THE FINE STRUCTURE OF THE RAT CEREBELLUM

II. The Stellate Neurons, Granule Cells, and Glia

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ABSTRACT

This paper describes the fine structure of the granule cells, stellate neurons, astrocytes, Bergmann glia, oligodendrocytes, and microglia of the rat cerebellum after fixation by perfusion with buffered 1 per cent osmium tetroxide. Criteria are given for differentiating the various cell types, and the findings are correlated with previous light microscope and electron microscope studies of the cerebellum.

INTRODUCTION

Though numerous studies on the fine structure of nervous tissue have been published in the past 10 years (3, 4, 8–10, 12, 13, 15–17, 26), with few exceptions they have been based on material fixed by immersion in a fixative solution, usually buffered osmium tetroxide. Fixation by immersion has long been known to produce serious artifacts at the light microscopic level (2), and even brief comparison of perfusion-fixed material with immersion-fixed material in the electron microscope reveals the severe changes produced by the latter method of fixation. These changes appear to be most marked in the glia.

In most electron microscope studies on the glia, the main criterion for differentiating astrocytes from oligodendrocytes has been the clear, empty cytoplasm of some of the cells. Many authors (4, 5, 9, 26) have identified these clear glial cells as astrocytes, though others (12, 13) have concluded that they are oligodendrocytes. In view of the known propensity of both cell types to swell in a variety of pathological conditions (18, 23), such a criterion is inadequate for cell identification in pathological material.

In 1962, Palay (17) introduced the method of fixation of neural tissues by perfusion with solutions of osmium tetroxide. The electron microscopic appearance of all the cell types in the nervous system was found to be different from that observed in immersion-fixed material. Most notable was the absence of any clear cells or of shrinkage or retraction spaces in the tissue. This improved method of fixation allowed the establishment of new criteria of cell identification in the nervous system, especially with regard to the glia. The purpose of this communication is to describe the fine structure of astrocytes, Bergmann glia, oligodendroglia, and microglia of the rat cerebellum, and to establish criteria for differentiating one type of cell from another and from the granule cells and stellate neurons.

MATERIALS AND METHODS

The material for this study was originally prepared for a study on the fine structure of the Purkinje cell of the rat cerebellum. Fixation was accomplished by a modification of Palay's perfusion method (10). The material was embedded in Epon 812 (11), sec-

tioned on a Porter-Blum microtome, and the sections were picked up on copper grids coated with formvar and carbon. The sections were stained with alkaline lead acetate (28) or uranyl acetate (27) and examined in an RCA EMU 3 E.

OBSERVATIONS

Granule Cells

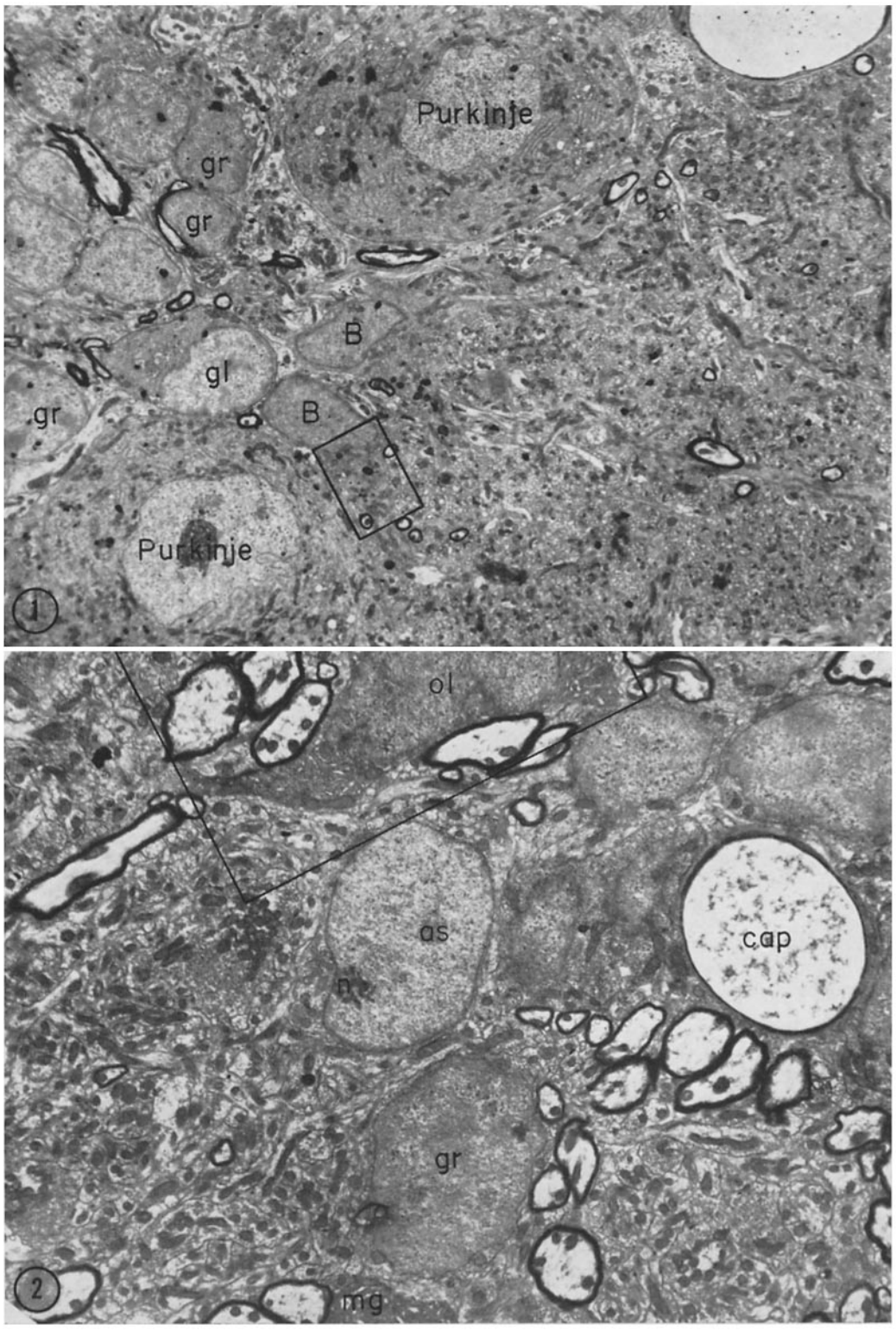
The granule cells (Figs. 1, 14) are small neurons whose perikaryon is about 7 to 10 μ in diameter. They form a distinct layer between the white matter and the Purkinje cell layer in the cerebellum. The majority of cells in this layer are granule cells with a few glial cells and stellate neurons interspersed among them. They have a large nucleus with only a thin rim of cytoplasm. Their dendrites, which are small and rather short, can rarely be traced for any distance from the cell body in thin sections. Dendritic canaliculi can usually be seen in the proximal dendrites. Their axons, which have been shown by light microscopy to pass upward to the molecular layer of the cerebellum, are unmyelinated and are also difficult to trace in thin sections. In the molecular layer they have been shown to split and run horizontally (7), thus forming the parallel fibers which are so numerous in electron micrographs of the molecular layer.

