of that cell. The perikaryal diameter is 8×10 μm.

Figure 68. Developing amacrine cell from the retina of an 108 day foetus. The apical process extends into the outer plexiform layer and there branches in two. A process of this kind was visible on only three of some 50 or more amacrine cells stained at this time of development. The processes in the inner plexiform layer had the appearance of a stratified amacrine cell such as that in figure 63. That is not necessarily to say that it would have developed into a stratified amacrine cell. The details of development of any kind of amacrine cell are mostly unknown.









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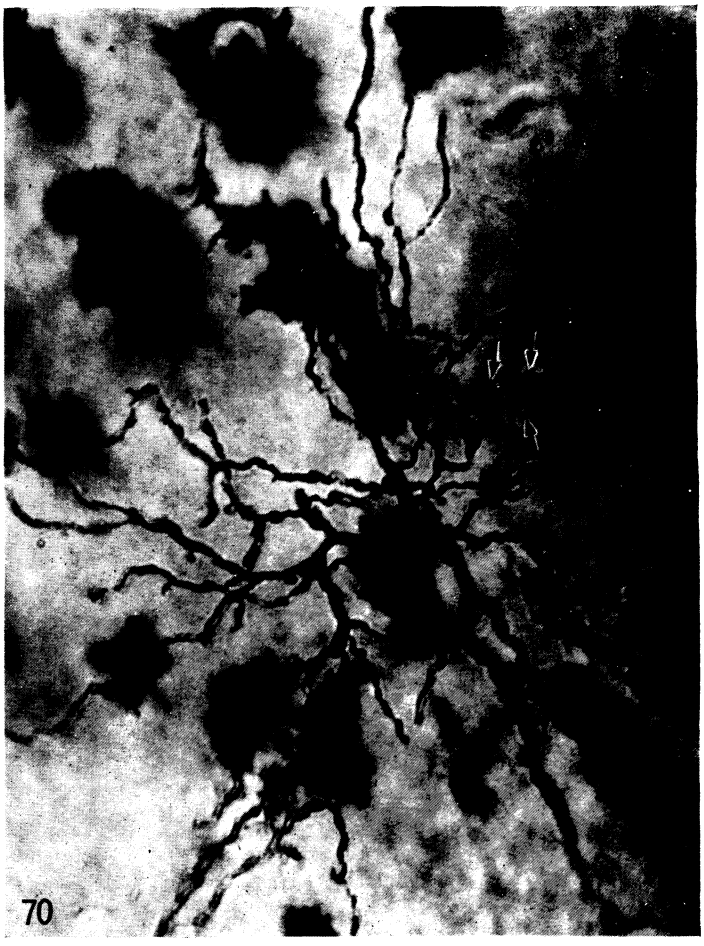
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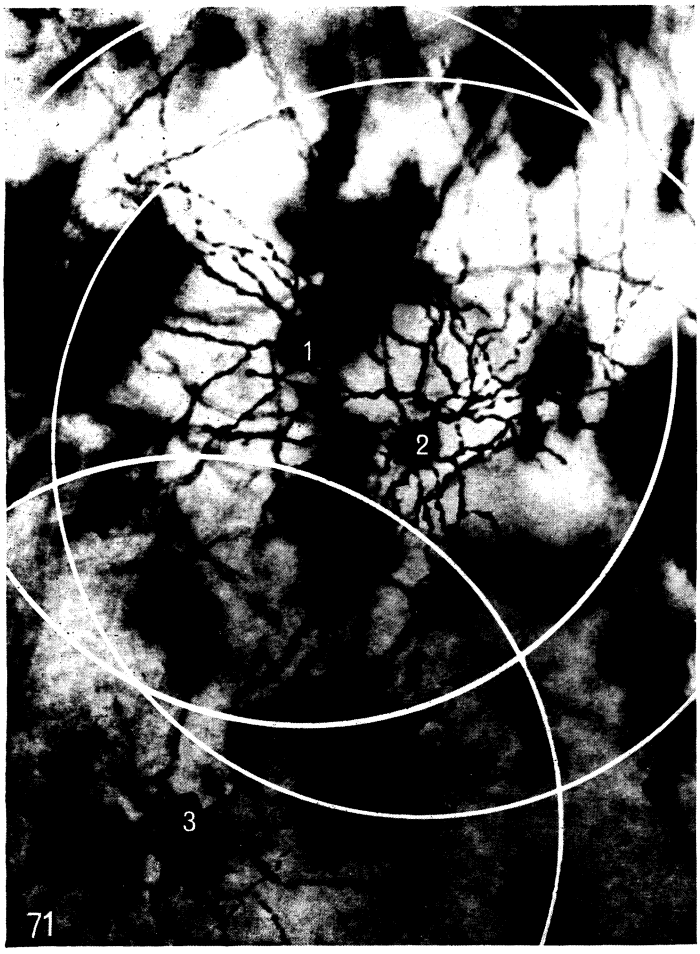
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Description of plate 41

Stratified amacrine cells of rhesus monkey and pigeon as seen in horizontal section. All Golgi–Colonnier procedure except figure 72. Magnification of figures × 800, except for figure 71 which is × 500.

Figure 69. Part of a large unistratified amacrine cell of rhesus monkey. There are three thick processes (about 2 μm in diameter) coming from the perikaryon which have thin branches (about 0·5 μm in diameter) off them (arrows). It is clear that this cell is different from that in figure 70 not only in its size but also in the size and pattern of branching of its processes. From the appearance of the cell it is easy to imagine that it may not be completely impregnated. After passing through the inner plexiform layer for about 100 μm all three processes sharply decreased in diameter to 0·5 μm and in a further 50 μm stopped abruptly as if underimpregnated. The cell is represented diagrammatically in figure 96, p. 168.

Figure 70. Unistratified amacrine cell of rhesus monkey of the kind shown in vertical section in figures 62 to 64, plate 40. The pattern of branching is quite different to that of the cell in figure 69. Arrows point to the occasional small spines that can be seen on the processes. The processes had a maximum diameter of spread of about 200 μm.

Figure 71. Three unistratified amacrine cells at a lower magnification than figure 70 to show that the processes of these cells overlap (see also figure 64, plate 40). The illustration also shows many of the criss-crossing fibres of the inner plexiform layer, some of which are not always attachable to a particular cell although they may be traceable for several hundred microns. The diameter of spread of the processes of these three amacrines is between 150 and 200 μm. Their fields have all been indicated as 200 μm. The picture obtained only shows the overlapping of these three amacrines. It must be remembered that there are presumably other unstained amacrines of the same kind as well as different kinds in the field of the picture. Such spatial relations are perhaps better indicated from the pigeon retina as shown in figure 72 and the summary, figure 96.

Figure 72. Horizontal section through one of the layers of unistratified amacrine cells of the pigeon retina as stained by Holmes (1947) procedure using 2,6-lutidine instead of pyridine (Blest 1961). As explained in the legend to figure 75, plate 42, probably only the neurofibrillar part of these cells stains; so that although they are comparable to those stained by Oliveira Castro (1966) in chicken they do not reveal the fine branches or, perhaps, even as many of the main branches, as he was able to observe by his Golgi procedure. The picture does show the relatively even spacing of a single kind of amacrine cell and the regular overlapping of the processes. The centres of the cells in this particular layer of unistratified amacrines (see figure 75, plate 42) were between 30 and 80 μm apart and processes from any one cell overlapped at least to the extent of the radius of the field of the adjacent cells, i.e. the processes extended as far as the level of the perikaryon of all the cells within 30 to 80 μm.

Such stratification has not been observed in primates and some other mammals such as the cat (see p. 173). Nonetheless, amacrine cells with stratified processes are found in primates and also cats, (B. B. Boycott unpublished observations). There are two possible explanations for this: (1) The processes of any particular type of amacrine or ganglion cell may not end in a constant plane of the inner plexiform layer. In that case they would not present a sufficiently organized system of processes to be detected by light microscopy. (2) The processes of similar types of stratified amacrines could be localized to the same stratum of the inner plexiform layer but the density of their branching might be insufficient to allow a layer to be revealed by light microscopy. Figure 64, plate 40, shows two stratified amacrine cells of the same kind whose processes are clearly in the same plane; and figure 71, plate 41, shows similar cells in horizontal section with their overlapping fields. They resemble comparable amacrine cells from a pigeon retina in figure 72 of the same plate. This suggests that the difference between primate retinae and those retinae that are observed by phase-contrast microscopy, as stratified, is indeed in the relative density of the processes. However, there are no data for vertebrates on the frequency of particular kinds of amacrine cells or on the density of branching of their processes. Therefore, either, or both, of the above possibilities could be true. These problems are discussed more fully on p. 173.

(*a*) *Unistratified amacrine cells* (figures 62 to 64, plate 40; plate 41). Unistratified amacrine cells of monkey are illustrated in vertical section in plate 40 and in horizontal section in plate 41. Their processes clearly occupy a plane within the inner plexiform layer. However, in general, unistratified amacrine cells (at least such as we have stained) have their processes in the scleral half of the inner plexiform layer. Those illustrated in figures 62 to 64, plate 40, and 69 to 71, plate 41, are the more commonly stained. The diameter of spread of their processes is usually about 100 to 200 μm. The cell in figure 62, plate 40, however had a diameter of spread of about 500 μm. In sectioned material, for much the same reasons as discussed for the horizontal cell axons on p. 141, it is clear that exact estimates of the diameter of spread of processes of these cells is difficult. Accordingly it is unclear whether there are two classes of unistratified amacrine cells, those of 100 to 200 μm in diameter of field and those of 500 μm; or a graded series of sizes of cells up to 500 μm in diameter and beyond. Nor have we determined whether there is a distribution of smaller to larger cells from the centre to the periphery of the retina. However, it is certain that those with dendritic diameters up to about 200 μm are to be found all through the central area and on to the foveal slope. Neither their processes, nor indeed those of other nerve cells, cross the foveal pit. As figure 64, plate 40, indicates the processes of individual cells of this kind overlap.

These unistratified amacrine cells invite comparison with those described by Oliveira Castro (1966) in developing chicken. As figure 70, plate 41, shows those of primates may, like those of birds (Oliveira Castro 1966), have small spines on their processes, although they appear not to be of a comparable density and we have not observed terminal tufts on the processes. These, however, as Oliveira Castro suggests, may be growth cones.

Besides these stratified amacrine cells which have been commonly observed in our material, there is a larger unistratified amacrine cell that has a different pattern of branching. This is shown in figure 69, plate 41. These larger amacrines have three main processes about 2 μm in diameter that run straight away from the perikaryon. The processes do not

branch dichotomously near the perikaryon as in the case of the smaller unistratified amacrines. When they do branch, the branches are fine processes about 0·5 μm in diameter that come directly off from the large process (figure 69, plate 41). When compared with the symmetry of the smaller stratified amacrines one may suppose that such cells should have four main branches. Observation of these large cells is difficult but they appear only to have three main branches, which, allowing for the distortion of a flat section of a curved structure, may be supposed to be disposed symmetrically as in figure 96.

We have probably not seen one of these large unistratified cells in its entirety, for in any plane of section or thickness of section that makes it possible to see them at all, they are likely to be cut. In addition, about 100 to 150 μm from the perikaryon the 2 μm diameter processes narrow down, often quite sharply, to 0·5 μm in diameter. The kind of whole mount technique used by Oliveira Castro (1966) will be necessary for their precise description. However, on the basis that the longest continuously intact pieces observed attached to a perikaryon were 400 μm and assuming that the cells are symmetrical, as all amacrines we have observed are, then even in the central area the diameters of the field over which the processes of such cells could spread would be as much as 1·0 mm. Not only are the processes of these cells frequently cut in sections, but they are also probably under-impregnated. Figure 69, plate 41, shows the main processes of such a cell with two fine processes clearly coming off the main process. If such fine processes failed to impregnate their absence would not readily be noted, and, like the horizontal cell axons (p. 139), the same problems of inadequate impregnation arise where the larger diameter processes narrow down. Although we can be sure that these cells exist and that their fields are large, we do not feel confident that we know their morphology. We have observed that the processes of these large amacrine cells overlap, as do those of all amacrines we have examined. The plane of branching of the large unistratified cells has always been immediately under the inner nuclear layer and in this way they may be compared with large stratified amacrines in other vertebrates; possibly, for example, the kind of cell shown in figure 52*c*.

(*b*) *Bistratified amacrine cells* (figure 59, plate 39; figure 66, plate 40). There appears to have been no previous description of a bistratified amacrine cell in a mammal. Cajal (1911) stated explicitly that he had never seen them in mammals but had done so in most other vertebrates. In the retina provided by Miss H. Kolb we have seen eight complete cells of this kind. Where their processes enter the inner plexiform layer they divide on a plane immediately under the inner nuclear layer and fan out to a field diameter of up to about 100 μm. (figures 59 and 66, plates 39 and 40). On these branches there are some small spines and fine processes that descend towards the layer of ganglion cell perikarya. These fine processes do not branch as they go through the inner plexiform layer, but do so when they reach the region near the ganglion cell layer (figure 66, plate 40). Here they become coarser and branch more frequently so that the branches of a single process overlap with the branches of other processes of the same cell. The diameter of their spread is about the same as that of the first layer of branches near the inner nuclear layer. Similar cells have been described by Cajal in birds and reptiles. It cannot yet be said whether or not, among mammals, they are unique to primates. It is possible that they have not been observed before in mammals because they do not stain at all readily.

(*c*) *Multistratified amacrine cells* (figure 65, plate 40). Neither Cajal, nor Polyak, nor

ourselves, have observed multistratified amacrine cells in mammals. That is to say cells with processes that branch in distinctive horizontal planes at more than two levels of the inner plexiform layer. Such cells have been seen quite commonly in other vertebrates (Cajal 1911). The cell shown in figure 65, plate 40, does not fit into the classification of amacrine cells here used. We have seen it quite frequently but generally only incompletely impregnated, rather as Polyak saw such a cell as that in figure 54*d*. Characteristically the processes originating from the perikaryon are quite thick; but at about the middle of the inner plexiform layer they divide into fine processes that form a band occupying about a third of the middle of that layer. In a sense such a cell could be classified as a stratified diffuse amacrine cell (p. 148). This would be confusing, however, because a characteristic of diffuse amacrine (and ganglion) cells is that their processes branch in or near the vertical plane of the retina. The processes of the stratified cells branch in a horizontal plane (p. 151) and that is what the processes of this cell do. Therefore, because this kind of cell occupies such a broad band of the inner plexiform layer, it is formally classified as a multistratified amacrine cell. It is almost certainly an amacrine cell and not the 'displaced parasol ganglion cell' Polyak suggested. Not just because it has not been observed with an axon but because it does not resemble certainly identified 'displaced' or other kinds of ganglion cells.

Introduction

Observations on ganglion cells

Polyak described six kinds of ganglion cells in the primate retina which he grouped as two classes. The 'monosynaptic' ganglion cells included only the midget ganglion cell. The 'polysynaptic' ganglion cells included five kinds of ganglion cells: the parasol, shrub, small diffuse, garland and giant ganglion cells. They are illustrated in figures 80 to 83. Polyak emphasized that, with the possible exception of the giant ganglion cell, all varieties of ganglion cells are present in every region of the retina from the central foveal region to the far periphery. We agree.

Midget ganglion cells (figure 8, plate 34; figures 76 to 79, plate 42; figure 87, plate 43).

In the central area of the rhesus macaque and human retina the most commonly stained ganglion cells are usually the midget ganglion cells first described by Polyak (figure 80). These are characterized by a single dendrite which goes straight into the inner plexiform layer. Polyak, however, thought that the middle of the inner plexiform layer was free of terminals of the midget ganglion cell dendrites (figure 76, plate 42). This corresponded with his observation that midget bipolar cell axon terminals end in the third of the inner plexiform layer just under the inner nuclear layer and the third just above the ganglion cell layer. This certainly appears to be true near the fovea and may correspond with the differences in mode of contact of the two different kinds of midget bipolar cells with a cone (p. 177).

The midget ganglion cell is apparently unique to primates and it has unique clearly defined synaptic relationships. Since its relationships are to some extent understood, it need not be fitted into the present descriptive classification of amacrine and ganglion cells (p. 146). It was one of Polyak's important discoveries that the midget bipolar axon terminals and the dendritic terminals of the midget ganglion cells come into nearly exclusive contact with

each other. The tip of the midget ganglion cell dendrite forms a few short branches (figure 8, plate 34 and figures 76 to 79, plate 42). In both Polyak's material and ours, fortunate coincidence of staining may show these closely entwined with the terminal swelling of a single midget bipolar cell (figure 77, plate 42). This junction is not, however, a single large synapse. Electron microscopy (Dowling & Boycott 1966) shows that there are a large number of points of synaptic contact between a midget bipolar terminal and the dendrites of a midget ganglion cell. It also shows that the bipolar axon terminal-ganglion cell dendrite synapse is always paired with another process, usually that from an amacrine cell (a dyad, figure 98). Because a midget bipolar cell is in postsynaptic contact with a single cone (p. 121) and because of the synaptic relationship described above, it is clear that a single midget ganglion cell could be specific to a single cone. That is to say a single midget ganglion cell could be colour-coded (see p. 165). The arrangement does not mean that a single cone is only specific to a single ganglion cell. Indeed that cannot be so, because any

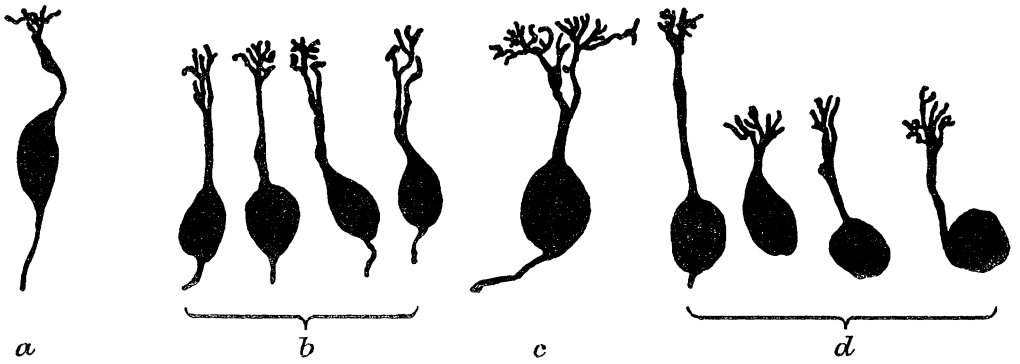


FIGURE 80. Midget ganglion cells of rhesus macaque retina (redrawn from Polyak: *a*, from figure 70*A*; *b*, from figure 70*C*; *c*, from figure 70*D*; *d*, from figure 69*C*). *a* is from close to the foveal pit; *b* are from the para- and perifoveal regions of the central area; *c* is from the near periphery; *d*, cells from the perifoveal region. The figure shows something of the relative sizes and variation in shape of these cells and the fact that they end at two different levels in the inner plexiform layer. See also plate 42.

one cone is connected to at least one, and possibly to more flat bipolar cells, as well as to at least one midget bipolar. In addition, because of the amacrine connexions through the dyads and the reciprocal contacts, such a unit is not isolated from the activity of the rest of the retina.

Unfortunately it is not possible to be certain whether or not other kinds of bipolar cells synapse with midget ganglion cells, or if midget bipolar cells synapse with other kinds of ganglion cells. Polyak thought that flat bipolar cells might synapse with midget ganglion cells and that branches from the midget bipolar cell terminals might end on any of his other types of ganglion cell. He also thought that the rod bipolar axosomatic synapse could be with the perikaryon of a midget ganglion cell (figure 98).

Figure 77, plate 42, shows such a close relationship between the midget bipolar axon terminal and the midget ganglion cell that it seems unlikely that either of the cells has synapses with another bipolar or ganglion cell. However, figure 78, plate 42 shows a midget ganglion cell dendrite that is not enclosing all the surface of the terminals of the bipolar

Description of plate 42

Vertical sections of ganglion cells of the primate retina stained by the Golgi–Colonnier procedure and amacrine cells of the pigeon retina (figure 75) stained by the Nonidez procedure. Braces indicate the dimensions of the inner plexiform layer. Magnification, × 800.

Figures 73, 74. Rhesus macaque. Two narrow-field diffuse amacrine cells a_1 and a_2 with overlapping processes. These cells are near the periphery of the central area. Their processes occupy the thickness of the inner plexiform layer and are closely entangled with the dendrites on one side of the ganglion cell. The ganglion cell is a unistratified ganglion cell so, clearly, some of the processes of the amacrine cells most probably synapse with other cells. The processes of the amacrine cells are so dense that they obscure the dendrite of the ganglion cell. Figure 73 shows the dendrites on the other side of the ganglion cell which must, therefore, contact other amacrine cells. Figures 73 and 74 illustrate rather well the observational difficulties of making meaningful estimates of the number and kind of amacrines, or for that matter bipolar cells, that a ganglion cell may contact.

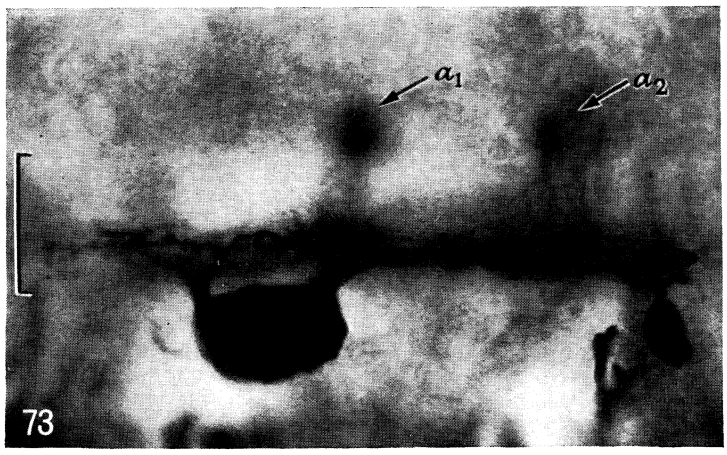
Figure 75. Vertical section of the retina of a pigeon outside the central area; hence the ganglion cell layer (*g*) is but one cell thick. The stain was Nonidez (1939) procedure. Like the Holmes stain figure 72, plate 41, Nonidez and other reduced silver procedures impregnate nerve cells with neurofilamentous components in the cytoplasm (Boycott, Gray & Guillery 1961; Gray & Guillery 1966). In the pigeon retina the result is to show five distinct layers within the inner plexiform layer. Layers 2 to 4 in the figure are certainly made up of the processes of unistratified amacrine cells and possibly correspond to the cells of the three layers described by Oliveira Castro (1966). The main processes from two amacrine cell perikarya to layers 3 and 4 are clearly shown here (cells a_3 and a_4). Layer 1 possibly has stained stratified amacrine cell processes in it and certainly has dendrites of displaced ganglion cells. Layer 5 is less distinct and often does not stain well. Some ganglion cell dendrites are stained and Cajal has described unistratified amacrine cells that could be in this layer. Between the layers there are numerous stained fibres that are dendrites of ganglion cells, axons of centrifugal fibres and possibly some amacrine cell processes. It is instructive to compare this picture with that of figure 52 taken from Cajal (1911) where at least in ox or dog retina there appears to be a layering of amacrine cell processes comparable to that shown here. Unfortunately the comparable cells in monkey do not react to reduced silver methods but this does not mean that there are no cells with processes organized into layers within the inner plexiform layer (see p. 173).

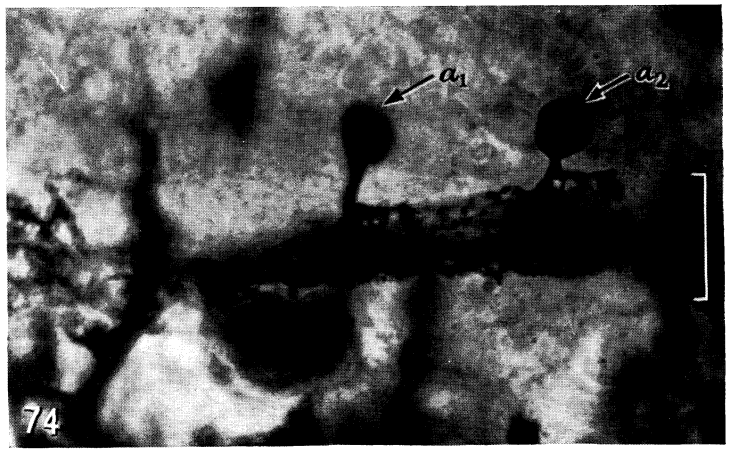
Figure 76. Two adjacent human midget ganglion cells just peripheral to the foveal slope. They show very well how, as Polyak said, the dendrites of the midget ganglion cells end at two levels within the inner plexiform layer. A similar difference can be seen in the two cells in figure 79. The arrow indicates an axon. The tip of the dendrites of both cells is split into two for about 18 μm and a midget bipolar terminal presumably fits into the groove. The diameter of the dendritic spread is between 4 and 5 μm and the perikarya are 14×14 μm.

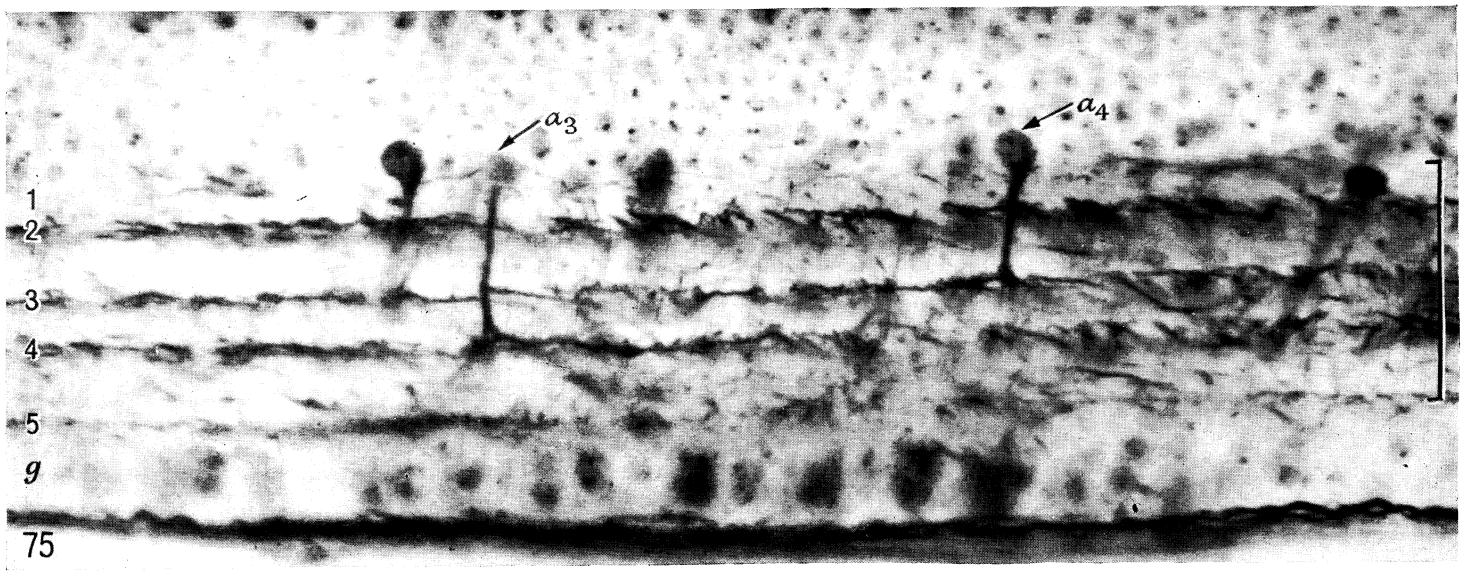
Figure 77. Rhesus macaque. Midget bipolar cell terminal and midget ganglion cell dendrite in close embrace. The cells are at the edge of the foveal slope. Perikaryon dimensions 10×12 μm.

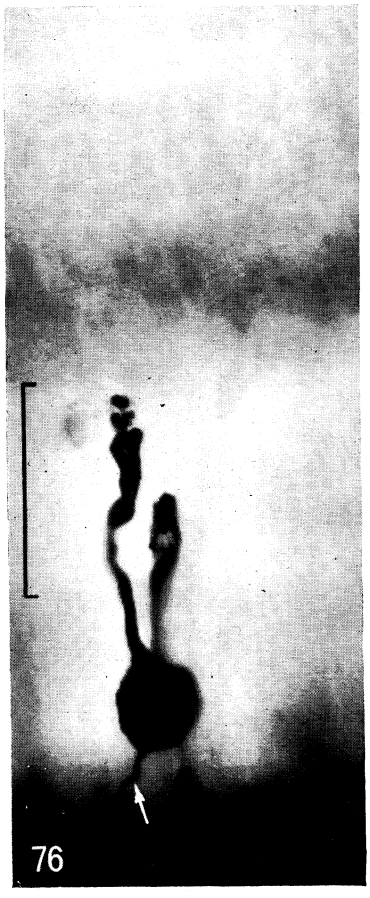
Figure 78. Rhesus macaque. Midget ganglion cell with its dendrites embracing a midget bipolar cell terminal. To the left is the terminal of another midget bipolar cell. For details see p. 157.

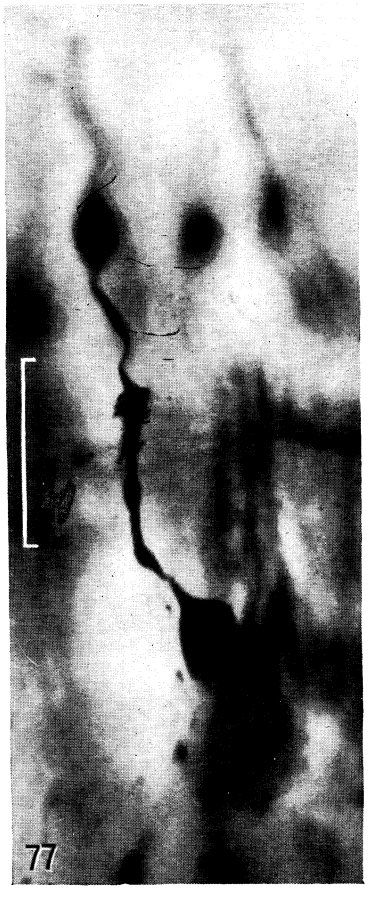
Figure 79. Rhesus macaque. Two midget ganglion cells with slightly wider dendritic diameters, rather like those shown by Polyak; see figure 80. The dendrites of one terminate near the ganglion cell layer and of the other near the inner nuclear layer. Axons can be seen on both cells.

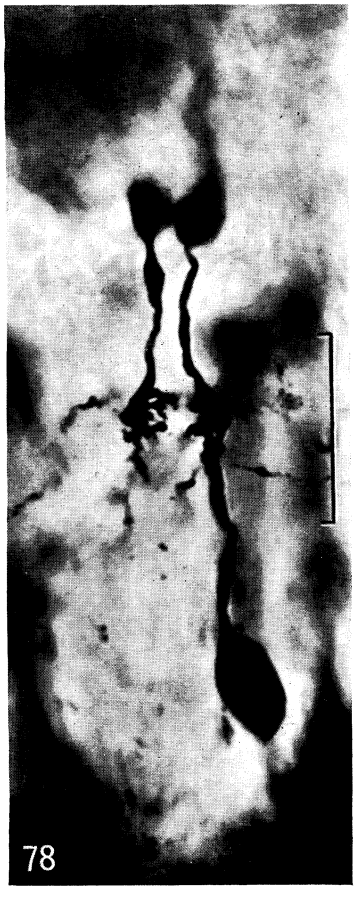


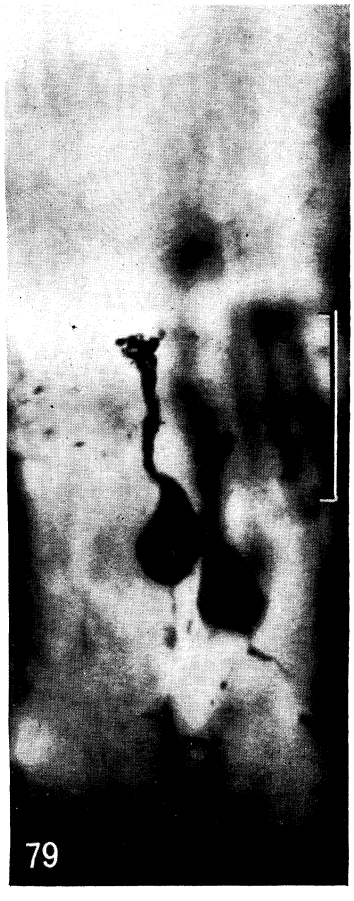












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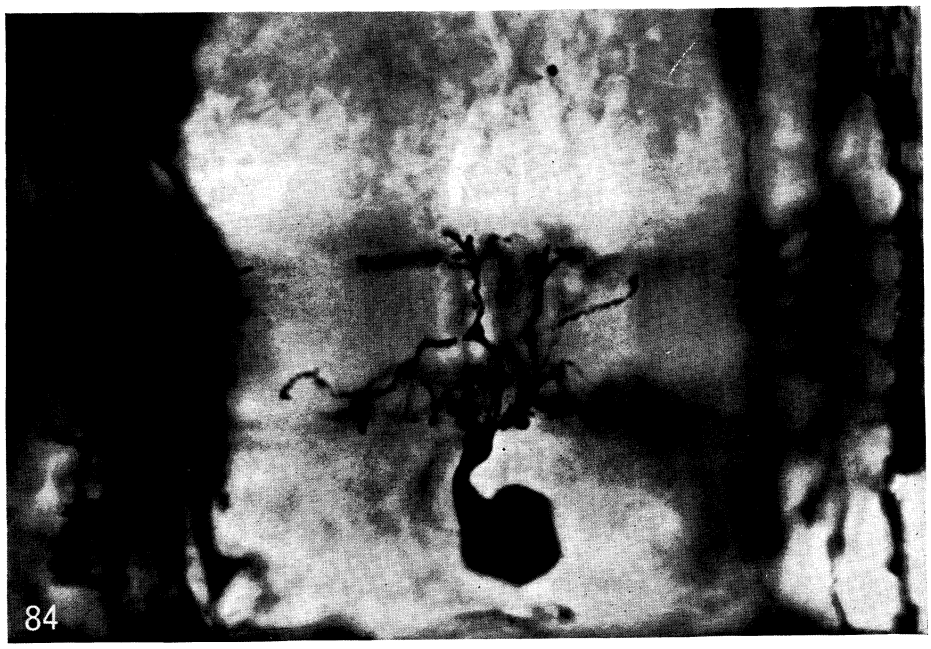
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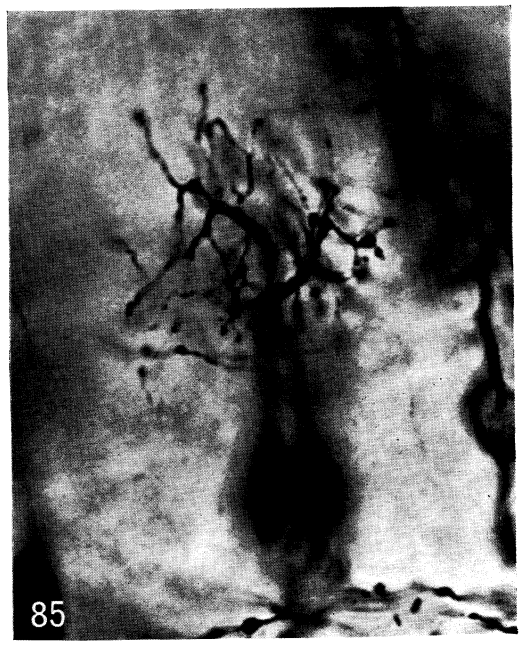
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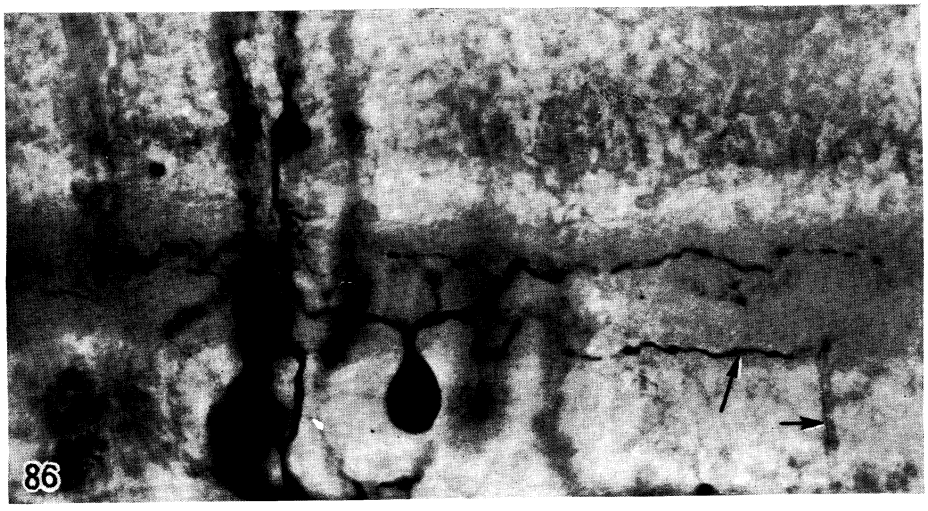
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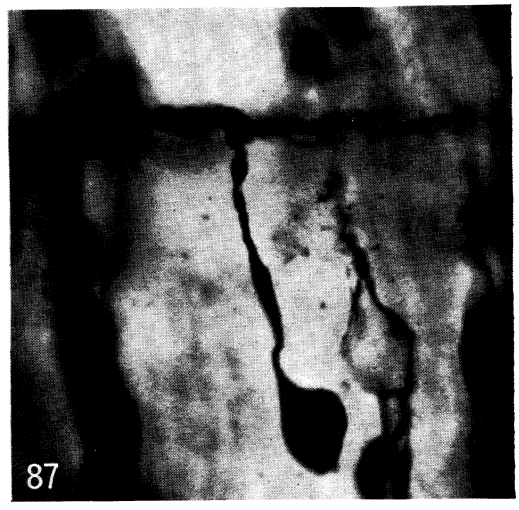
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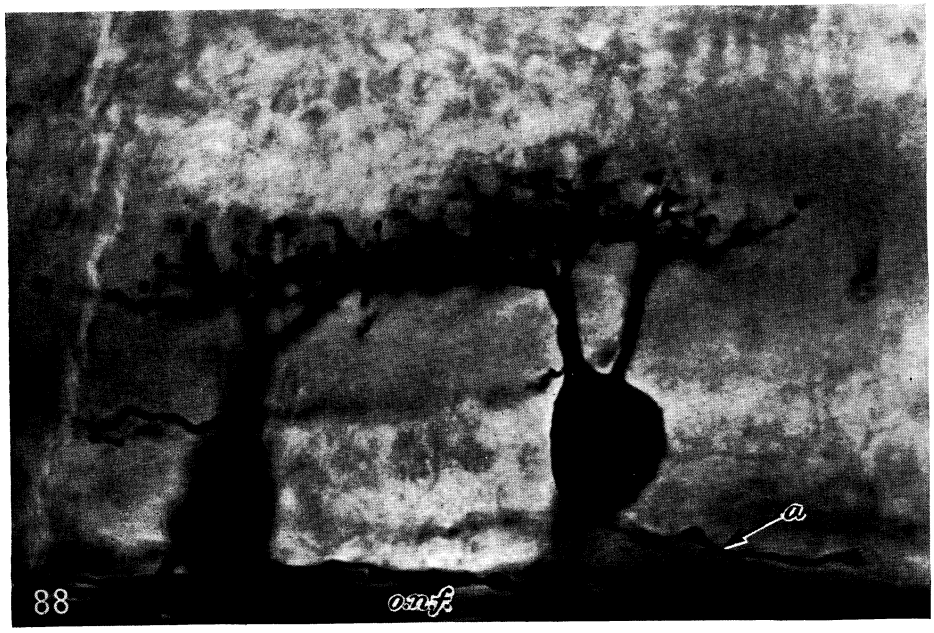
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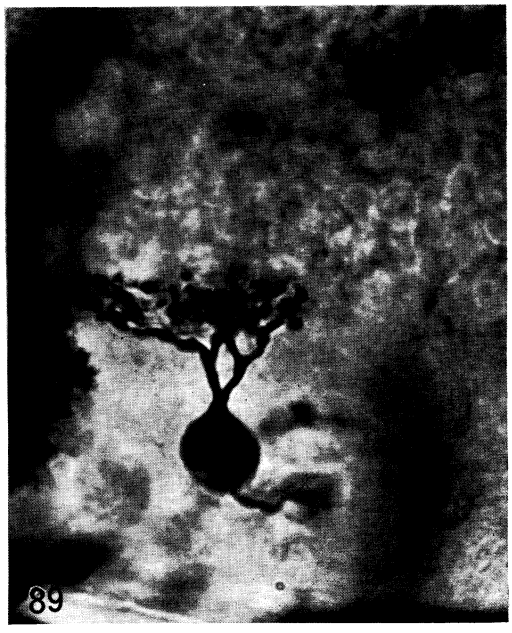
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cell. Beside it there is another midget bipolar terminal with processes (described by Polyak) that could well be in contact with another ganglion cell, either midget or of some other kind. There are many examples of this kind of difficulty throughout the retina; although useful guesses may be made, it is often not possible to decide definitively what kinds of bipolar cells synapse with what kinds of ganglion cells.

Not all the midget bipolar cells have axon terminal branches as compact as those illustrated in figures 8 and 11, plate 34, and figure 78, plate 42. Some have a more divided axon as shown in figure 15, plate 34. Polyak's data (figure 80) show the variation in the dendritic diameters of the midget ganglion cells. As Polyak supposed, those with the narrower diameters tend to be nearer the fovea, but even at the periphery of the central area the dendritic diameter of a midget ganglion cell apparently never gets much larger than 10 μm. Of course if it did it would no longer be recognizable as a midget ganglion cell. It might resemble, for example, the stratified diffuse ganglion cell of figure 89, plate 43.

Description of plate 43

Vertical sections of rhesus macaque retina to show the various types of ganglion cells. All at $\times 800$ except figure 86 which is $\times 500$; and all stained by Golgi–Colonnier.

Figure 84. Diffuse ganglion cell seen directly from the side. The dendritic spread is between 50 and 60 μm and the dimensions of the perikaryon are 15×15 μm.

Figure 85. Diffuse ganglion cell observed slightly obliquely. One of the perikarya is that of a midget ganglion cell but all the processes visible are from this one diffuse ganglion cell. The dendritic spread is 45 μm and the perikaryon is 10×20 μm. Diffuse ganglion cells stain quite often in the primate retina and they are common in other vertebrates.

Figure 86. A unistratified ganglion cell with dendrites running in the third of the inner plexiform layer near the inner nuclear layer. The lower process, arrowed on the right, that comes off the dendrite is presumed to be an axon because after passage through the inner plexiform layer it comes out through the ganglion cell layer (arrow) and into the optic nerve. Presumably there could be synapses on the axon but we have no evidence of this or data on the likelihood that an axon of this kind of cell would always take this course. Dimensions; perikaryon, 13 μm $\times$ 20μm; dendritic diameter 200 μm.

Figure 87. Midget ganglion cell with its apical process reaching up very close to the processes of an unistratified amacrine cell (such as in figure 63, plate 40) the perikaryon of which is just visible out of focus to the left and close to a strand of glia.

Figure 88. Two stratified diffuse ganglion cells with overlapping dendrites. These are towards the periphery of the central area. The combined diameters of their dendritic fields was 110 μm. That of the cell on the right was 70 μm, on the left 60 μm. The overlap of these fields was 20 μm. The perikaryon on the left was 17 μm $\times$ 23 μm, on the right 15 μm $\times$ 25 μm. *o.n.f.*, optic nerve fibres; *a*, axon.

Figure 89. Stratified diffuse ganglion cell from the periphery of the retina. Its dendritic diameter is 35 μm, smaller than those of the two cells in figure 88. Its perikaryon size is 11 μm $\times$ 11 μm. In view of the fact that the axon terminals of the midget bipolar cells may spread more widely in the periphery, up to 15 μm, possibly more, it is conceivable that this cell has synaptic relationships different from those cells in figure 88 with which it is classified. It could also be held to resemble a midget ganglion cell such as that described by Polyak (see figure 80*c*, p. 157) which is from about the same region.