In well fixed preparations, the chromatin material is evenly distributed throughout the nucleus with no clear areas, though the pattern of granularity may be a little coarser toward the center of the nucleus in some cases (Figs. 1 and 12). In poorly fixed specimens, the chromatin is clumped at the periphery of the nucleus with clear areas in the center. An indentation in the nucleus is frequently seen and is commonly located near the region at which the cell cytoplasm is expanded to accommodate the Golgi apparatus. In many cases, the fold appears to extend into the region of the nucleus in which the nucleolus is located. The cytoplasm consists of a thin rim containing free RNP particles, a few mitochondria, and membranes of endoplasmic reticulum. On one side of the cell there is an expansion of the cytoplasm which contains the Golgi apparatus, a few membranes of the granular endoplasmic reticulum, and often a multivesicular body and one or two dense bodies (Figs. 3 and 4). The Golgi apparatus is composed of agranular membranes and large and small vesicles and is similar to that in other neurons, though it is less extensive than that in most larger neurons. The dense bodies differ from those seen in the Purkinje cell in that they are generally smaller, and often contain numerous very small dense particles.

Subsurface cisterns of the type seen in the Purkinje cell (10) or of the type seen in the acoustic ganglion (24) are observed occasionally (Figs. 3 and 4). The plasma membrane may be in close apposition to the plasma membranes of other granule cells over much of its surface, or it may lie in apposition to plasma membranes of astrocytes, oligodendrocytes, or a Purkinje cell or to granule cell dendrites. Some sections show a granule cell bordered on one side or even completely surrounded by thin dense processes consisting of little more than a double membrane (Fig. 11). These processes are probably derived from

FIGURE 1. This micrograph of the Purkinje cell layer of the rat cerebellum also includes a small portion of the granule cell layer and molecular layer. Two Purkinje cells, two Bergmann astrocytes (*B*), and several granule cells (*gr*) are shown. The orientation of the cytoplasm of the Bergmann astrocytes toward the molecular layer is apparent. The enclosed portion of Bergmann astrocyte cytoplasm is shown at higher magnification in Fig. 8. The cell marked (*gl*) has not been identified with certainty but has the cytoplasmic characteristics of an oligodendrocyte. It is larger than the usual oligodendrocytes and has a paler nucleus. It is believed to be an unusually large oligodendrocyte (18, 22) or an incompletely differentiated glial cell. × 2,400.

FIGURE 2. This micrograph shows an oligodendrocyte (*ol*), a granule cell (*gr*), an astrocyte (*as*), and a microglial cell (*mg*) for comparison. The marked density of the oligodendrocyte and the microglial cell relative to the granule cell and astrocyte is apparent. A higher magnification of the oligodendrocyte within the square is shown in Fig. 9. The nucleolus (*n*) of the astrocyte is easily seen. *Cap*, capillary. × 5,300.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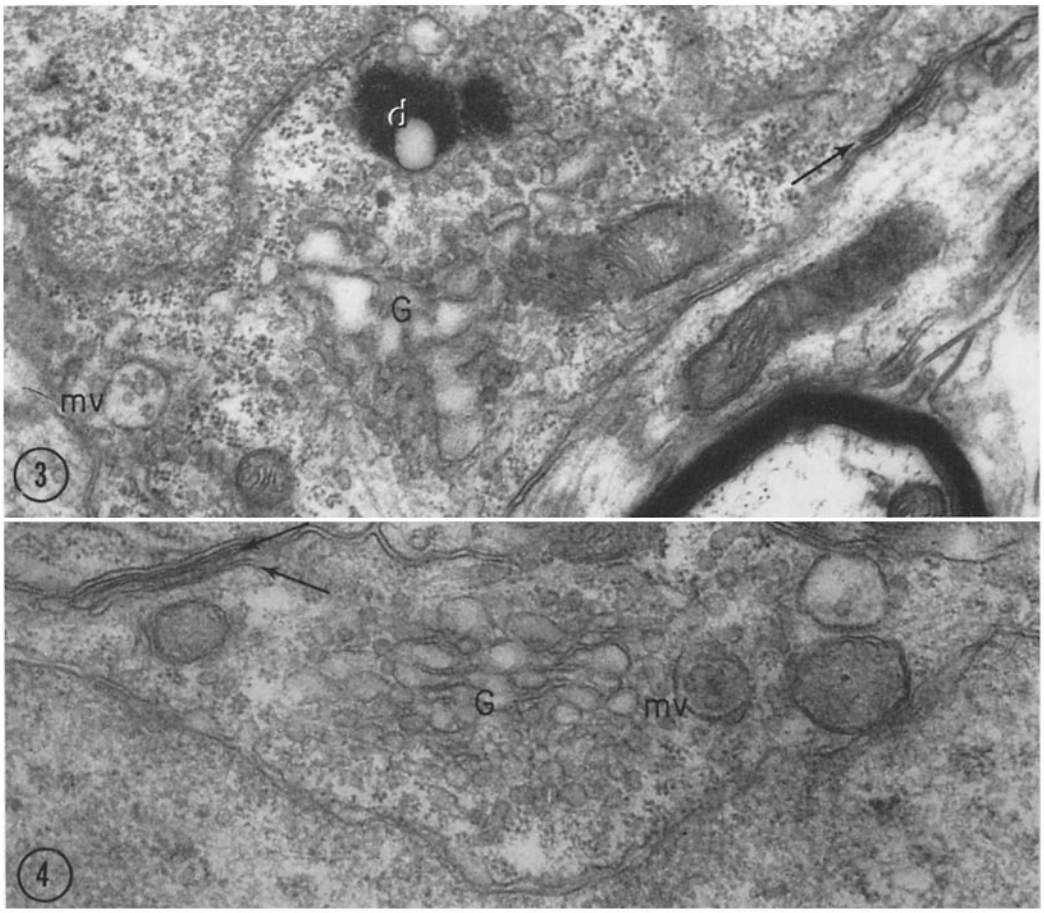


FIGURE 3. In this micrograph of the Golgi region of a granule cell, the nucleus is at the upper left. A multivesicular body (*mv*), a dense body (*d*) containing a vacuole, and the Golgi apparatus (*G*) are shown. A subsurface cistern is apparent at the upper right (arrow). RNP particles can be seen scattered through the cytoplasm. × 28,500.