However, like Polyak, we have seen midget ganglion cells in the periphery of the retina that could well have come from the central area. We do not know what relationship, if any, these have to the wider spread of many of the axon terminals of the midget bipolar cells (figure 30). Such observations further illustrate the present limitations of light microscopy as a means of estimating the synaptic contacts and functional relationships between cells in the inner plexiform layer. Since the midget bipolar cell must be colour-coded it might seem unlikely that flat bipolars and midget bipolars would be connected to the same ganglion cell. Polyak has suggested that the farther midget bipolar and midget ganglion cells are from the fovea the more likely they are to have wider connexions. This would agree with the sharp change in visual acuity from the fovea outwards across the retina. Towards the edge of the central area the relationship of the cones to the midget bipolar cells may vary somewhat (p. 124). It is also in this region of the retina that the

a *b* *c*

FIGURE 81. *a*, diffuse ganglion cell ('shrub' Polyak); *b*, diffuse stratified ganglion cell ('parasol' Polyak) from the perifoveal region of rhesus macaque (redrawn from Polyak's figure 69*C*); *c*, is a displaced ganglion cell from rhesus macaque retina. The long process is the axon that runs to the optic nerve fibre layer (redrawn from Polyak figure 87). See also plates 43, 44.

midget bipolar terminals may spread out, thus presumably raising the probability that a midget bipolar cell might synapse with more than one ganglion cell. However, the frequent appearance in Golgi material, particularly near the fovea, of midget bipolar cell axon terminals in the close embrace of midget ganglion cell dendrites does show that many midget ganglion cells are directly connected to only one bipolar cell. Such a unit, it must be repeated, would not be isolated from other retinal cells, because there are lateral connexions to other cells through the amacrines.

Diffuse ganglion cells (figures 84, 85, plate 43).

Diffuse ganglion cells have been described in all other vertebrates that have been examined (Cajal). Figure 81 *a* shows their general form in primates as described by Polyak who called them shrub ganglion cells.

The diffuse ganglion cells we have observed in the central area have perikarya between about 8 and 16 μm in diameter, and dendritic diameters between 30 and 75 μm. Those nearer the fovea had smaller dendritic fields; those further away had larger. Figures 84 and 85, plate 43, show the general form of the branching of the dendrites. Figure 85

shows particularly well, as Polyak described, how from quite thick dendrites branches ascend and descend in a seemingly erratic manner towards or away from the inner nuclear layer. The branching tends to occur in or near the vertical plane. The finer tips of the dendrites may branch into a small bunch of fine terminals. Polyak considered that these cells could be postsynaptic to all his varieties of bipolars.

Giant ganglion cells

Polyak described another kind of ganglion cell which he called a giant ganglion cell and which in the present scheme of description might be regarded as a large diffuse ganglion cell or, on the same arguments as for the multistratified amacrine cell (p. 155), a multistratified ganglion cell. One of Polyak's examples is represented in figure 82. The perikarya are large and the branches go 'for long distances in all directions' of the inner plexiform layer. From his figure it can be estimated that the diameter of dendritic spread

FIGURE 82. Giant ganglion cell from just outside the central area. (redrawn from figure 70*E* of Polyak). Close examination of Polyak's drawing suggests that this cell might have its dendrites more in a plane of the inner plexiform layer than its present appearance suggests (cf. figure 83*b*). It would then be a unistratified ganglion cell represented by him in an oblique view.

is about 250 to 300 μm. This diameter is comparable to that of the unistratified ganglion cells (p. 163), so presumably the term 'giant' referred to the perikaryon diameter which seems to be about 30 μm. He regarded the giant ganglion cell as a cell especially characteristic of the area of the retina surrounding the central area. We have not observed such a ganglion cell and so have nothing to add to Polyak's description.

Stratified diffuse ganglion cells (figures 88, 89, plate 43; figures 90, 92 and 93, plate 44).

Like the stratified diffuse amacrine cells, stratified diffuse ganglion cells do not fall neatly into the classification of stratified and diffuse cells. They are, however, very commonly stained. They usually have a single dendrite about 3 to 4 μm in diameter which goes straight to one of three levels in the inner plexiform layer and there branches freely and frequently within about a third of that layer. The most commonly stained cells of this kind in our material are those with their branching in the third of the inner plexiform layer nearest the inner nuclear layer. We have seen some with dendrites branching in the middle third of the inner plexiform layer, and Polyak describes some branching in the third of that layer nearest the ganglion cell perikarya. These branches give a characteristic flat-topped appearance to the cell which led Polyak to call them parasol ganglion cells

(figure 81 *b*). Like the stratified diffuse amacrine cells the plane of branching of the dendrites tends to be in or near the vertical plane (see p. 151). Near the fovea the diameter of the field formed by the branching is about 40 μm (figures 92, 93, plate 44). Towards the periphery of the central area, cells with twice that diameter may be found (figure 88, plate 43). These two cells show how the dendrites of such ganglion cells may overlap and perhaps, therefore, share bipolar and amacrine cells in common. That illustration also shows that towards the edge of the central area these cells may have more than one dendrite coming from the perikaryon to make the terminal arborisation. The cell in figure 89, plate 43, is from the periphery of the retina and since its dendritic diameter is about 40 μm suggests, by comparison with others, that there is not necessarily a regular progression of increasing dendritic diameters from the centre of the retina to the periphery (see, however, details in the figure legend). Polyak apparently supposed these ganglion cells to synapse with any kind of cone bipolar but not with rod bipolars. Ganglion cells of this general form have been described in most other vertebrates (Cajal).

Unistratified ganglion cells (figure 86, plate 43; figures 90 to 93, plate 44).

Polyak described two kinds of ganglion cells which clearly can be classified as stratified ganglion cells. He regarded both of them as synapsing with all his kinds of bipolars. The two kinds of cells are shown in figure 83.

Description of plate 44

Unistratified ganglion cells and displaced ganglion cells in vertical and oblique sections. All stained Golgi–Colonnier; all rhesus macaque except figure 94 which is human. Magnification $\times 800$.

Figure 90. Unistratified ganglion cell probably of the kind Polyak called a garland ganglion cell. Above it are the processes of a diffuse stratified ganglion cell as in figure 88, plate 43. Dimensions of the former: perikaryon 15 μm $\times$ 15 μm; dendritic diameter 200 μm; the latter: perikaryon, 15 μm $\times$ 15 μm; dendritic diameter, 100 μm.

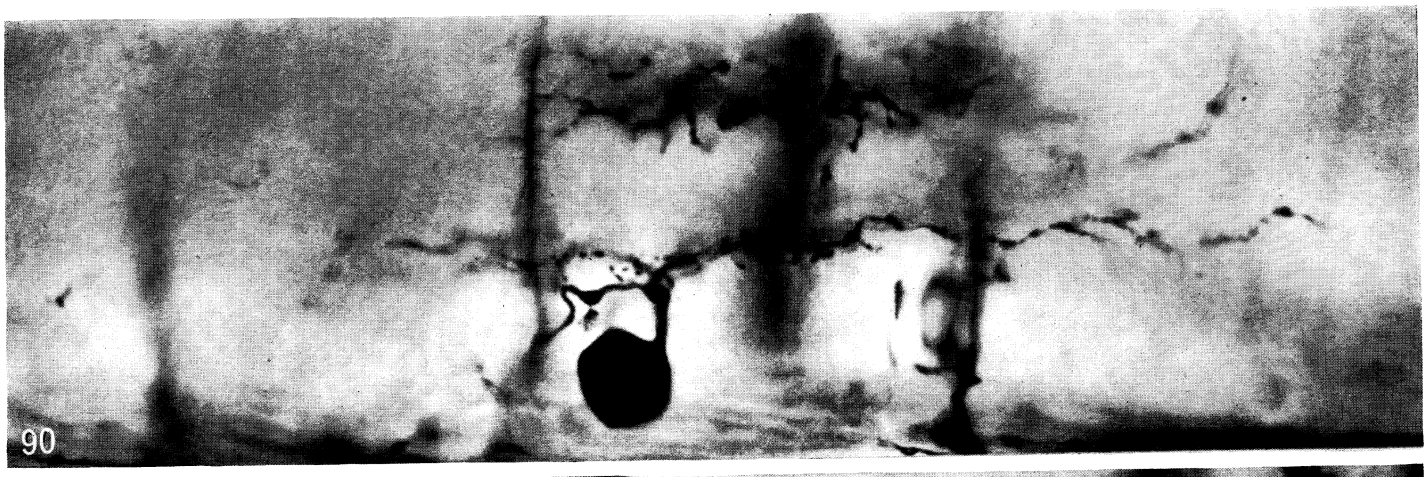
Figure 91. Unistratified ganglion cell close to the foveal slope. To the left, out of focus, is a midget ganglion cell (*m*). To the right and very near one of the dendrites is the axon terminal of a rod bipolar cell (*t*). The section was oblique. The dendritic diameter of the cell was 300 μm.

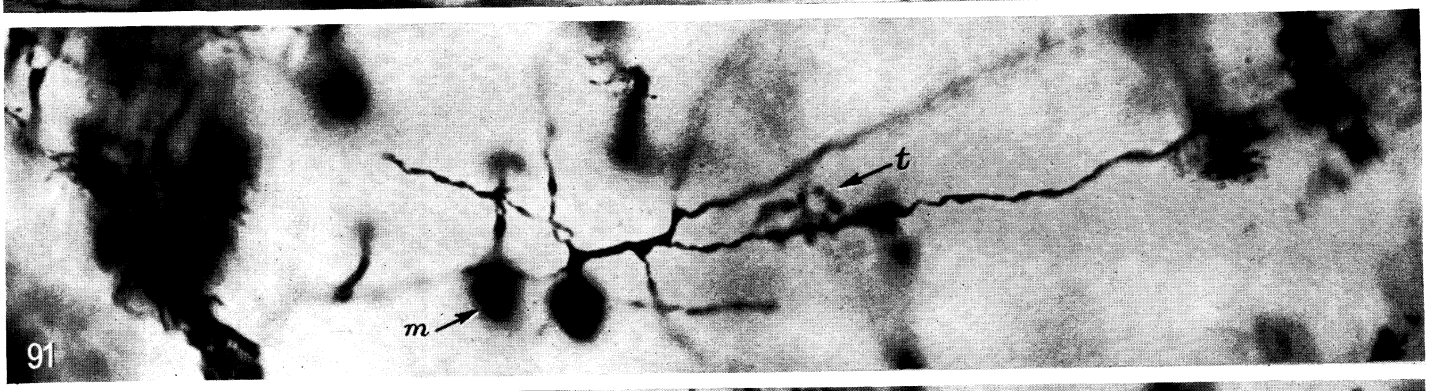
Figure 92. Horizontal section showing the tops of two stratified diffuse ganglion cells near the foveal slope and on the right the tops of two midget ganglion cells (*m*). Part of a third stratified diffuse cell is visible bottom right; all three of these cells have a dendritic diameter of 40 to 50 μm. The diameters of the midget bipolar cells' dendritic fields are 10 μm.

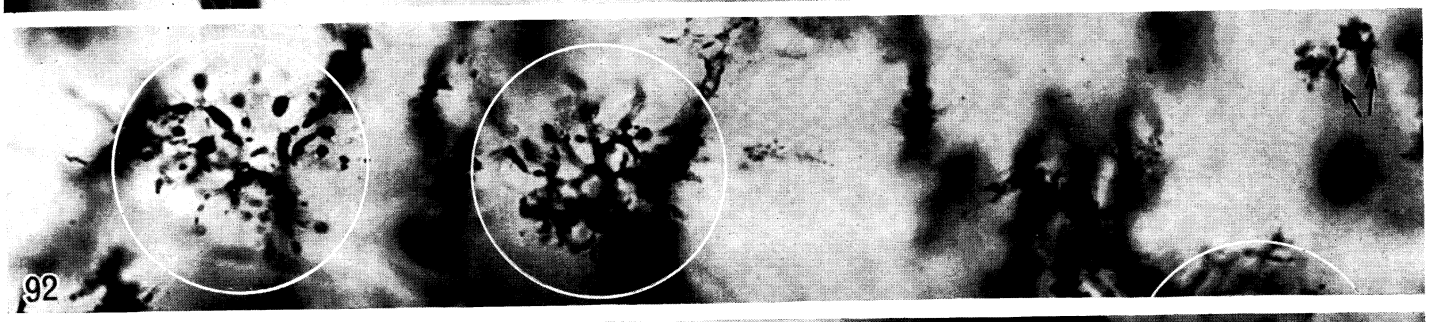
Figure 93. Horizontal section along the foveal slope showing three kinds of ganglion cells. In the centre is a unistratified ganglion cell (as in figure 86, plate 43 and figure 83) with a dendritic diameter of 200 μm. Above it is a stratified diffuse ganglion cell (as in figure 88, plate 43) with a dendritic diameter of 40 μm. Indicated by arrows is the position of the tops of midget ganglion cells which are just in focus and whose dendritic spreads are 10 μm.

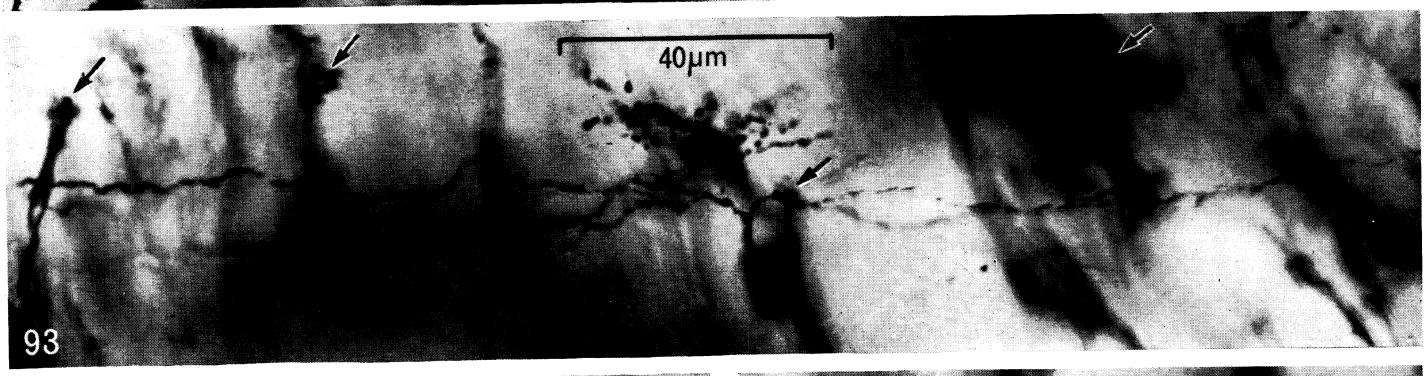
Figure 94. Vertical section showing two displaced ganglion cells from the human retina. The axons (*a*) run out into the optic nerve fibre layer and the dendritic systems of the two cells partly overlap. The diameter of dendritic spread of each was 50 μm and both perikarya were 15 $\times$ 20 μm.

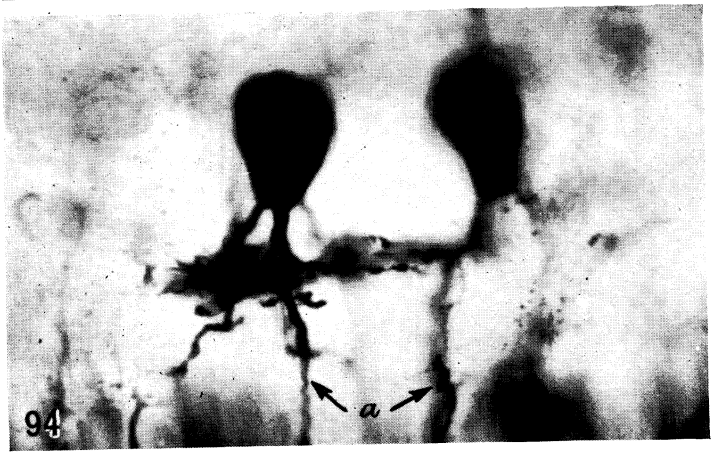
Figure 95. Vertical section showing a displaced midget ganglion cell from rhesus macaque retina. The arrow points to the axon (*a*).

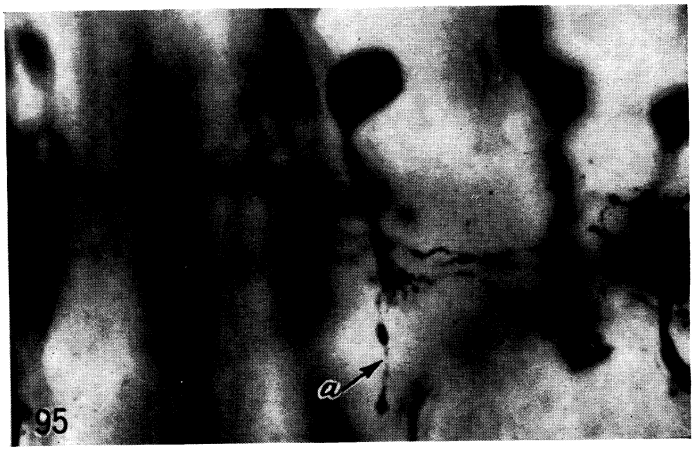












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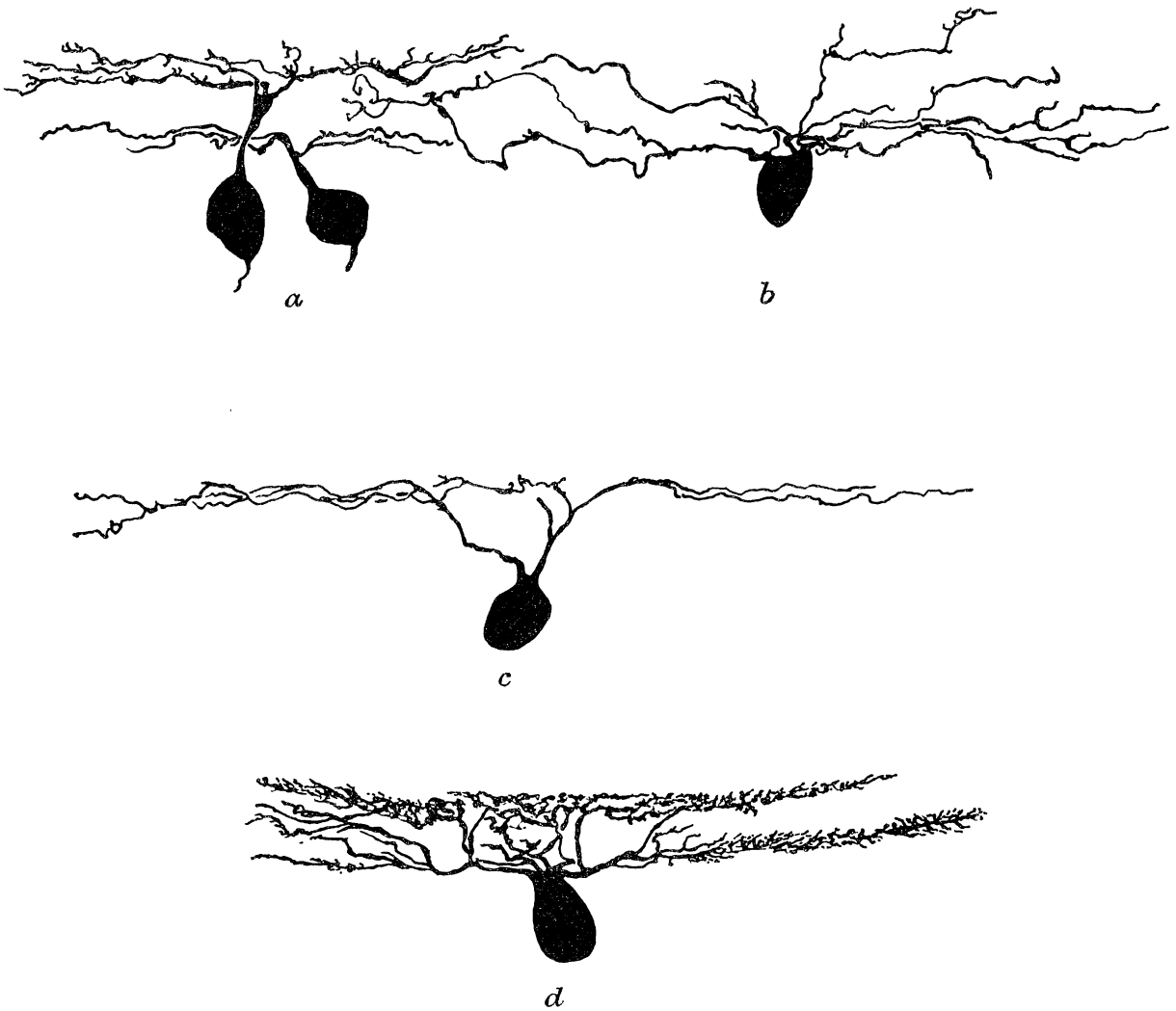

FIGURE 83. *a*, two unistratified ganglion cells ('small diffuse' Polyak); *b*, oblique view of the same kind of cell. (*a*, redrawn from figure 70*D* of Polyak and *b*, from figure 69*B*). The cells are from near the edge of the central area of rhesus macaque retina. The oblique view (*b*) shows very well how, unless very careful attention is paid to the orientation of a section, a cell can seem to have its dendrites spread through the inner plexiform layer instead of in a plane. Two stratified ('garland', Polyak) ganglion cells *c* and *d* from the periphery of the central area of rhesus macaque retina (*c*, redrawn from figure 75*B*; *d*, from figure 75*A* of Polyak). *c*, is unistratified; it is possible that *d* should be regarded as a bistratified ganglion cell. See also plates 43 and 44.

Figure 86, plate 43, shows a cell with a dendritic diameter of 200 μm. The dendrites of this particular cell are predominantly near the inner nuclear layer. It is a cell that Polyak would probably have called a small diffuse ganglion cell (figures 83*a*, *b*). Figure 90, plate 44, shows a cell that Polyak might have regarded as his garland ganglion cell (figure 83*c*). Its dendrites lie near the ganglion cell layer; in the figure the dendrites branching above it are from a stratified diffuse ganglion cell. On the whole we have found it difficult to distinguish between Polyak's small diffuse and shrub ganglion cells. Essentially the former has smooth dendrites and the latter has dendrites with numerous 'twigs and runners of considerable thickness' going in an irregular manner through quite long

distances of the upper two-thirds of the inner plexiform layer. It may be that we have not stained the two types clearly. However, from his description and our observation it is clear that, if there are two kinds, they are both unistratified ganglion cells.

Unistratified ganglion cells like these are found with dendrites at any level in the inner plexiform layer. Like the unistratified amacrine cells they do not appear to be organized so that bands of dendrites of a given type of ganglion cell are formed in the inner plexiform layer. Like the stratified amacrines, stratified ganglion cells are found all over the central area. Figure 93, plate 44, shows a unistratified ganglion cell with a dendritic diameter of 200 μm on the foveal slope and shows how ganglion cells of different dendritic diameters may be found within a small region of the retina. A more elaborate study is needed to characterize these cells. Multistratified ganglion cells are common in some vertebrates (Cajal) but none have been described for primates, so that they may be considered rare or absent. However, the cell described by Polyak (figure 83*d*) could be considered a bistratified ganglion cell.

Displaced ganglion cells (figure 7, plate 33; figures 94, 95, plate 44).

Displaced ganglion cells, or Dogiel's cells, are quite commonly found in amphibian, reptilian and avian retinae (Cajal 1911). Cajal did not describe them in mammals. Polyak has examples from all three of his primates (figure 81*c*). We have stained previously-described displaced ganglion cells only in man (figure 94, plate 44). The cell in figure 95, plate 44, from rhesus macaque appears to be a displaced midget ganglion cell.

Polyak's pictures of displaced ganglion cells in primates (figure 81*c*) and those of ours in man (figure 94, plate 44) show a large perikaryon with dendrites forming a field of about 50 μm or so in diameter, with an axon coming off a dendrite.

Displaced ganglion cells are regarded as ganglion cells because of the structure of the perikaryon (figure 7, plate 33) and because they possess a process that is presumed to be an axon since it crosses the inner plexiform layer into the optic nerve fibre layer. As Polyak points out, it is not known whether this axon goes to the brain or loops through the optic nerve fibre layer and back into the inner plexiform layer. The 'association ganglion cells' recently described by Gallego & Cruz (1965) would, from their description, appear not to be displaced ganglion cells. The number and importance of displaced ganglion cells in the functioning of the retina are entirely obscure. The name 'displaced' rather implies that they are some sort of morphogenetic accident and this may well be true for the displaced midget ganglion cell of figure 95, plate 44. However, displaced ganglion cells have been described from retinae of every class of vertebrates except possibly fish (Cajal). Whatever their morphological and functional significance they cannot be ignored, if only because they could possibly be a source of the spikes that have been recorded from the inner nuclear layer by Brown & Wiesel (1959).

Discussion

Vertical pathways in the primate retina

As far centrally as the inner plexiform layer the two classes of receptors of the primate retina have separate bipolar pathways. The rod spherules synapse only with rod bipolar

cells, while the cone pedicles synapse with two distinct kinds of cone bipolar cells, the midget bipolar and the diffuse cone or flat bipolar cell. A midget bipolar cell usually connects to only one cone; a flat bipolar cell to about seven cones. Each cone is connected to at least one midget bipolar and at least one, and perhaps more, flat bipolar cells.

Recent physiological evidence also suggests that rod and cone pathways in primates are exclusive until the inner plexiform layer. Records from those retinal ganglion cells in rhesus monkey that are connected with both rods and cones show that they can respond to only one type of receptor at any one time. Such a ganglion cell may give a photopic or a scotopic response but it will never give both together, even when photopic and scotopic stimuli are presented simultaneously (Gouras & Link 1966). The latency of the threshold cone responses (*ca.* 50 ms) is faster than that for the rods (*ca.* 150 ms) so that the cone response reaches the ganglion cell first and fires it, while the rod response is inhibited. Examination of the *b*-wave of the electroretinogram rather than the ganglion cell response shows that there is no suggestion of interaction or inhibition between rod and cone responses to any combination or sequence of flashes. The *b*-wave of the electroretinogram is apparently a summation of the activity of cells in the inner nuclear layer, probably the bipolar cells (see Gouras & Link 1966). This suggests that the inhibition of the rod response occurs between the bipolar cells and the ganglion cell and, thus, separate pathways must exist to the inner plexiform layer.

A single rod bipolar cell may be postsynaptic to between about 10 and 50 rods over an area of about 500 μm^2. Adjacent rod bipolar dendrites overlap (figure 23, plate 35) so that it is possible that a single rod could be in synaptic relationship to more than one rod bipolar. The midget bipolar cells on the other hand are, in general, in synaptic relationship with only one cone. They have from 10 to 25 or more points of contact with that cone. The number varies with the size of the cone pedicle and the number of triads it contains. The other cone bipolars, the diffuse or flat bipolars, make synaptic contact with as many as seven cones. The site of synaptic contact is on the cone base and like the midget bipolars they probably contact each cone several times (inset to figure 98). The fields of midget bipolars obviously cannot overlap with each other. Those of flat bipolars do so on the foveal slope; but we cannot yet say if they do so elsewhere in the retina. They do, of course, overlap the midget bipolar cells. Nor can we say whether any one cone synapses with more than one flat bipolar.

Thus rods and cones are connected so that they converge onto their respective bipolars. The cone connexions differ from those of the rods in that there is, in addition, an exclusive pathway for each cone as far as the inner plexiform layer. It is not clear why the cones should have two pathways. Certainly the midget bipolar cells must be colour-coded because a single cone contains only one of three cone pigments present in the primate retina (Marks, Dobelle & MacNichol 1964; Marks 1965; Brown & Wald 1964). The midget bipolar system could also provide for the high visual resolution of the cone system particularly near the fovea. The cone pathway through the flat bipolar cells might well, therefore, be supposed to provide for a brightness or luminosity function.

For the reasons discussed on p. 156 it has not been determined with certainty what types of bipolar cells end on what types of ganglion cells. Experimentally it has been shown that both rods and cones can influence one and the same ganglion cell, so that it is likely

that both rod bipolar and flat bipolar axon terminals connect with the same ganglion cells (Gouras 1966, 1967; Gouras & Link 1966), but there are also ganglion cells that respond exclusively to cone stimulation (Wiesel & Hubel 1966). Occasional stained pairs show the midget ganglion cell dendrites tightly embracing a midget bipolar cell terminal (figure 77, plate 42) and thus many, perhaps all, of the midget bipolars synapse only with midget ganglion cells. In other instances (figure 78, plate 42) it looks as if the midget bipolar terminal, while predominantly in contact with the dendrites of one midget ganglion cell, could nonetheless have some contacts with the dendrites of other midget ganglion cells, or ganglion cells of a different kind, or even both. It is not established whether any other ganglion cells sometimes synapse exclusively with the axons of midget biopolar cells or flat bipolar cells, or whether midget ganglion cells may synapse with bipolar cell axons other than those of midget bipolars. It is hard to see, at present, how definitive statements could be obtained anatomically. What is clear, however, is that the primary direct vertical input into a midget ganglion cell is often from just one midget bipolar cell and this is likely to be the most common arrangement.

Such anatomical conclusions are supported by experiments which show that in the central portion of the primate retina there are many cells with receptive field centres that are very small (Hubel & Wiesel 1960; Wiesel & Hubel 1966). The smallest receptive field centres may have a size of the order of magnitude of a single cone. Previously we have presented arguments (Dowling & Boycott 1966) that the centres of the concentric receptive fields of retinal ganglion cells are mediated by the direct vertical pathways of the retina, while the peripheral part of the receptive field is mediated through the amacrine cells.* Thus the smallness of the receptive field centres in the central retina of primates appears to correspond to the 'private' pathways of the central cones.

Amacrine cells *Lateral pathways in the primate retina*

The interneurons of the retina (the amacrine and horizontal cells) must provide the anatomical basis for an explanation of such important retinal phenomena as receptive field organization, adaptation pools, Mach bands, etc. Of the two types of interneurons it is easier to suggest roles for the amacrine cells such as those mentioned above. In the primate retina, electron microscopy shows that the synapses between the bipolar terminals and the amacrine cell processes are among the most numerous of the synapses in the inner plexiform layer. The amacrine cell processes are both pre- and postsynaptic to the bipolar cell axon terminals and thus there is a reciprocal relationship that allows for the possibility of feedback between the amacrines and the bipolar terminal. The amacrine cells also synapse with each other and with the ganglion cell dendrites and some of the ganglion cell somata. In Golgi preparations amacrine cells can be classed into diffuse and stratified categories. Plates 39 to 42 and figures 52 to 54 and 96 to 98 summarize what is known about the shapes of primate amacrine cells. Aside from the details of their synaptic contacts within the inner plexiform layer we are almost entirely ignorant as to the

* Recently Rodieck (1967) has independently and for different reasons reached similar conclusions in the cat.

spatial relationships of these cells with the other cells of the retina and with each other, since their sites of synaptic contact cannot be identified by light microscope methods.

The Golgi method sometimes shows that there is extensive overlapping of the fields of amacrine cells of the same kind (e.g. figure 64, plate 40; figure 71, plate 41). Figure 96 also gives an impression of how the fields of different kinds of amacrine cells overlap. Any one kind of amacrine cell we have described may be found in any part of the central area of the retina, but the Golgi method does not reveal what proportions of the particular kinds are represented in a given volume of retina. However, it is our impression that in the primates here investigated the narrow-field diffuse amacrine cells are the most numerous and the long-ranging large amacrine cells the least numerous. And this is confirmed in thin sections such as those of figures 5 and 6, plate 33. It is also our impression that any one part of the retina has a proportion of all the kinds of amacrine cells or their processes that are to be found in the retina as a whole and that the relative proportions of the different elements are much the same, at least all over the central area. There is certainly no vertical segment of the inner plexiform layer where there are only diffuse amacrines or stratified amacrines, or only samples of particular kinds of these. That is not to say that there are no differences at all in the relative proportions of the processes of the different types of amacrine from the fovea outwards across the retina. It is to say, that if they occur they are not obvious with present methods of observation. The most likely possible differences in proportions of different amacrine cell types would be if there were particular amacrines associated with the rod or the cone systems. The relative proportions of these cells changed from the fovea outwards, yet we have found no evidence to suggest differences in amacrine cell types between the foveal slope and the parts of the retina where rod bipolar terminals are found. Indeed it might be argued that since there are no exclusively rod-responding ganglion cells (Gouras & Link 1966; Wiesel & Hubel 1966) this should not be expected. As with the amacrines, significant differences in the ganglion cell types have not been seen from the centre of the fovea outwards across the central area of the retina, although there are, of course, a greater proportion of midget ganglion cells nearer the fovea than away from it because of the greater cone density.

The density of branching of the narrow-field diffuse amacrine cells is greater than that of the wide-field diffuse amacrines and the stratified amacrines. The smaller stratified amacrines apparently branch more than the larger stratified amacrines (figure 96). Although the distribution of the sites of synaptic contact on amacrine cell processes is unknown, it seems likely that the frequency of branching is correlated with the frequency of synaptic sites. With this assumption it may be supposed that the small diffuse amacrine cells make more synaptic contacts per unit area of retina than the larger amacrines. The largest amacrines may make as many or more synaptic contacts spread over a wider area; we do not know; but they presumably have fewer synaptic contacts per unit area of retina.

It is tempting to suppose that the stratified amacrine cells, i.e. those with the greater lateral extents, are the ones that, together with their amacrine-to-amacrine contacts, are responsible for the organization of the peripheral opponent fields (Dowling & Boycott 1966). They might also be supposed to link sets of bipolars and ganglion cells. The small diffuse amacrine cells could be supposed to be mostly concerned with contributing to the

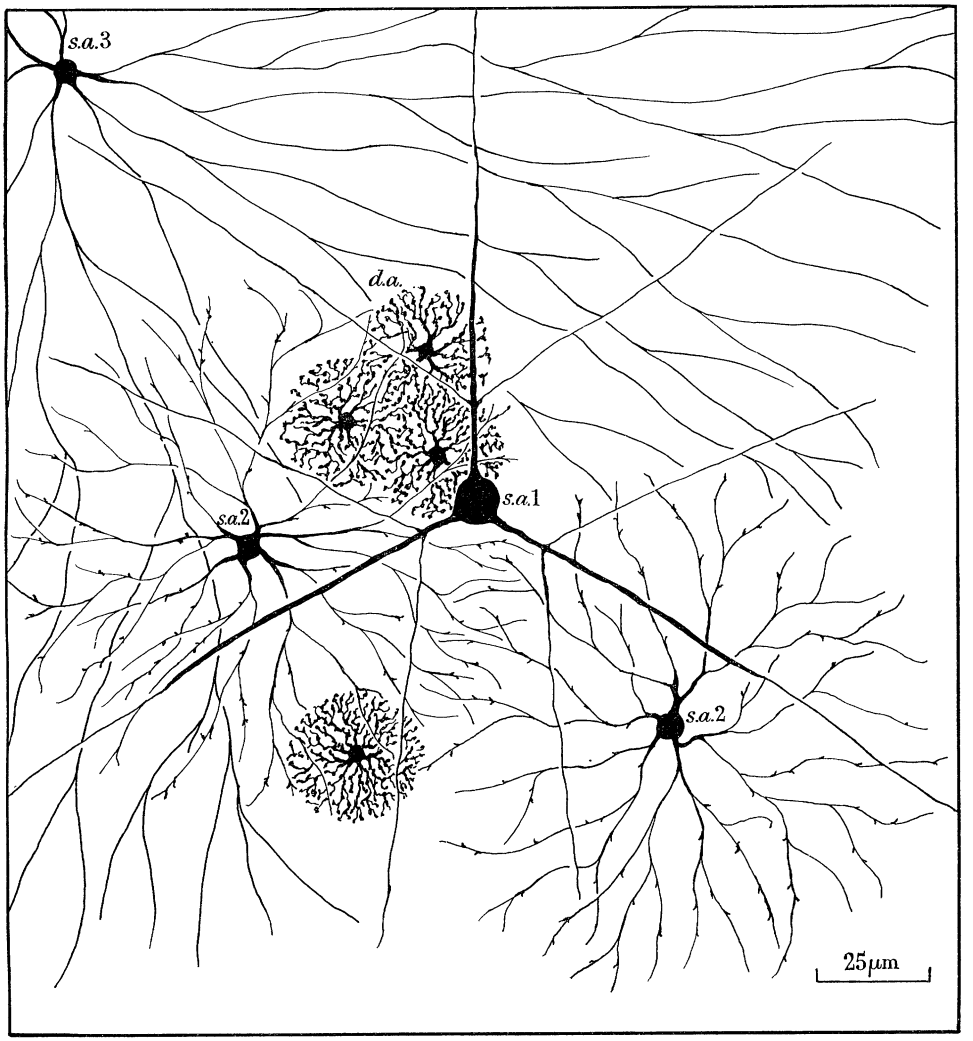

FIGURE 96. Diagram of amacrine cells from the central area of the rhesus macaque retina as they might be observed in a flattened piece of retina with the inner nuclear layer uppermost. The unistratified amacrine *s.a.*1 is the largest kind of amacrine and is illustrated in figure 69, plate 41. The unistratified amacrines *s.a.* 2 are of the kind shown in figures 70 and 71, plate 41 and figures 63 and 64, plate 40. The unistratified amacrine *s.a.*3 is of the kind shown in figure 62, plate 40. The diffuse amacrines *d.a.* are illustrated in figures 56, 58 and 60, plate 39. None of the other amacrine types are shown. The purpose of the diagram is to show something of the relative extents of the dendritic fields of the three most commonly stained types of amacrine cell and to try to convey visually their possible spatial relationships. Analysis of cell types and field sizes in Golgi-stained material does not give much information as to the relationships of populations of cells of the same or different kinds. At present in vertebrate retinae the relative proportions and distribution of the different morphological kinds of cells are unknown. The area of the retina in the rectangle of this diagram is about 46500 μm^2. Assuming the diffuse amacrines (*d.a.*) are evenly spaced, as illustrated by the group of three, then, if they were all drawn, there would be between 60 and 70 cells of this kind within the area of the diagram. There would be about six to eight unistratified amacrines (*s.a.*2). The distances between the perikarya of the unistratified cells (*s.a.*2) are estimated from figure 64, plate 40, figure 71, plate 41, and the sort of appearance obtained in figure 5, plate 33. The result is a distance between perikarya of about 75 μm. The

(*continued on facing page*)

dyads and forming the reciprocal contacts onto the bipolar terminals. In other words, perhaps the diffuse amacrine cells could be concerned particularly with local interactions such as the neural adaptation that has been suggested to be mediated by the reciprocal contacts (Dowling & Boycott 1966; Dowling 1967). In frogs and birds narrow-field diffuse amacrine cells are numerous and have many very fine branches (Cajal 1911; B. B. Boycott, unpublished). It is likely that they provide many, if not most, of the processes responsible for the large numbers of serial synapses that are to be found per unit area in these retinae (Kidd 1962; Dowling 1968). These serial synapses are particularly interpolated between the bipolar terminals and the ganglion cell dendrites, and their presence is correlated with the greater complexity of the physiological responses of ganglion cell units of frog and bird retinae (Dowling 1968).

We have shown that there are synapses between amacrine cells (Dowling & Boycott 1966). Presumably these can be between amacrine cells of the same type. A subjective estimate of this can be obtained from the unistratified amacrine cells of the pigeon retina shown in figure 75, plate 42, and figure 72, plate 41. Figure 74, plate 42, also suggests that adjacent diffuse amacrines may well synapse together. Whatever the functional difference between the two classes of amacrine cells, it must be that the two kinds are interconnected. This might well occur directly between the two kinds of cell. However, one of the major difficulties of understanding retinal structure is to imagine why there are so many shapes of amacrine and ganglion cells. Thus, as a matter of pure guesswork, it may further be supposed that cell types such as the bistratified amacrine cells and the wide-field diffuse amacrines are the shape they are because their rôle is to integrate stratified and diffuse amacrine cell systems. It is difficult to imagine at the moment how any of this can become better than speculation. Perhaps a way to begin would be through a careful comparison of the shapes and distribution of the cells in physiologically simply organized retinae such as

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two cells drawn here are about 100 μm apart, so there could be another cell of the same type in between. The distances between the diffuse amacrines (*d.a.*) are estimated from pairs of stained cells such as figure 74, plate 42, and are roughly 25 μm. The diameter of spread of the processes is given as about 25 μm for the diffuse amacrines and 125 μm for the unistratified amacrines.

There are in the primate retina, however, unistratified amacrine cells *s.a.*3, resembling those of the *s.a.*2 type, but the diameter of spread of their processes is about 400 to 500 μm. A cell of this kind is illustrated in figure 62, plate 40; a quadrant of only one is shown here. It is clear from this diagram that there is a whole range of overlapping of the processes of the different kinds of cells. And that to be complete the diagram should have included processes from cells with perikarya outside the field. To have drawn these and all the cells in the fields would have made the diagram impossibly complex.

The largest amacrine cell in the monkey retina is represented by *s.a.*1. This cell appears in figure 69, plate 41, as asymmetrical and we have certainly not observed the full extent of these cells. However, allowing for the distortion of the retina in sectioning and flattening it, it is apparent that it could have the symmetry shown here. This symmetry is maintained by the fact that the finer branches come off the main process at an angle of about 55°. As figure 69 shows, the finer branches probably have further branches coming off at much the same angle. They have not been shown here because they have not been regularly stained.

those of primates, and the more complexly responding retinae of frogs and birds. Ability to identify under the electron microscope different ultrastructural features for the processes of different amacrine cell types would be a most important asset. A beginning becomes conceivable with the observation in the frog retina that there are some amacrine cells with granular and others with agranular synaptic vesicles (Dowling 1968) which may be correlated with the results of fluorescence microscopy (Ehinger 1966; Laties & Jacobowitz 1966).

From considerations of rod-cone interactions (p. 164) and the known colour-coding of the retina, it is clear that there is segregation of bipolar pathways as far as the ganglion cells. It is, therefore, possible that there are groupings of amacrine cells that are relatively independent of one another. This is clearly suggested in a pigeon retina by the four or five different layers of unistratified amacrine cells (figure 75, plate 42). Yet, we have not been able to recognize amacrines that we can imagine as more likely to be associated with rod bipolar cells than with cone bipolars and vice versa. Nor can we recognize features of amacrine cells that could suggest that there may be colour-coded amacrine cells anatomically distinguishable from non-colour-coded amacrines. Something of this kind would appear to be necessary for the integration of colour-coded information into known opponent fields (De Valois 1965; Wiesel & Hubel 1966).

Horizontal cells

Roles for the horizontal cells of the primate retina are more difficult to suggest. It is now likely that there are not more than two kinds of horizontal cell in the primate retina. The evidence from light microscopy that the dendrites of the type A cells form the lateral elements of the triads of the cone pedicles is reasonably good. Since the known physiology (Gouras 1966; see also page 165) suggests that the rod and cone pathways are independent as far as the inner plexiform layer, it follows that the type B cells may synapse with the rod spherules. In the goldfish Stell (1967) has shown that external horizontal cells contact cones, while the intermediate horizontal cells contact only rods. It is not known whether the two or more types of horizontal cells (inner and outer) in mammals are thus specifically connected. (By using the methods described in the Appendix, page 177, the prediction of the position of the dendrites of the type A horizontal cells has so far been confirmed, but the type B cells have not been shown to go to rods. However, in the summary, figure 98 page 174, the two kinds of cells have been represented on the hypothesis that one type (A) is connected exclusively to cones and the other type (B) exclusively to rods.) By electron microscopy it has been observed that the processes of horizontal cells end as lateral elements in the cone triads and possibly also in a similar position in the rod spherules (insets to figure 98). With the type A horizontal cells it has been possible to show that there are about seven groupings of dendritic terminals thus representing the innervation of seven cones. Some type A horizontal cells may perhaps go to six cones, some to nine, but where the horizontal cells are clearly isolated and unequivocally observable, seven is the number; a number that is about the same as the number of cones contacted by one flat bipolar cell. It is also clear that any one cone is in contact certainly with two and possibly with as many as four horizontal cells. It is doubtful if more than four horizontal cells contact a cone, because there is not room for them to do so. From the variation in

number of terminals on one 'A type' cell (table 3, p. 136) it is possible to imagine that the number of horizontal cells contacting different cones is variable. Figure 97 shows schematically the possible relationship of four type A horizontal cells to one cone. In this figure the cells are arranged symmetrically so that their axons extend in directions at right angles to each other. It is difficult to see why in the outer plexiform layer of a primate retina there is such an asymmetrical relationship; an asymmetry that is as true for the B type as the A type horizontal cells. No such asymmetry is found in the processes of the amacrine cells of the primate inner plexiform layer, although occasional apparently asymmetrical amacrine cells have been described in birds by Cajal. The axons of both kinds of horizontal cell run in the outer plexiform layer at some distance from the terminals of the receptors. They are often to be observed between the primary branches of the apical

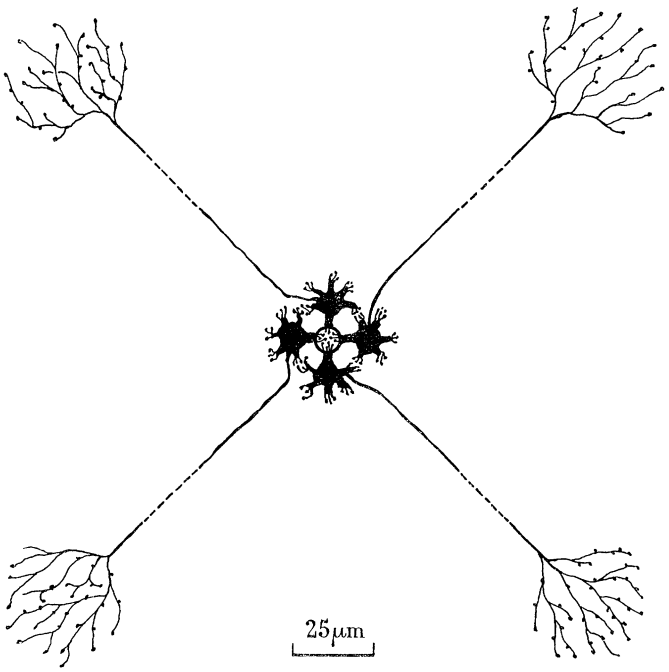

FIGURE 97. Diagram to show the possible relationship of one cone of 8 μm in diameter (circle in the centre of the field) to four type A horizontal cells. The axons are represented on the assumption, for which there is no evidence, that they leave the cells in directions at right angles to each other. In this way the changes occurring in one cone might be communicated symmetrically across the retina. The dotted lines show that it is unknown how far the axons run. The field of branching of the type A axon terminals is also unknown (see figure 32*B*). It is also an assumption that if the length of the horizontal cell axons were known they would be the same.

dendrites of the rod bipolar and flat bipolar cells (figure 98). In addition, when the axon terminals are examined they are observed to rise towards the receptors from the horizontal cell axon, see figures 48 to 51, plate 38. There are, therefore, no synapses to the photoreceptors along the length of the axon and none can be observed by electron microscopy within the outer plexiform layer. Thus it is unlikely that information can be received from, or sent to, the receptors which a horizontal cell axon passes. No direct data exist as to the lengths of horizontal cell axons of either type, although it seems that they may often be at

least as long as a millimetre. Considering the spreads of the proximal and distal processes of both kinds of horizontal cells it would seem that there are wide areas between the axon terminal expansions and the perikaryon of a horizontal cell that are not in functional contact with other cells. The anatomical evidence, therefore, is that primate horizontal cells do not form relationships that could provide a functionally continuous lateral network in the outer plexiform layer. If horizontal cells contacted one another as amacrine cells do, then this would be less of a difficulty. But no horizontal cell to horizontal cell contacts have been identified in a primate. Yet, if these observations are true, it is most difficult to imagine what function there might be for lateral interaction between restricted areas of the retina that are widely spaced apart. Speculation is not helped by the fact that we do not know the area over which the axon terminals of a single cell spread. In figure 97 the axons have been represented so that they do not overlap. Yet for all we know the axons could branch so that they do overlap. It is reasonably certain that different pieces of axon may innervate the same area (see figure 32*B*).

In cat and rabbit retinae, horizontal cell to horizontal cell synaptic contacts have been seen, but in both of these retinae many of the horizontal cells are distinctively different from those of primates (Dowling *et al.* 1966). They are large cells, with numerous thick and fine processes that have a lateral extent of between 300 and 500 μm. It is said that these cells do not have an axon (Leicester & Stone 1967; Gallego 1965). Their processes form an extensive network over the entire field subsumed by the spread of the main branches. Thus they appear capable of innervating any of the receptors in the area over the cell. With their horizontal cell to horizontal cell contacts it is possible to envisage in these animals a continuous network mediating lateral interaction across the entire outer plexiform layer.

Since the monkey, compared with the cat, has an apparently distinctly different horizontal cell arrangement it might be supposed that the physiology of the retinae would be very different in the two species. The majority of work carried out so far on receptive fields of the ganglion cells suggests that the organization is basically similar in the cat and the monkey (Rodieck & Stone 1965; Wiesel & Hubel 1966). The majority of ganglion cells of both species show a similar centre-surround antagonistic arrangement. The one major difference that has been found is that monkey ganglion cells are often colour-coded and cat ganglion cells are not. It is, then, conceivable that the differences between the horizontal cells of primates and other mammals might be related to differences in distribution of colour vision. A further possibility, of course, is that monkey horizontal cells do form a continuous lateral network in the outer plexiform layer but that it has not yet been recognized.

Stratification in the inner plexiform layer of vertebrates and the localization of synapses

Observation by light- and phase-contrast microscopy of the inner plexiform layer of vertebrates reveals some species with little or no stratification in that layer. Man (plates 32 and 33) and the rhesus monkey are examples; the cat and the white rat are others (Brown & Major 1966; Brown 1965). Frogs, pigeons and chickens have several distinctive strata in the inner plexiform layer that are easily observed by these methods (Cajal 1892, 1911; Brown & Major 1966). The former group have relatively simple ganglion cell receptive field responses

(see above, and Brown 1965), the latter group have complex ganglion cell responses (see Lettvin *et al.* (1959), Maturana *et al.* (1960) for frogs; Maturana (1962, 1964) and Maturana & Frenk (1963) for birds). In their 1960 paper and further in 1961, Lettvin *et al.* correlated such distinctive stratification with the presence of ganglion cells whose dendrites are varied in their morphology and branch so that they include uni-, bi- and multi-stratified ganglion cells. As Brown (1965) and Brown & Major (1966) point out, those retinae with simpler physiological responses do not have multistratified ganglion cells as defined by Cajal.

In the rabbit, however, such a correlation between structure and function breaks down. Barlow & Hill (1963), Barlow, Hill & Levick (1964), Barlow & Levick (1965) showed that there are cells in the retinae of rabbits that are selective for the direction of movement of targets and for the velocity of targets in their receptive fields. Levick (1965, 1967), within the visual streak of the rabbit's retina, has found 'orientation-selective' cells, 'local-edge-detectors' and 'uniformity-detectors', thus giving the rabbit retina, to date, a total of eight classes of ganglion cells with specific responses. So that 'in the diversity of function represented by the optic nerve output, the rabbit even exceeds the extravagance described for the frog' (Levick 1967). Yet, as Brown & Major (1966) point out, and preliminary observations of our own (B.B.B.) suggest, multistratified ganglion cells are absent from the rabbit retina and no stratification of the inner plexiform layer is obvious.

It is difficult to know whether the shapes of cells determine their connectivity and thus their function (Maturana *et al.* 1960) or whether the necessary connectivity determines their shape. As discussed in the introduction (p. 113) it is hard to decide what the significant parameters of nerve cell shape may be. It is clear, however, that in a search for working hypotheses it is the definition of the working units and the principles of their connexions rather than the shapes they adopt to achieve those connexions that are at present more likely to be important for understanding function. Dowling (1968) has shown a significant difference in the inner plexiform layer between the frog and the bird, on the one hand, and the cat and two primates on the other. The former have a much greater proportion of synapses between amacrine cells and these are often interposed as serial synapses between the bipolar terminals and the ganglion cell dendrites. Preliminary examination of rabbits has shown that they too have more of such synapses per unit area than in the physiologically simpler retinae of the cat and the monkey (M. Dubin & J. E. Dowling, in preparation). At present this would seem to be a more significant correlation between structure and function than the shapes of the cells concerned.

Thus the shapes of the cells, and in consequence the extent to which their processes are organized into strata in the inner plexiform layer, may reveal only that in those retinae with very large numbers of synapses per unit area groupings of classes and functional units of cells are packed on particular planes. For example, although exact measurements have not been made, in the layer of bird unistratified amacrine cell processes of figure 72, plate 41, the diameter of spread of the processes is quite different from other unistratified amacrine cell layers (figure 75, plate 42) and each group of stratified amacrines differs from the other in this respect. Their shape and position, as such, may be irrelevant to their mode of functioning; the layering and sizes may represent only grouping of particular sets of unistratified amacrines in relation to particular functional units.

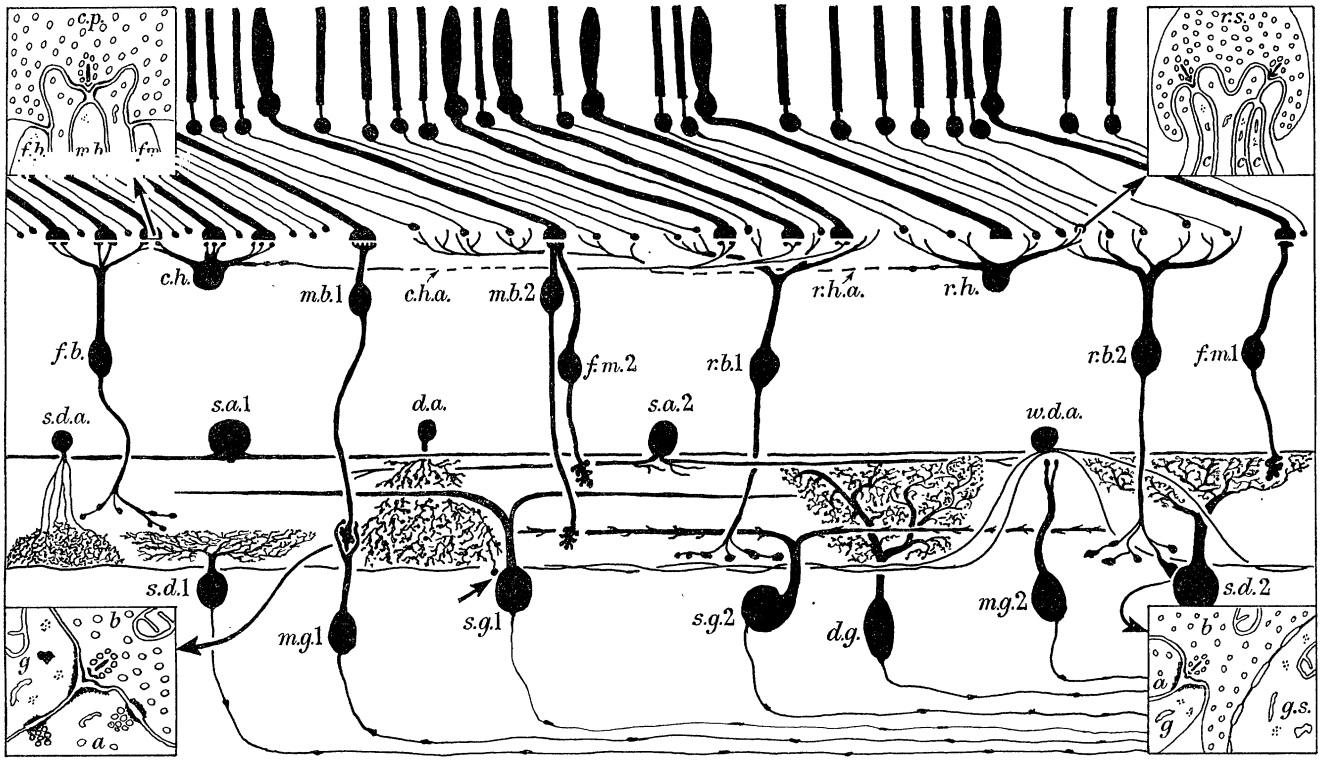

FIGURE 98. Diagram to show the main kinds of nerve cell identified in the rhesus monkey and human retina. It is of a vertical section about 2 mm from the centre of the fovea of a rhesus monkey. The lateral extent of the drawing is about 450 μm and all the cell types illustrated would probably be present in such a piece of retina. The diagram does not represent the proportions in which they would be found; for the most part these are unknown. However, for example, it is likely that each cone pedicle makes synaptic contact with at least one midget bipolar cell (*m.b.*1) and there would also be many more diffuse amacrine cells (*d.a.*) than shown here (see legend to figure 96). For reasons of clarity some distortion of the cells and the proportions of the layers has been made. Therefore, for example, the outer nuclear layer is thinner and the outer plexiform layer thicker than is natural. The layers and dimensions of the retina are given in figure 1, plate 32, and table 1. All the cells are diagrammatic representations of what might be observed in a Golgi preparation, therefore no outer segments are drawn on the receptors since these very rarely stain with Golgi procedures. For clarity the connexions of the nerve cells with the cone pedicles and the rod spherules are separated and no interreceptor contacts are shown. The inset *top left* is a diagram of an electron micrograph to show how the cones (*c.p.*) contact their nerve cells. The inset *top right* shows how nerve cell processes invaginate into a rod spherule (*r.s.*) These two diagrams and the two below also show that in any representation of the light microscopy of the retina from Golgi material the connexions of the cells cannot be represented precisely since they are too small to be resolved. The inset *lower left* represents a dyad in which a bipolar cell *b* is presynaptic to a ganglion cell dendrite *g* and an amacrine cell process *a*. The amacrine cell process has a reciprocal contact back on to the bipolar terminal and an amacrine to ganglion cell dendrite synapse. The inset *lower right* shows that the rod bipolar cell axosomatic contacts may consist of 'close contacts' on to the ganglion cell perikaryon (*g.s.*) (though which types of ganglion cell is unknown). This inset also shows that these same bipolar terminals have dyad contacts. There are many such dyad contacts around each of the axon terminals of all the kinds of bipolar cell. The top right inset is redrawn from Missotten (1965), the other three from Dowling & Boycott (1966); further synapses as revealed by electron microscopy are summarized in the text.

(*continued on facing page*)

The cell *c.h.* is a TYPE A HORIZONTAL CELL drawn to show that such cells go to more than one cone (figure 32*A*, p. 134). The type A horizontal cell axon (*c.h.a.*) extends for an unknown distance (hence broken line) perhaps to synapse with a number of cones (figure 32*B*, p. 135). The cell *r.h.* is a type B horizontal cell that is represented as postsynaptic to a large number of rod spherules, although the evidence for this is less certain than for the connexions of the type A cells (see page 137). The axon of a type B horizontal cell is shown entering rod spherules *r.h.a.* an unknown distance away. There is one axon to each type of horizontal cell. The FLAT BIPOLAR CELL (*f.b.*) is shown as in synaptic relationship with more than one cone. It is concluded (p. 128) that such cells usually synapse with seven. Their probable synaptic relationship with part of a cone pedicle is shown in the upper left inset (*f.b.*). The terminals of the flat bipolar cell axons in the inner plexiform layer resemble those of the rod bipolar cells but the swellings are not usually as large as the rod bipolar axon terminals. They often end in the middle of the inner plexiform layer and they never form axosomatic junctions. The MIDGET BIPOLAR CELL (*m.b.*1) shows that the apical dendrites of such cells have several points of contact with a single cone pedicle and the upper left inset shows that these are as invaginations into the centre of a triad. The axon terminal in the inner plexiform layer is, in this region of the retina, about 5×5 μm and is often in synaptic relationship with a midget ganglion cell. The flat midget bipolar cell (*f.m.*1) is a newly discovered cell (see p. 177) that is connected to only one cone but which has synaptic contacts only with the base of the cone pedicle. The cells *m.b.*2 and *f.m.*2 illustrate that a single cone can be in contact with both kinds of midget bipolar. It is not known, however, if all cones are connected to two midget bipolar cells (see p. 180). The ROD BIPOLAR CELLS (*r.b.*1 and *r.b.*2) have dendrites that reach up into the rod spherules. Since these are stacked upon each other the dendrites of rod bipolars and rod horizontal cells, unlike those nerve cells connected to cones, end on several levels. The dendrites of the rod bipolar cells presumably form the central elements (*c*) of the rod spherule (upper right inset), while the remaining processes are the dendrites and axons of horizontal cells. The axon terminals of a single rod bipolar vary from very small to the largest of any of the bipolar cells. The terminals end mostly in the third of the inner plexiform layer near the ganglion cell perikarya and may form an axosomatic junction which is a 'close contact' onto the ganglion cell perikaryon as shown in the bottom right inset.

The UNISTRATIFIED AMACRINE CELL (*s.a.*1) has a large perikaryon and three main processes that extend immediately under the inner nuclear layer and bear infrequent finer branches (see figure 96). Its total diameter may be as much as a millimetre and here its main processes are used to delimit the inner nuclear layer from the inner plexiform layer. The type of unistratified amacrine cell (*s.a.* 2) also has its processes in the third of the inner plexiform layer nearest the inner nuclear layer. The diameter of the lateral extent of the processes of this kind of cell is usually between 100 and 200 μm. The processes branch dichotomously on a plane and fairly frequently (figure 70, plate 41). However, for diagrammatic purposes, that, as with other unistratified amacrine and ganglion cells, has not been drawn, except at the beginning and the tips of the cell. Bistratified and multistratified amacrine cells have not been drawn here. The NARROW-FIELD DIFFUSE AMACRINE CELL (*d.a.*) has branches all through its field within the inner plexiform layer. The cell illustrated has a large number of branches but the number of processes varies rather widely in these amacrines. In addition, some have rather varicose processes, while others have smoother processes. Narrow-field diffuse amacrines are numerous throughout the retina. They may make amacrine axosomatic contacts with the ganglion cell somata as shown here on *s.g.*1. The STRATIFIED DIFFUSE AMACRINE CELL (*s.d.a.*) resembles cells of the previous type except that the branching is confined to the upper, middle or lower thirds of the inner plexiform layer. The processes tend to be smooth, but the frequency of branching is rather variable (figure 55, plate 39, and figure 67, plate 40). The WIDE-FIELD DIFFUSE AMACRINE CELL (*w.d.a.*) shows that in these cells several branches arise from the perikaryon but that they branch very little if

(continued on following page)

at all as they pass towards the ganglion cell perikarya where they branch loosely and run parallel with that layer for as much as 500 μm. Thus the processes of these cells may have a diameter of lateral spread of as much as a millimetre. In this diagram these processes are used to mark the boundary between the ganglion cell perikarya and the inner plexiform layer.

The MIDGET GANGLION CELLS are distinguished by a single dendrite which in this region of the retina is unbranched in the inner plexiform layer except at its tip where it branches to embrace the terminals of midget bipolar cells. The branching of midget ganglion cells tends to be either in the lower (*m.g.* 1) or in the upper (*m.g.* 2) third of the inner plexiform layer. Midget bipolars with dendrites invaginating into the centres of triads (*m.b.* 1 and *m.b.* 2) probably synapse with midget ganglion cell dendrites in the layer where *m.g.* 1 ends, while those midget bipolar cells with flat contacts on the cone pedicles (*f.m.* 1 and *f.m.* 2) synapse with midget ganglion cells such as *m.g.* 2 (for details see p. 177). Two UNISTRATIFIED GANGLION CELLS are shown here. The cell *s.g.* 1 probably corresponds to Polyak's 'small diffuse' ganglion cell (figure 83 *a*); *s.g.* 2 probably corresponds to Polyak's 'garland' ganglion cell (figure 83 *c*, *d*). The dendrites of both cells branch on a plane, although this is not represented here. The exact stratum of branching probably differs for individual cells of the same type. The dendrites of the cell *s.g.* 1 appear to be smooth, but those of *s.g.* 2 bear spines, the distribution and density of which is unknown. The DIFFUSE GANGLION CELL (*d.g.*) has dendrites which branch at all levels in the inner plexiform layer from the ganglion cell perikaryon layer to the inner nuclear layer. The dendritic distribution is analogous to that of the narrow-field diffuse amacrine cell *d.a.* STRATIFIED DIFFUSE GANGLION CELLS (*s.d.* 1 and *s.d.* 2) are commonly stained in Golgi procedures but the details of their dendritic branching are difficult to observe. The branches of an individual cell occupy only about one-third of the thickness of the inner plexiform layer in a manner analogous to that of a stratified diffuse amacrine cell *s.d.a.* The cells correspond to Polyak's 'parasol' ganglion cell (figure 81 *b*). DISPLACED GANGLION CELLS are found in the primate retina (figures 94, 95, plate 44) but have not been drawn here.

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APPENDIX

A SECOND TYPE OF MIDGET BIPOLAR CELL IN THE PRIMATE RETINA

By Helga Kolb, B. B. Boycott, and J. E. Dowling

Electron microscopy of the retina shows that neural processes contact cone pedicles in two ways. They may penetrate into the cone pedicle in invaginations or make superficial contacts on the base of the pedicle. In the primate, three processes penetrate into each invagination and this arrangement has been called a triad (Missotten 1965). The central element of the triad has been identified as being from a midget bipolar cell, while the lateral elements have been shown to be horizontal cell processes. The neural processes making superficial contacts on the pedicle base are believed to be the apical dendrites of flat bipolars (Missotten 1965; Stell 1965*b*; Dowling & Boycott 1966).

The main paper develops and correlates these findings with the light microscopy of Golgi-stained retinae. For example, in the central area of the primate retina, the number of apical processes that can be counted on a midget bipolar dendrite closely matches the number of invaginations into a cone pedicle of the central area (p. 124). This suggests that a midget bipolar extends processes to the centre of every invagination of a single pedicle, which agrees with the findings so far obtained by electron microscopy.

To further our knowledge of the contacts in the outer plexiform layer of the primate retina, one of us (H.K.) has undertaken a study of Golgi-stained retinae of rhesus monkey by electron microscopy. For this purpose both the Golgi–Colonnier (Colonnier 1964) and the Golgi rapid triple impregnation (Stell 1965*a*) methods have been used (see methods in main paper p. 112). The results to date substantially confirm the above description and will be presented in full elsewhere (H. Kolb, in preparation). However, in the course of this work a new type of midget bipolar has been found and will be described here.

Polyak (1941) was the first to recognize the midget bipolar as a neuron exclusive to one cone, and described it as characterized by a single small dendritic terminal (bouquet) that exactly fitted, in size, the base of the cone pedicle. The present study now subdivides midget bipolars into two distinct types. The apical processes of the new type of midget bipolar make superficial contacts on the base of the cone pedicle. They never penetrate into the invaginations of a cone pedicle as do the processes of the previously described bipolar. This new cell will be called the flat midget bipolar to distinguish it from the previously described type of cell, which we shall refer to as an invaginating midget bipolar.

Figure 101, plate 45, is an electron micrograph of a rhesus monkey retina fixed with osmium tetroxide and shows the configuration of the triad of processes invaginating the cone pedicle with the associated synaptic ribbon and vesicles. Processes making superficial contacts on the cone pedicle are marked by arrows. Figure 102 shows an electron micrograph of a Golgi-stained process from a midget bipolar that was identified by light microscopy as a type with processes penetrating into the cone pedicle. By comparison, figure 103 shows a quite different situation. This is an electron micrograph of the terminals of two apical processes of a flat midget bipolar and it can be clearly seen that they lie at the

side of the central process of the triad and do not enter the pedicle. They make the superficial type of contact. Figure 104 again shows flat midget contacts, and, by comparison with figure 101 above it, clearly shows that the point of contact is superficial to the cone base, identical to the arrowed processes in figure 101.

Analysis of serial sections of the invaginating and flat midget bipolar terminations on the cone pedicle reveals that the invaginating midget bipolar has an apical process to fit in to every triad as the central element, whereas the flat midget bipolar has double the number of apical processes, all of which make superficial contacts. Both types of midget bipolar contact only a single cone pedicle. In most cases the flat midget bipolar endings lie adjacent to the point of entry of the invaginating midget into the triad (figures 103 and 104), but

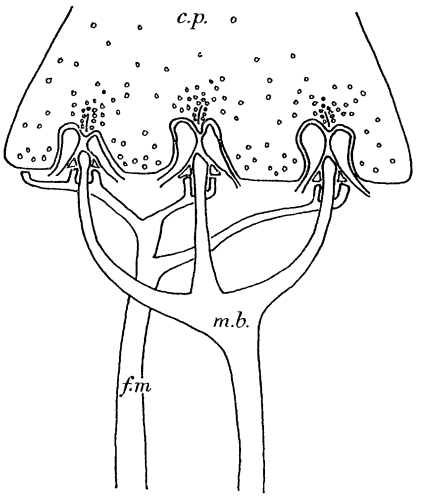


FIGURE 99. Diagram to illustrate that one kind of midget bipolar (*m.b.*) forms the central process of each of the invaginations into a cone pedicle (*c.p.*), while another midget bipolar (*f.m.*) makes only surface contacts onto the same cone pedicle base. The cone pedicle drawn is about 9 μm in diameter and would, therefore, contain about 25 triads; only three have been shown here (and clearly the diagram only be can approximately to scale). Each invaginating midget bipolar (*m.b.*) would, therefore, have as many contacts with the cone pedicle as there are triads. The flat midget bipolar (*f.m.*) has at least twice that number, and the points of contact are mostly on parts of the cone pedicle base between the points of entry of the invaginating midget bipolar processes and the horizontal cell processes. The lateral processes in the triads are of horizontal cells.

they may occasionally make contact at some distance from a triad. The apical processes of the flat midget are smaller in diameter than the conventional midgets, i.e. about 0·1 μm as compared with about 0·2 μm for invaginating midget processes.

Following discovery of the flat midget bipolar by electron microscopy, we tried to see if the two types of midget bipolar can be recognized by light microscopy. In both new material and that used for the preceding paper this has been possible; and from examination of over 300 cells at least one in every three midget bipolars could be identified as a flat midget bipolar. Figure 105, plate 45, shows a light micrograph of an invaginating midget bipolar. The dendritic terminal contains clearly defined apical processes. The cell body lies high in the inner nuclear layer and the axon passes straight down to terminate in the lower portion of the inner plexiform layer. By comparison, figure 106 shows a flat midget

bipolar situated within 500 μm of the invaginating midget of figure 105. The cell body lies lower in the inner nuclear layer and its longer dendrite passes up to the outer plexiform layer to form a flattened dendritic terminal. Individual apical processes cannot be resolved. The axon passes down to end just within the inner plexiform layer at a much higher level than the axon terminal of the conventional midget bipolar.

The shape of the dendritic terminal is the most obvious difference between the invaginating and the flat midget bipolars, see figures 107 to 112. Figure 107 is an invaginating midget bipolar (as are those of figure 15, plate 34, and figures 24 to 26, plate 35), and six apical processes can be distinguished in one plane of focus. Figures 108 and 109, plate 45, are of flat midget bipolars and show the flattened shape of the dendritic terminals. The main dendrite often branches at a lower level in the outer plexiform layer than does the main dendrite of the invaginating midget bipolar, and the subbranches all pass up vertically or obliquely to form a flattened or dome-shaped aggregation of processes that synapse with the same cone pedicle (figures 108 and 109). Electron microscopy shows that the branches subdivide into many small apical processes that turn to run horizontally under the cone pedicle. Individual apical processes can only rarely be distinguished by light microscopy. Electron microscopy of a flat midget bipolar, however, shows that there are about twice as many apical processes when compared with the processes on the invaginating midget bipolar. Also they are of a finer diameter. Thus the appearance of the terminal of a flat midget bipolar as a single mass under the light microscope is not surprising. Figures 111 and 112, plate 45, show different focusses of two cells, an invaginating midget bipolar and a flat midget bipolar, lying 5 μm from each other. In one plane of focus (figure 111) the invaginating midget dendritic terminal is clearly defined, and consists of distinct apical processes each of which corresponds to the central element of the triad in size (0·2 μm). Some of the apical processes (e.g. to the left of the arrow, figure 111, plate 45) subdivide, suggesting that they pass to two closely neighbouring triads, a fact often observed by electron microscopy. In the other plane of focus the flat midget terminal is to be seen and it appears as a solid mass with a few distinguishable horizontally running processes. In figures 107 and 111 part of the cell body of the invaginating midget bipolar is to be seen, whereas in none of the photomicrographs of the flat midget is it visible at the same level, again showing that the cell body of this type of midget lies lower in the inner nuclear layer for this type of cell. Figure 8, plate 34, of the main paper shows a typical flat midget bipolar cell at magnification $\times 800$.

The differences between the invaginating midget bipolar and the flat midget bipolar are summarized in figure 99, which is a diagram combining the electron microscope and light microscope findings just described.

The discovery of the flat midget bipolar raises the important question of whether some or all cones connect with more than one midget bipolar cell. On a few occasions we have observed by light microscopy two stained midget bipolars that overlap to such a degree that they are almost certainly innervating the same cone pedicle (figure 110, plate 45, and figure 11, plate 34). It is difficult to decide in such cases whether one cell is an invaginating midget bipolar and the other a flat midget bipolar, but this seems to be the likely inference. The different levels of the cell body and axon terminations (see figure 11, plate 34) are suggestive evidence that these are in fact an invaginating and a flat midget bipolar

contacting the same cone pedicle. The evidence is that there is no type of bipolar other than the invaginating midget that extends processes into the invaginations of the cone pedicle. Therefore, every cone pedicle in the primate must be connected to a conventional midget bipolar, but at present, we can only say with certainty that some of the cones

Description of plate 45

All the illustrations on this plate are from the retinae of rhesus monkey.

Figure 101. Electron micrograph of a portion of a cone pedicle showing the arrangement of the triads with associated synaptic ribbons and synaptic vesicles. Processes making superficial contacts are arrowed. Osmium-tetroxide fixation. × 22800.

Figure 102. Electron micrograph of a triad with a central process from an invaginating midget bipolar impregnated with Golgi stain. Golgi rapid triple impregnation method (Stell 1965*a*). × 22800.

Figure 103. Electron micrograph of a triad with the superficial contacts from apical dendrites of a flat midget bipolar impregnated with Golgi stain. The processes lie on either side of a process of an invaginating midget bipolar. Golgi rapid triple impregnation method (Stell 1965*a*). × 49000.

Figure 104. Electron micrograph of two triads and with Golgi-impregnated superficial contacts from a flat midget bipolar. Compare with figure 101. The arrowed stained processes correspond to the arrowed processes in figure 101. Golgi rapid triple impregnation method (Stell 1965*a*). × 24600.

Figure 105. Invaginating midget bipolar cell. The apical dendrite is approximately 6 μm in diameter. The cell was between 1·0 and 1·5 mm from the centre of the foveal pit. Golgi–Colonnier staining. × 1700.

Figure 106. Flat midget bipolar cell. The apical dendrite is approximately 6 μm in diameter and the cell was between 1·0 and 1·5 mm from the centre of the foveal pit. Golgi–Colonnier method. × 1700.

Figure 107. Dendritic terminal of an invaginating midget bipolar, showing the small distinct apical processes. Note the cell body is visible. Golgi–Colonnier. 1·0 to 1·5 mm from the foveal pit. × 3000.

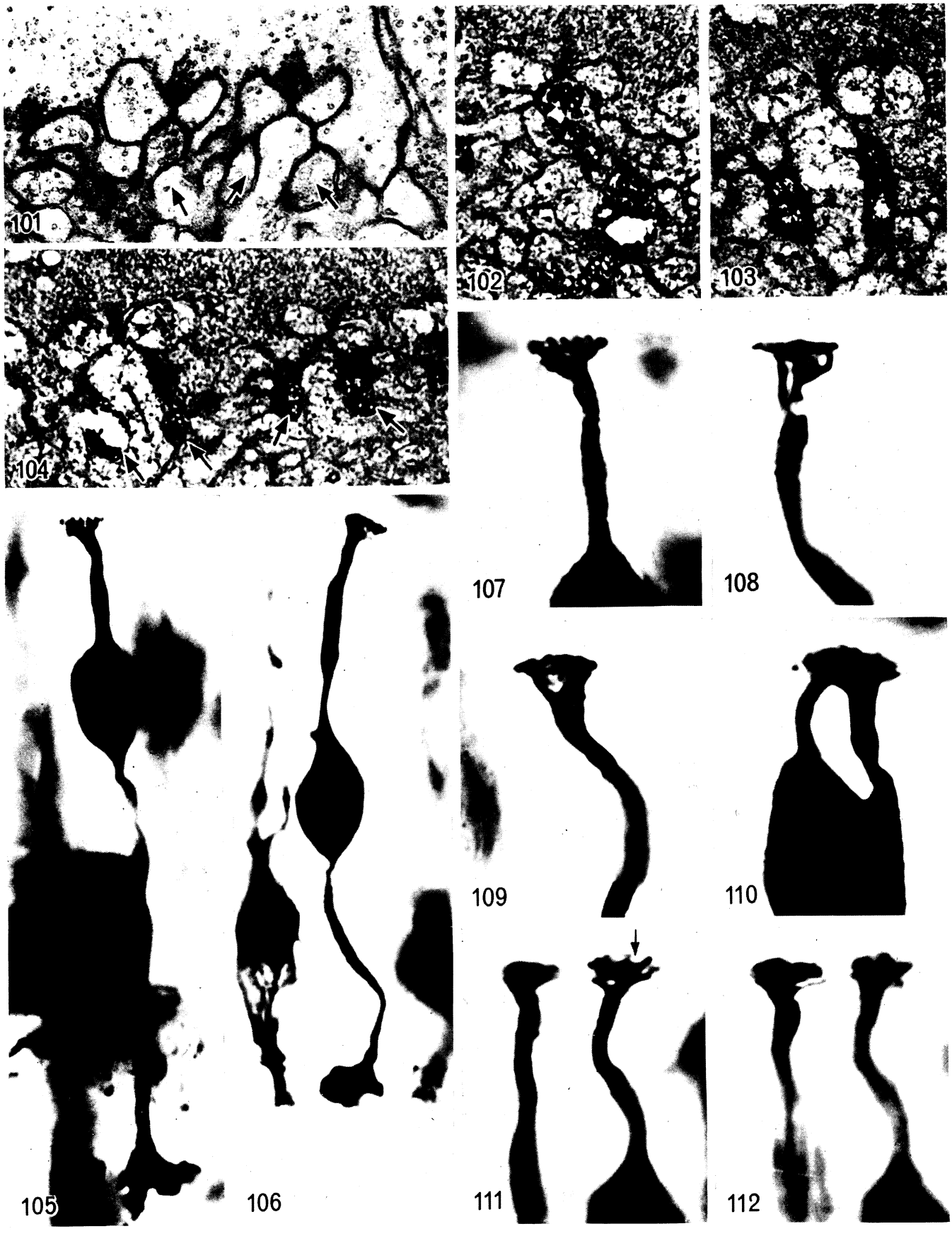
Figure 108. Dendritic terminal of a flat midget bipolar; its top arranged in line with figure 107 the perikaryon is not visible. Golgi–Colonnier method. × 3000.

Figure 109. Dendritic terminal of a flat midget bipolar lying within 1·0 mm of the foveal pit. Golgi rapid triple impregnation method (Stell 1965*a*). × 3000.

Figure 110. Two midget bipolars overlapping to such an extent that they must be innervating the same cone pedicle. Note the cell body of the one bipolar lies high and suggests that this one is an invaginating midget bipolar while the other one may be the flat midget bipolar type. This is an enlargement of the cells shown in figure 11, plate 34, of the main paper. Golgi–Colonnier method. × 3000.

Figure 111. An invaginating midget bipolar is in focus in this photo-micrograph while the neighbouring flat midget bipolar is out of focus. Note the distinct apical dendrites on the terminal and the branching into two of one of the processes to the left of the arrow. The view is slightly oblique. Golgi rapid triple impregnation method. × 3000.

Figure 112. The same field as the previous picture but the flat midget bipolar is now in focus. The cells are separated by 3 to 4 μm. Note the dense flat appearance of the dendritic terminal of this cell due to the lack of resolution of the processes. The cells are from within 1·0 mm of the foveal pit. Golgi rapid triple impregnation method. × 3000.



(*Facing p.* 180)

connect with the two types of midget bipolar cell. Whether all cones have such an arrangement or whether some cone pedicles connect with more than one flat midget bipolar cannot be decided at the present time. The different levels at which the axons of the two types of midget bipolars terminate in the inner plexiform layer may have some significance. Polyak (1941) noticed this and stated that the number of midgets with short axons was equal to the number with long axons but made no distinction of cell types in this observation. The two types of midget bipolar may be contacting two different types of ganglion cell, although midget ganglion cells whose dendritic trees end at a high level or a low level in the inner plexiform layer appear to be otherwise identical.

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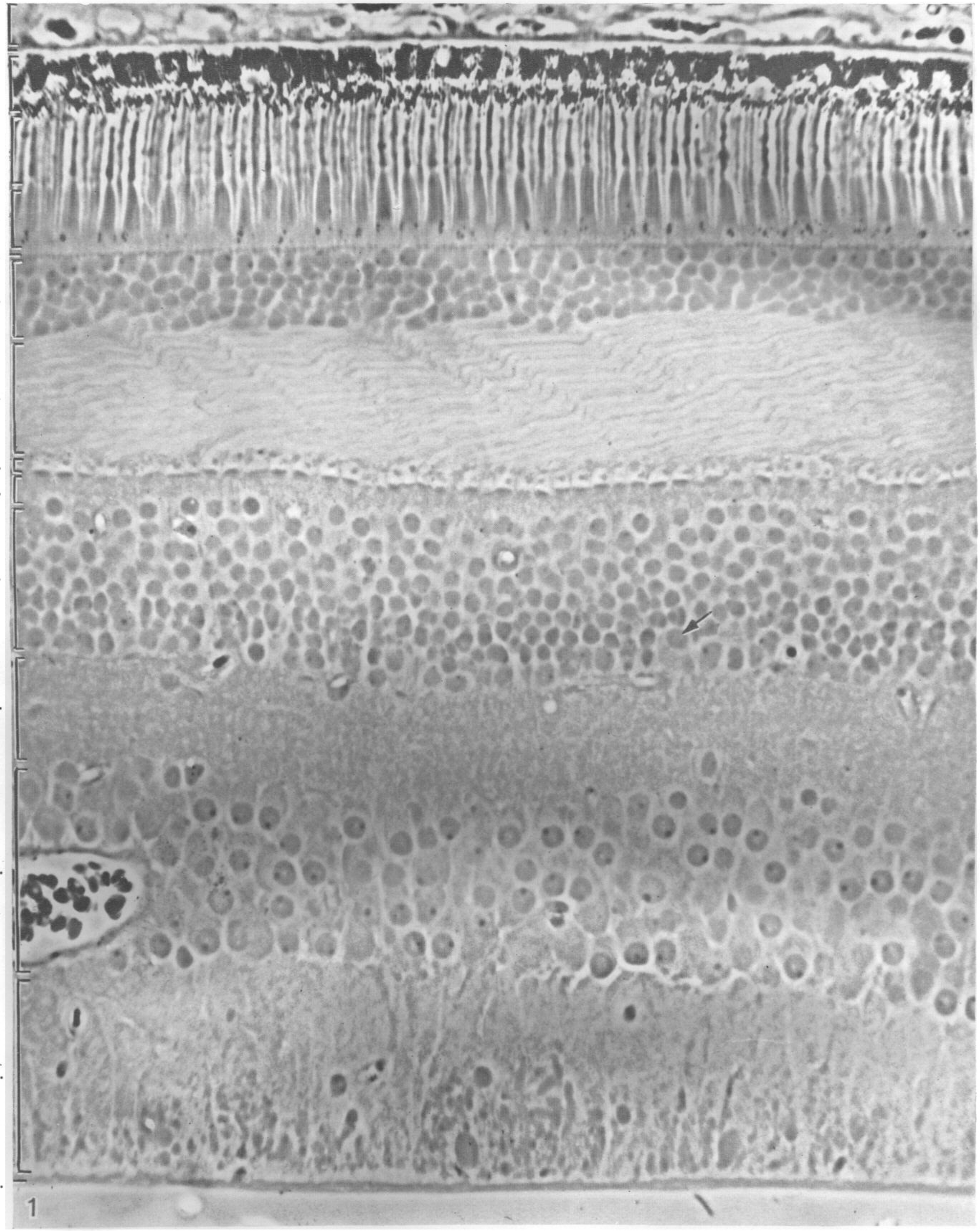
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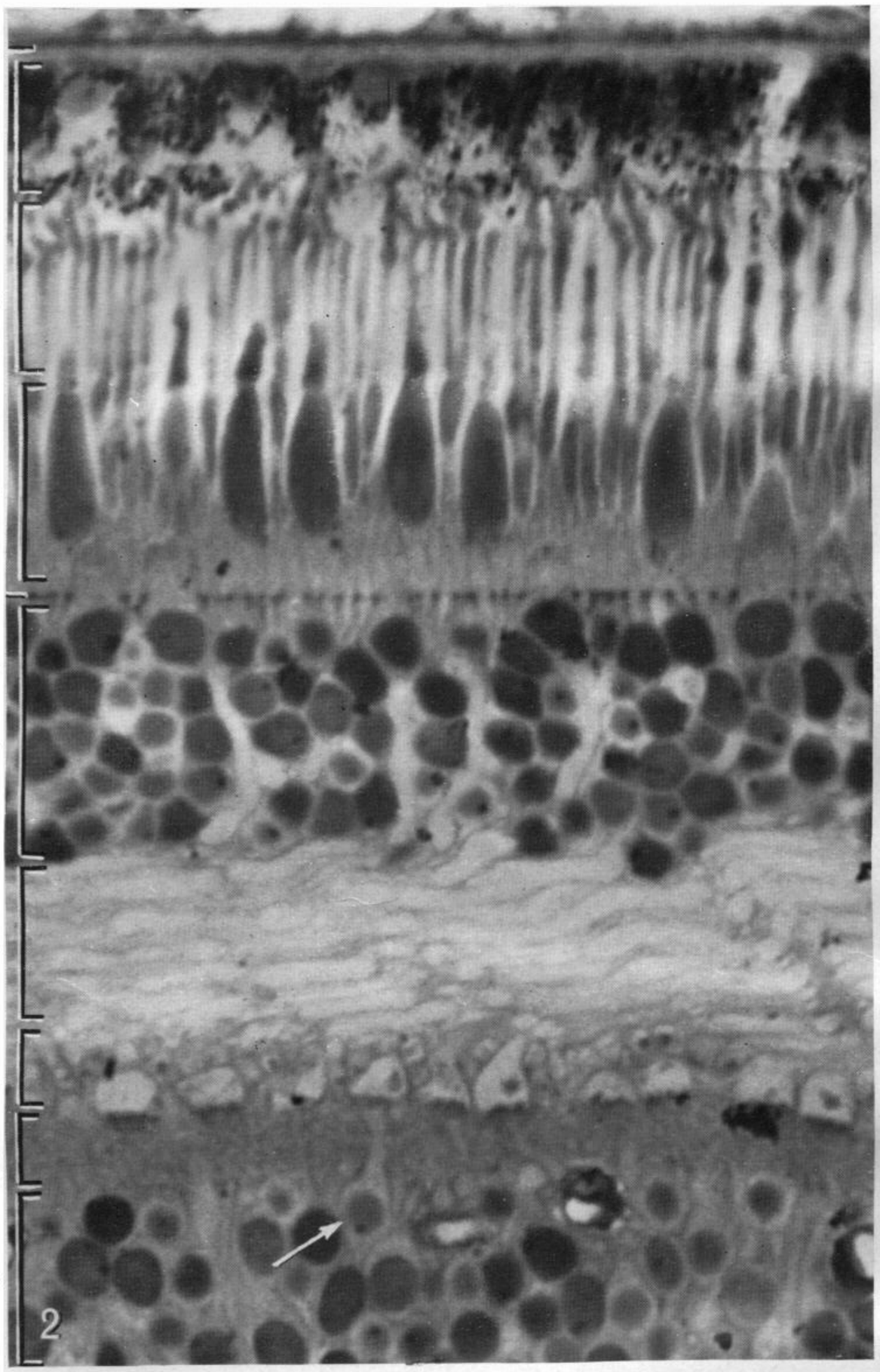