FIGURE 4. This micrograph of granule cell cytoplasm shows the Golgi apparatus (*G*), a multivesicular body (*mv*), and two mitochondria. The nucleus is at the bottom. At the upper left is a subsurface cistern (upper arrow) beneath which is a second flattened cistern (lower arrow). This arrangement is less common than that shown in Fig. 3. × 28,500.

oligodendroglia. Axosomatic synapses have not been seen, but synapses between the dendrites and the mossy fiber endings are numerous (Fig. 9). They consist of an asymmetrical thickening of the membranes which are separated by a synaptic cleft of about 300 A (8) as opposed to the usual distance of 200 A between apposed membranes. Within the synaptic cleft, a thin layer of dense material is seen. These synapses are described in detail by Gray (8) who also described dendro-dendritic attachment plaques.

Stellate Neurons

The term "stellate neuron" in this paper is used in its broad sense to include all the neurons of the cerebellar cortex other than the granule cells and Purkinje cells. The bodies of these cells vary from 6 or 7 μ to perhaps 20 μ in diameter, the ones in the granule cell layer being, in general, larger than those in the molecular layer.

Small stellate neurons (Fig. 5) are scattered throughout the molecular layer. They have a

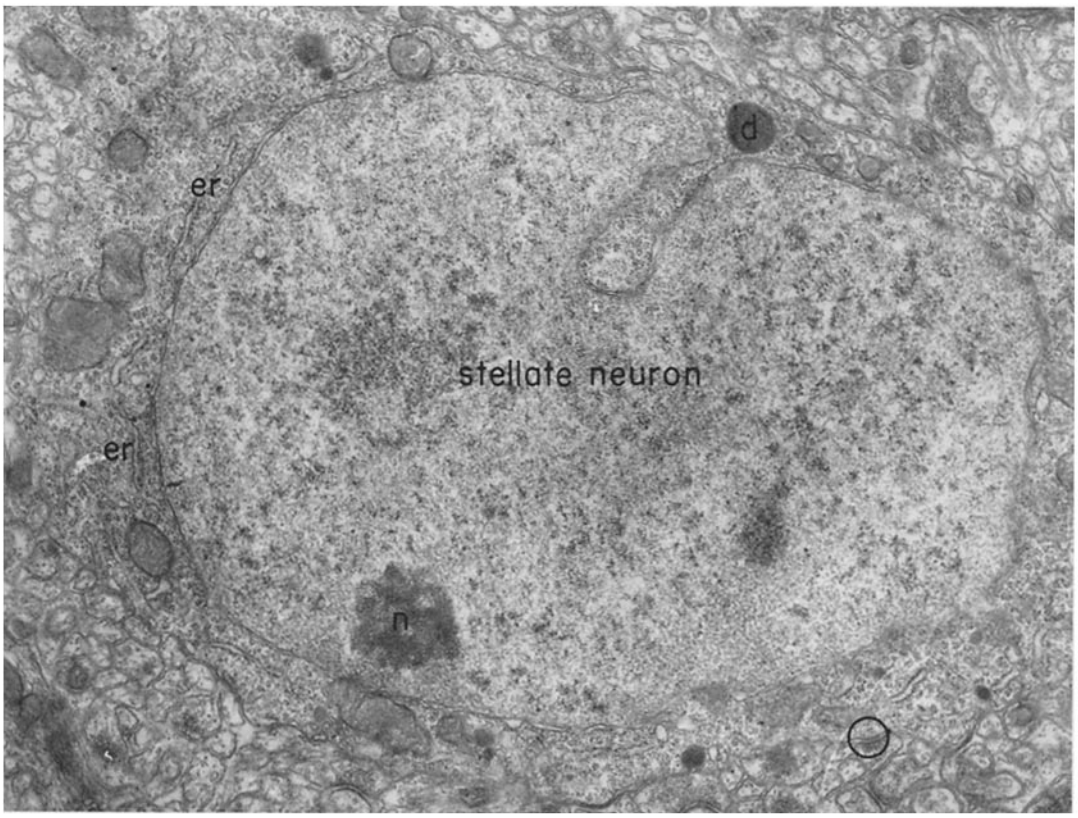


FIGURE 5. This micrograph shows a small stellate neuron in the molecular layer. A deep fold is seen in the double nuclear membrane, and a nucleolus (*n*) is shown in the nucleus. A dense body (*d*) is located at the base of the fold in the nuclear membrane. Numerous membranes of the granular endoplasmic reticulum (*er*) are apparent. At lower right is an axosomatic synapse (circled). × 12,000.

round-to-oval nucleus which is delineated by a double membrane showing a moderate number of nuclear pores. The nuclear membrane usually has one or more deep folds. A small nucleolus is seen often in the nucleus and stands out in contrast to the otherwise homogeneous nuclear material. The cytoplasm consists of a thin rim which is expanded on one side where the Golgi apparatus is located. Elements of the granular endoplasmic reticulum are scattered throughout the cytoplasm. Several mitochondria are usually seen, along with a few dense bodies. The stellate neurons have few processes, and it is usually not possible to trace one of these for more than a few microns. Dendritic canaliculi may be seen in the proximal cell processes. Axosomatic synapses are numerous; several may be visible in any given plane of section. They consist of a slight thickening of the pre- and postsynaptic membranes with a cluster of synaptic vesicles located on the presynaptic membrane. The separation of the pre- and postsynaptic membranes is slightly greater than the usual 180- to 200-A space between other membranes in the nervous system. Usually, no dense line is seen between the pre- and postsynaptic membranes.

In the larger stellate neurons, the nuclei have a convoluted double nuclear membrane and a prominent nucleolus. The cytoplasm is abundant and contains a large Golgi apparatus, abundant elements of the granular endoplasmic reticulum, numerous mitochondria, dense bodies which resemble those seen in the Purkinje cell, and some large dendrites which have numerous dendritic canaliculi. Axosomatic synapses similar to those seen on the smaller stellate neurons are seen frequently. Axodendritic synapses were not identified in this study, but this is probably because of the difficulty of tracing the dendrites out to their

smaller branches. The largest of these stellate neurons resemble Purkinje cells in most respects.