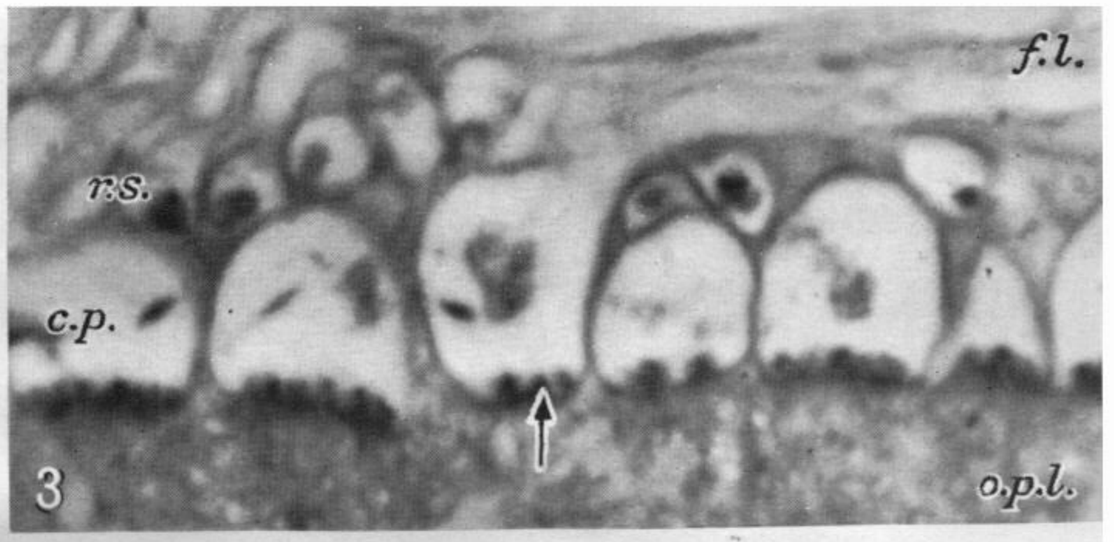

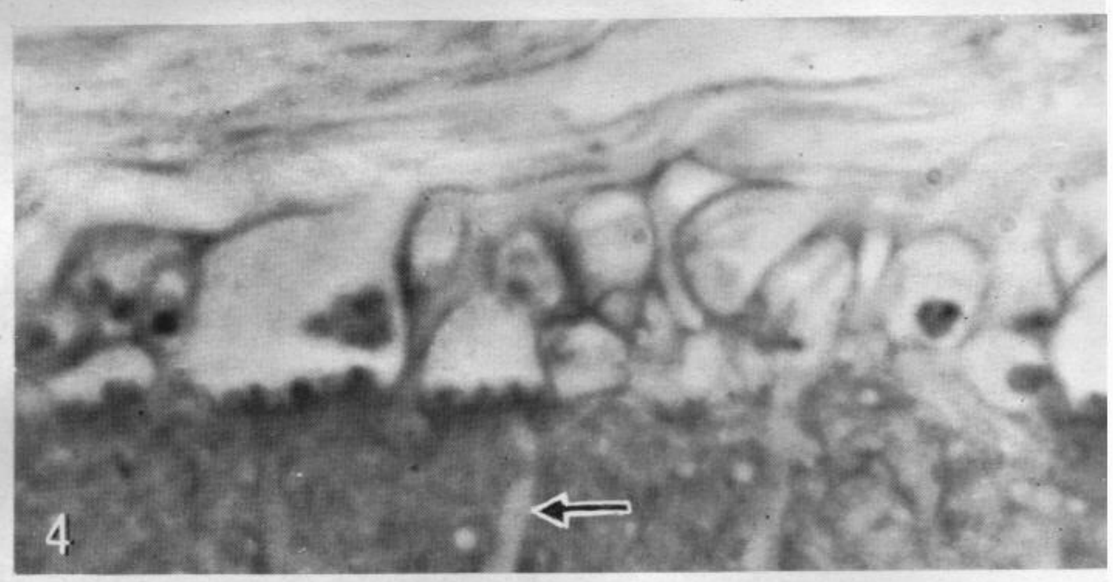

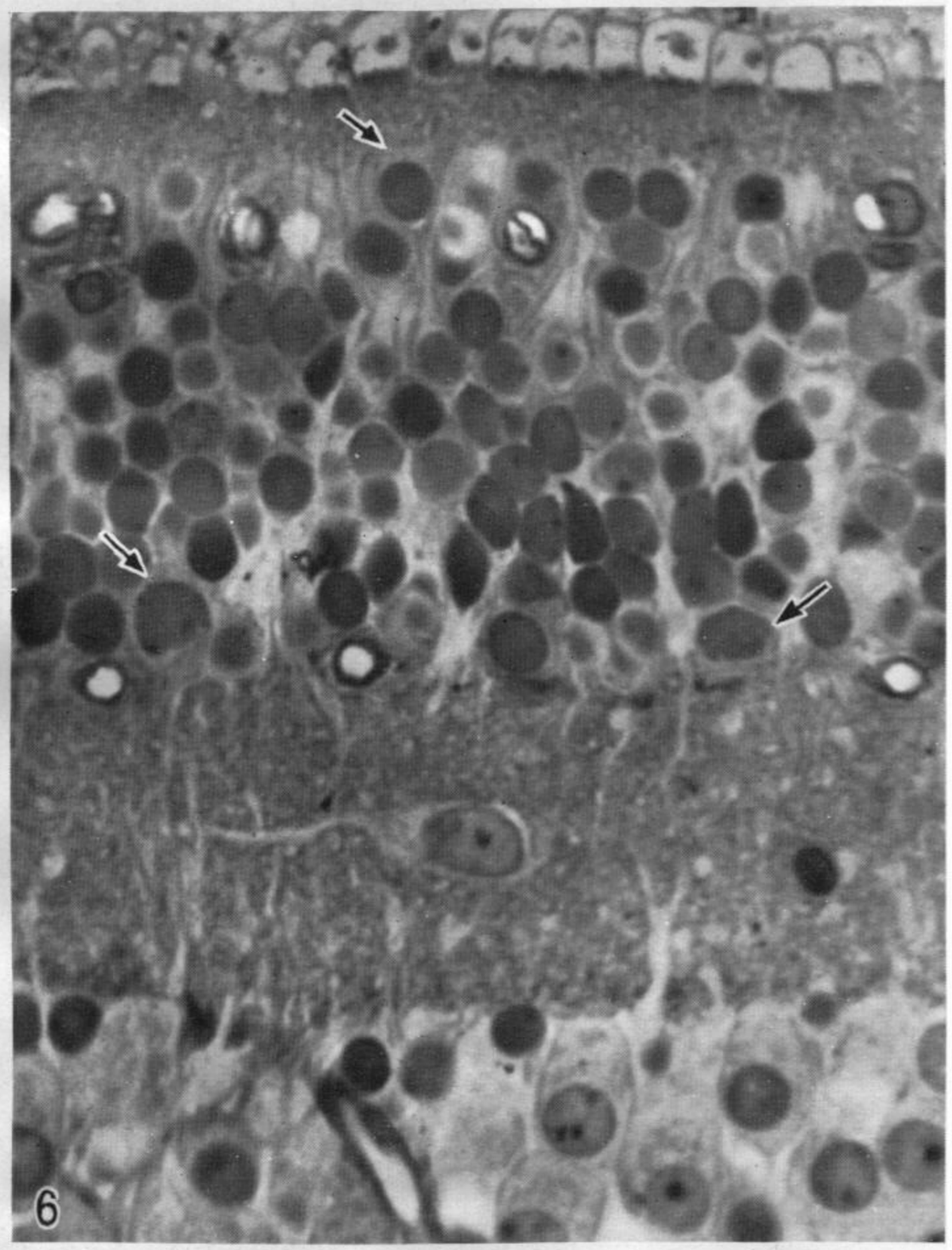

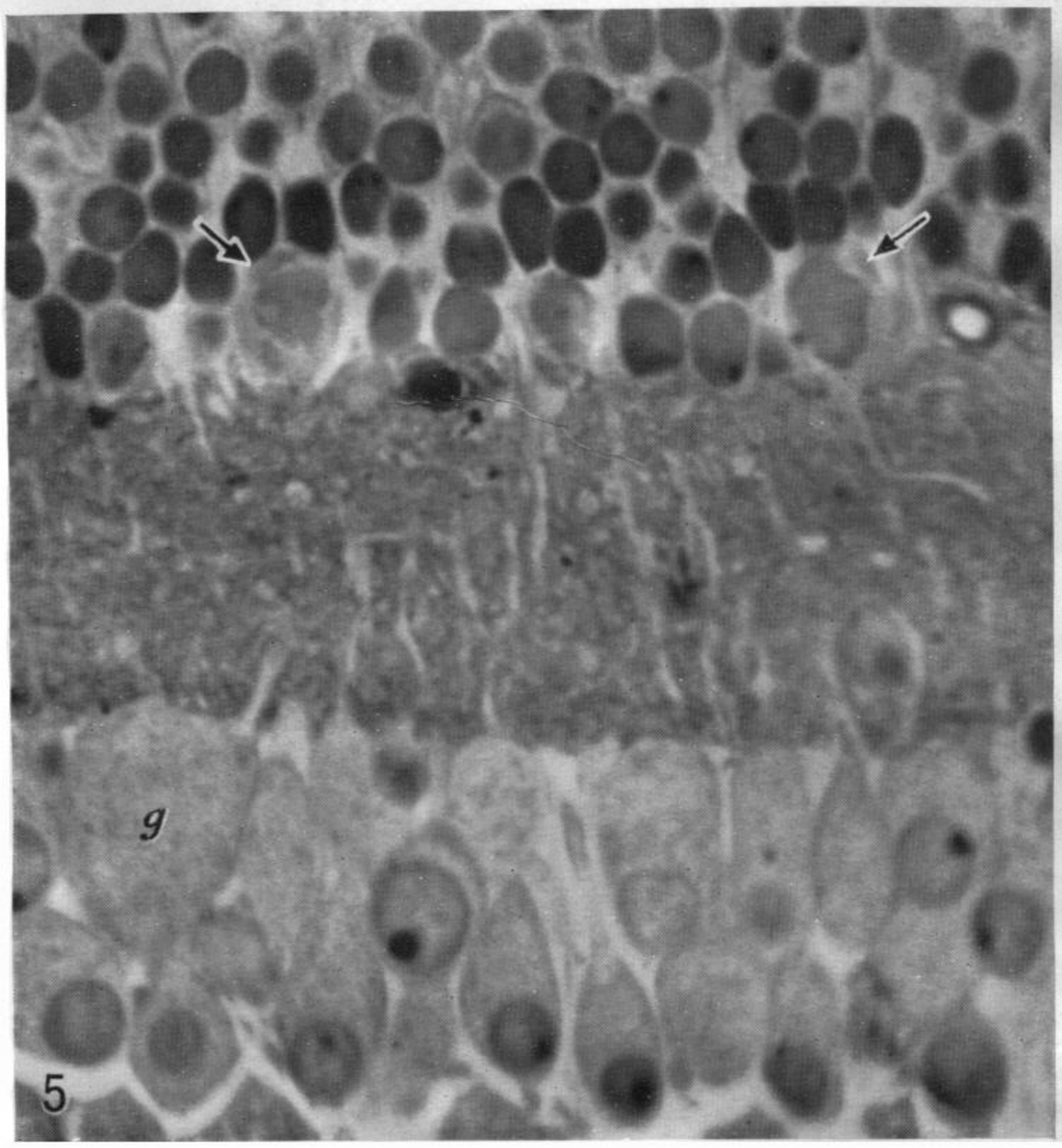

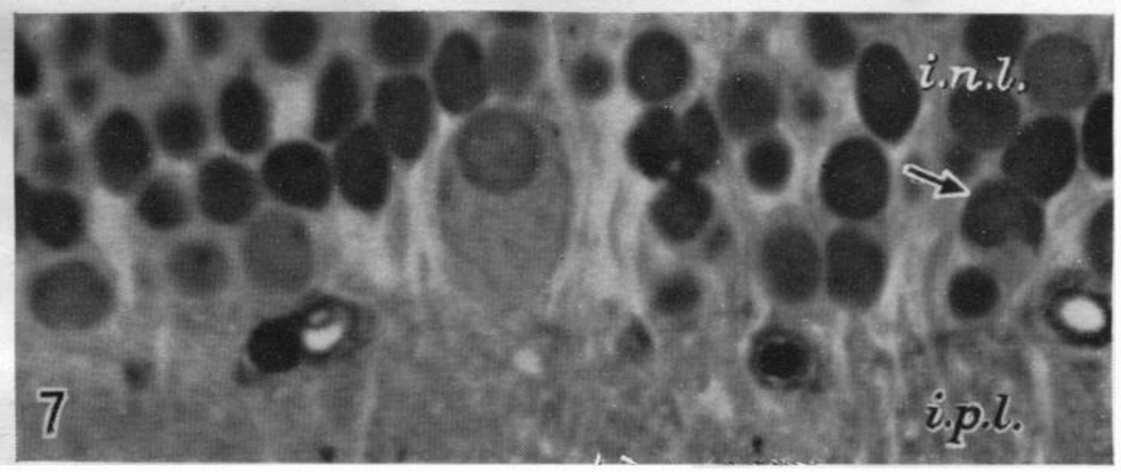

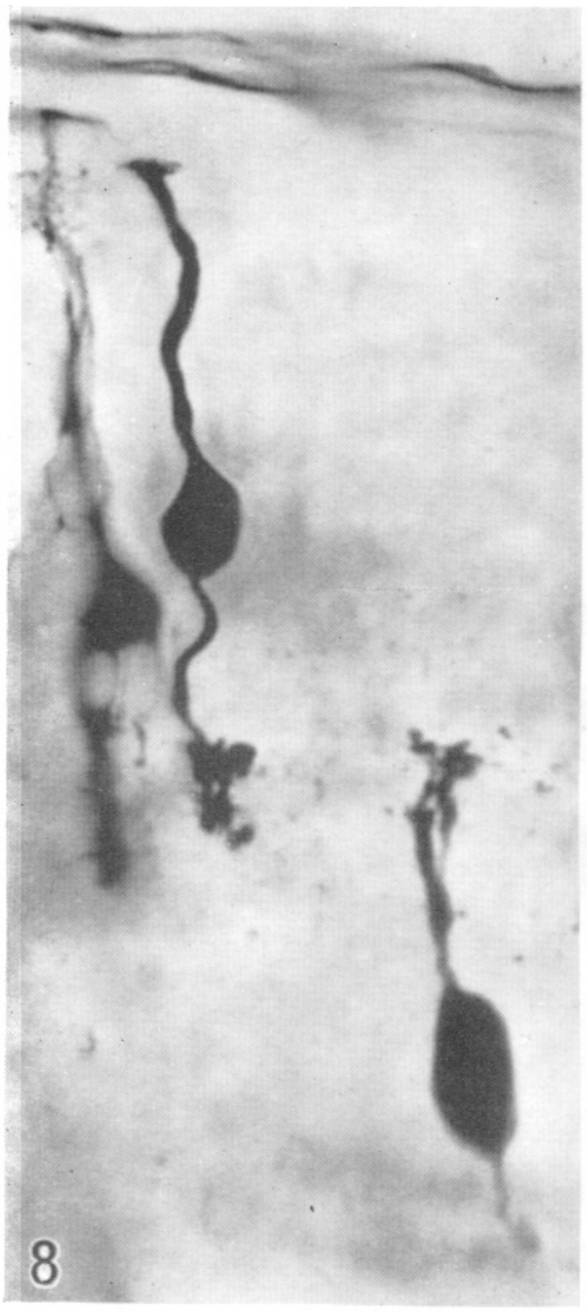
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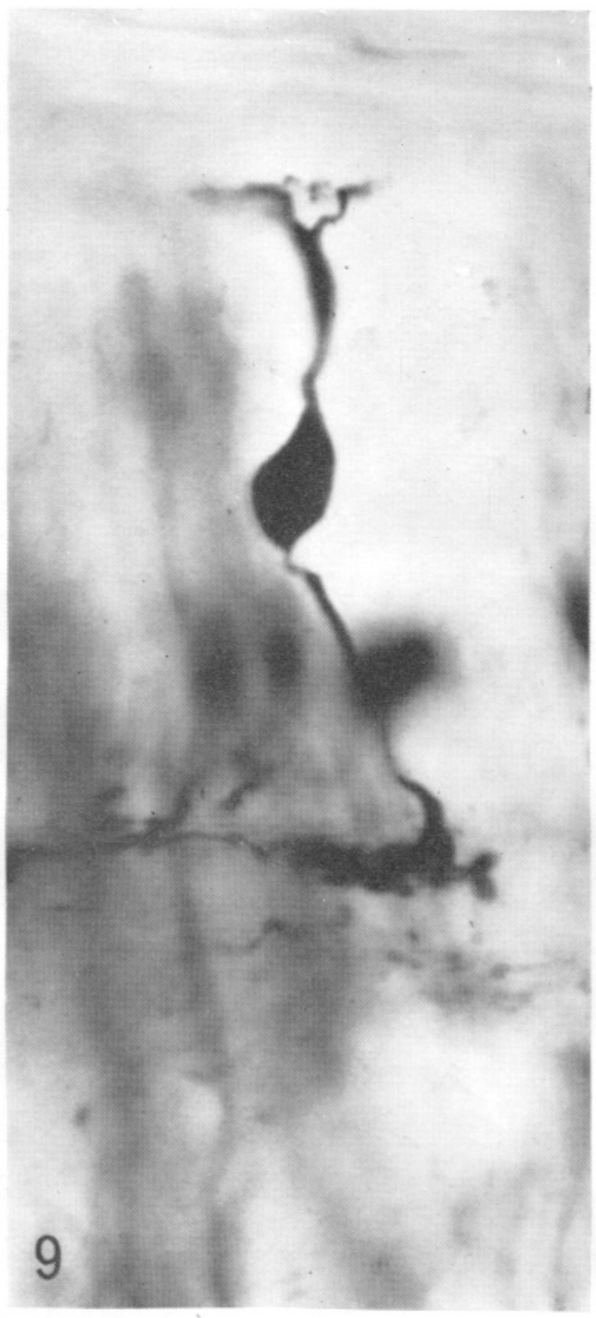
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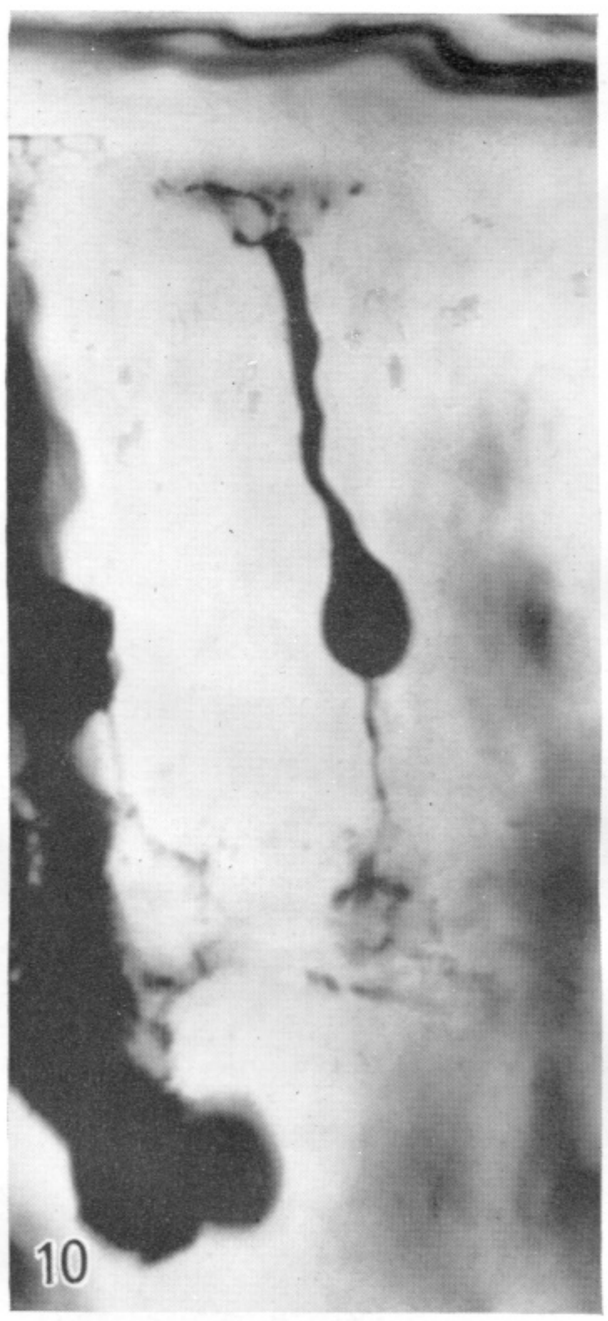
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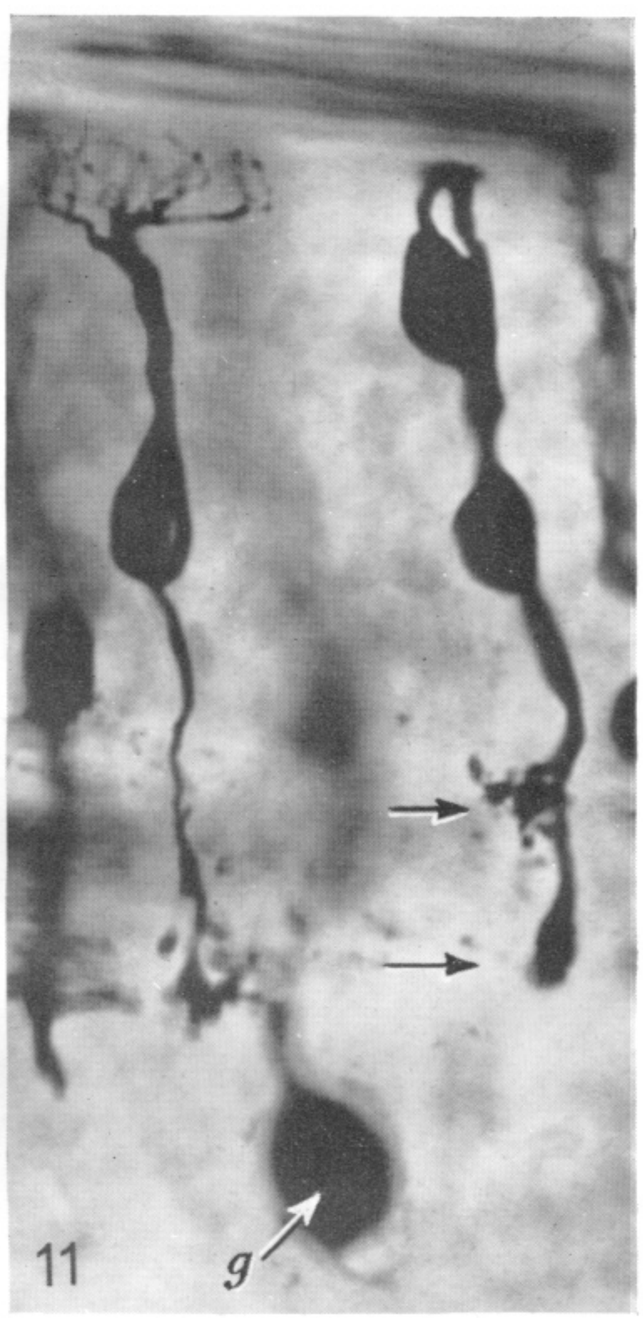

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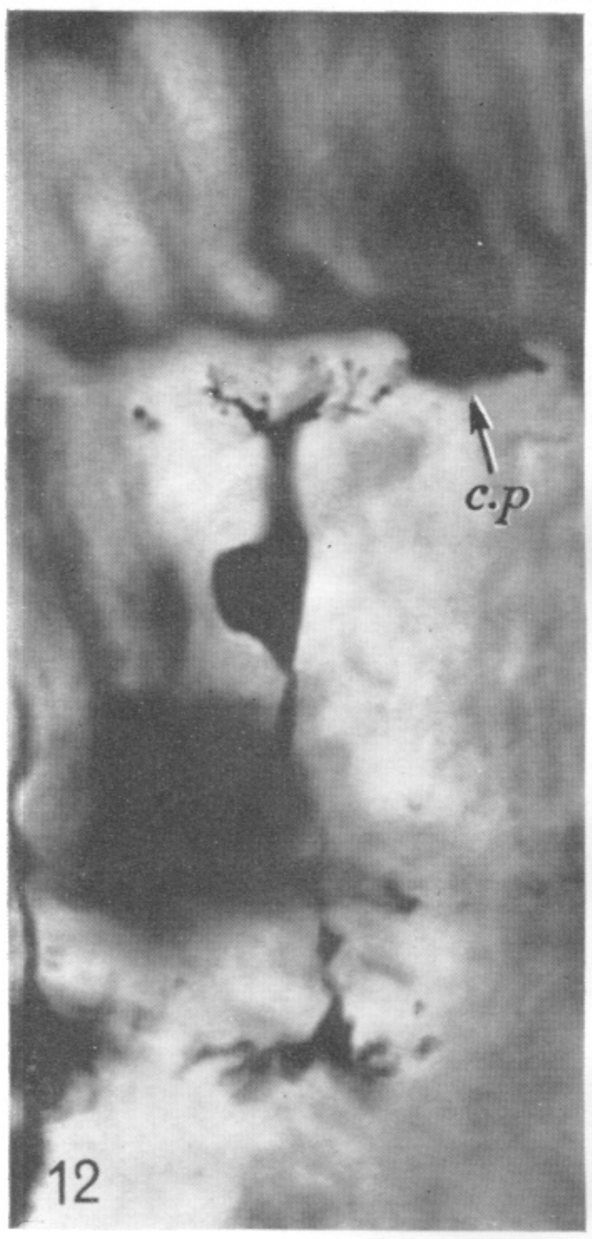

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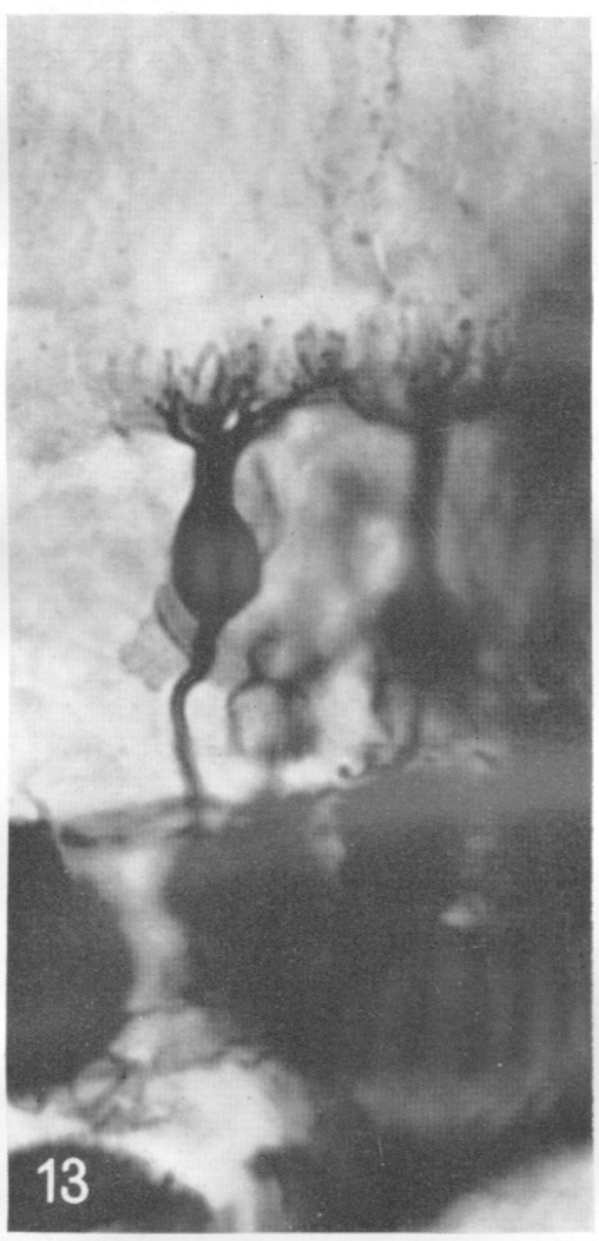
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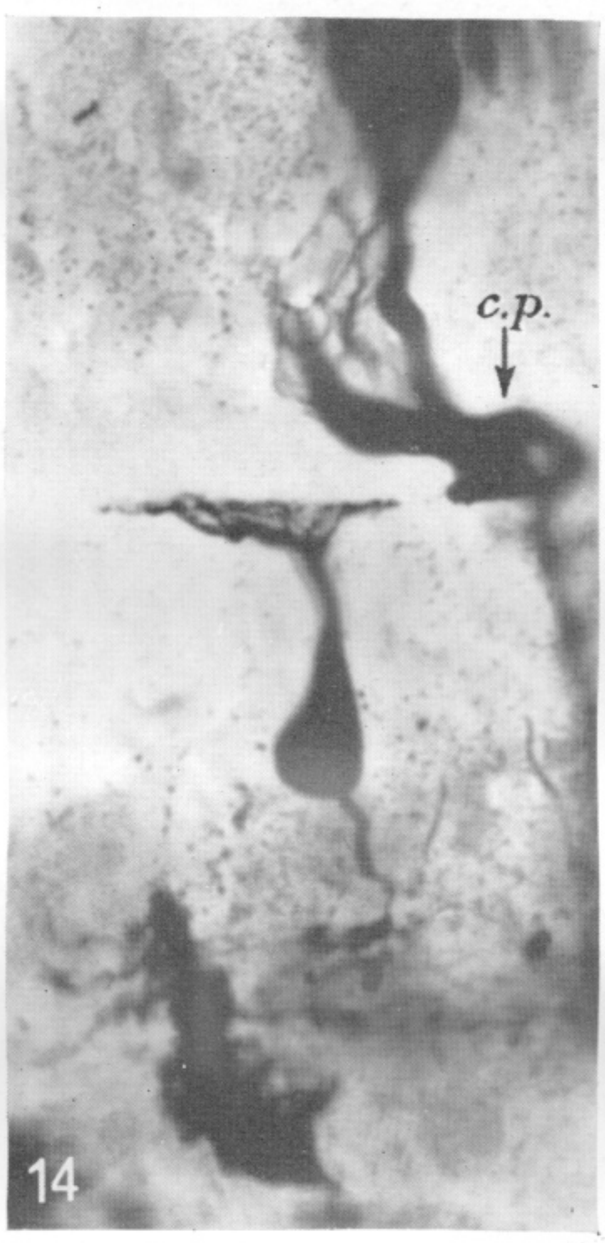

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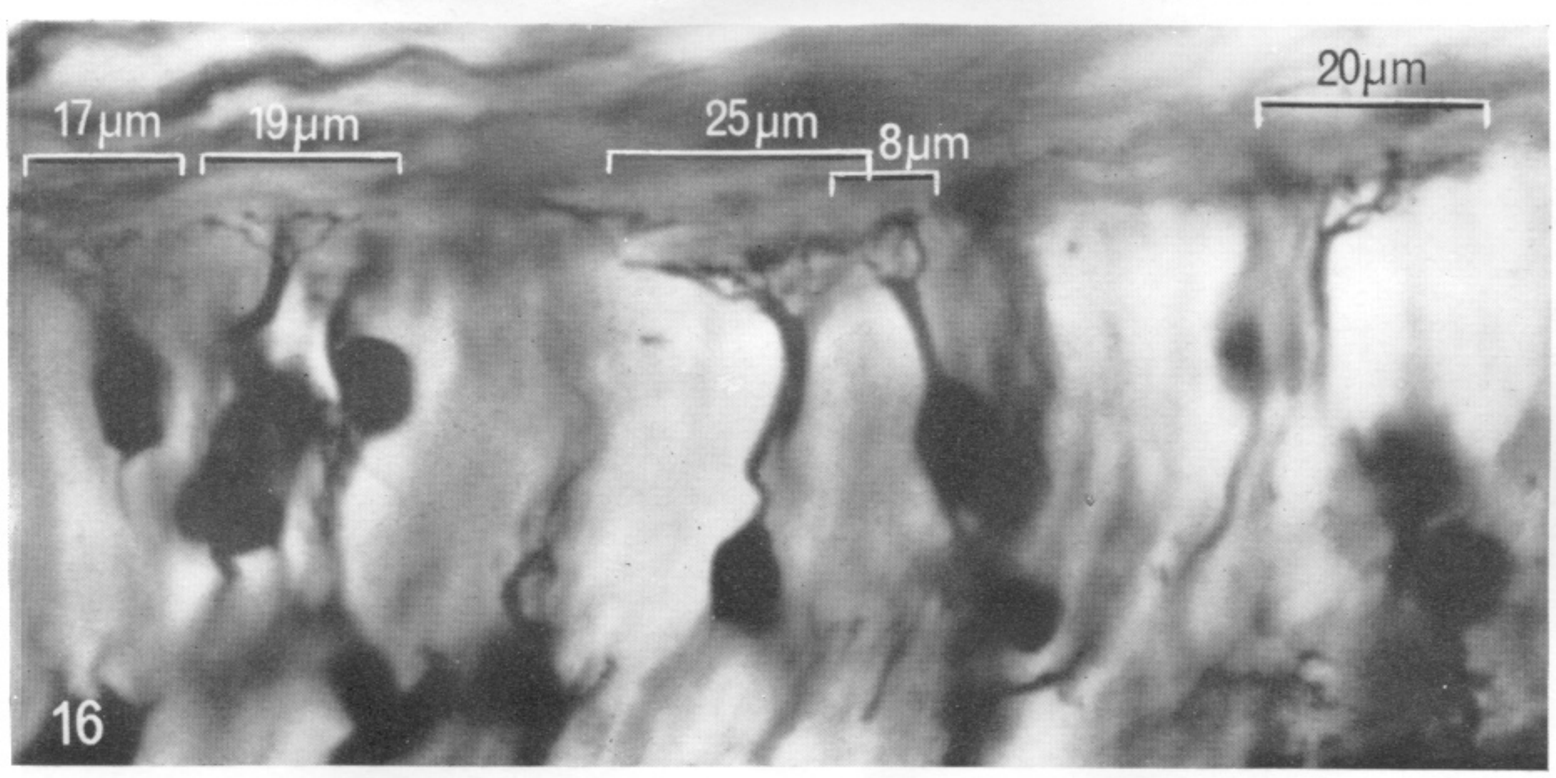

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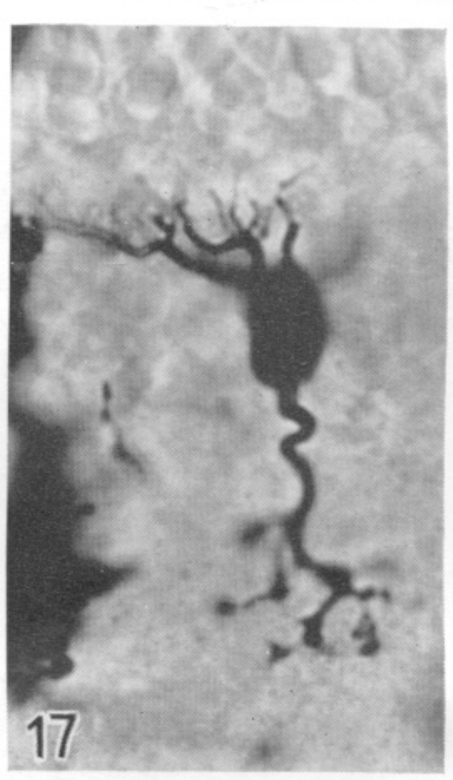
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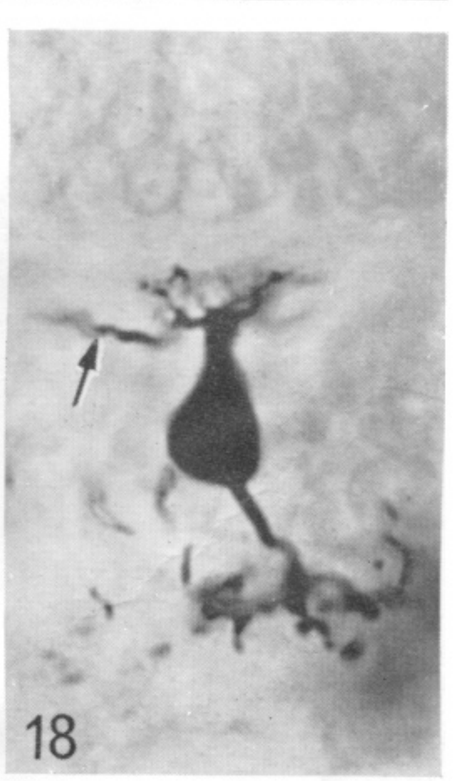
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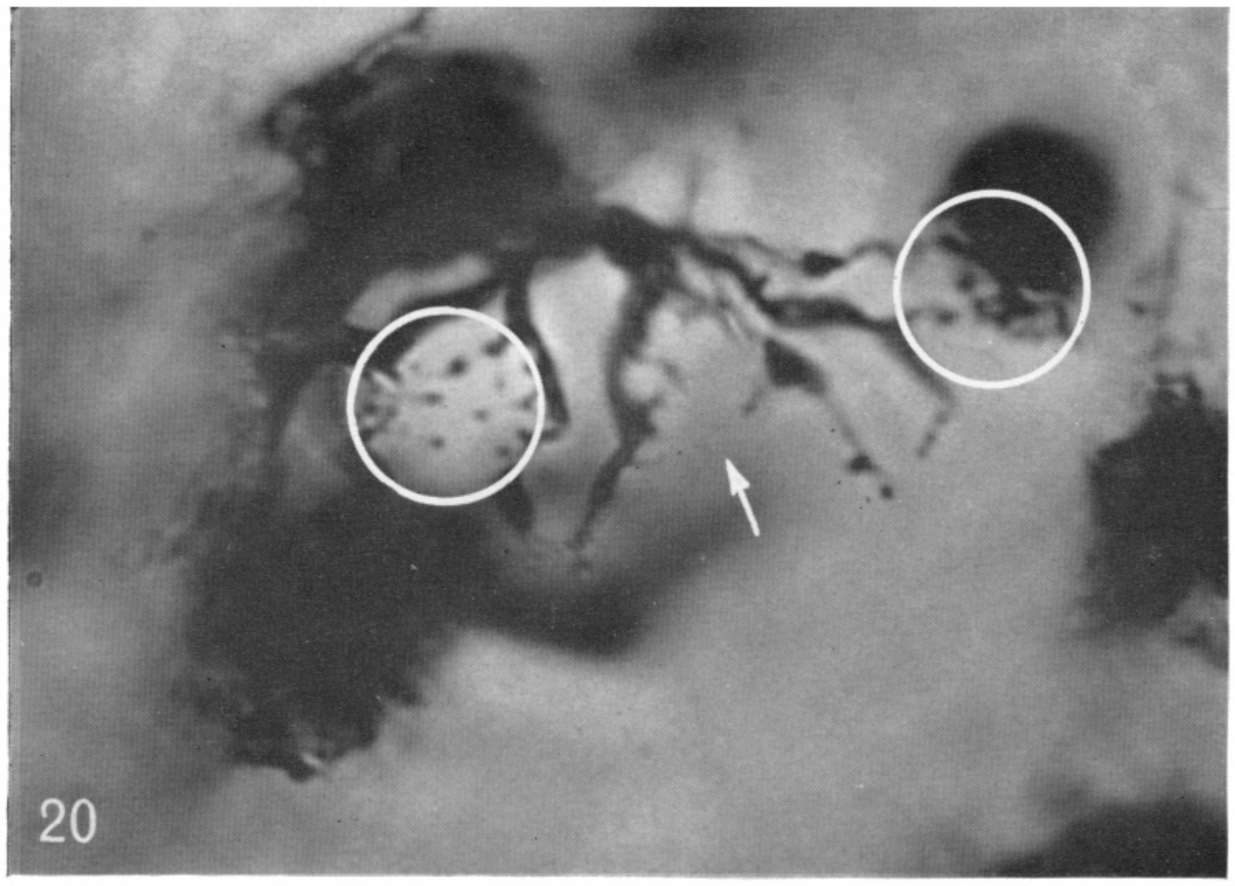
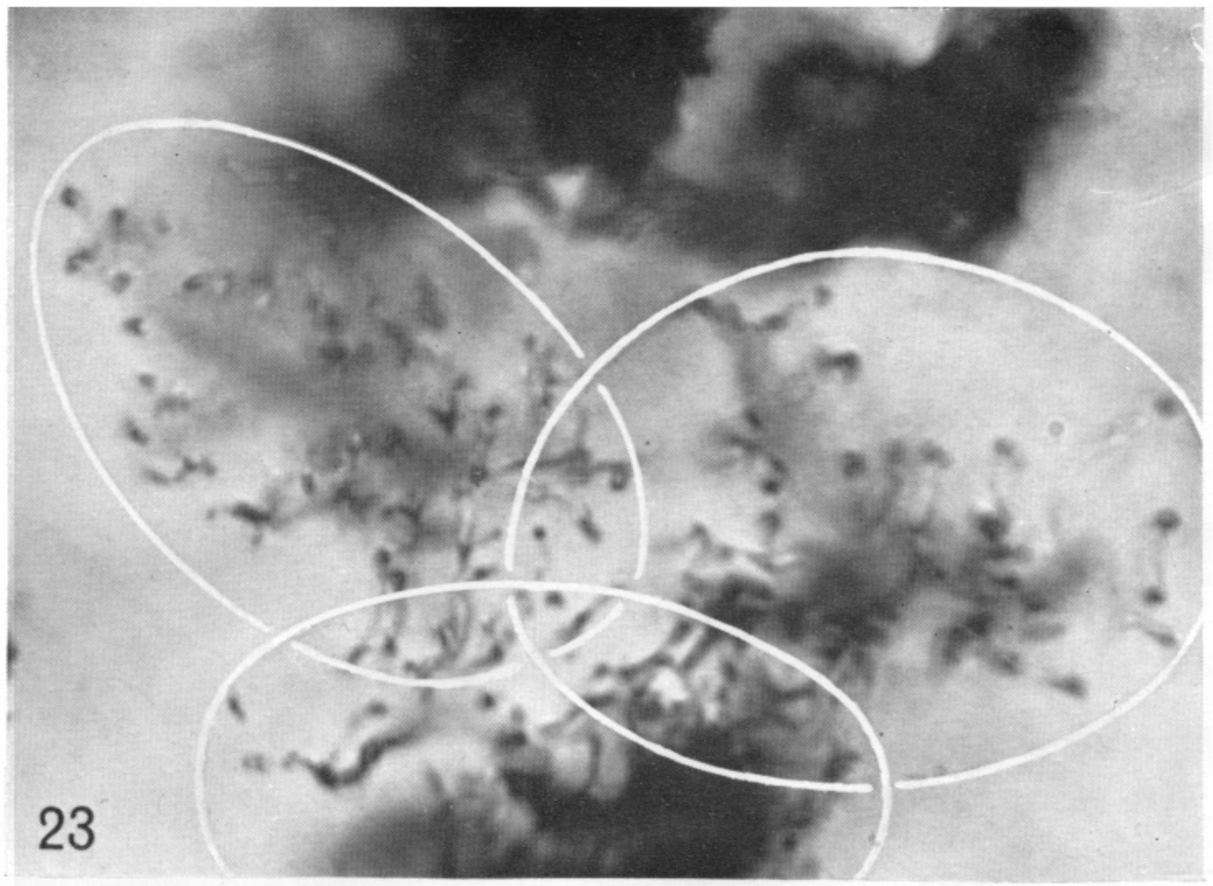
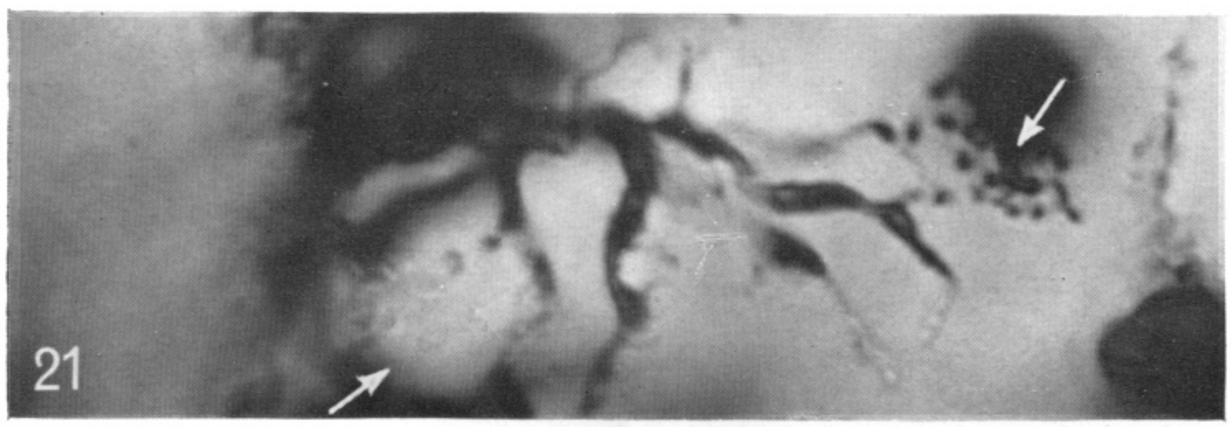
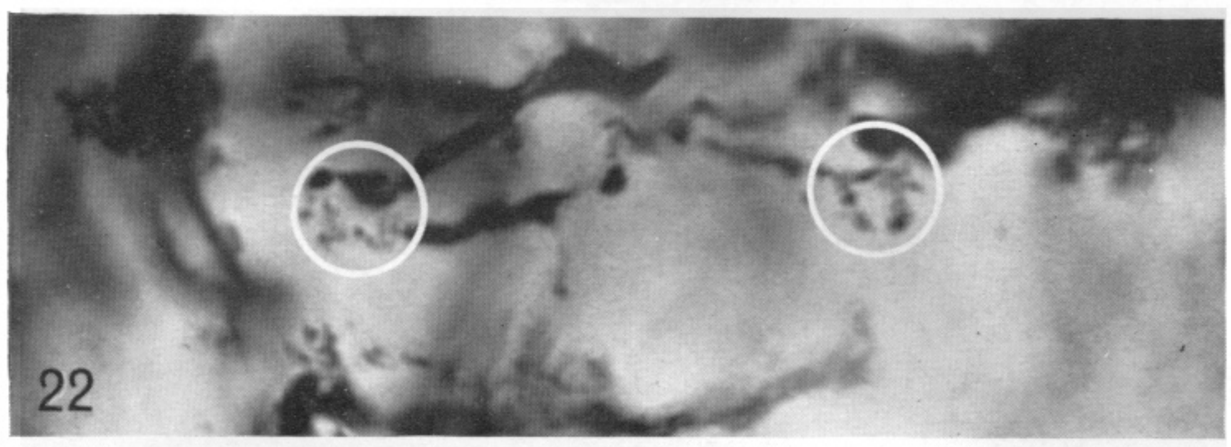
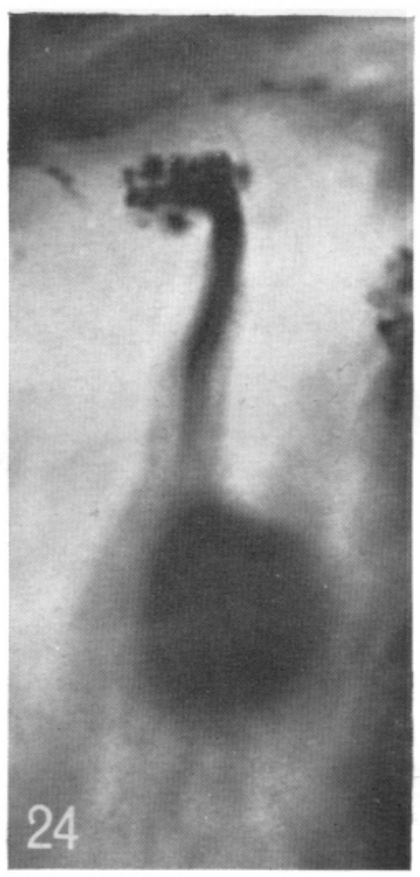
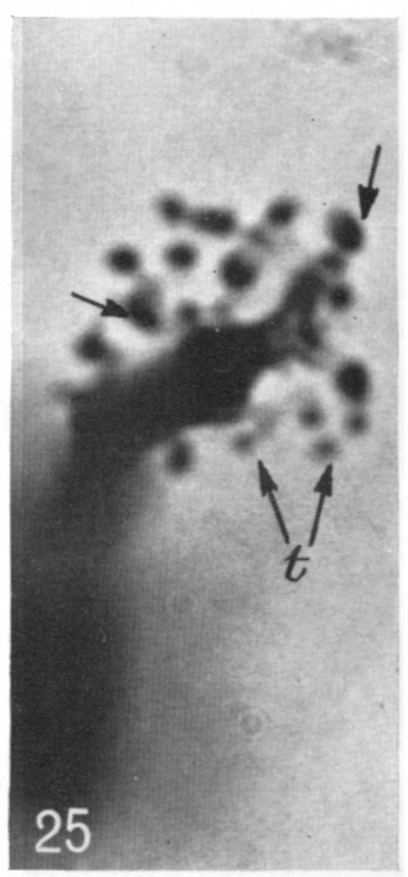


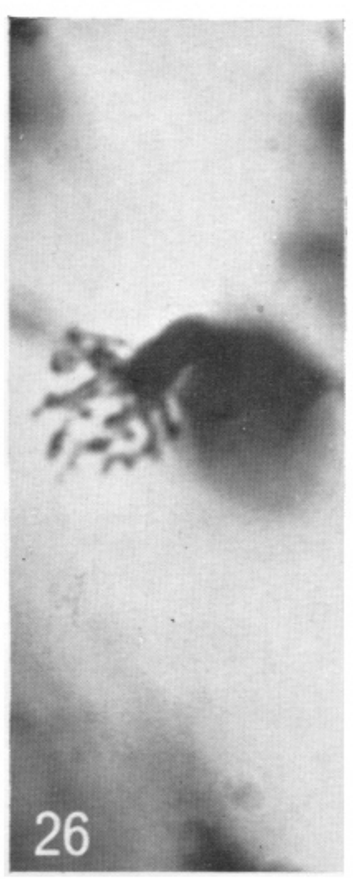


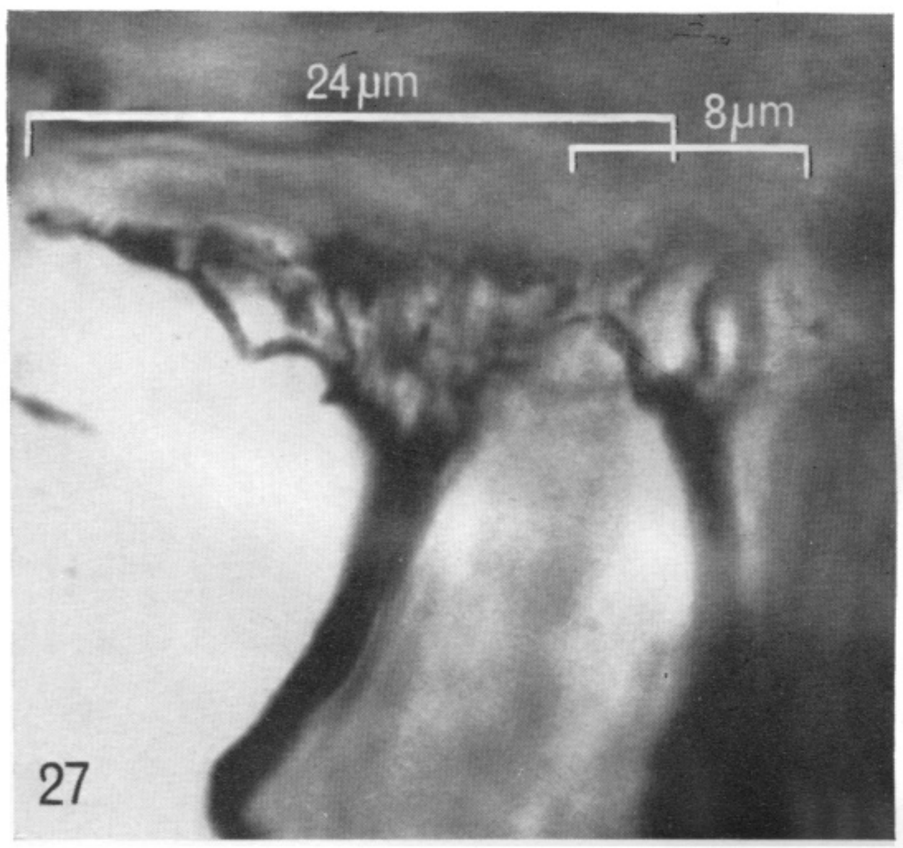


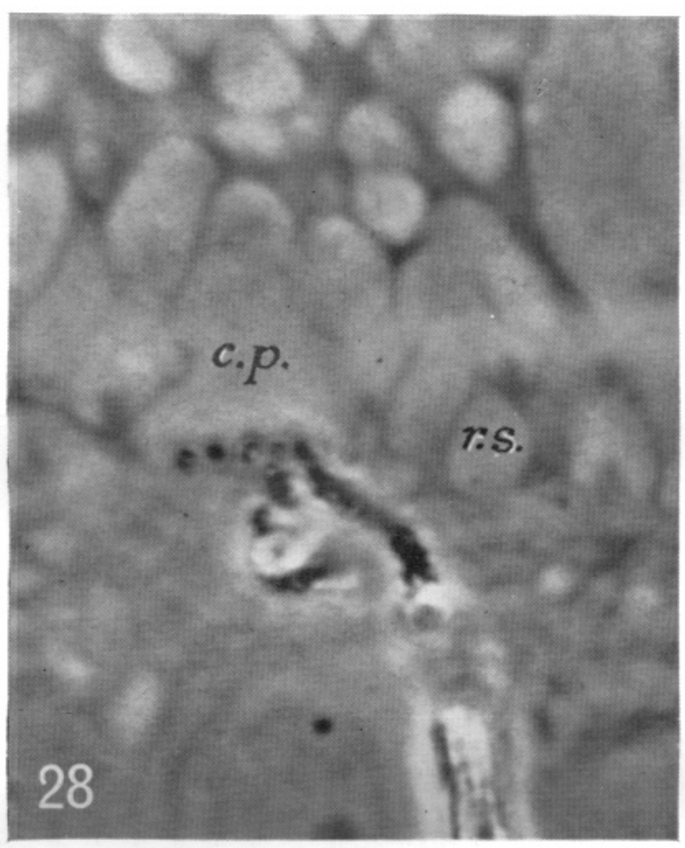


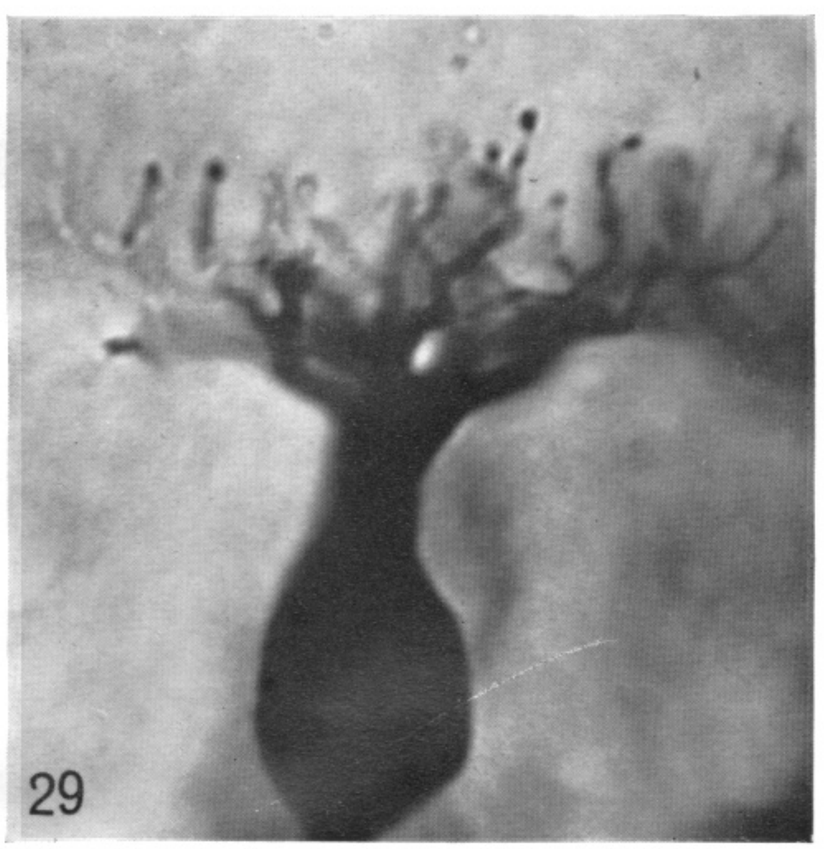






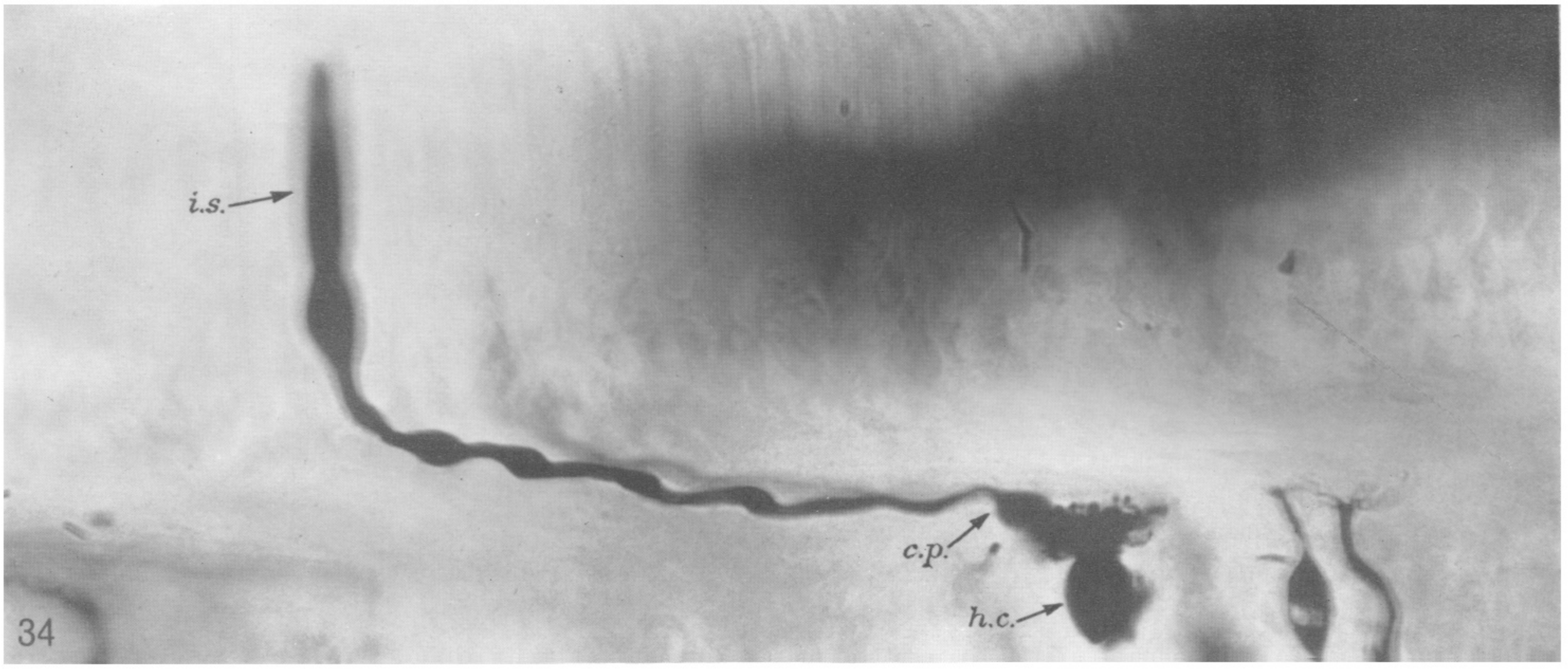






Bipolar cells of rhesus macaque retina as observed in vertical and horizontal sections. All are stained by the Golgi–Colonnier procedure and the magnification is × 2000 except for figure 25 at × 4000.

FIGURE 20. Horizontal section showing a flat bipolar cell with the dendrites branching in a plane and terminating in fine processes that are distributed so that they can be thought of as corresponding to the diameter of the base of a cone pedicle. The circles represent bases of cone pedicles 7 to 8 µm in diameter. This picture does not show the whole of the top of the flat bipolar but its dendritic spread is 35 µm. We have never regularly stained all the fine branches of



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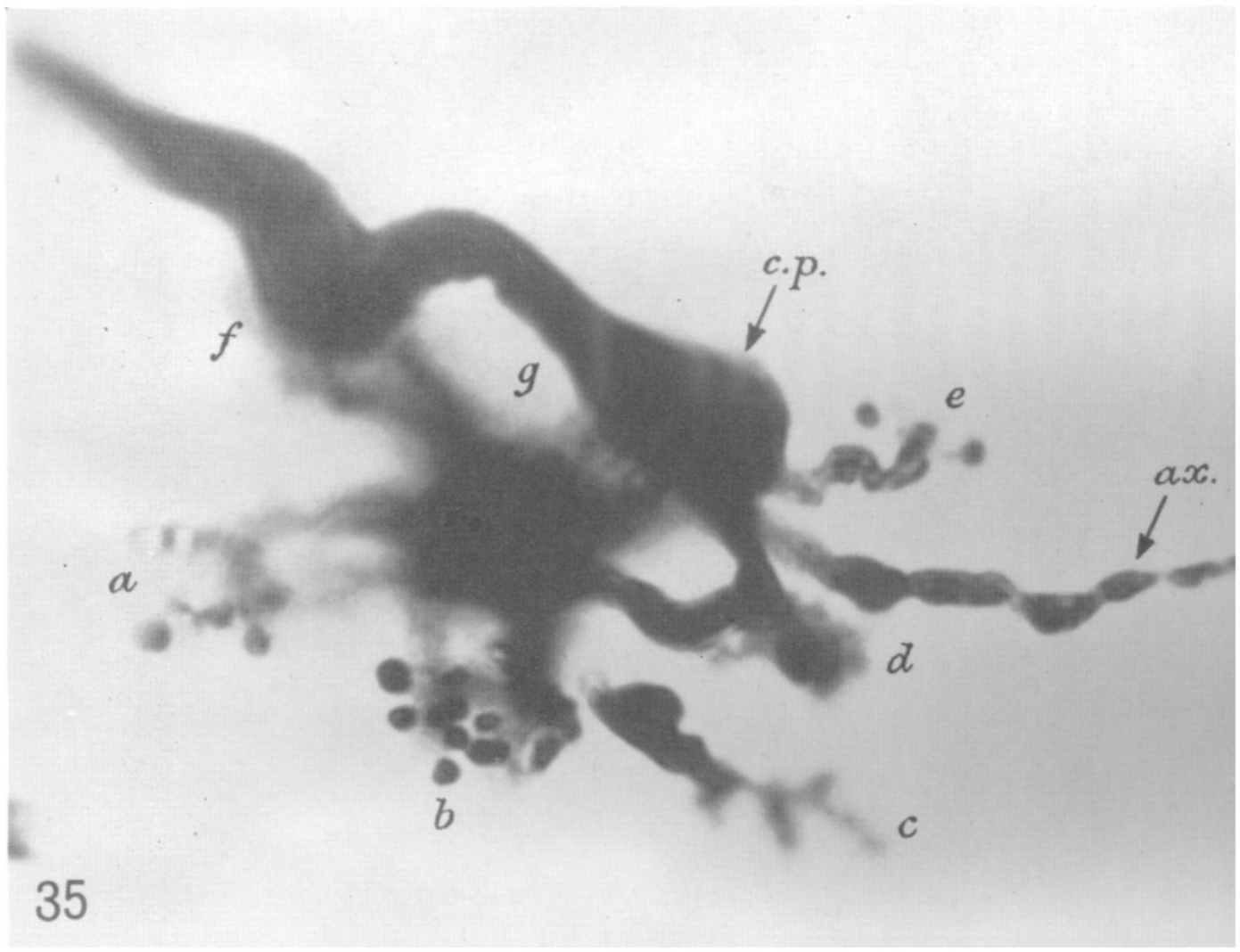

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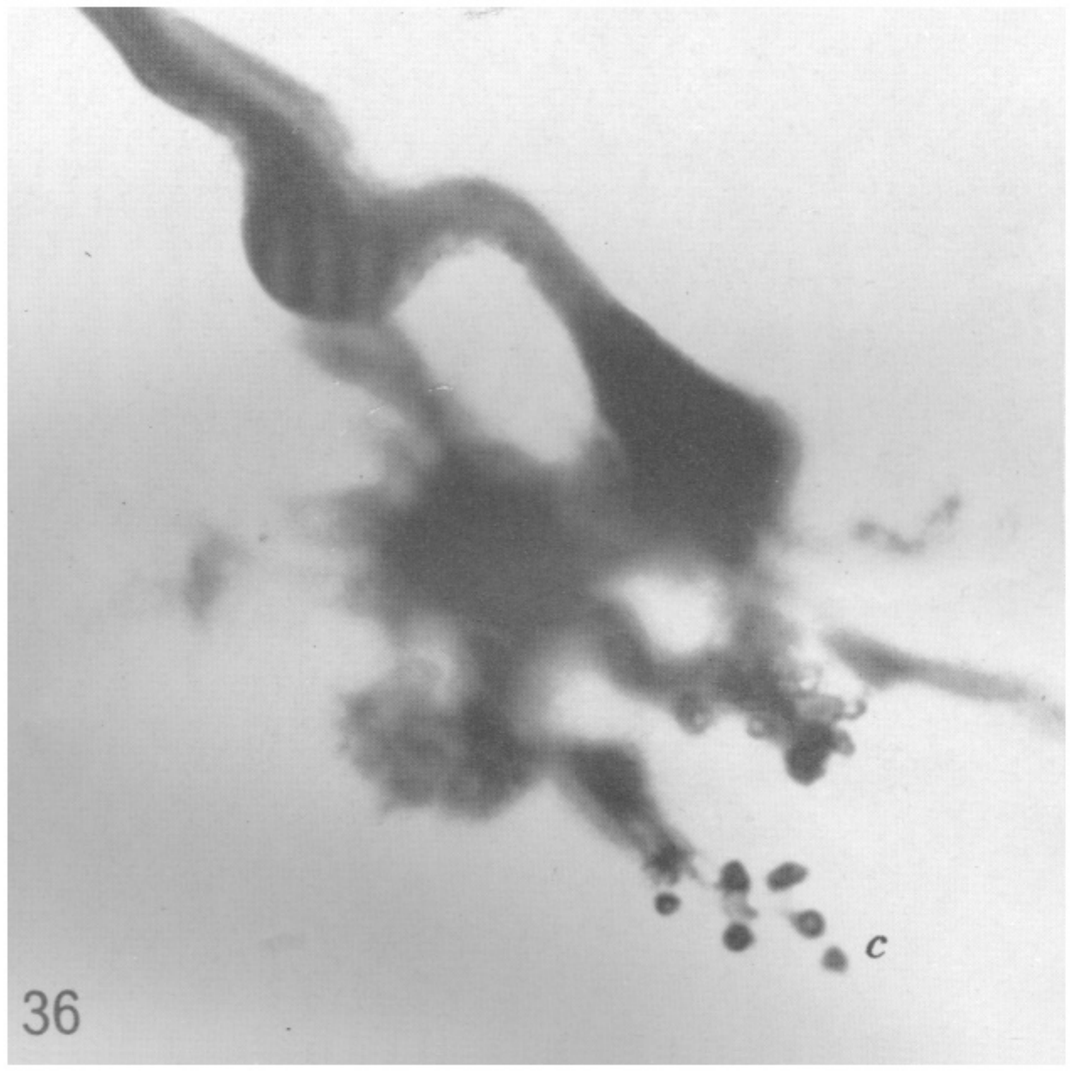

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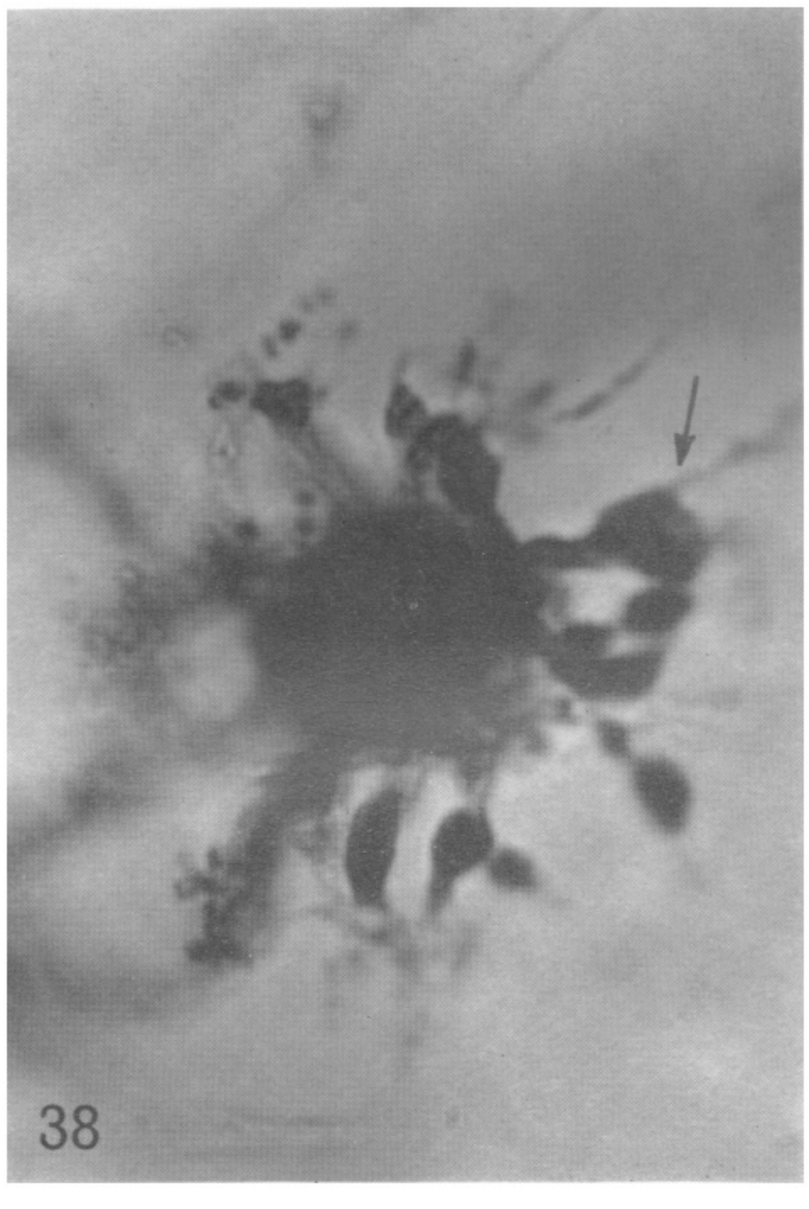

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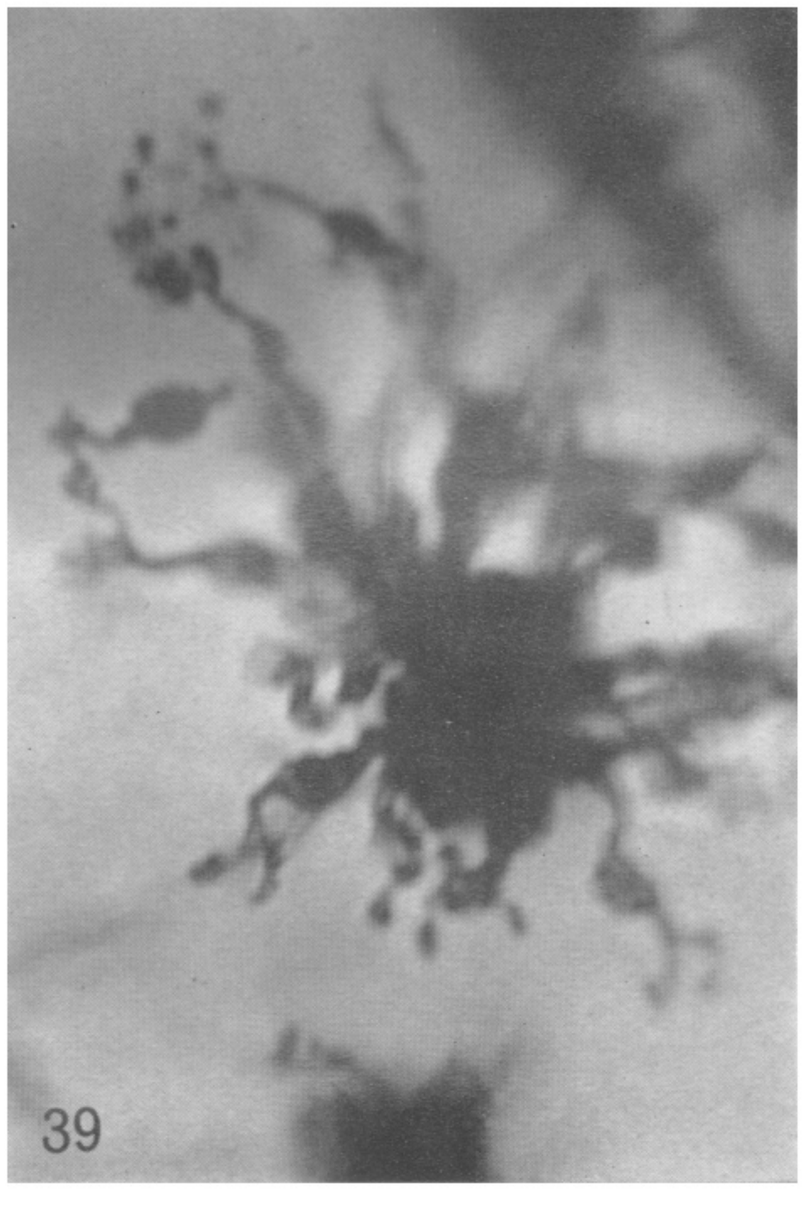

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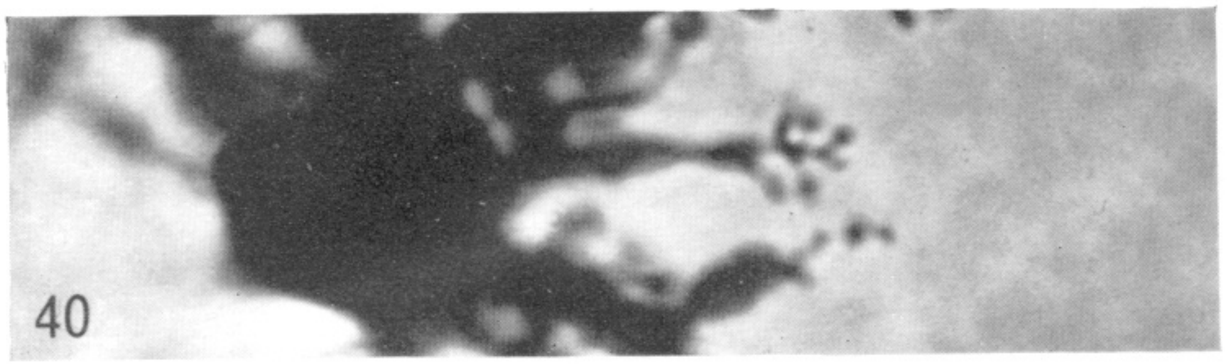

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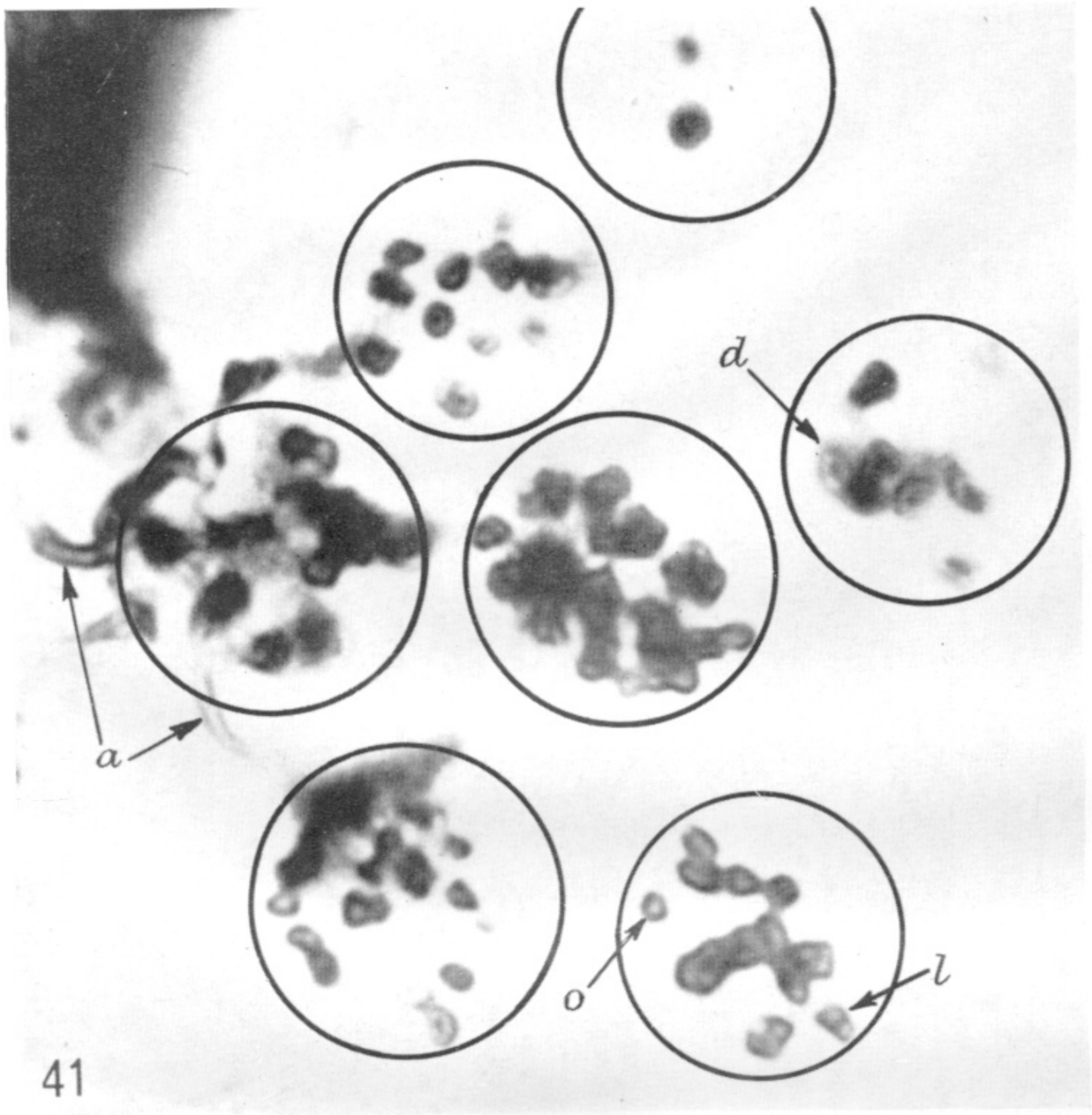

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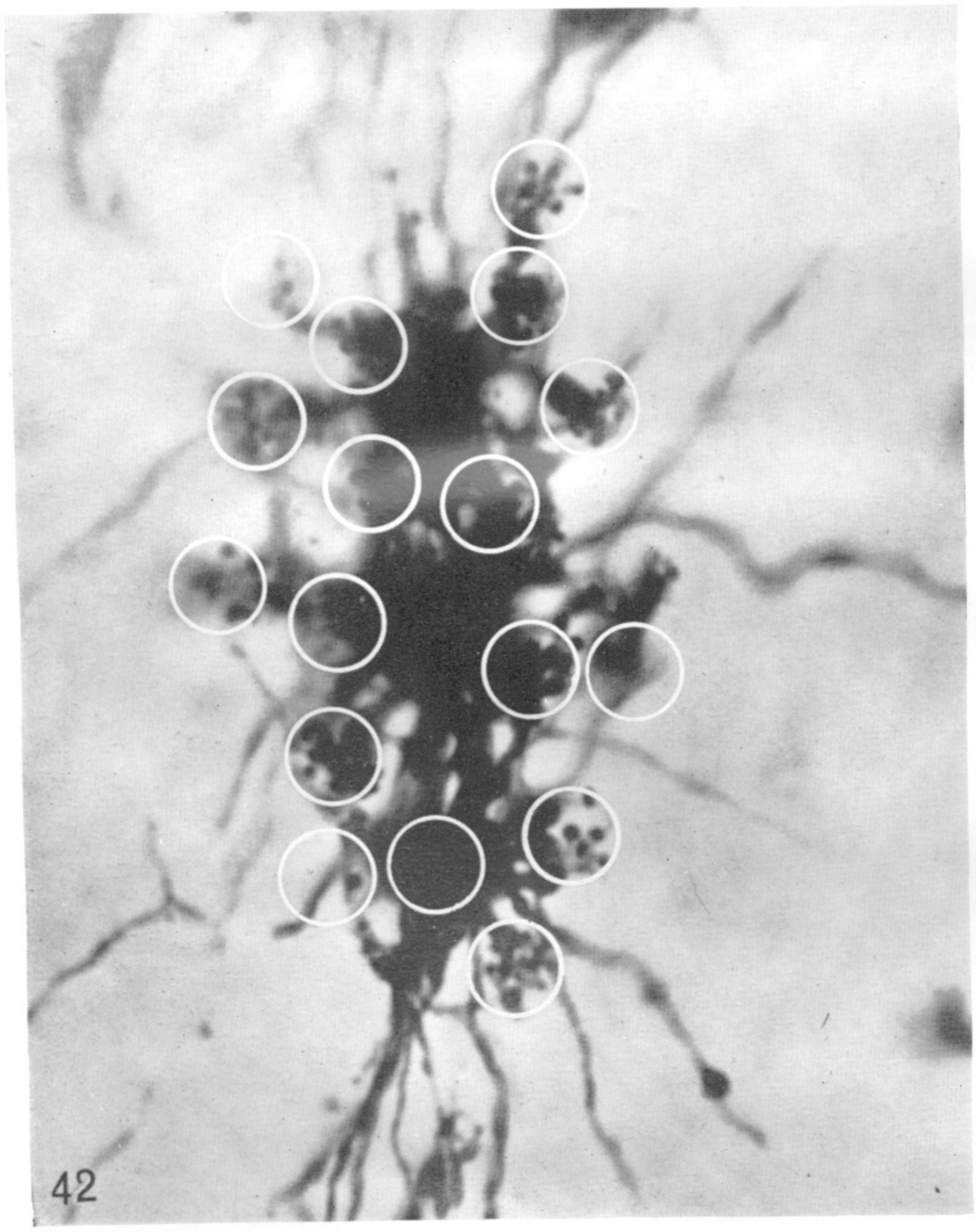

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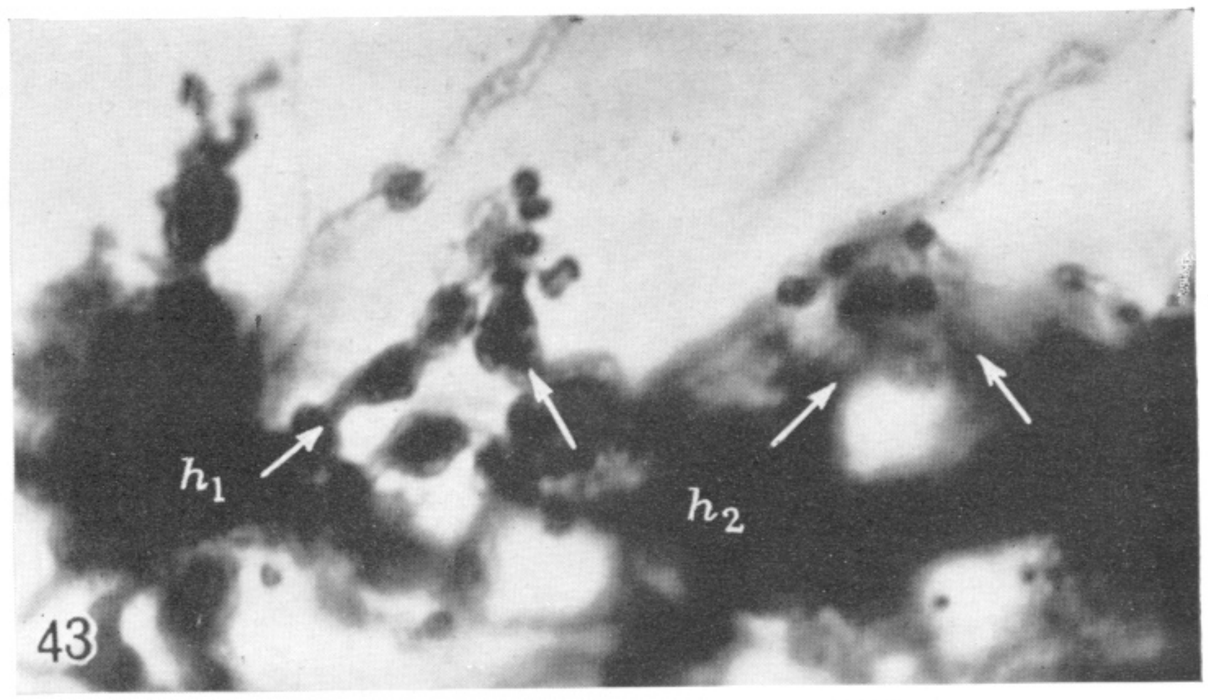

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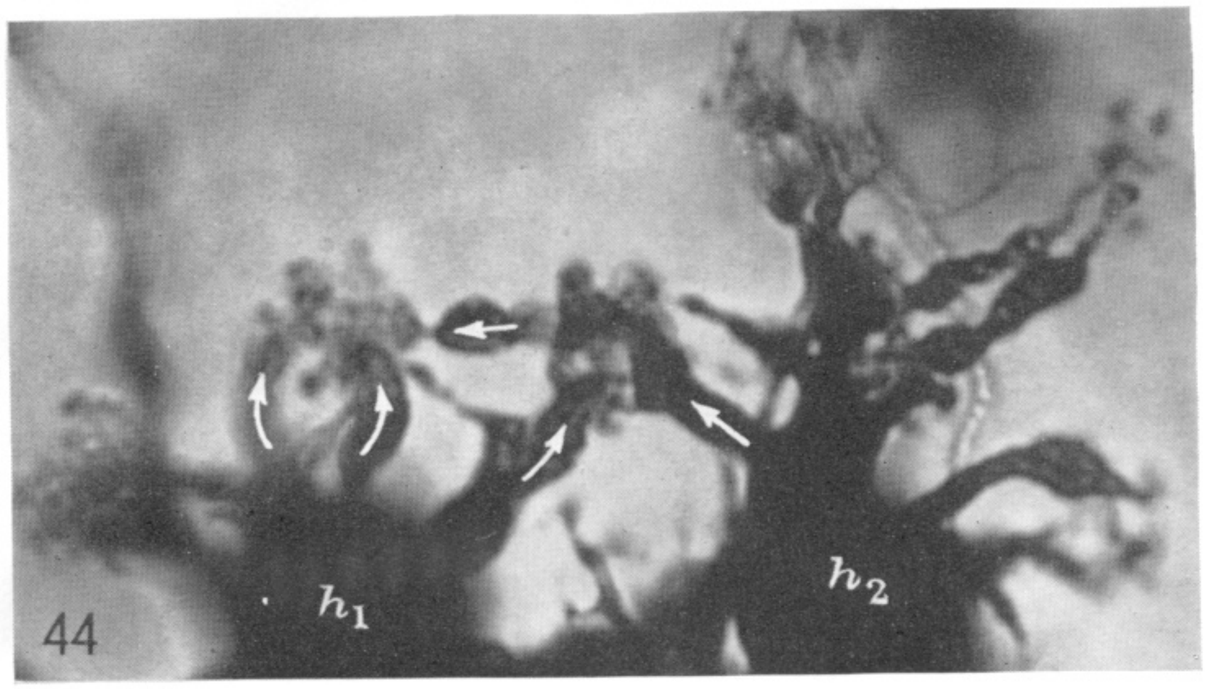

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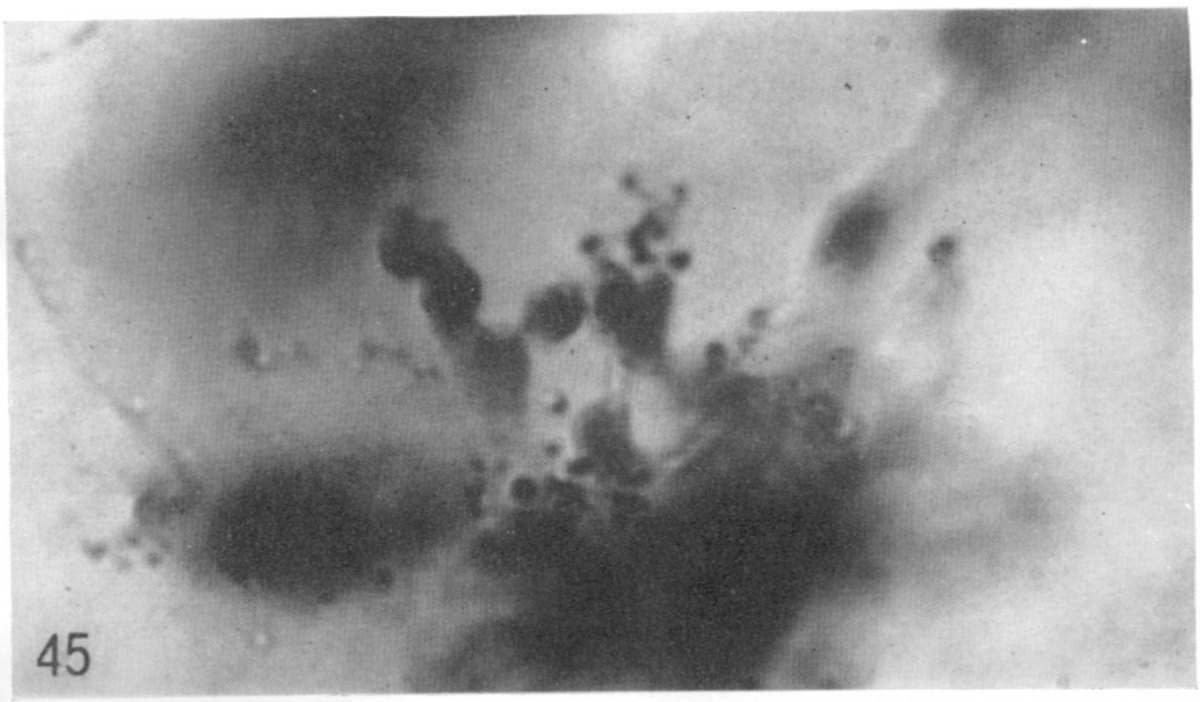