Astrocytes

The astrocytes of the cerebellar cortex and the underlying white matter have similar cytological characteristics with the exception of the Bergmann astrocytes (Fañanas cells, Golgi epithelial cells) which are a distinct group and will be treated separately. The remaining astrocytes (Figs. 6 and 7) have a nucleus which is usually oval though it may be round or semilunar. Invaginations of the nuclear membrane have not been observed. The nuclear chromatin, which is evenly distributed, is more finely granular and less dense than that of the granule cells or oligodendrocytes. A small nucleolus is often seen in the nucleus. The cytoplasm is less dense than that of other cells in the cerebellum; it contains few scattered RNP particles, scattered tubular elements of the granular endoplasmic reticulum, a variable number of dense bodies, a small Golgi apparatus, some mitochondria, and an infrequent multivesicular body. The dense bodies vary considerably in number, size, and shape from one cell to another. They are usually enclosed within a membrane and contain finely granular dense material. Sometimes there is a vacuole within these dense bodies. Glial fibrils are usually visible running in bundles through the cytoplasm and into the cell processes.

The processes of the astrocytes in the granule cell layer are generally smaller than those of the astrocytes of the white matter and seem to contain fewer glial fibrils. Otherwise, the astrocytes in the two locations do not differ from each other in appearance (compare Fig. 6 with Fig. 7).

Bergmann Astrocytes

The Bergmann glia which are located in the Purkinje cell layer and lower molecular layer are very numerous (Figs. 1, 2, 8, and 15). Their nuclei resemble those of the other astrocytes in the cerebellum and are located on the side of the cells adjacent to the granule cell layer. The main cytoplasmic mass is located on the side toward the pial surface. The cytoplasm is more dense than that of the other astrocytes and contains more RNP particles. The Golgi apparatus is small. The granular endoplasmic reticulum is poorly defined and is of the tubular variety. Multivesicular bodies are sometimes seen. The number of dense bodies (which are similar to those in other astrocytes) is quite variable; they may be clustered near the nucleus or near the base of the processes. The mitochondria in these cells are denser than in most cell types and their cristae are difficult to see.

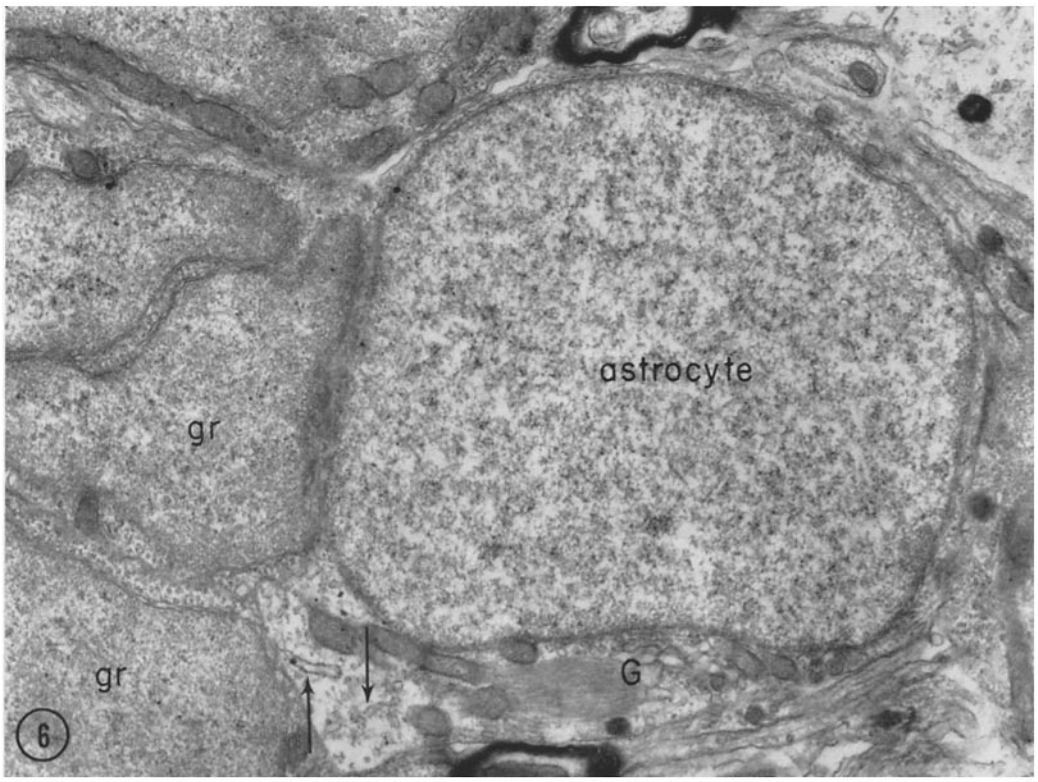
The processes of these cells have been shown by light microscopy to extend upward and end in a cone-shaped expansion of the pial surface (18, 20, 22, 25). In some sections they can be traced for a distance from the cell body even though they are very slender. The main branches have a number of small side branches which pass between the parallel fibers of the molecular layer. Some of the astrocytic processes contain particles 300 to 450 A in diameter which stain heavily with alkaline lead acetate; they appear to be glycogen particles. The astrocytic processes which end in the cone-shaped foot processes on the pial surface often contain glial fibrils which are not seen in the cell body or proximal processes of Bergmann glia. In some instances, a process runs along the surface of a Purkinje cell dendrite for some distance; in this situation such a process may be difficult to differentiate from a climbing fiber. The cone-shaped ending on the pial basement membrane usually contains mitochondria, tubular elements of the granular endoplasmic reticulum, and glial fibrils.

Oligodendrocytes

The oligodendrocytes (Figs. 9, 10, 11, and 16) are slightly smaller than the astrocytes or granule

FIGURE 6. This micrograph shows an astrocyte in the granule cell layer of the cerebellum. Granule cells are seen at the left (*gr*). The density of the astrocytic nucleus is less than that of the granule cells. The Golgi apparatus (*G*) and a few membranes of the granular endoplasmic reticulum (arrows) are shown. Just below and to the left of the Golgi apparatus is a bundle of glial fibrils. × 14,000.

FIGURE 7. This astrocyte from the white matter of the cerebellum contains many more glial fibrils (*gf*) in its cytoplasm than does the one shown in Fig. 6. A process filled with glial fibrils can be seen at lower left. The relatively small Golgi apparatus is seen near the bottom (*G*). × 11,000.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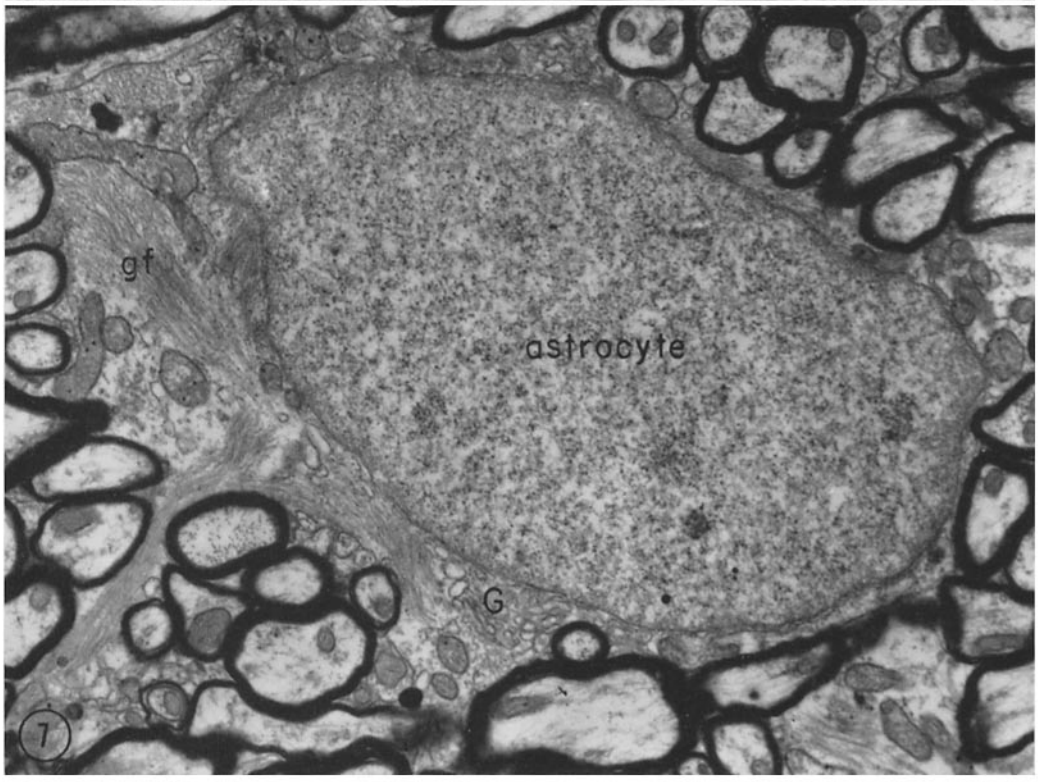
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cells. They are most numerous at the junction of the white matter and the granule cell layer. A few cells may be scattered in the granule cell layer, especially near the Purkinje cells. They have a round or slightly oval nucleus; its chromatin is evenly distributed but may be slightly clumped at the periphery. The density and texture of the nucleus is very similar to that of the granule cell, but invaginations of the nuclear membrane are rarely seen.

The cytoplasm is usually attenuated and consists of a moderately thin rim which is expanded on one side where the Golgi apparatus is located. In the case of the interfasicular oligodendroglia, which have a little more cytoplasm than the oligodendroglia in the cortex, there is usually an expanded area of cytoplasm at both ends of the cell. The cytoplasm is more dense than that of any other cell type in the cerebellum except the microgliocyte. The endoplasmic reticulum is arrayed around the nucleus, and the clear cisternae stand out prominently against the dense cytoplasm, as do the cisternae of the Golgi apparatus. The mitochondria, larger than those in surrounding neurons (17), have a dense matrix, and their cristae do not stand out well because of this over-all density. The density of the cytoplasm is largely due to the high concentration of free RNP particles. Multivesicular bodies and dense bodies are seldom seen.

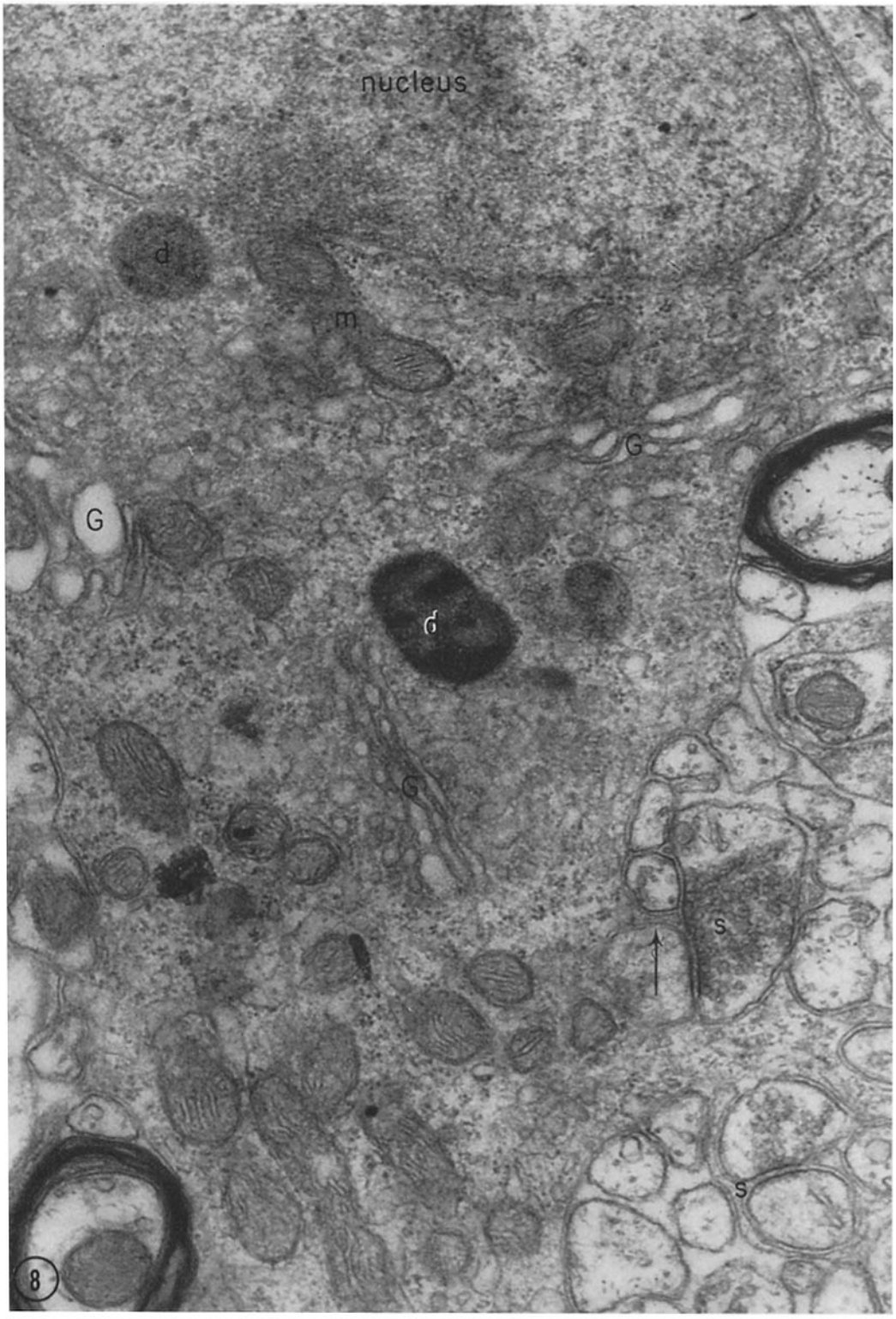
The oligodendroglial cell processes are of two types. The larger processes contain tubules which resemble the dendritic canaliculi. That these are less clearly defined may be due to the greater density of the cytoplasm or to the character of the tubules themselves. The smaller processes (Figs. 10 to 12) consist of little more than an outpouching of the plasma membrane with little, if any, cytoplasm between the membranes which appear fused at times. These latter processes are applied closely to the plasma membrane and, when they are viewed in sections which pass obliquely through the process, one has the impression that the plasma membrane is thickened (Fig. 10). On occasion, a process from an oligodendrocyte may run completely around the cell one or more times to form a lamellar structure like myelin. More usually, the process separates from the plasma membrane at some point; sometimes it can be traced to the region of a myelinated axon where it appears to join the myelin sheath. These processes may also be seen along the plasma membrane of a granule cell.