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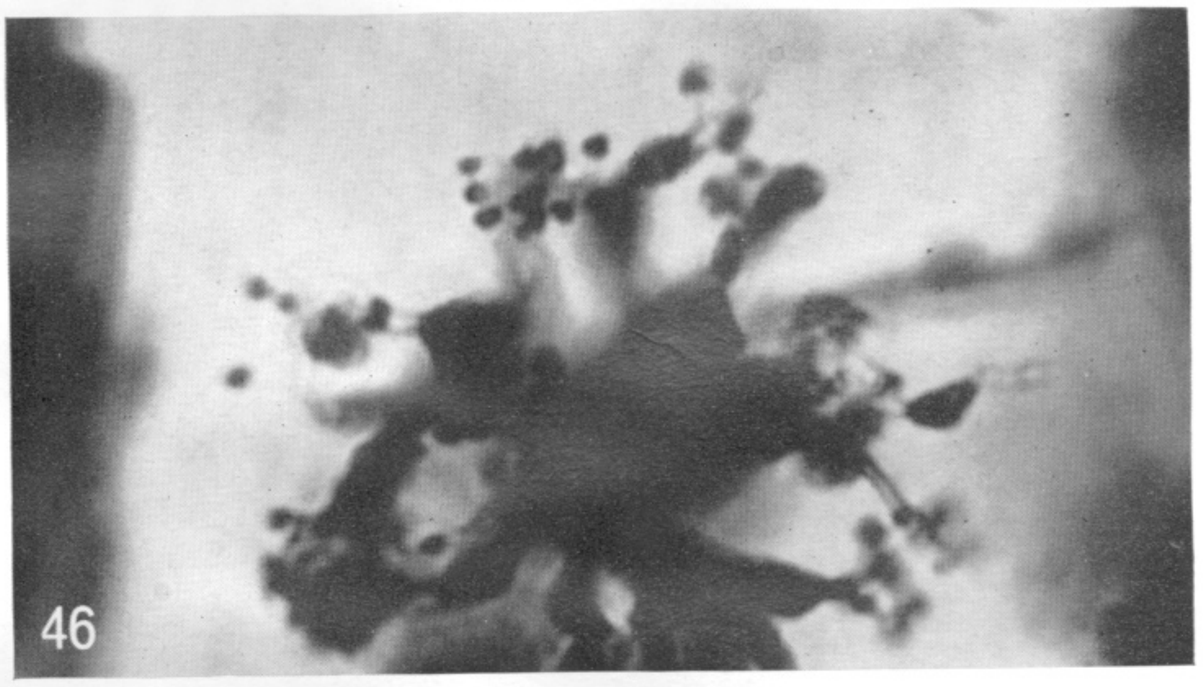

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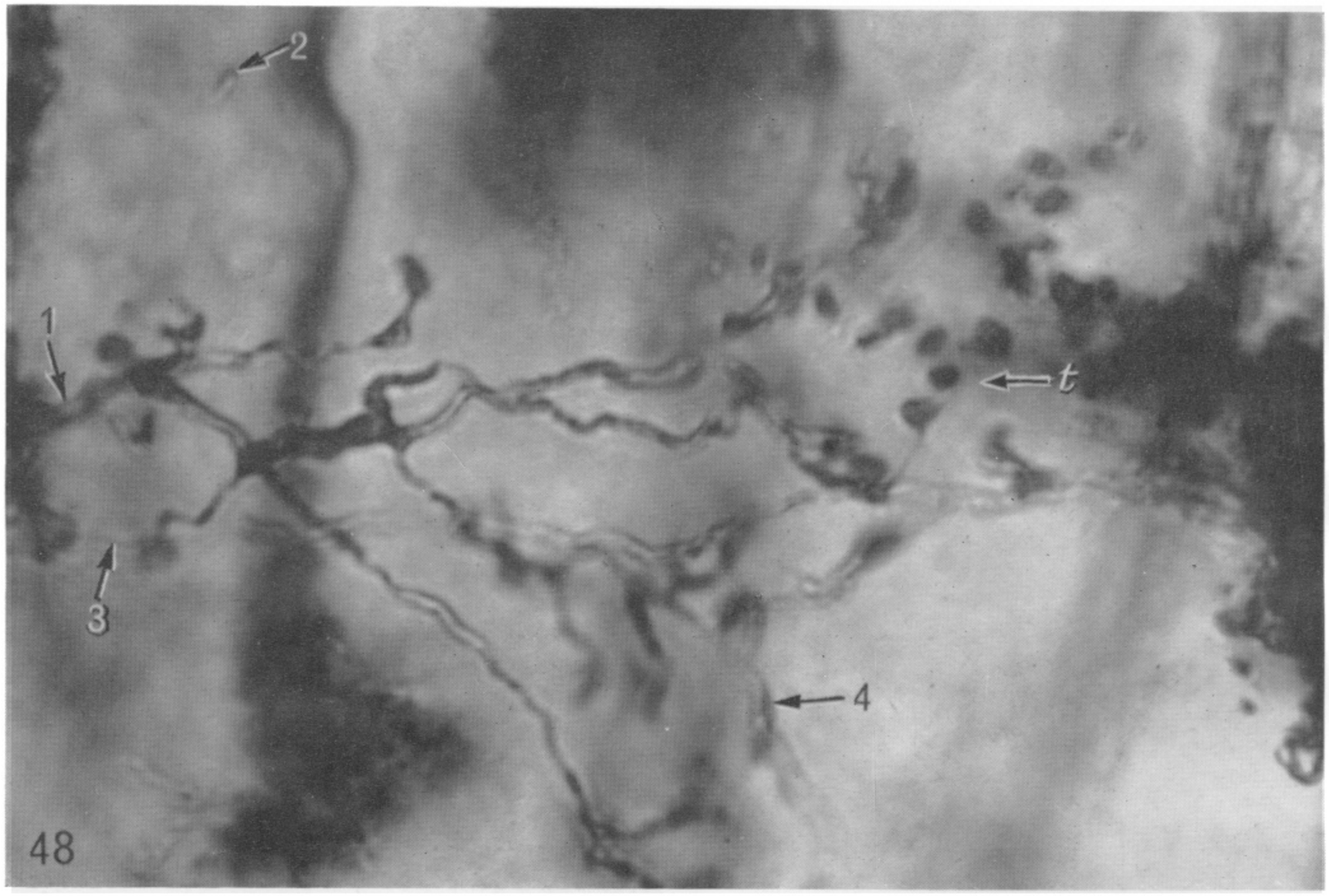

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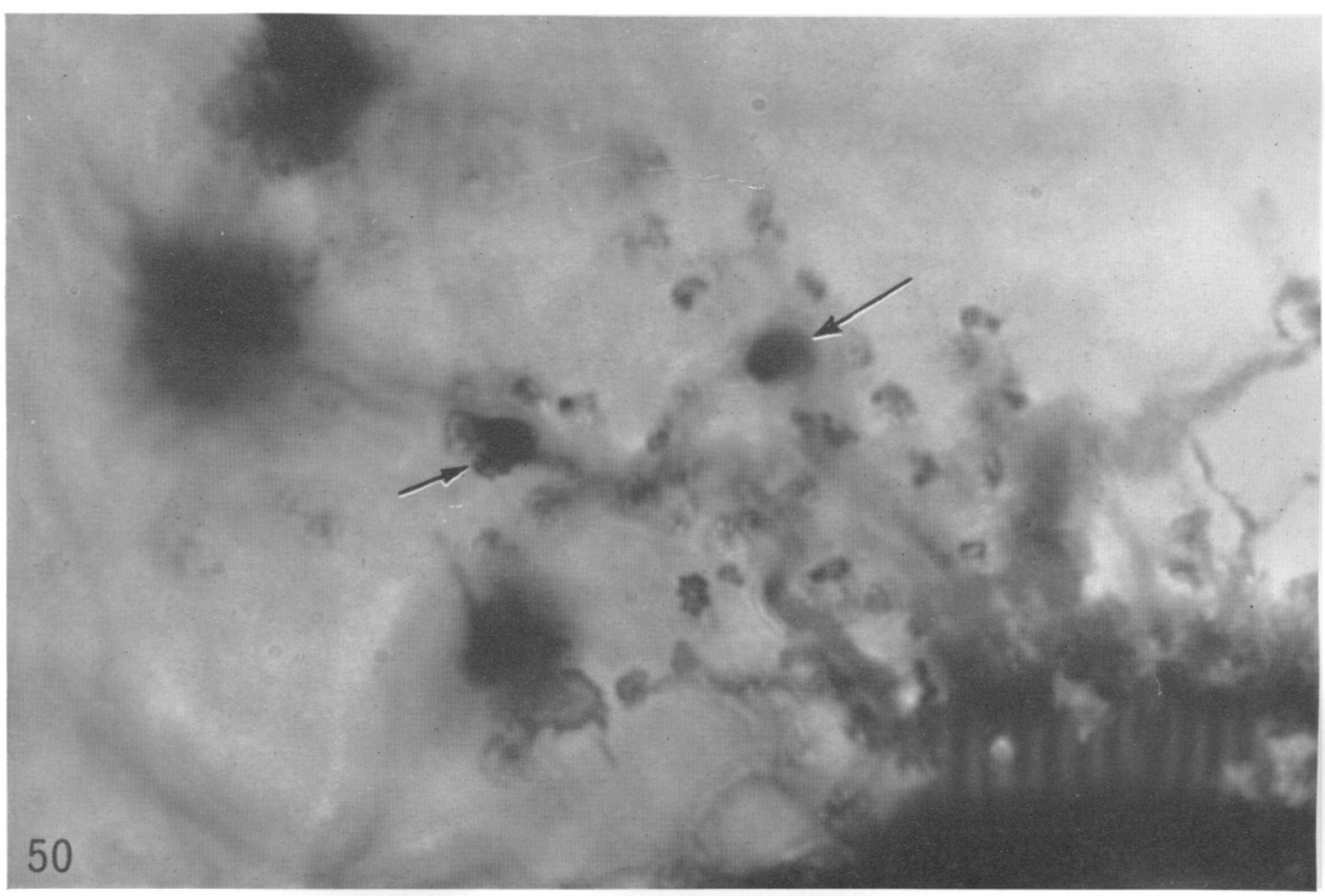

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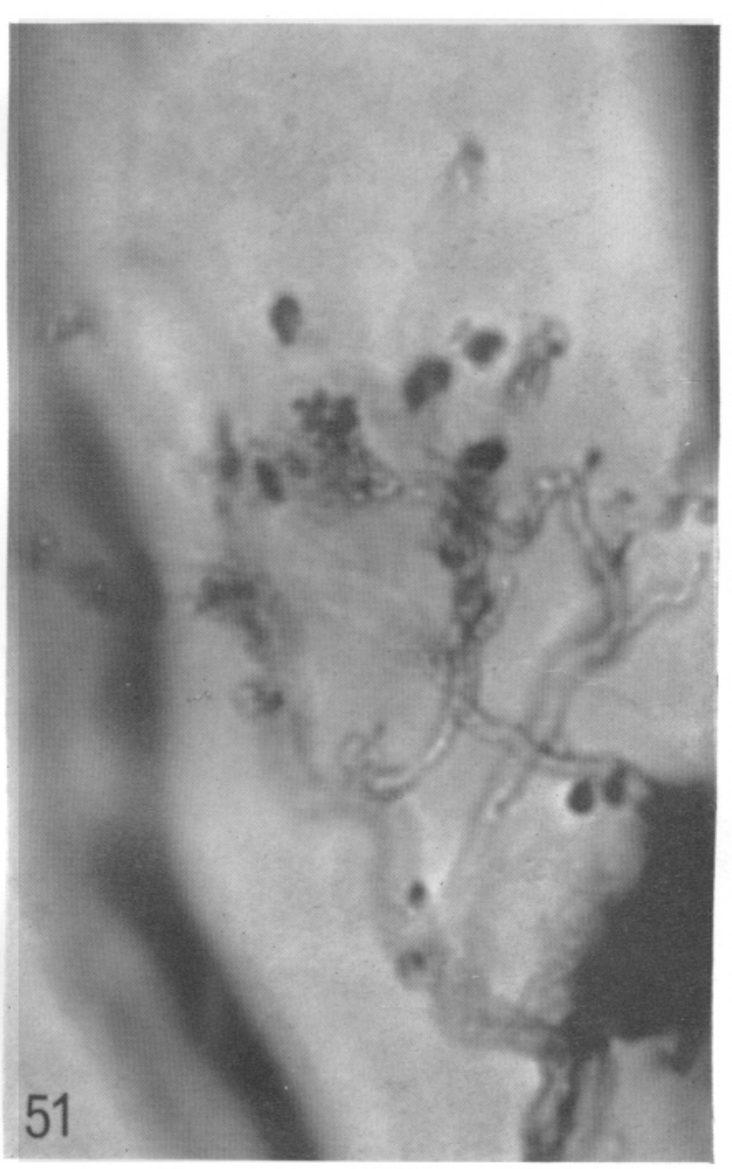

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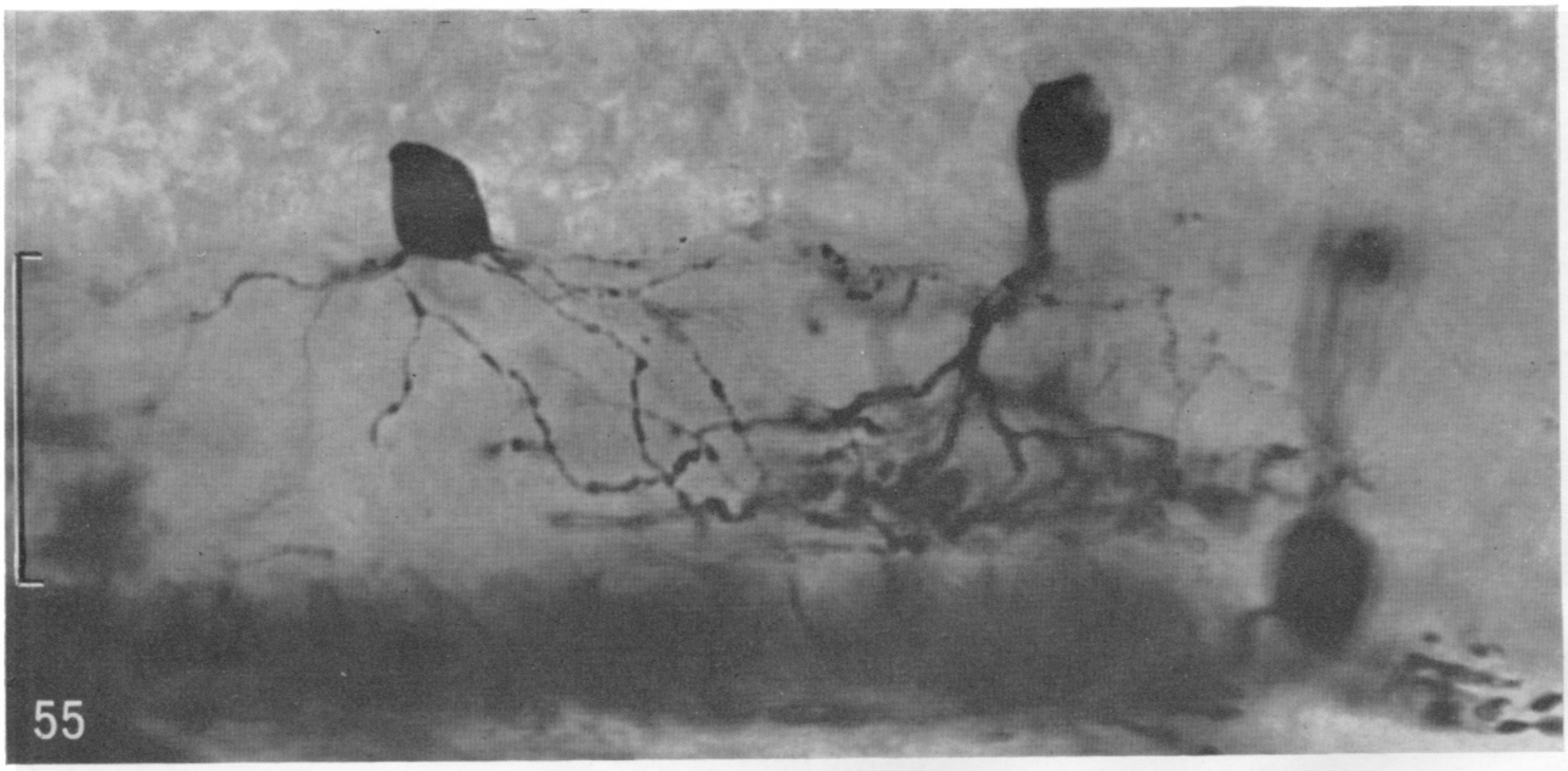
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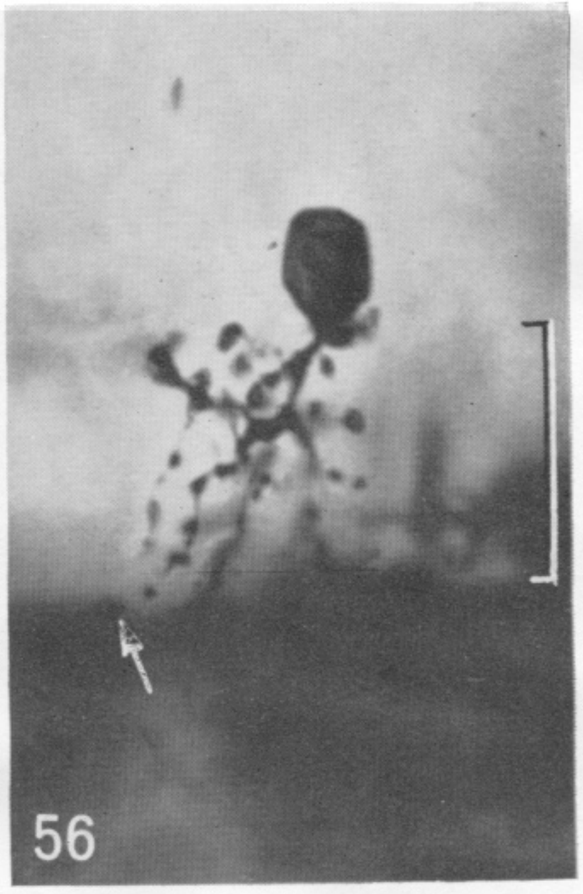
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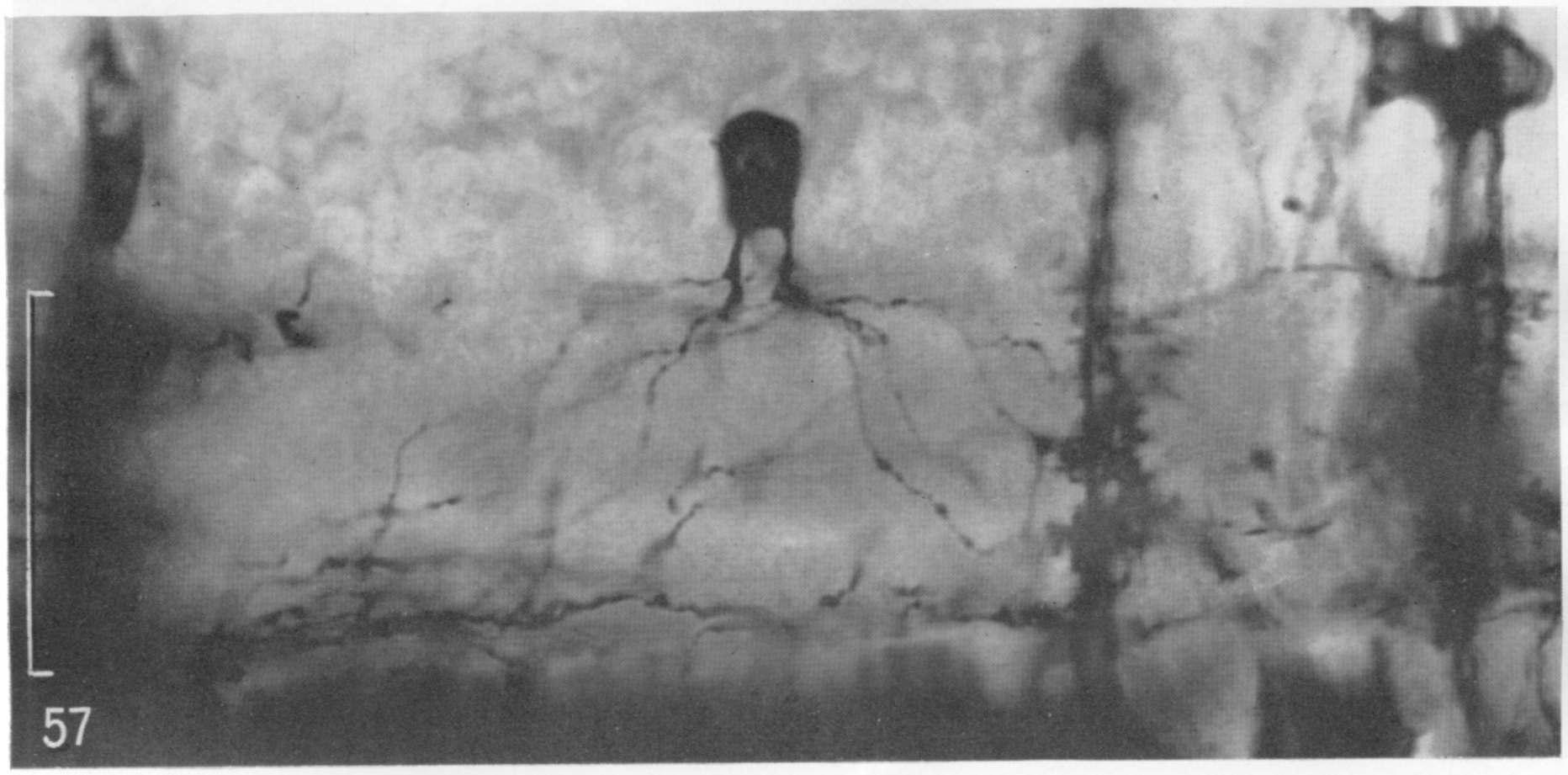
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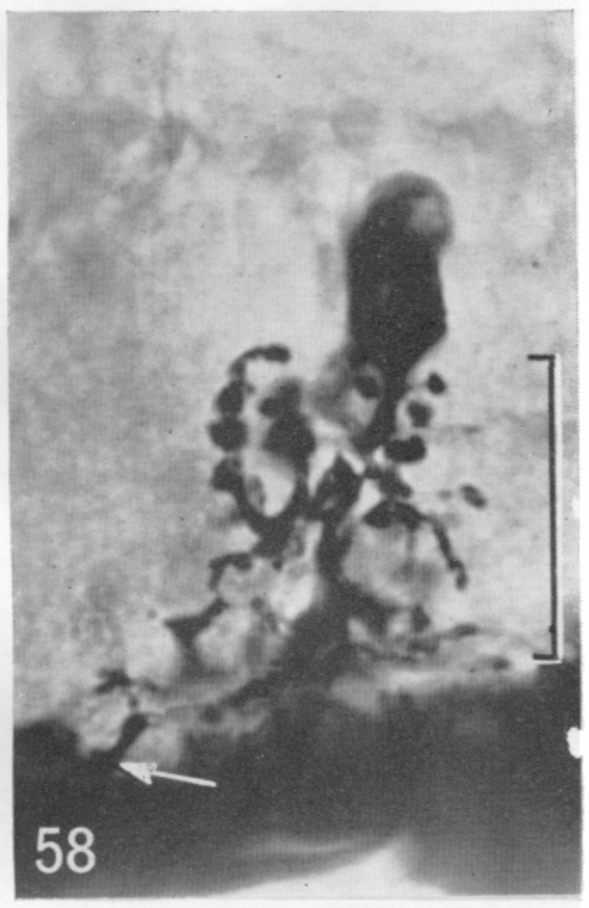
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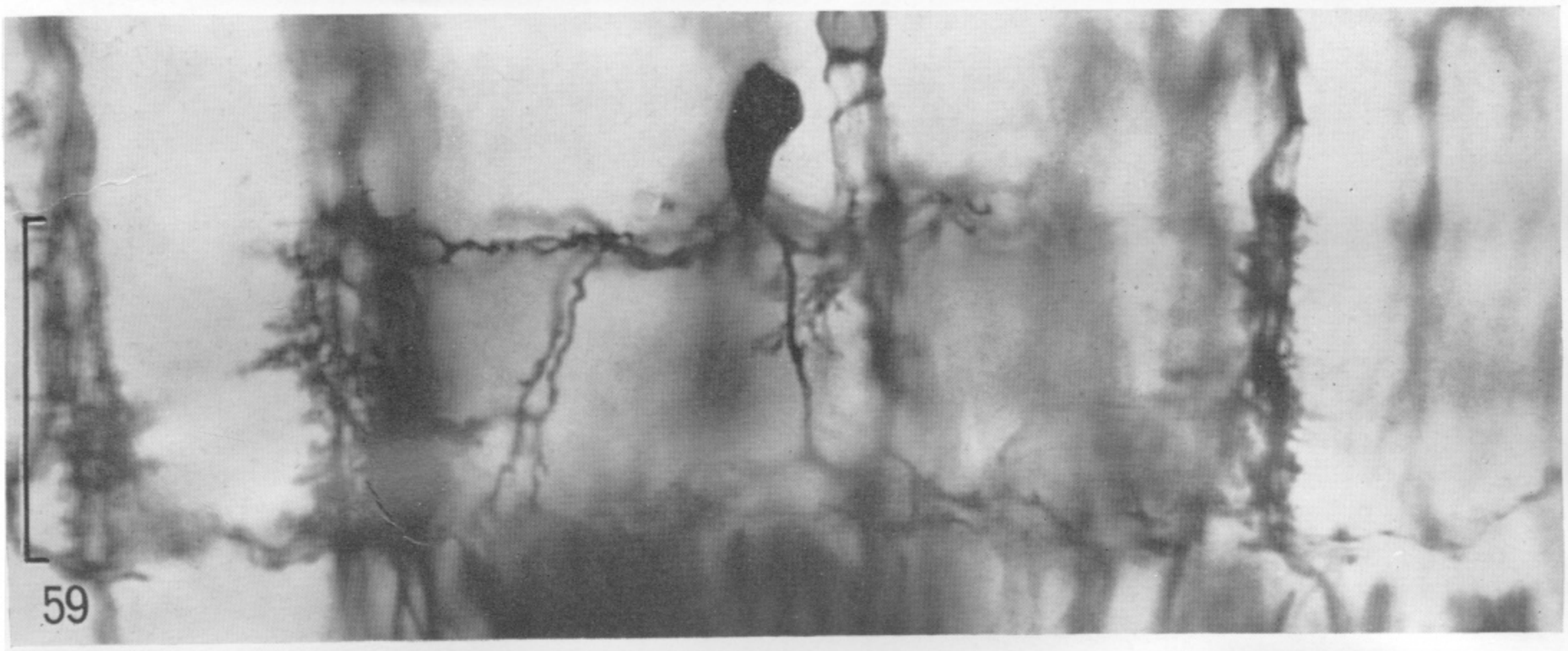
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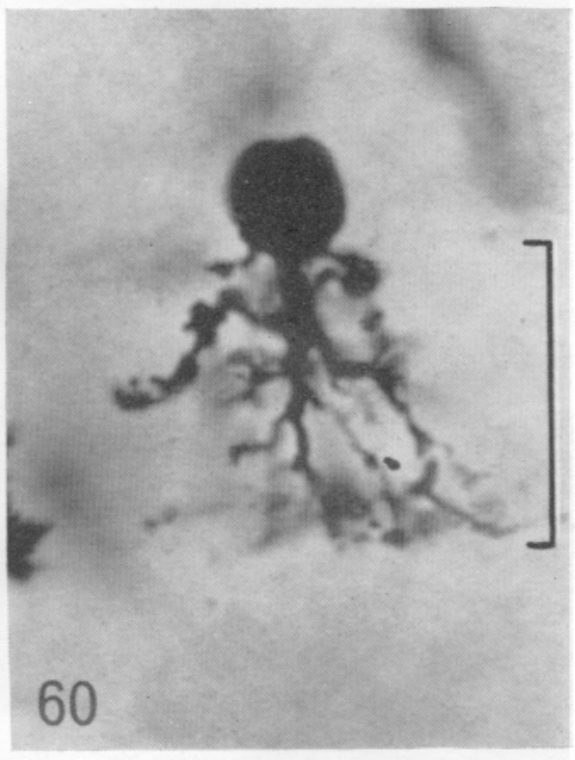
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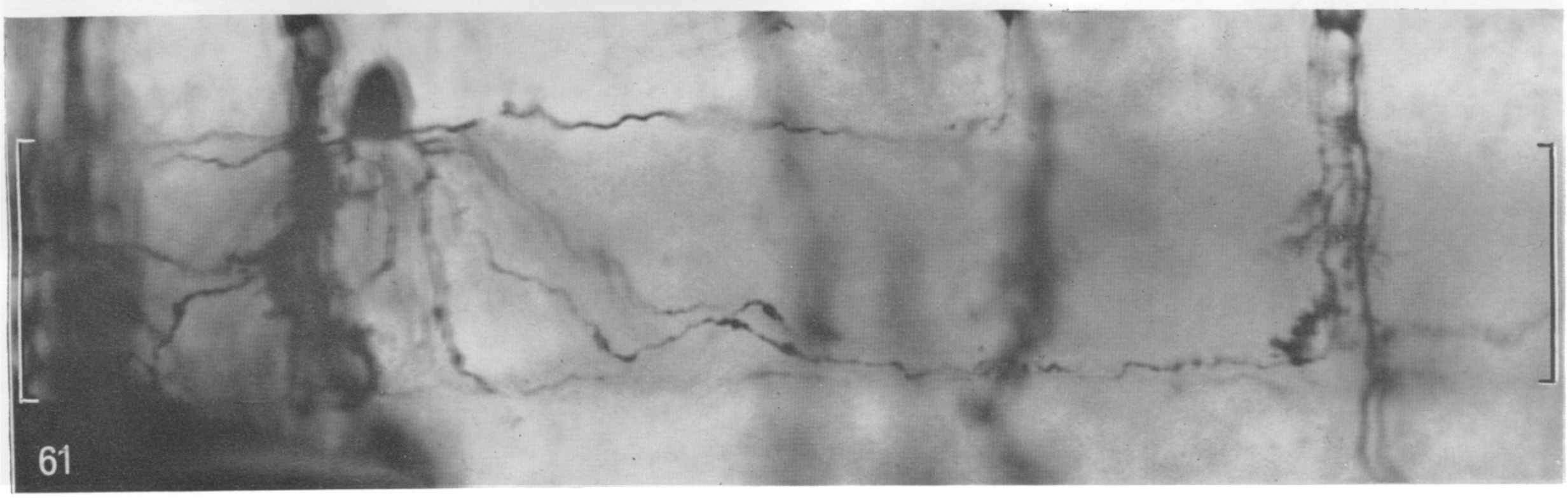
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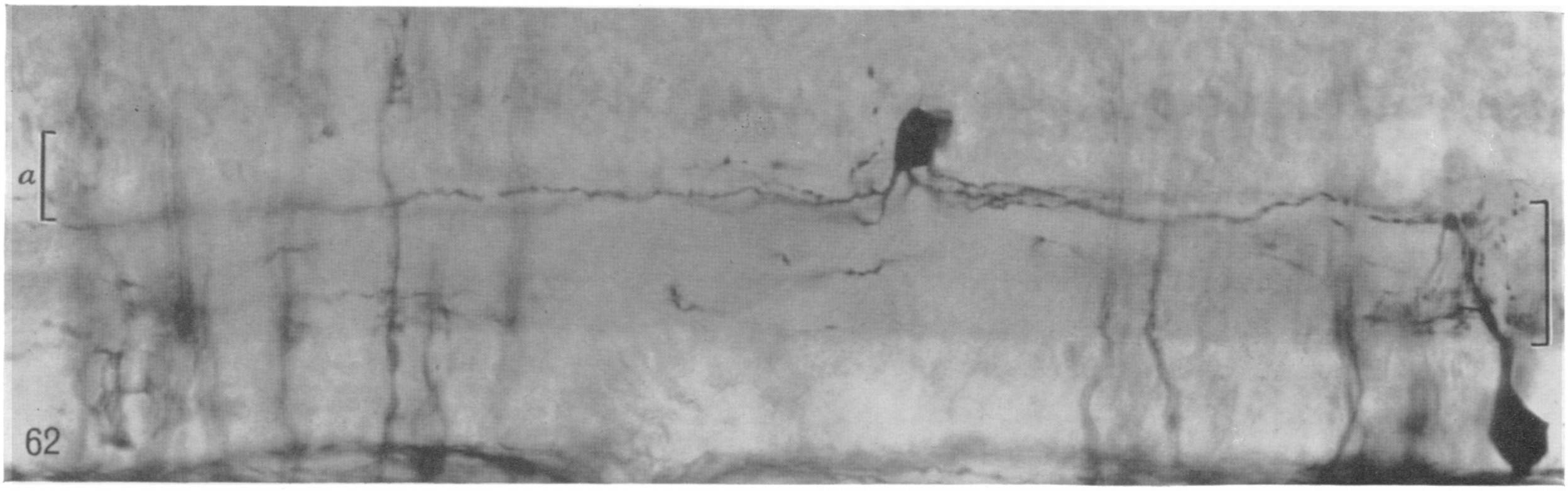

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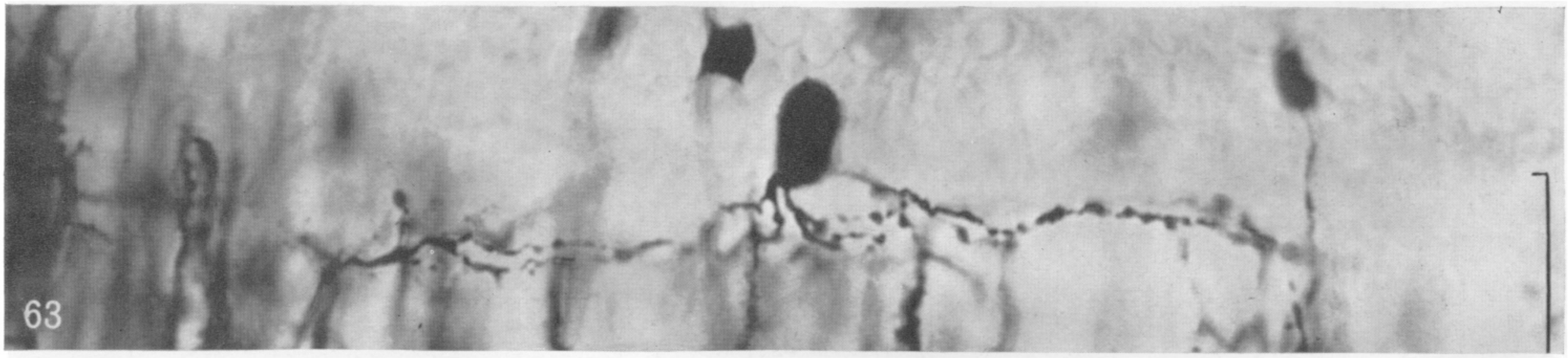

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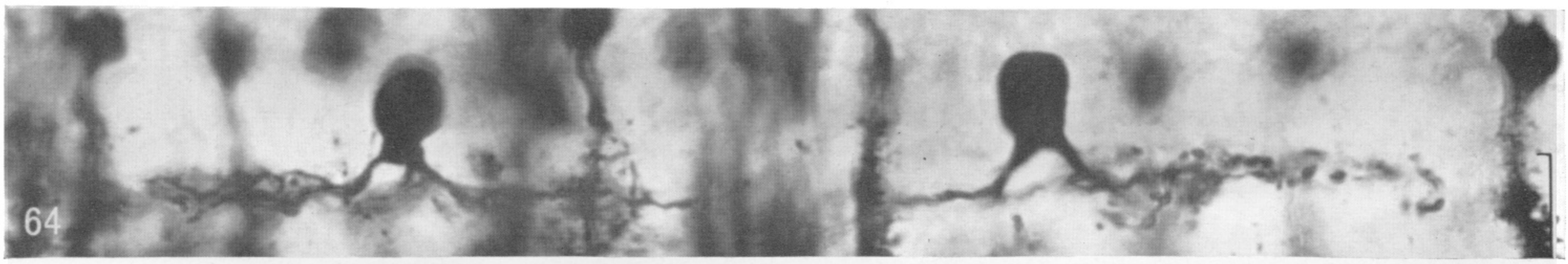

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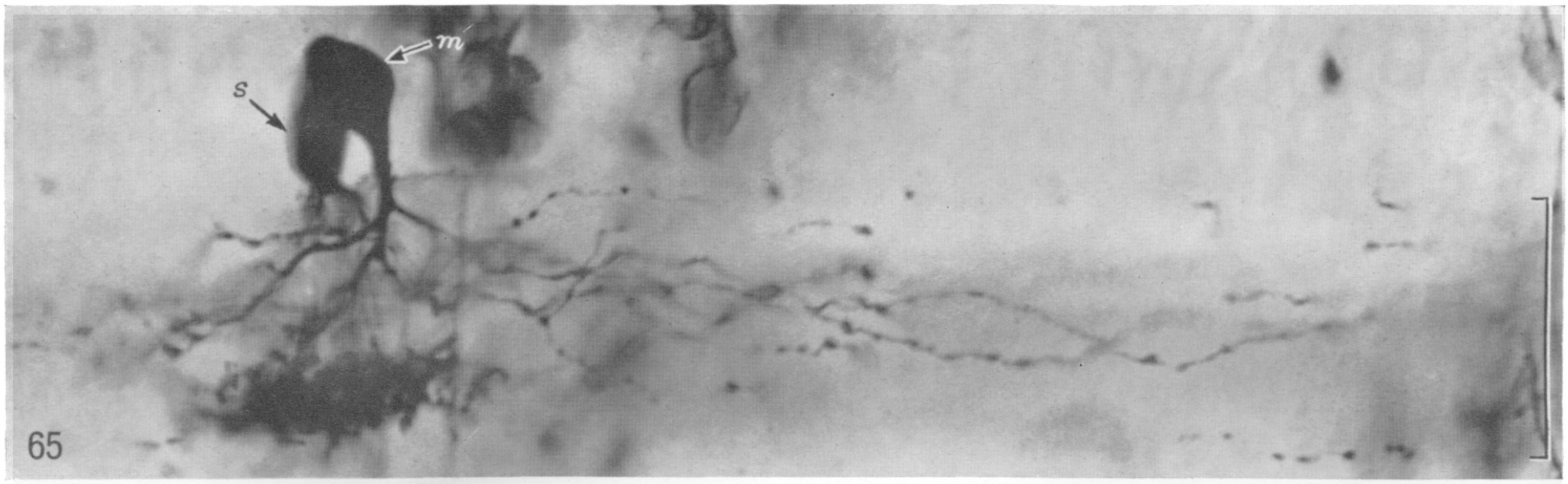

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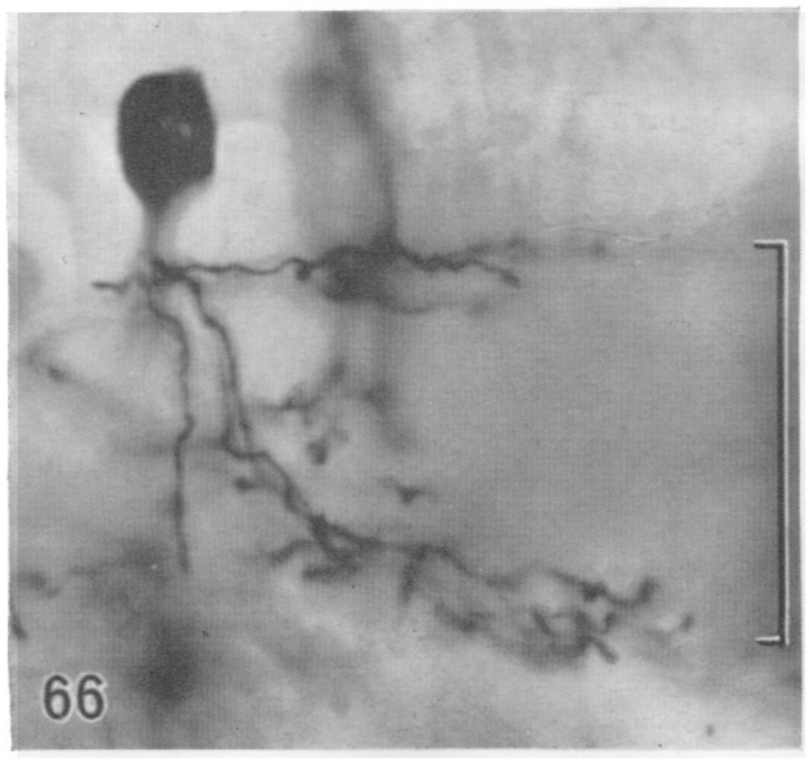

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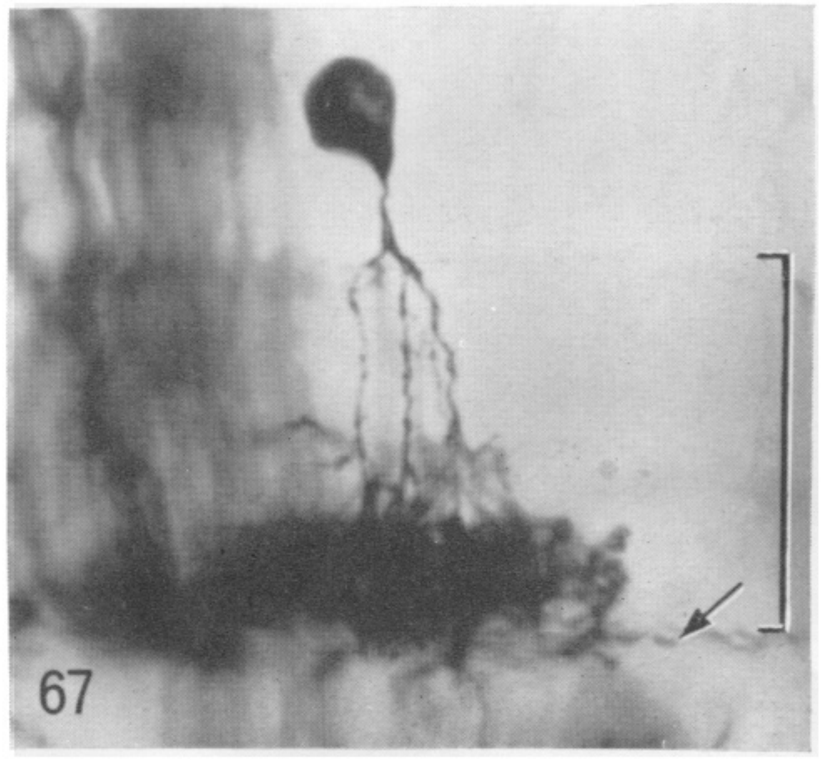

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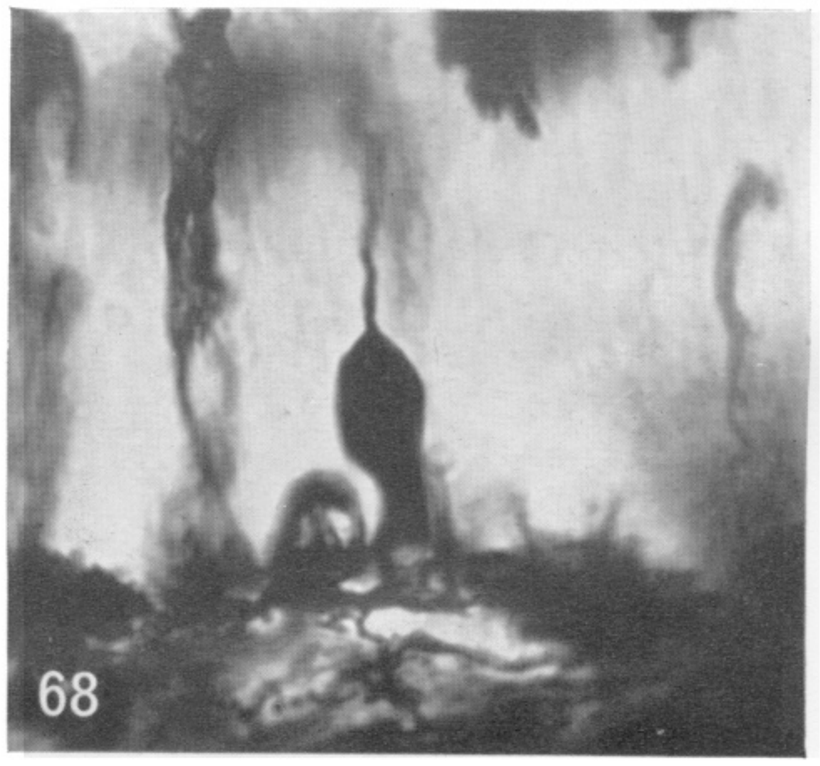