Sometimes, larger cells are seen which resemble the small oligodendrocytes in their cytoplasmic characteristics. The nuclei of these cells, which are probably incompletely differentiated glial cells or unusually large oligodendrocytes (18, 21), are larger and paler than those of ordinary oligodendrocytes (Fig. 1).

Microglia

Microglia are rather scarce in the cerebellum though they appear to be more common in older animals. They are dense cells which usually have an elongated nucleus and a very dense cytoplasm (13, 17). The nucleus is dense, particularly about the periphery, but the central nucleoplasm may be more granular with a few light areas between the granules. The cytoplasm is abundant, and thick processes are frequently seen. Within the cytoplasm are elements of the granular endoplasmic reticulum and RNP particles. The Golgi apparatus is large and often stands out as a light area in the dense cytoplasm. The mitochondria are fewer in number and seem to be smaller than those in surrounding neurons. One or more dense bodies may be present. These usually contain irregular and very dense globules of material, some lighter material, and cleft-like spaces. They appear to be surrounded by a poorly defined membrane and are usually located at the cell border. A small amount of extracellular space may be seen in a few places about the cell, particularly about its thick processes.

FIGURE 8. This micrograph shows at a higher magnification the cytoplasm of one of the Bergmann astrocytes shown in Fig. 1. Mitochondria (*m*), RNP particles, Golgi appartus (*G*), and dense bodies (*d*) are shown. A small process of the type that probably gives these cells their feathery appearance in certain light microscopic preparations is shown at the arrow. No glial fibrils are present in the cell body of these cells, and the cytoplasm is more dense than that of the other astrocytes of the cerebellum shown in Figs. 6 and 7. At the lower right are two axodendritic synapses (*s*) in the lower portion of the molecular layer. × 28,000.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DISCUSSION

The smaller cellular elements of the rat cerebellar cortex are characterized by a small round or oval nucleus and scant cytoplasm. The identification of the various cell types by light microscopy depends largely upon the situation of the cell body and the character and distribution of their processes, criteria which are not fully applicable to electron microscopic material. In thin sections, the processes of a cell are only occasionally seen in continuity with the cell body and it may be difficult to determine the exact location from which a section was taken. It is therefore necessary, to a considerable extent, to depend upon cytological features for cell identification in an electron micrograph. Some of these features, such as cytoplasmic density and concentration of RNP particles, require comparison of adjacent cells; other features are more evident and do not necessitate direct comparison.

Identification of the oligodendrocytes among the granule cells of the cerebellum is difficult at low magnifications because of their similar size and shape. Separation of the two cell types is facilitated considerably by the uranyl acetate stain which stains RNP particles heavily and accentuates the difference in cytoplasmic density of the two types of cells. The oligodendocrytes then stand out as dark cells among the granule cells.

The frequent occurrence of very narrow, dense, oligodendroglial processes, which often lie parallel to the oligodendroglial plasma membrane for some distance, is of particular interest. Several of these processes have been seen to terminate in close relationship to a myelin sheath. This may be regarded as evidence in favour of the view that the myelin sheaths are normally formed by spiral wrapping of oligodendroglial processes (1, 14, 19). It seems probable that the myelin sheaths retain their connection to the oligodendrocytes in adult animals, but these processes are so tenuous that it is difficult to trace them from the oligodendrocyte to the myelin sheath. This author's conception of this relationship, similar to that proposed by Bunge *et al.*, is illustrated in Fig. 16.