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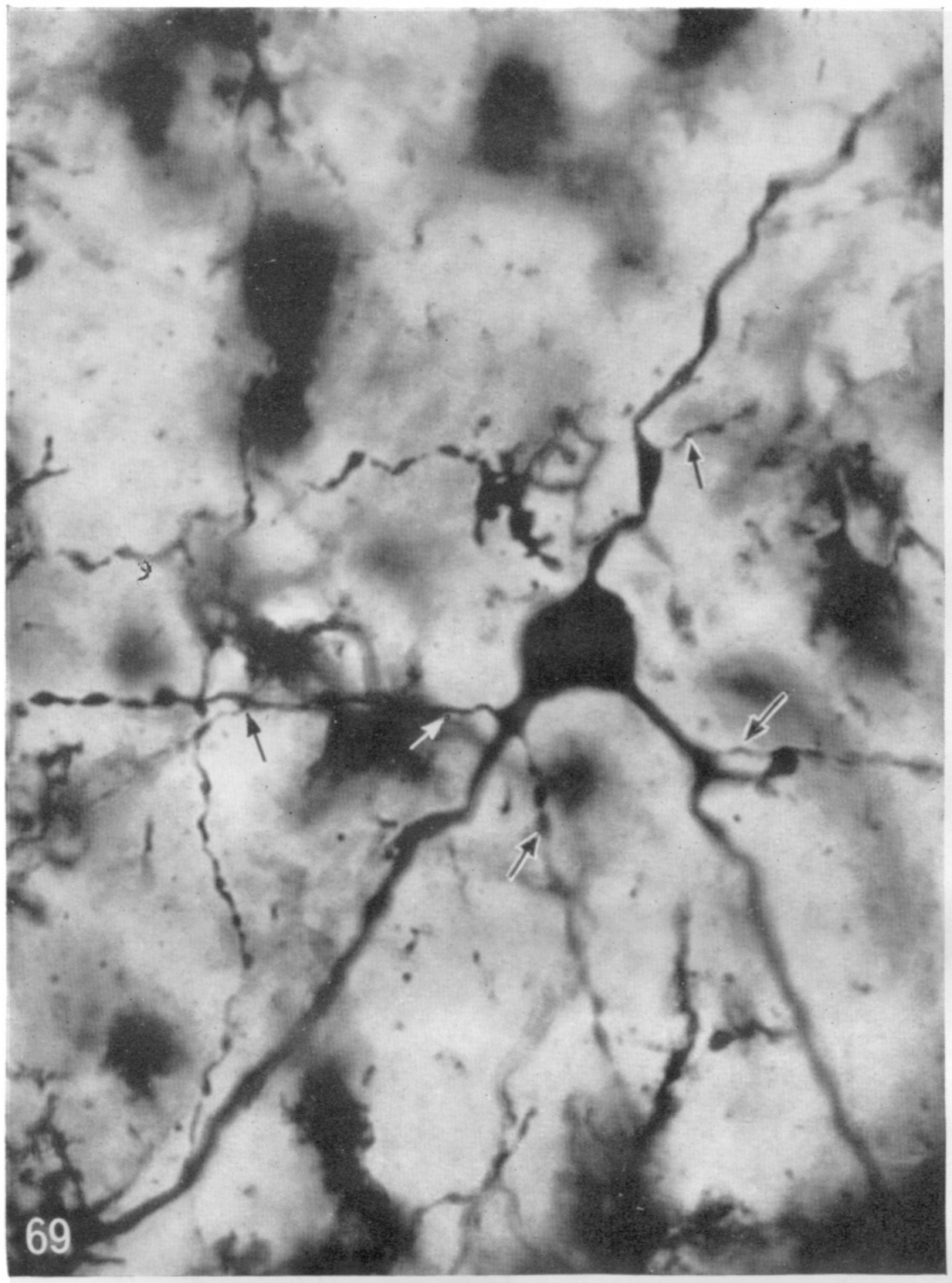

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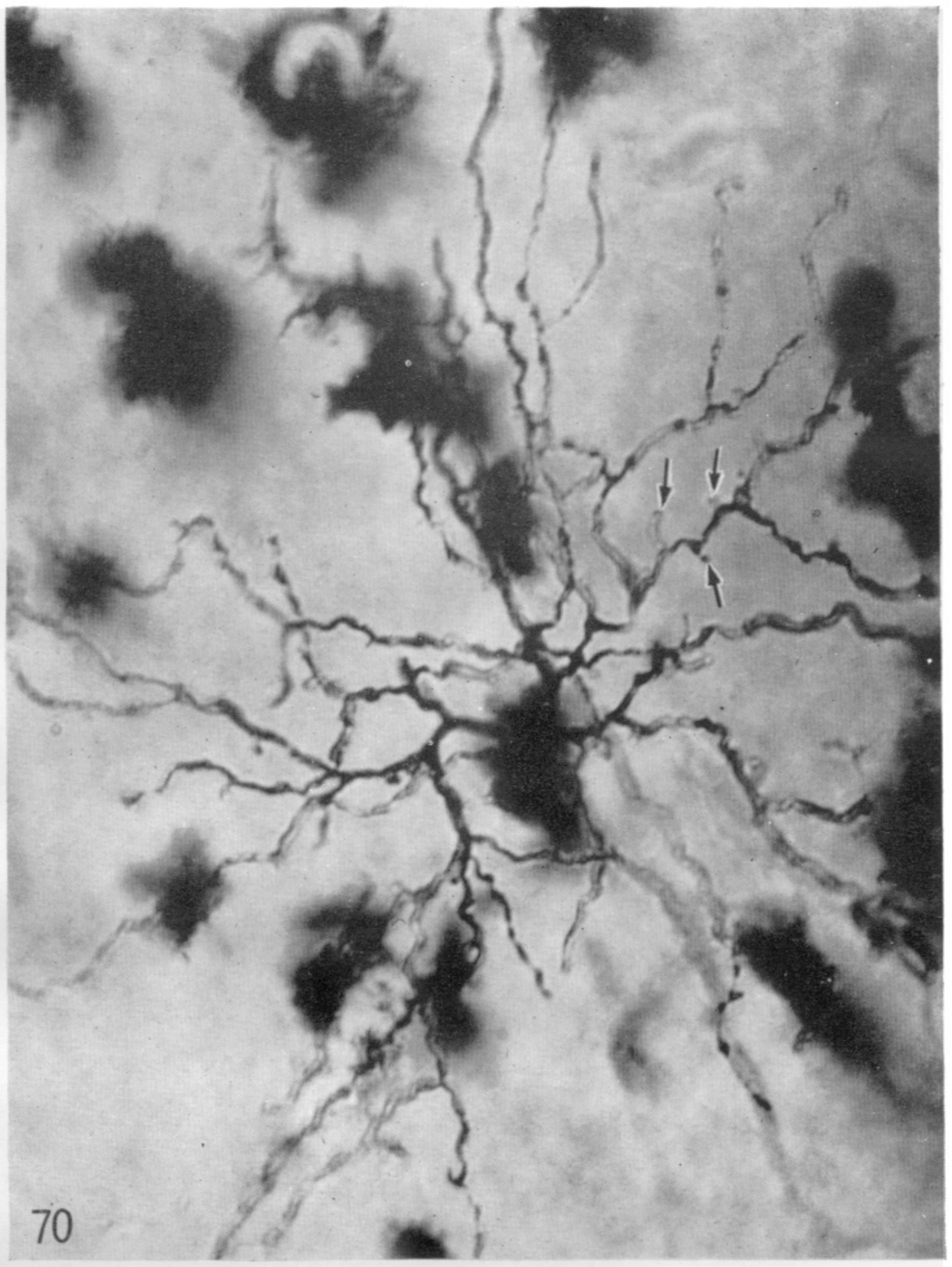

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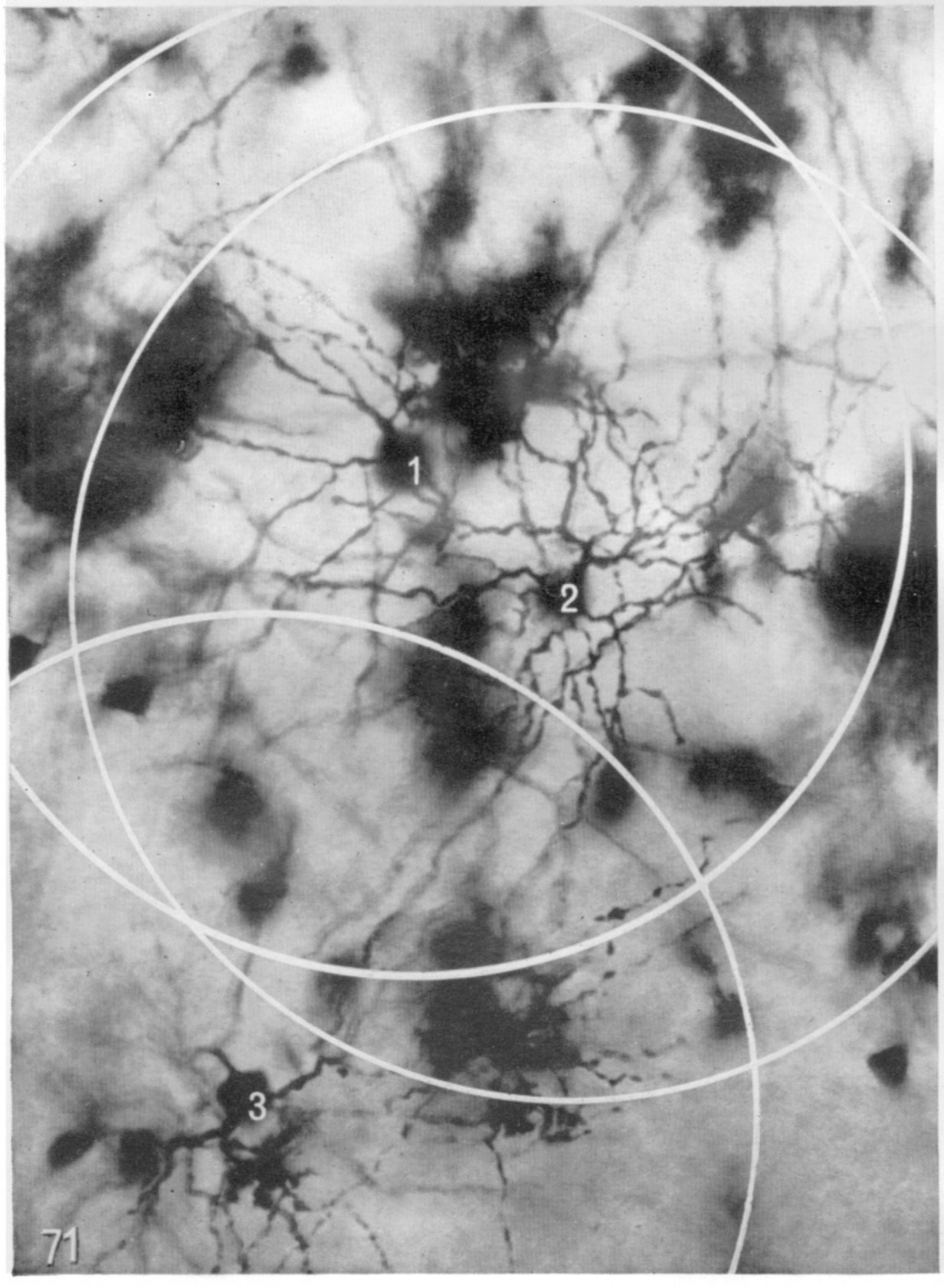

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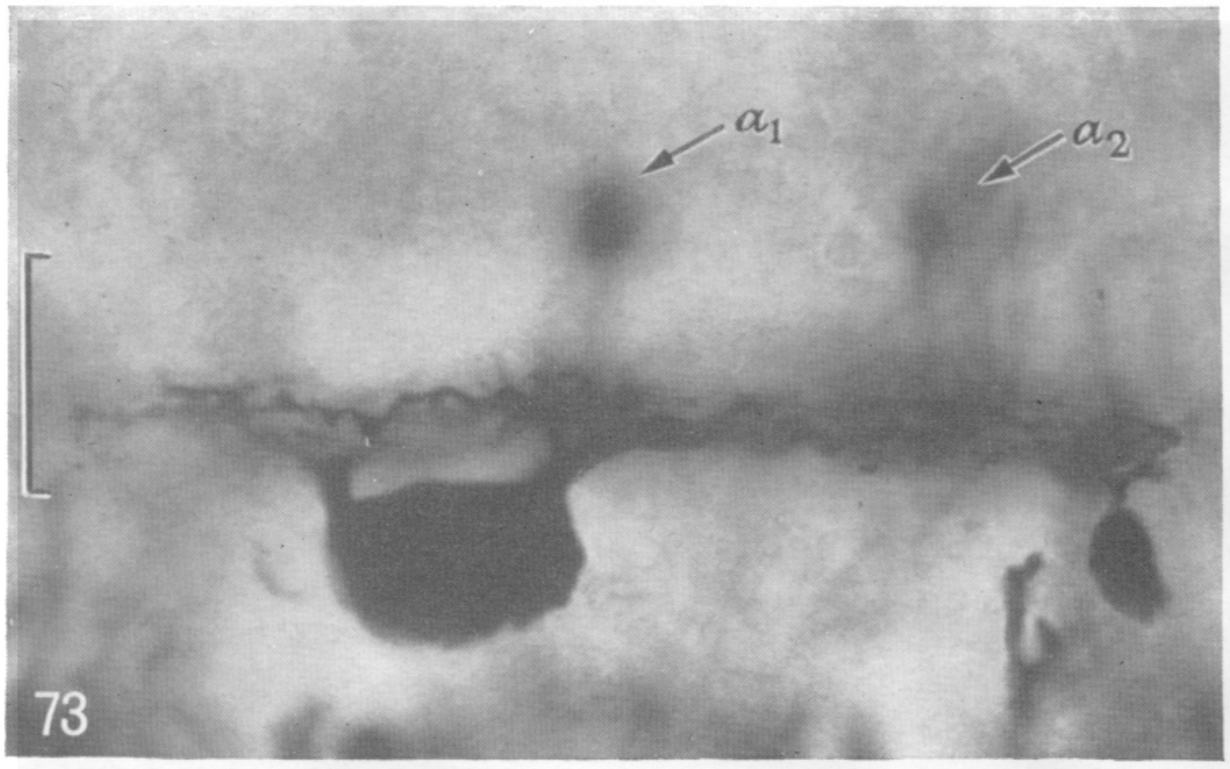

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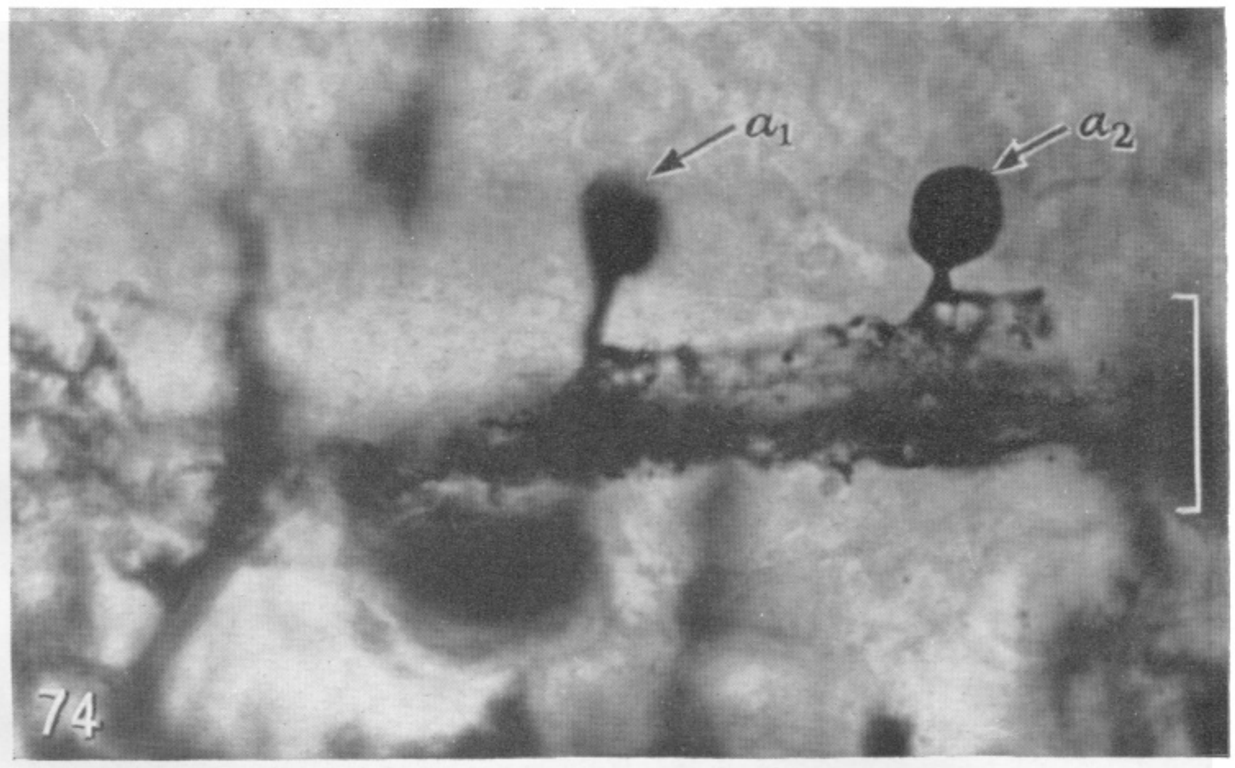

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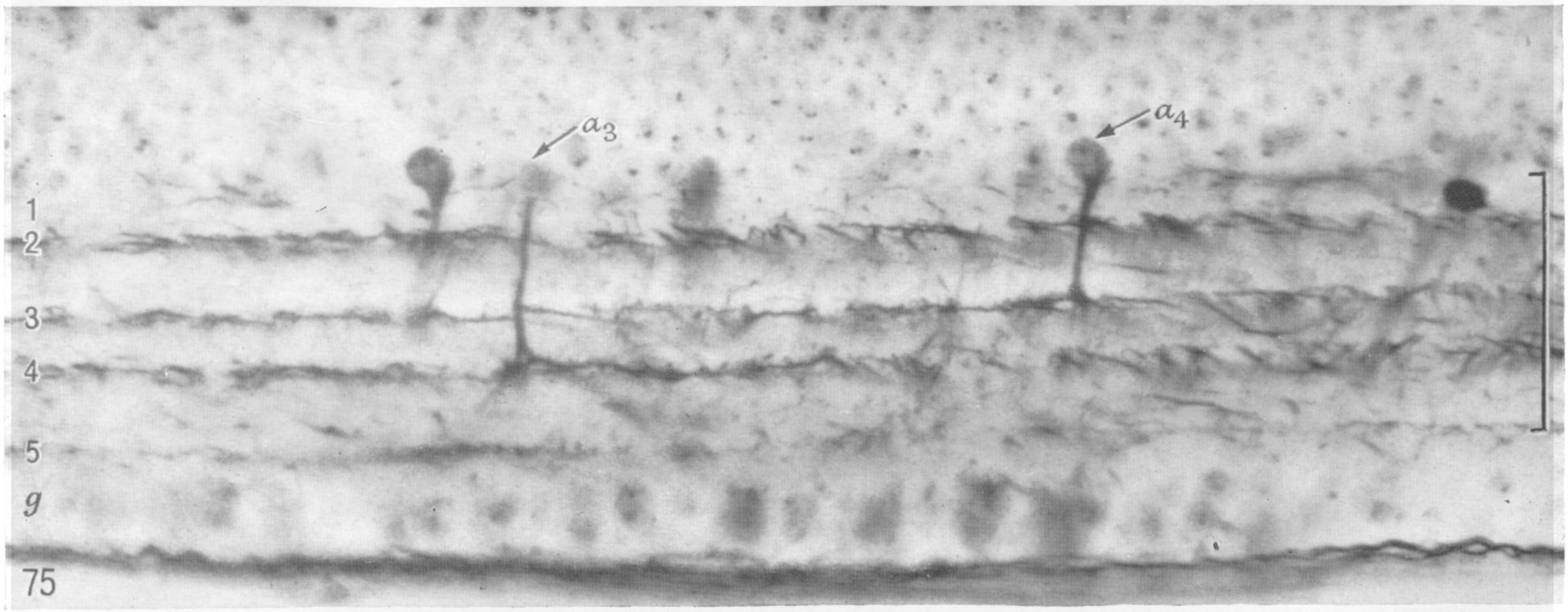

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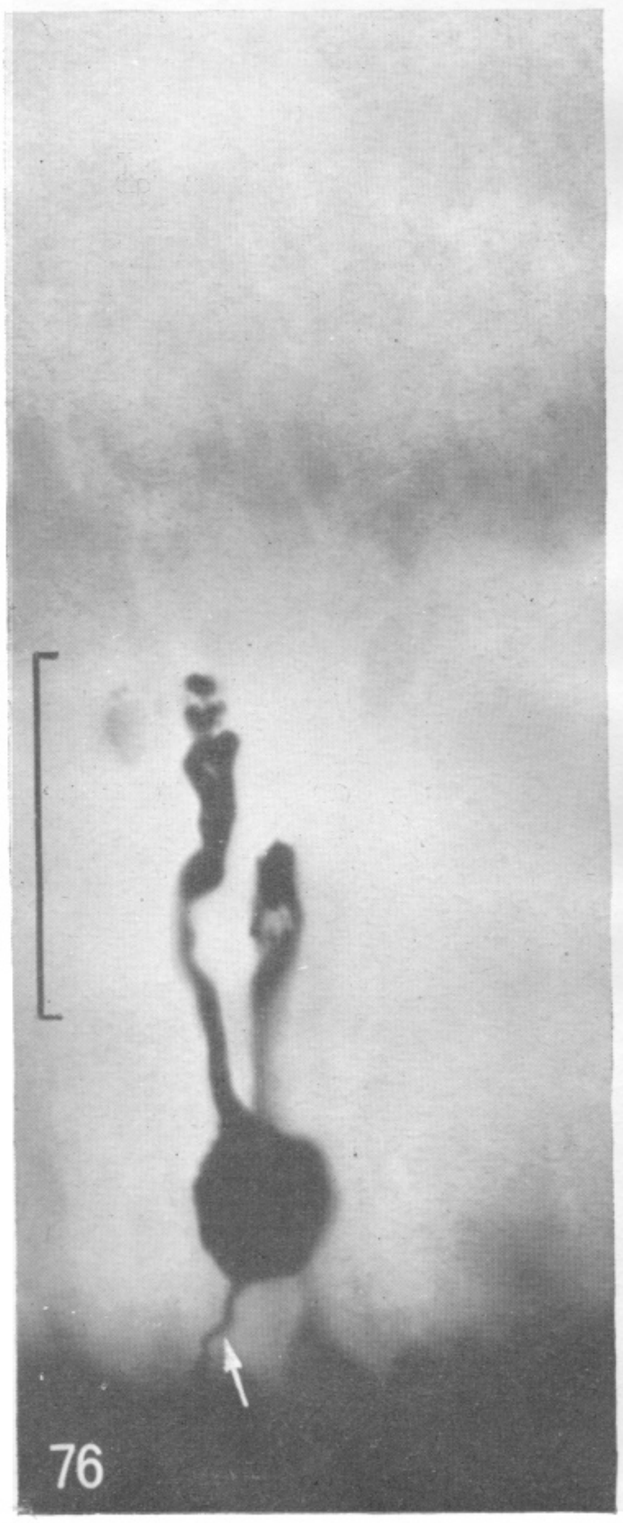

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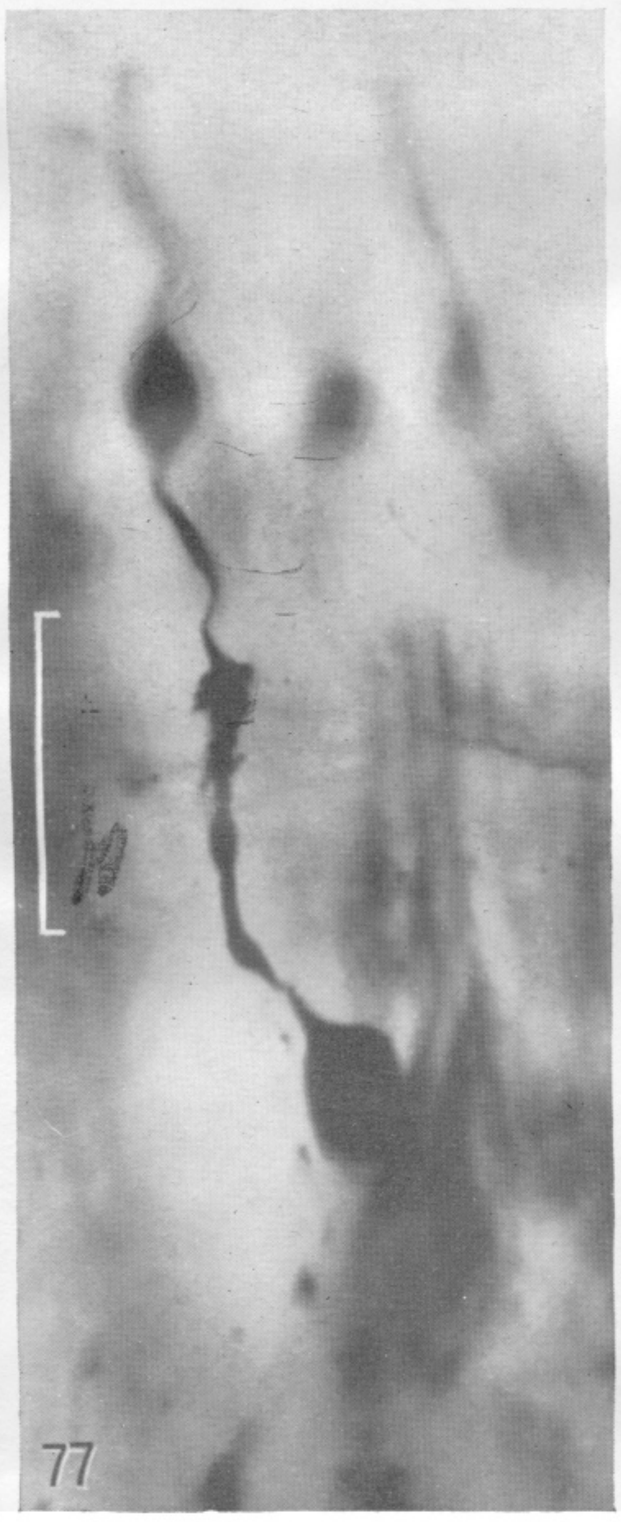

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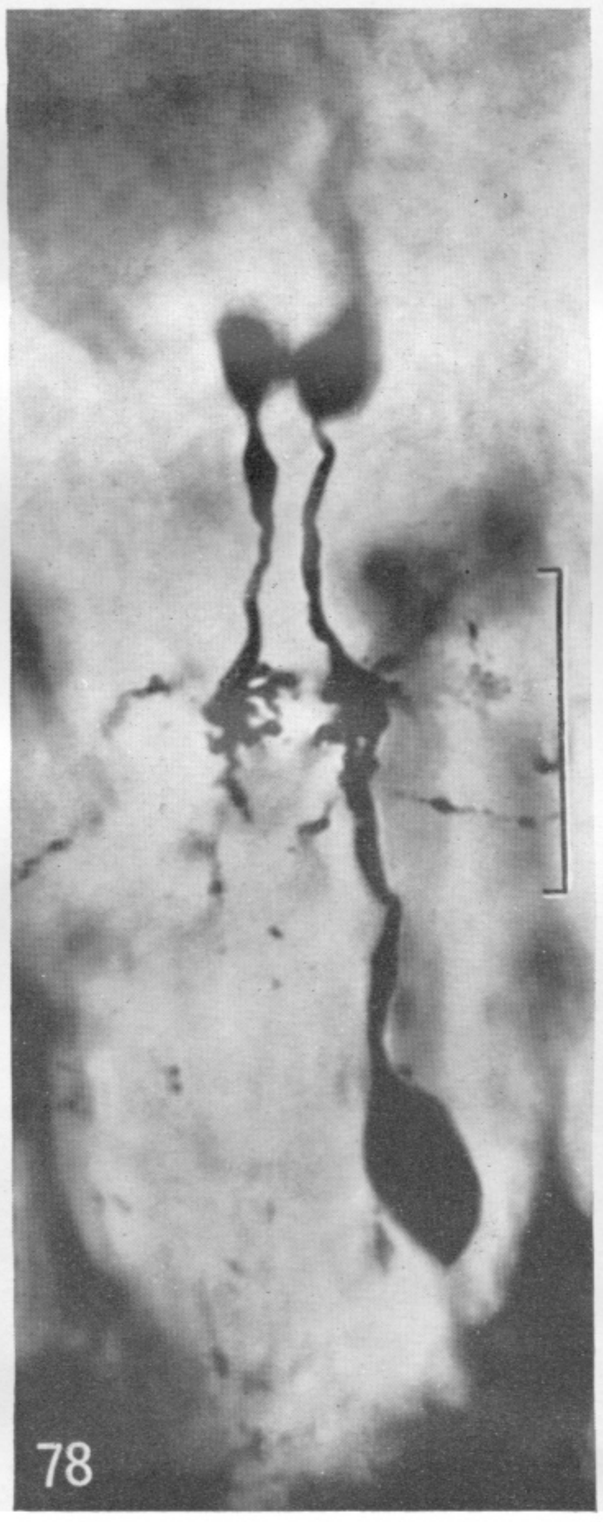

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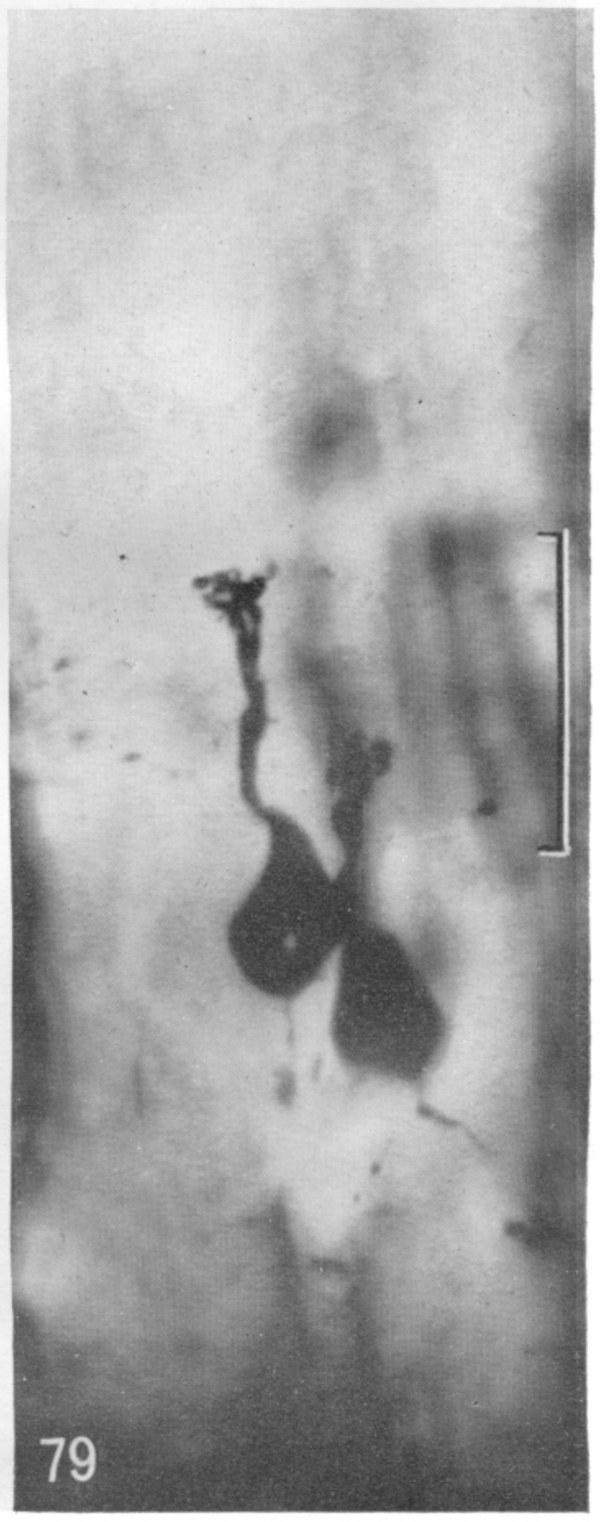