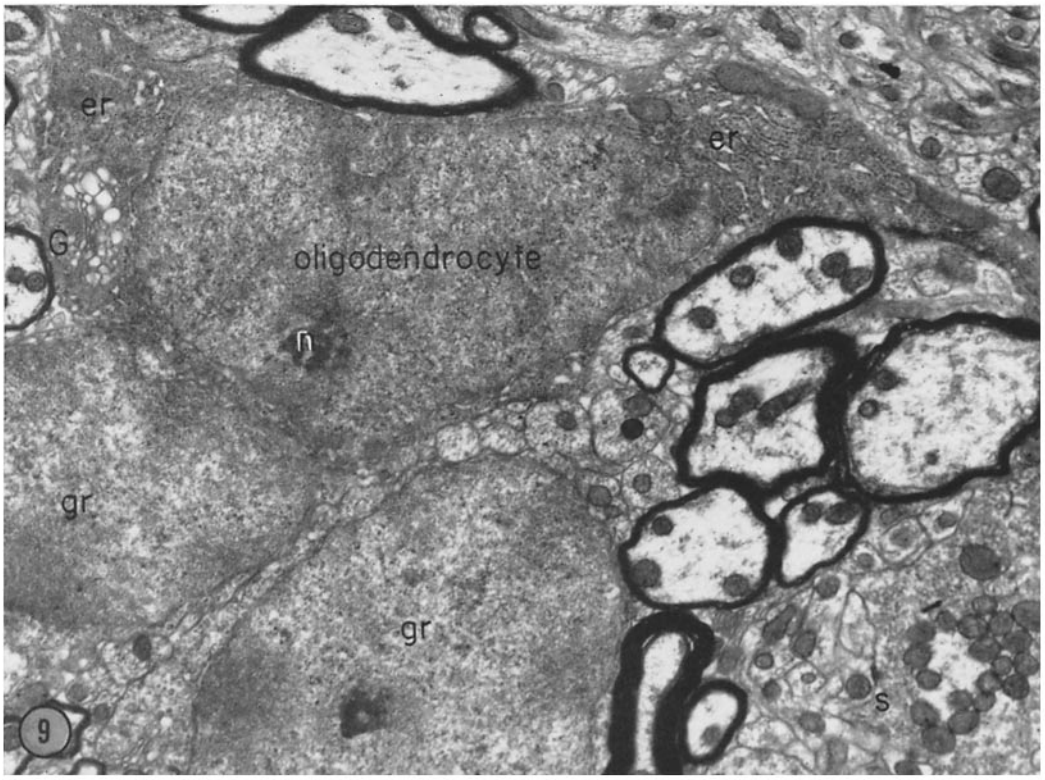
The astrocytes in the cerebellum are so sufficiently distinctive, because of the density and texture of their nuclei, that they can be recognized even at low magnification. The absence of cytological differences between the astrocytes of the white matter (which in most animals are fibrous) and those in the granule cell layer (which in most animals are protoplasmic) probably reflects the poor differentiation of these glial types in the rat. The description of these cells in the rat, therefore, may not be applicable to animals with more fully differentiated glia. It is interesting in this regard that Farquhar and Hartmann (5) found no differences between fibrous and protoplasmic astrocytes in immersion-fixed material from rats and humans.

Multivesicular bodies and dense bodies together with occasional transitional forms were present in all of the cell types described. The dense bodies varied considerably in size, shape, and number from one cell to another and from one cell type to another. They were most numerous in astrocytes and, in these cells, may correspond to the pigment granules described by Penfield (18). It seems likely that all of these dense bodies fall into the general category of lysosomes, but proof of this must await enzymatic histochemical study at the electron microscopic level.

Identification of the various cell types in this study was facilitated by careful orientation of tissue blocks so that sections were taken either at right angles to the folia or parallel to the folia. Such orientation, which is not possible in material fixed by immersion in osmium tetroxide, was particularly useful in recognizing the cell processes in the

FIGURE 9. This micrograph shows an oligodendrocyte and portions of two granule cells (*gr*). The prominent granular endoplasmic reticulum (*er*) and marked cytoplasmic density are apparent. The Golgi apparatus (*G*) stands out against the dense cytoplasm. The nucleolus (*n*) is shown in the nucleus. At the lower right is a portion of a mossy fibre ending which synapses with a granule cell dendrite at *s*. This cell is seen at lower magnification within the square in Fig. 2. × 10,000.

FIGURE 10. A thin process runs around the bottom and left side of the oligodendrocyte shown in this micrograph. In some places it is cut obliquely and appears as a thickening of the oligodendrocyte plasma membrane. The Golgi apparatus (*G*) and prominent endoplasmic reticulum (*er*) are also shown. The relatively large size of the mitochondria is apparent. × 13,500.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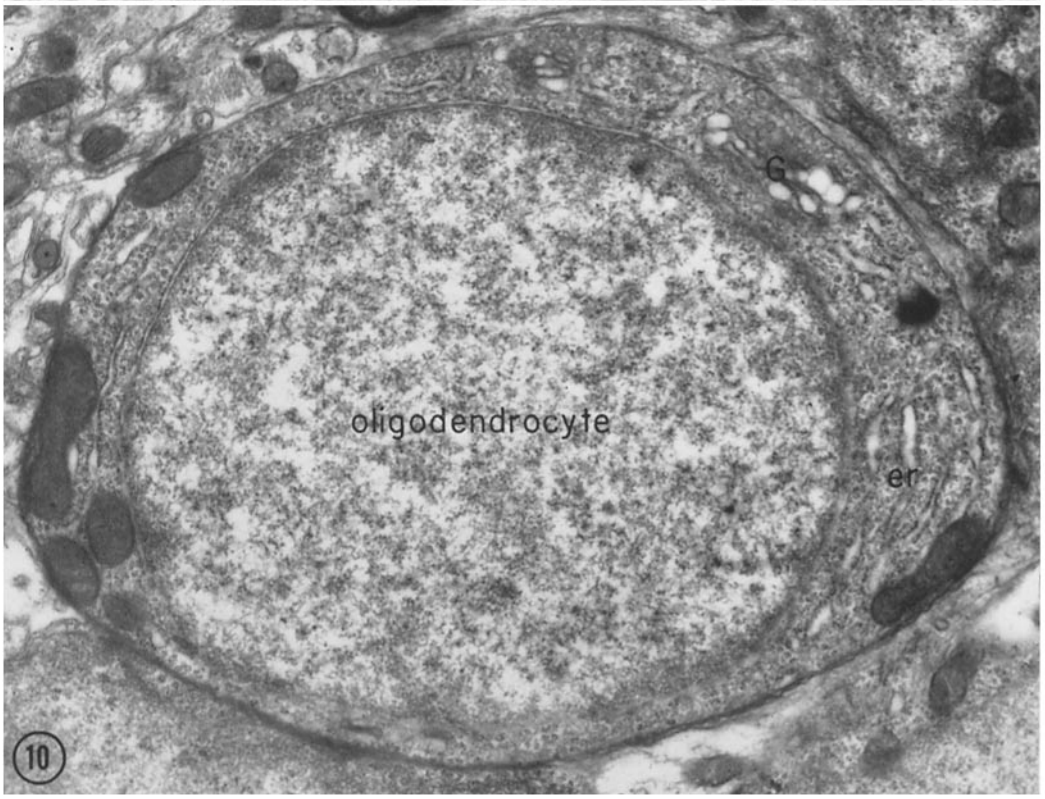
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region about the Purkinje cell layer and in following the processes of the Bergmann glia.