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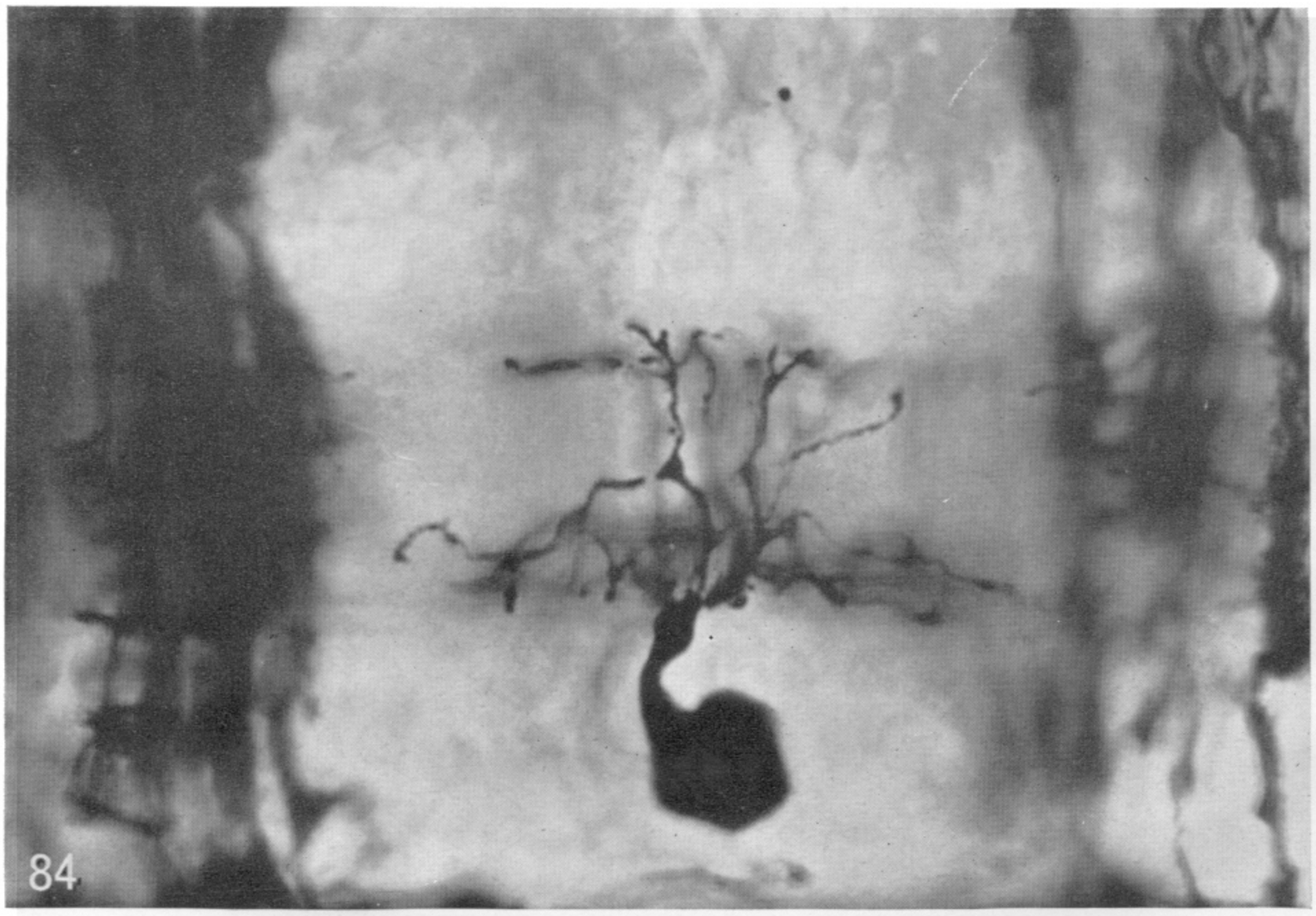
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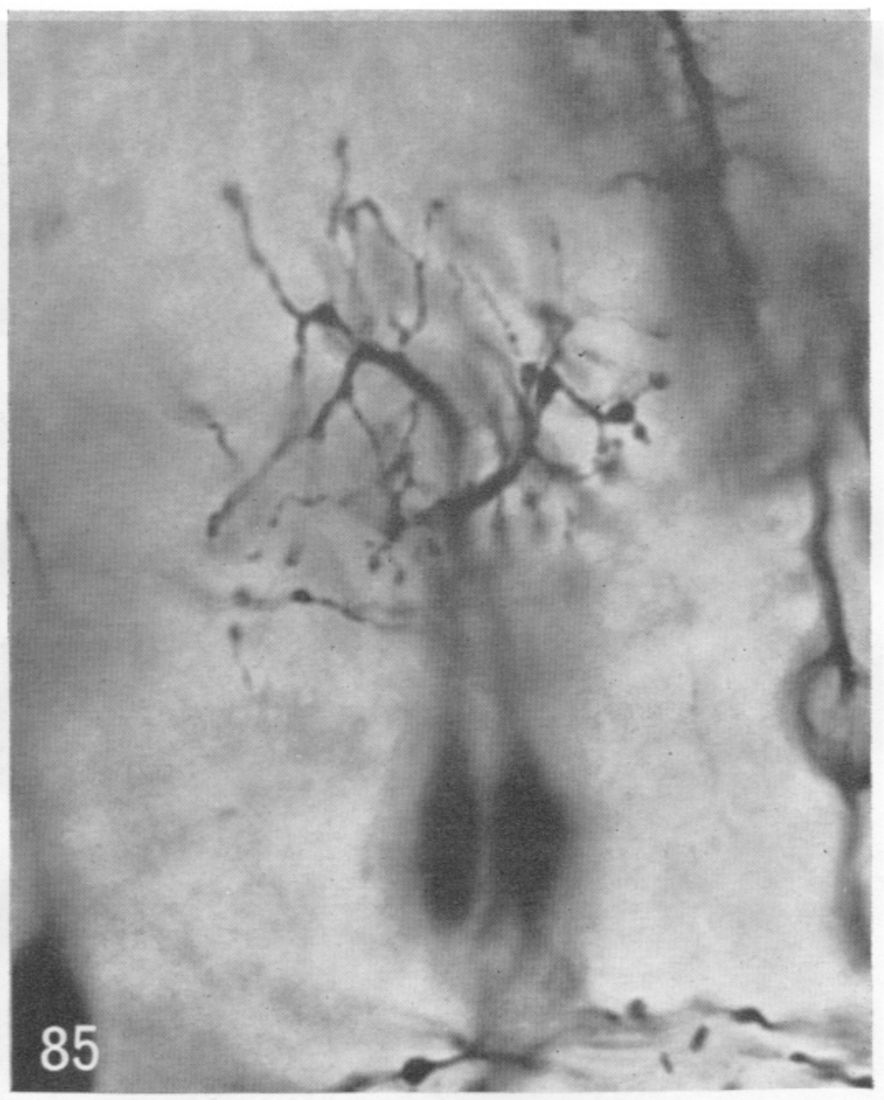
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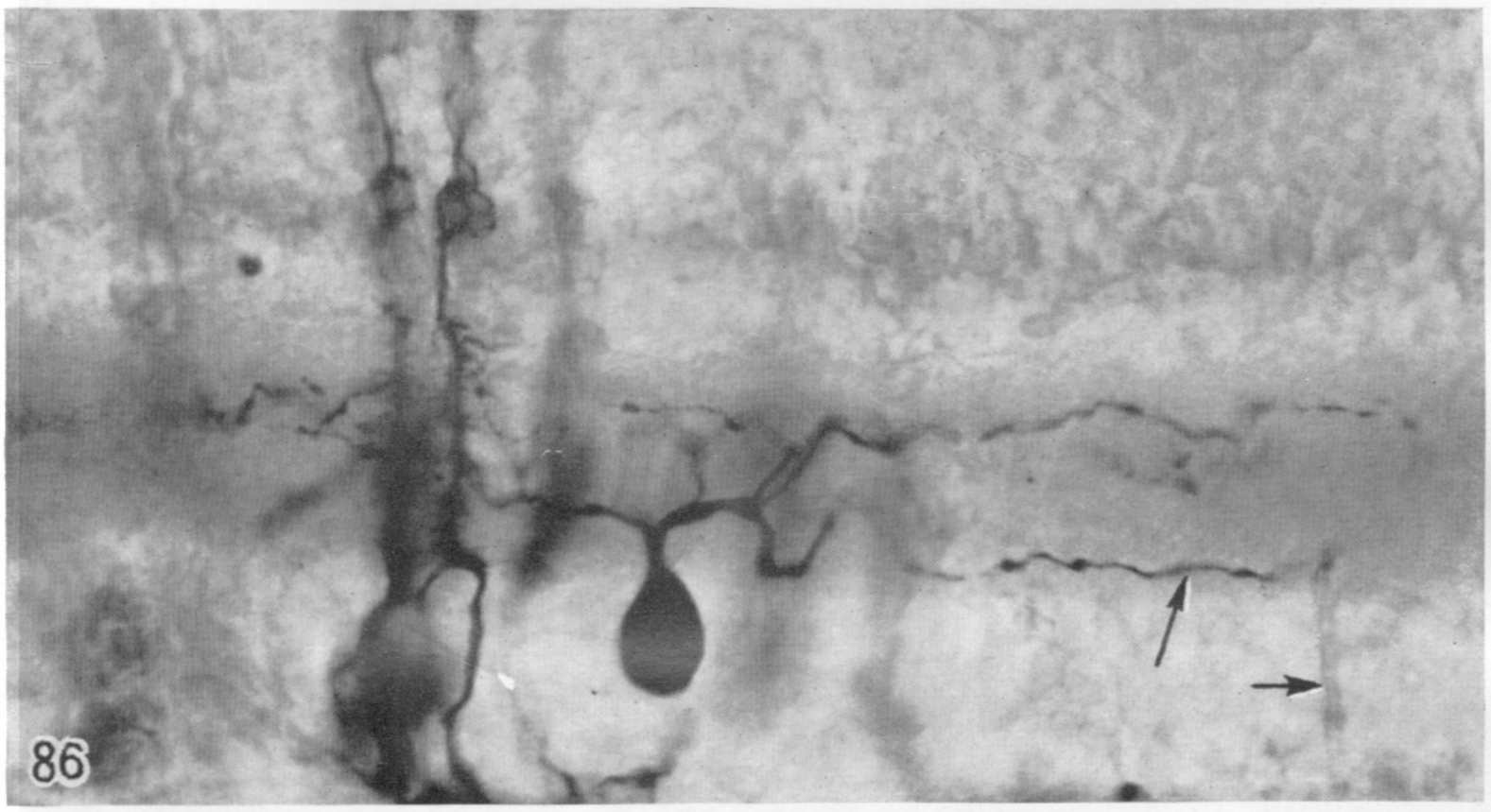
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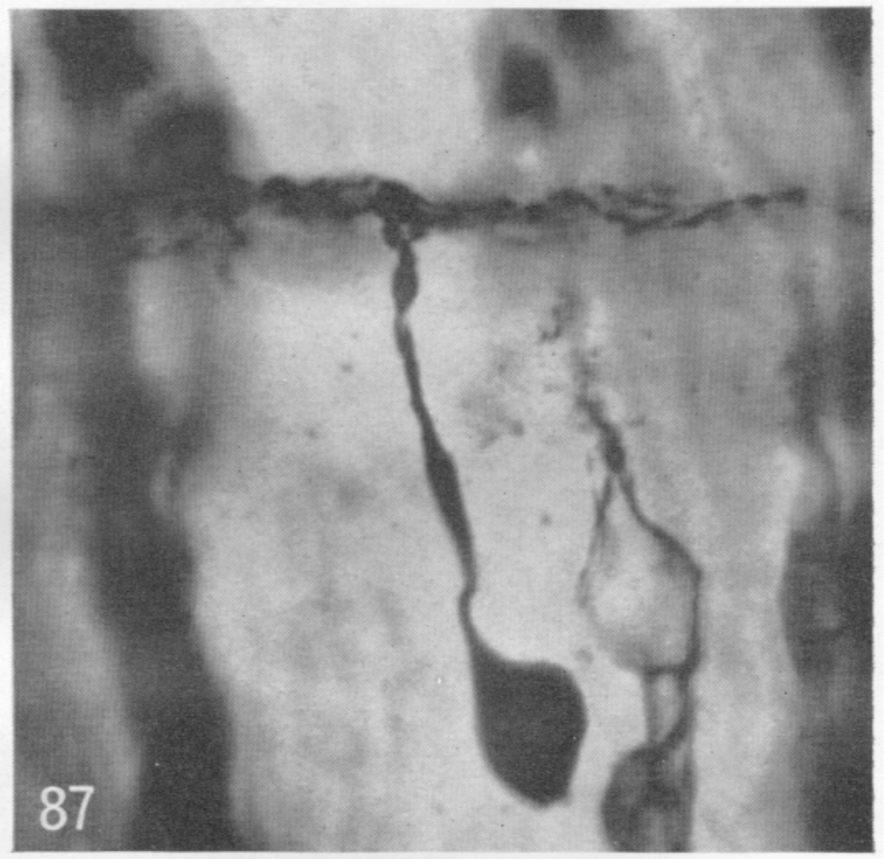
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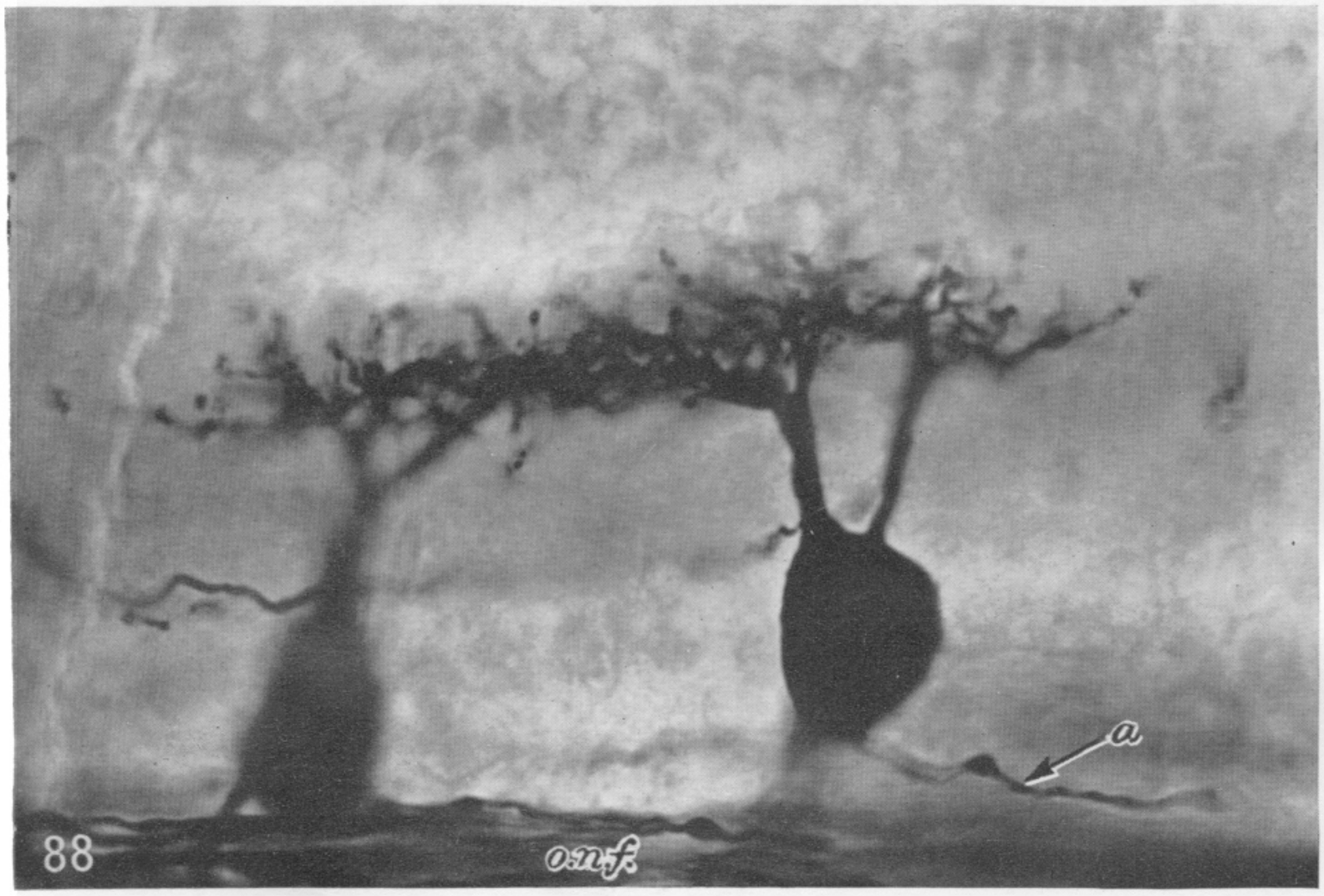
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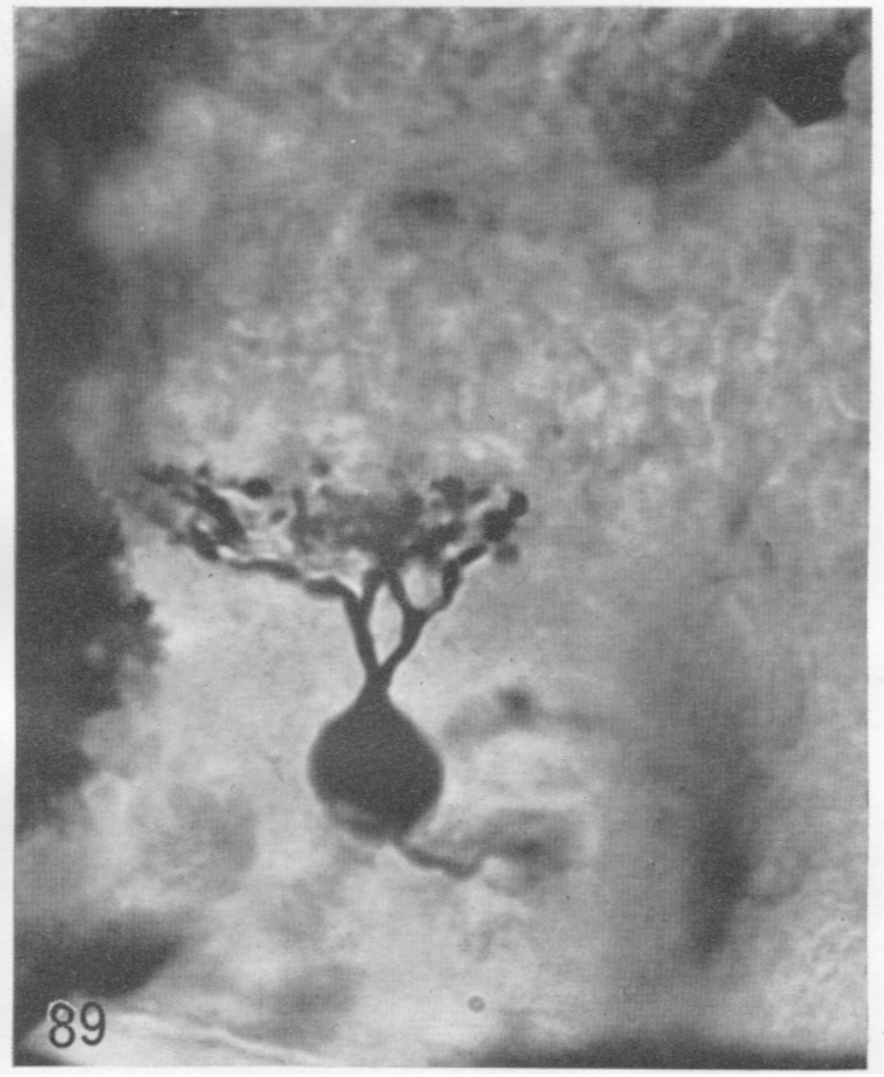
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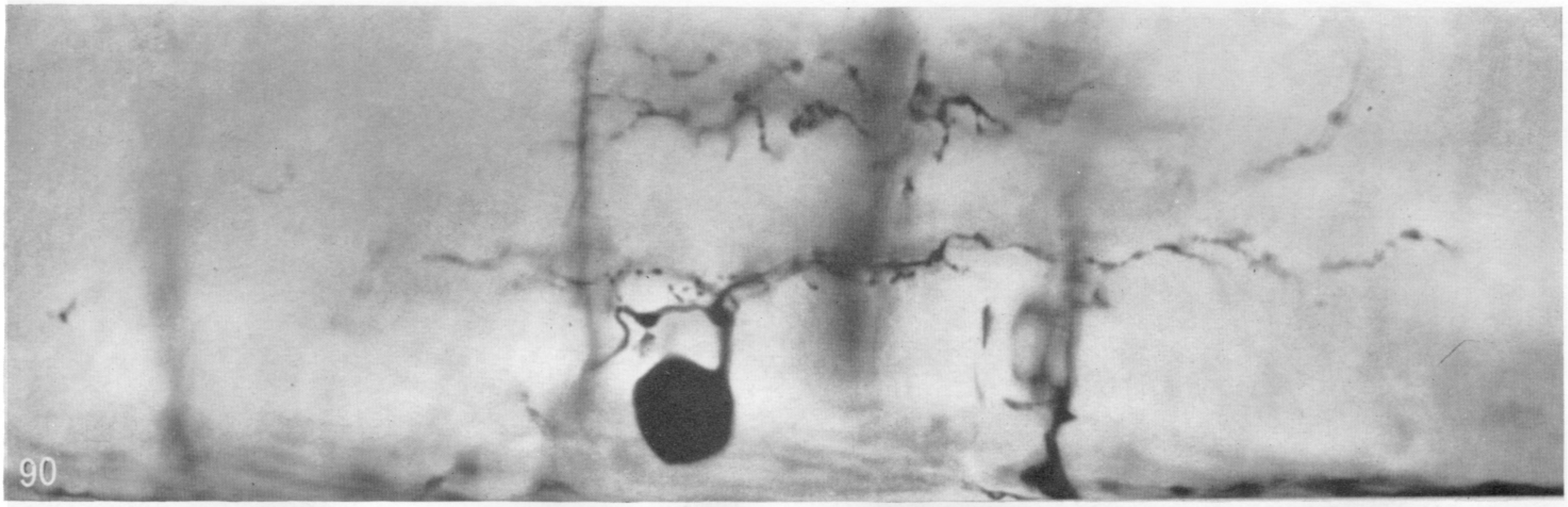
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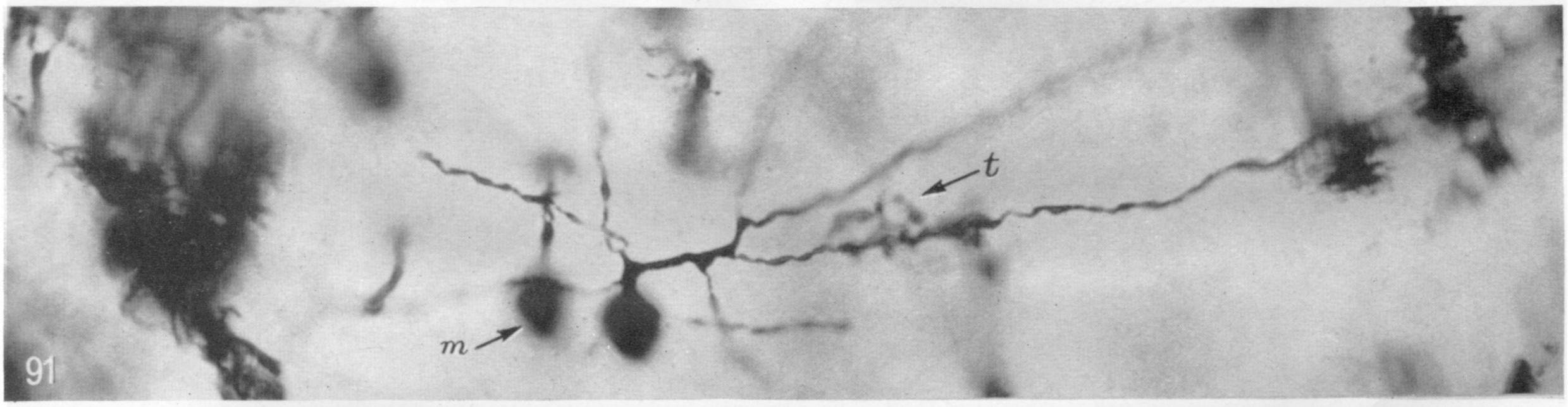
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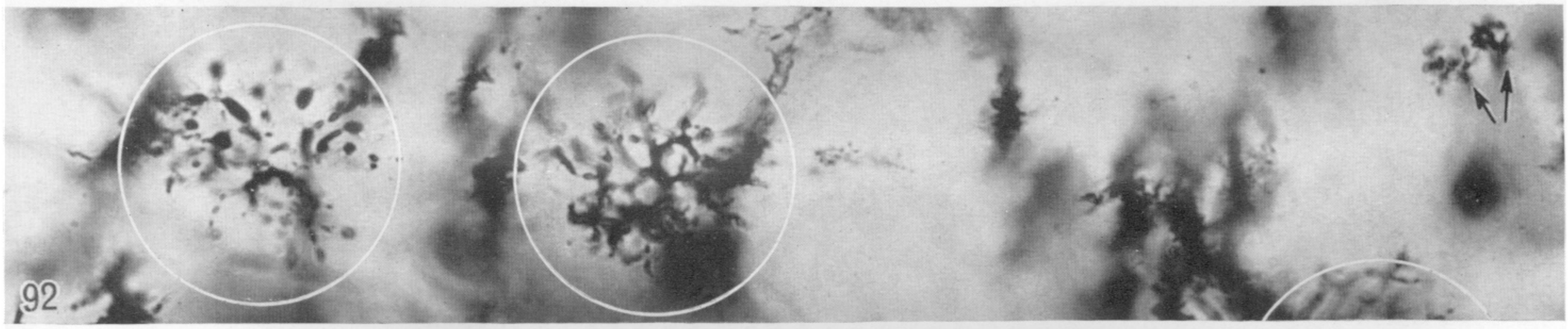
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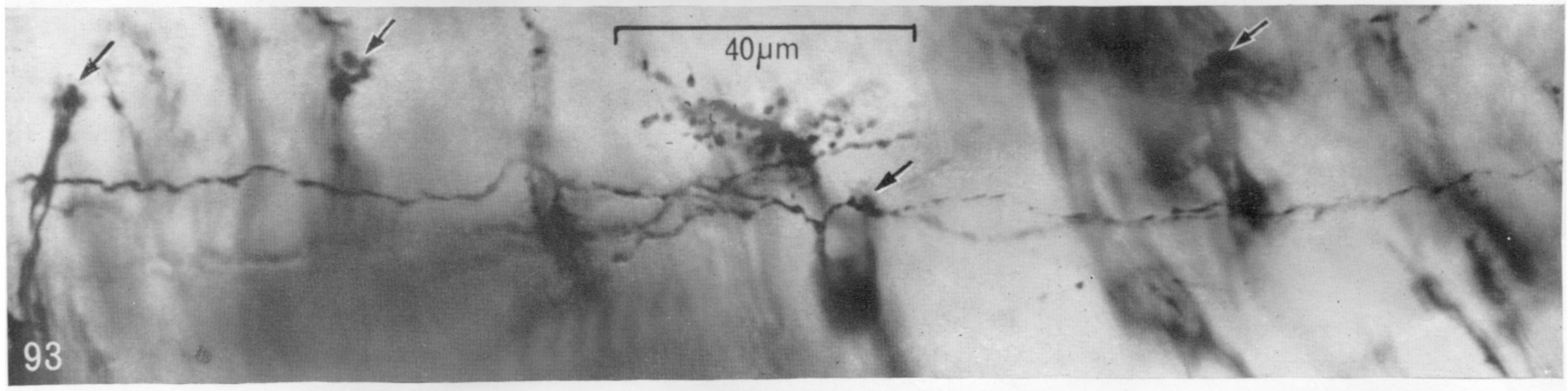
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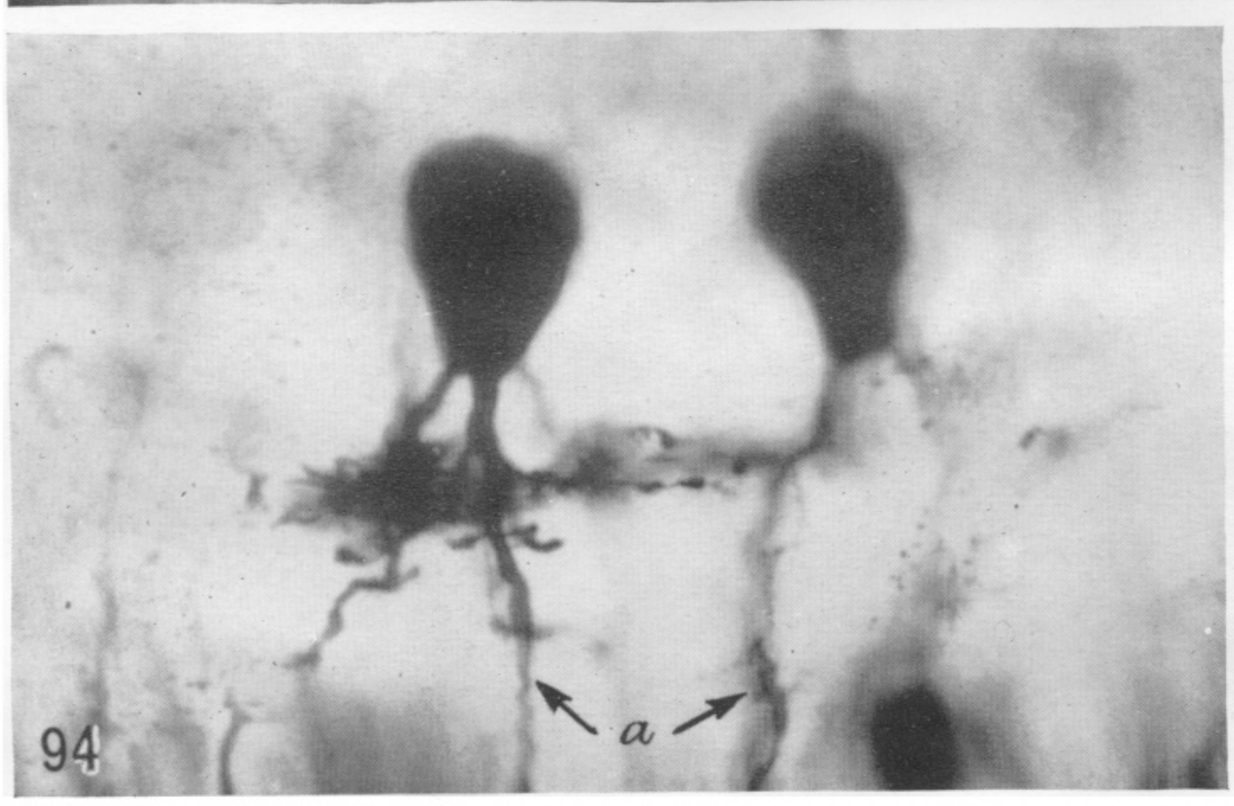
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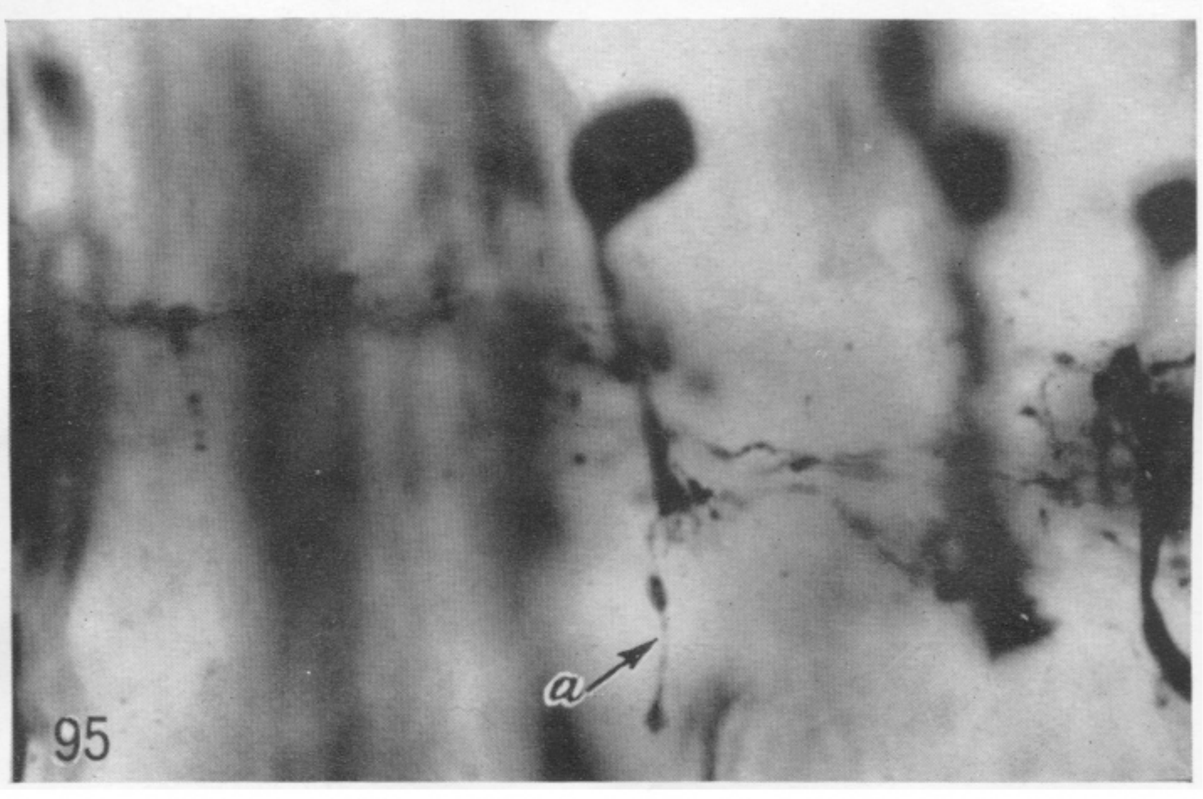
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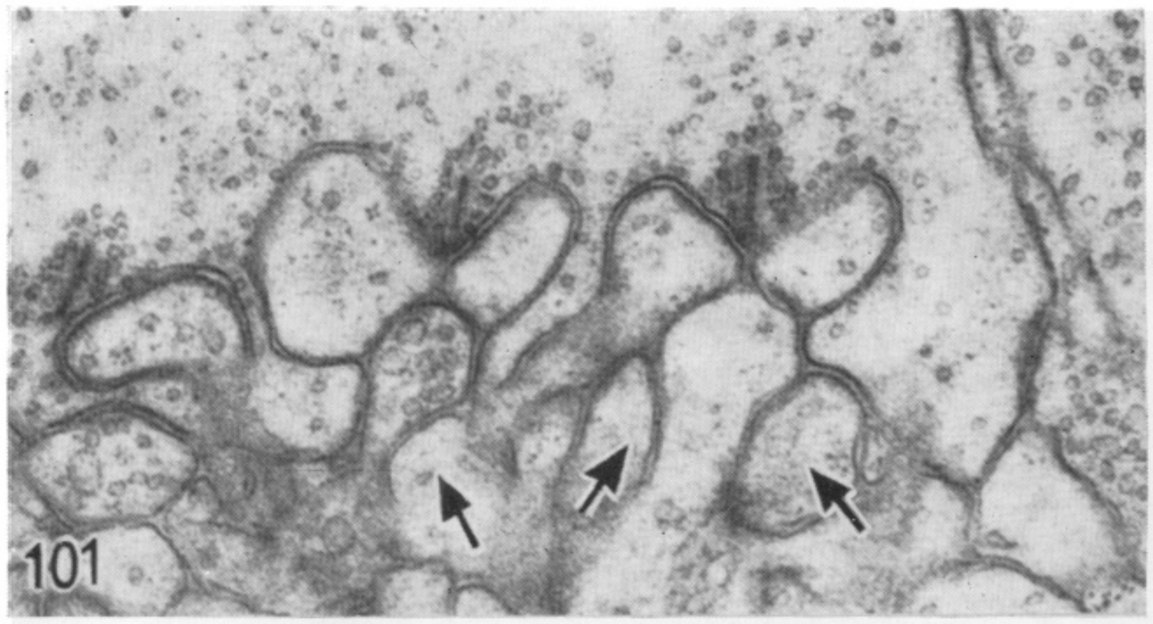
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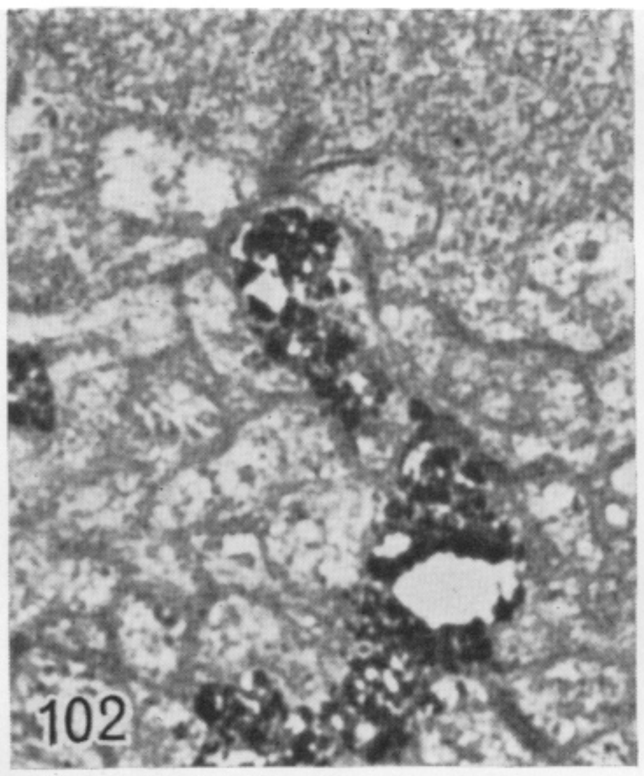
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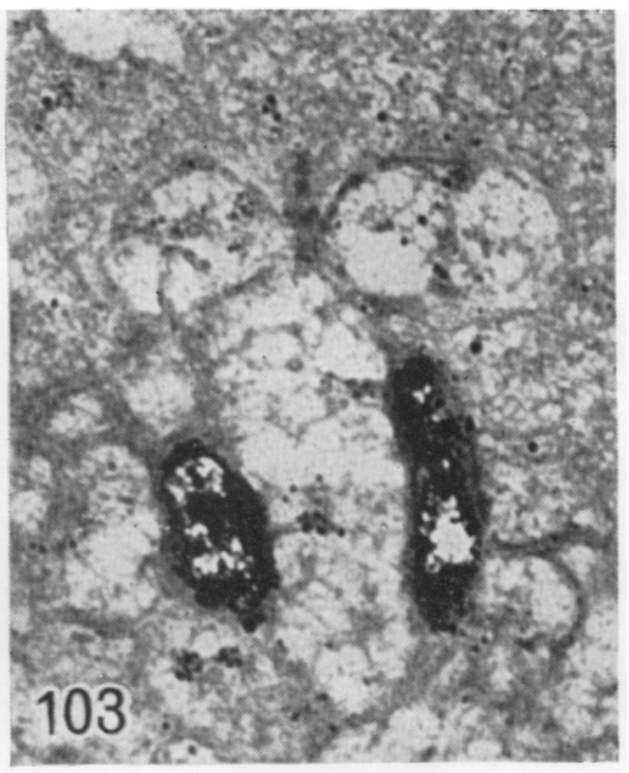
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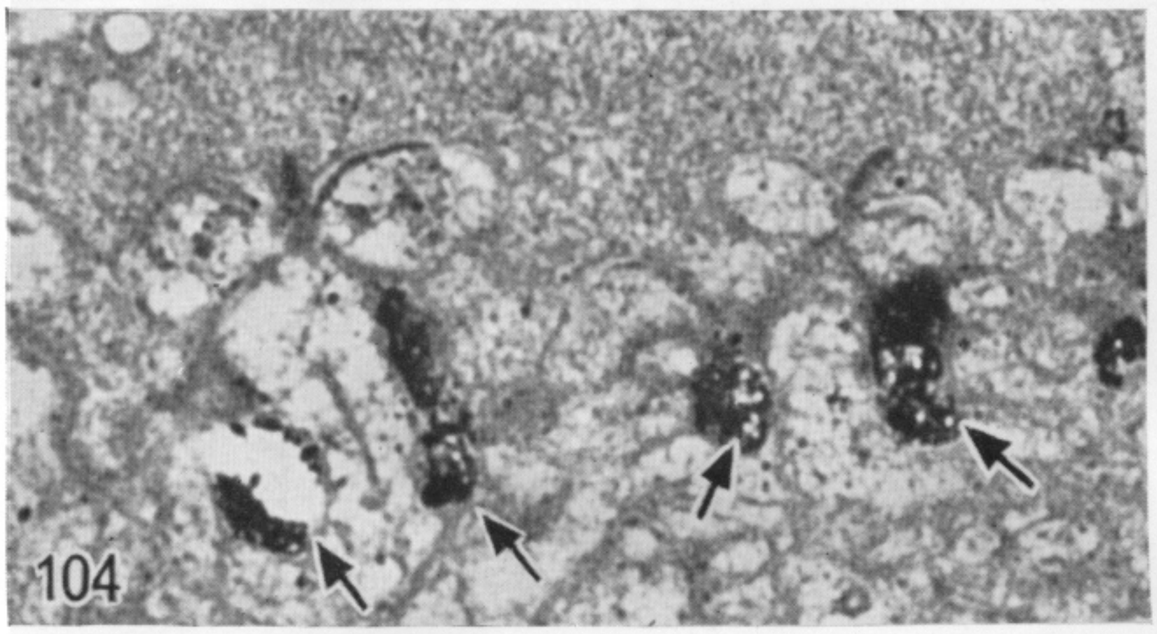
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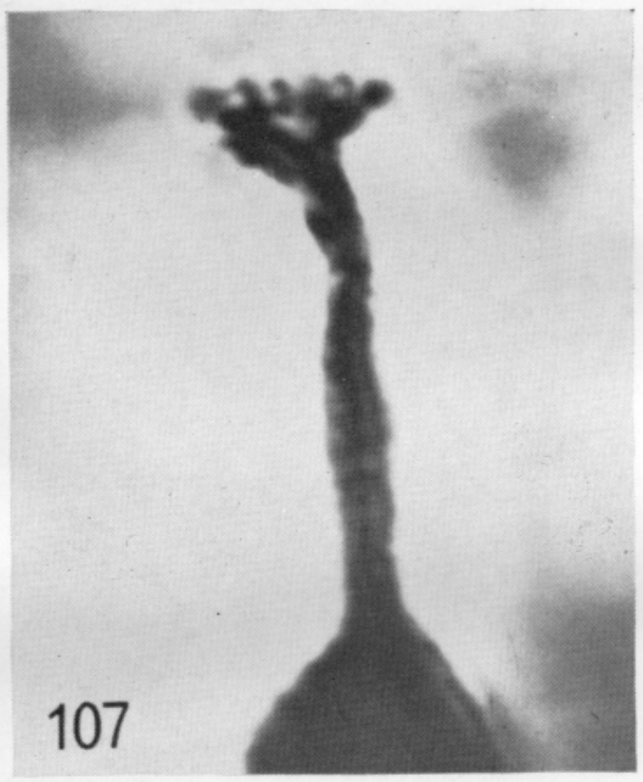
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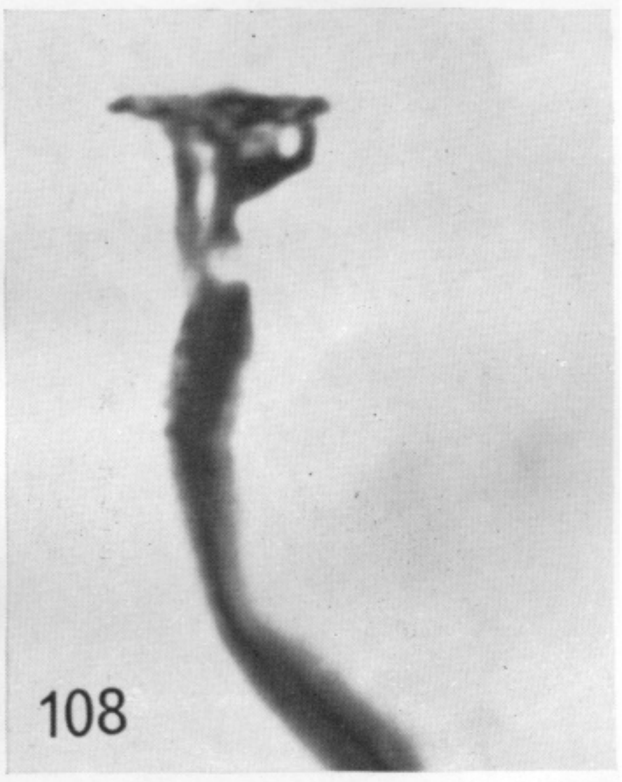
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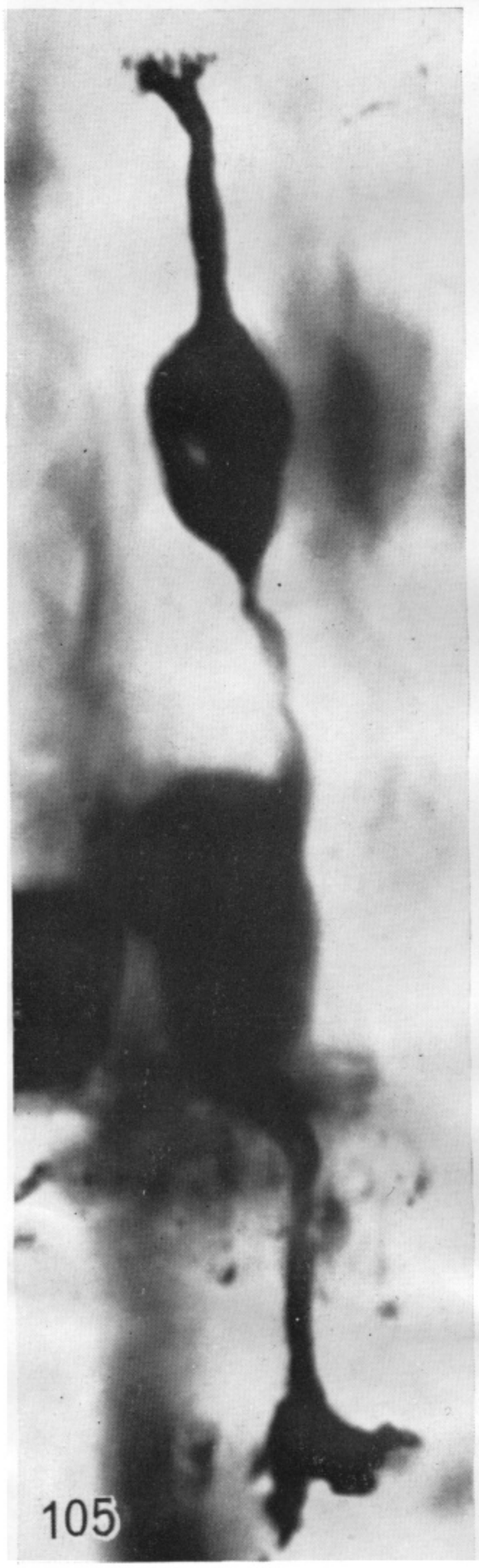
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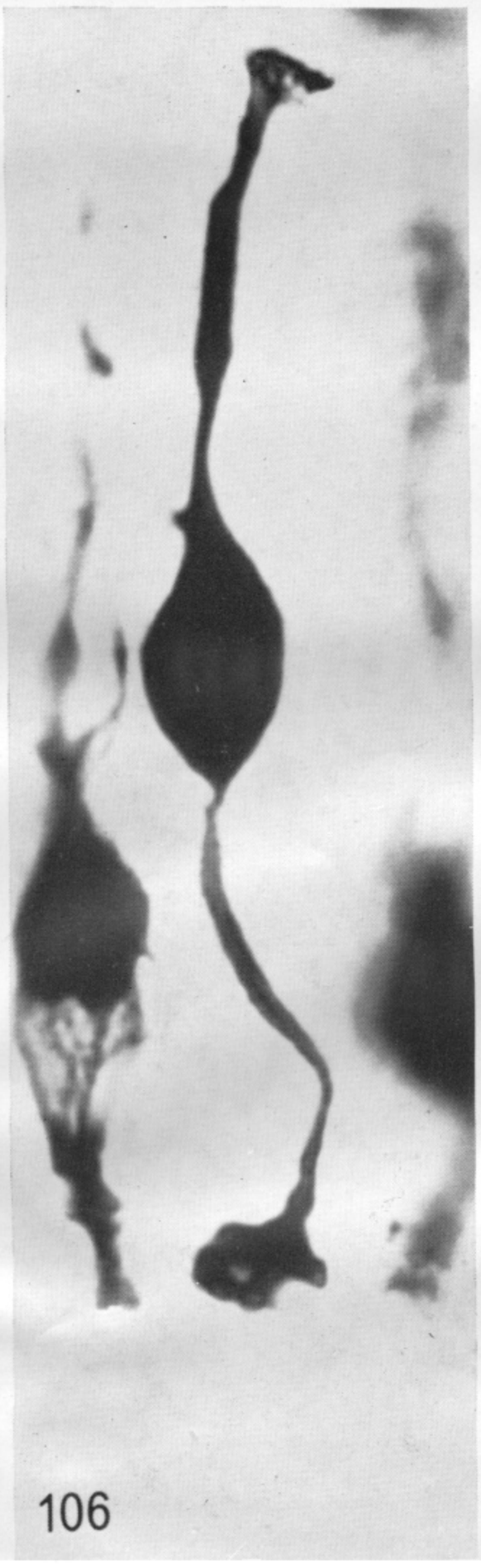
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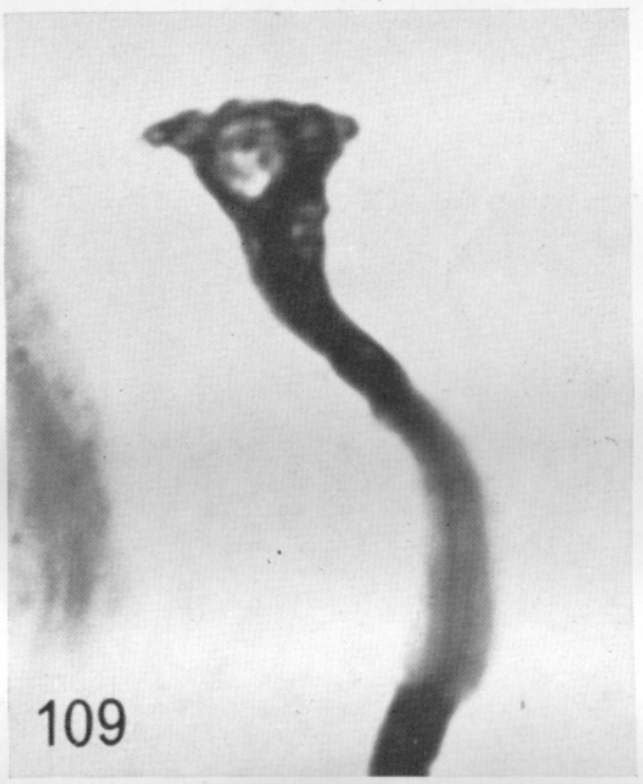
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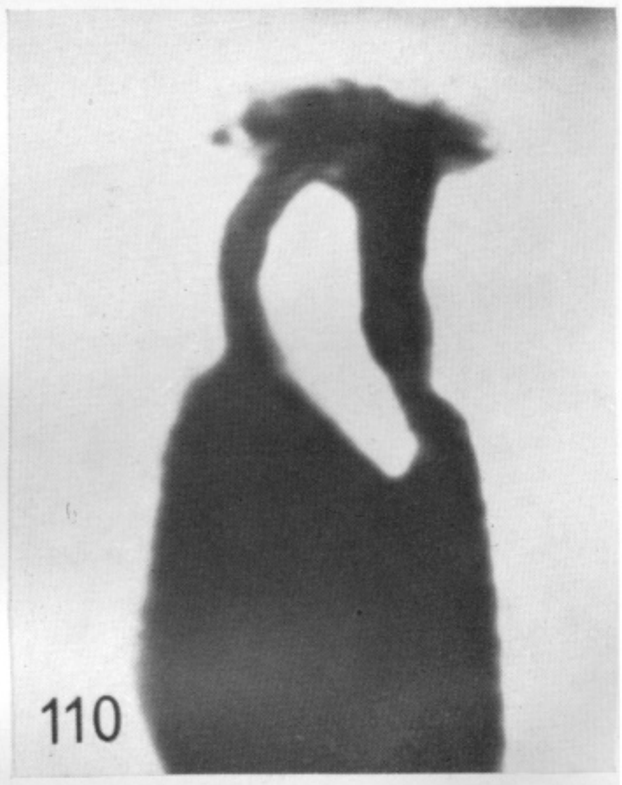
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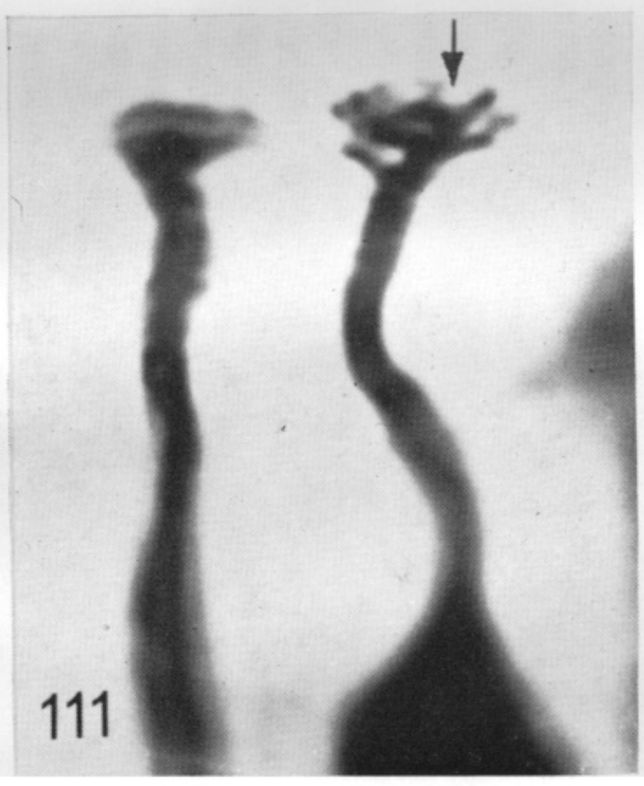
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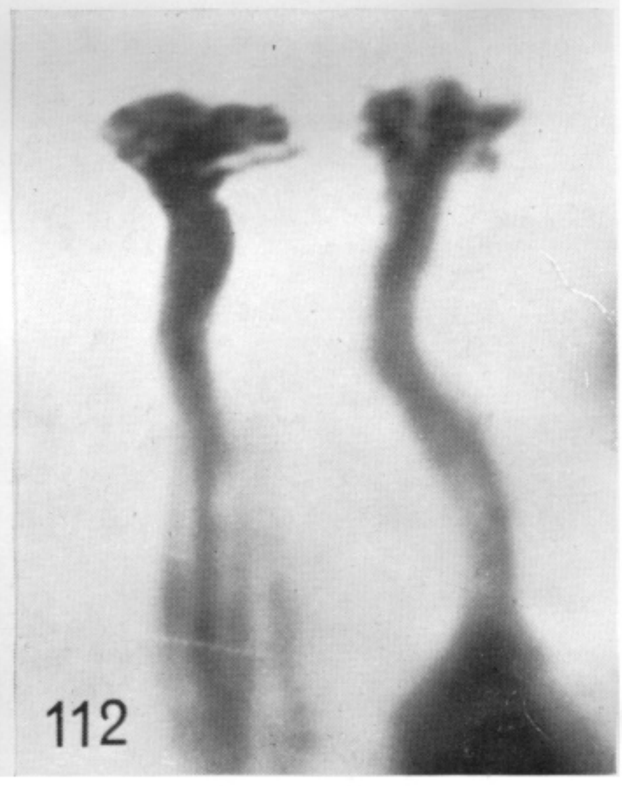
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