By means of special staining techniques with light microscopy, the stellate neurons of the cerebellar cortex have been subdivided into a number of cell types, including basket cells, small stellate neurons of the molecular layer, larger stellate neurons of the granule cell layer, and a group of cells located just beneath the Purkinje cell layer, the intermediate cells of Lugaro (6). With the electron microscope, it is possible to separate the large from the small stellate neurons on the basis of size and cytological features described, though occasional cells of intermediate size are seen. It has not been possible in this study to separate the small stellate neurons of the molecular layer from the basket cells, though the basket cell processes are easily recognized where they pass around the Purkinje cells. Lugaro cells are scarcely seen even by light microscopy, and only a few cells were seen which seemed to fit the criteria of location (just beneath the Purkinje cell layer) and orientation of processes (horizontal) which would suggest that they were Lugaro cells. These cells did not appear to differ cytologically from other large stellate neurons in the granule cell layer.

Microglia were readily identified by their great density and their shape. The presence of a small amount of extracellular space about some of these cells was of particular interest, in view of the absence of extracellular space in other parts of the tissue. Two explanations of this situation are possible: the cells could be more susceptible to shrinkage during fixation and processing than the other cells of the cerebellum, or the space could represent a space present in the living animal. If the latter is the case, the space may be related to the mobility of these cells. In order to move, the microglial cell must separate neuronal and glial cells and processes which normally are closely apposed to each other and may be adherent to each other. It may be that the microglia secrete some substance which decreases the adherence of those cell processes. Also, if the microglia are significantly less adherent than the other cell types, shrinkage artifacts would tend to concentrate about these cells.

The main advantages of perfusion fixation as seen in this study, in agreement with the findings of Palay *et al.* (17), are the absence of glial swelling, the homogeneous distribution of the nuclear chromatin, the absence of light and dark cell artifacts, and the possibility of orienting tissue blocks which makes possible the study of cellular relationships in a way impossible without orientation of sections.

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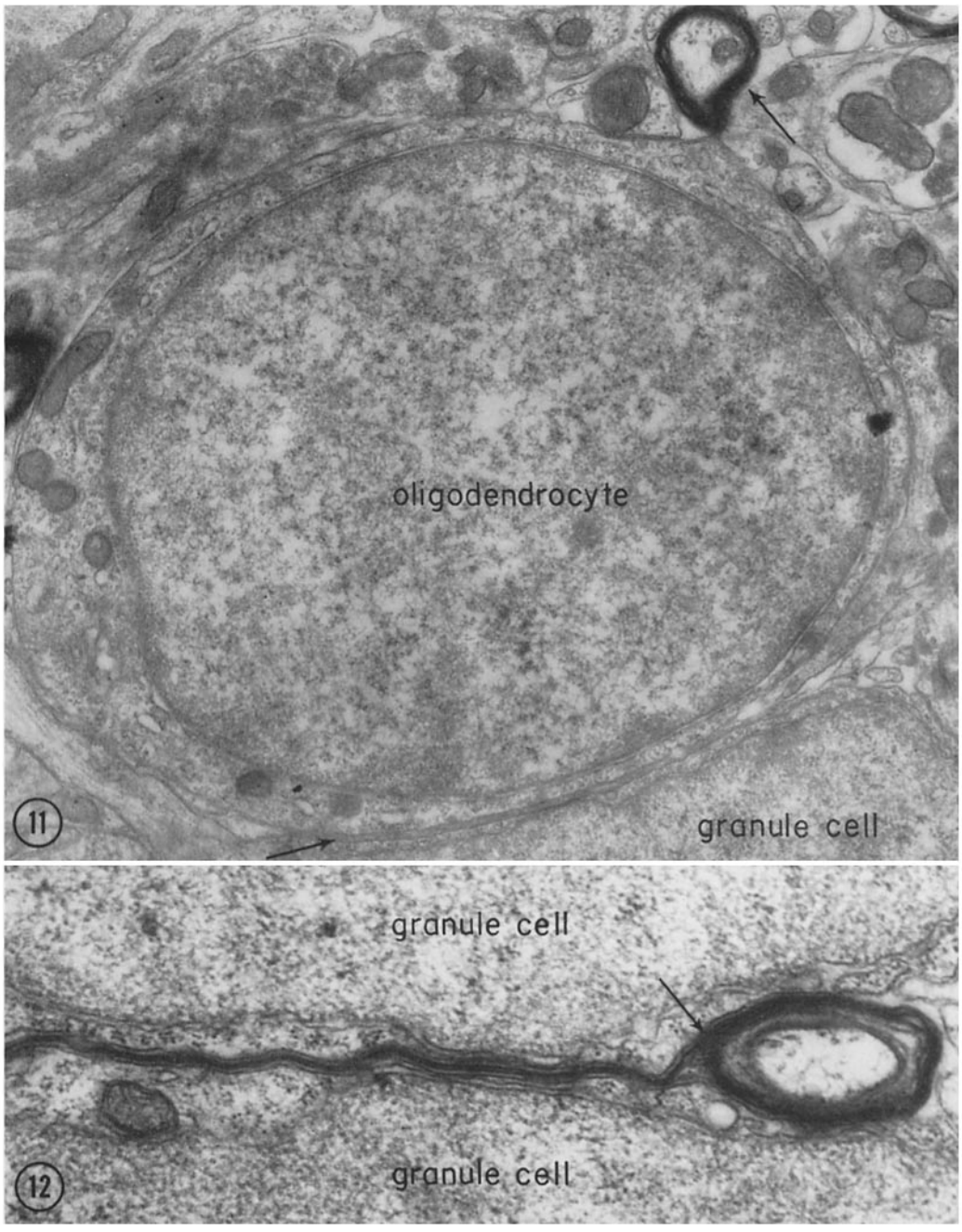
For references, See pp. 292 and 293.

FIGURE 11. A small process of the oligodendrocyte shown in this micrograph can be traced for a short distance along the edge of a myelin sheath (upper arrow). The process is partially obscured by the obliquely cut myelin and, because of the obliquity of the cut, it is not possible to definitely demonstrate the connection of the process with the myelin sheath. A very thin process can be seen at the bottom (arrow) running between the oligodendrocyte and a granule cell. × 14,000.

FIGURE 12. This micrograph shows a cell process consisting of little more than a double membrane passing between two granule cells to end in a meylin sheath (arrow). A small amount of retained cytoplasm is apparent adjacent to the myelin sheath just below the arrow. This process is probably of oligodendroglial origin. × 28,500.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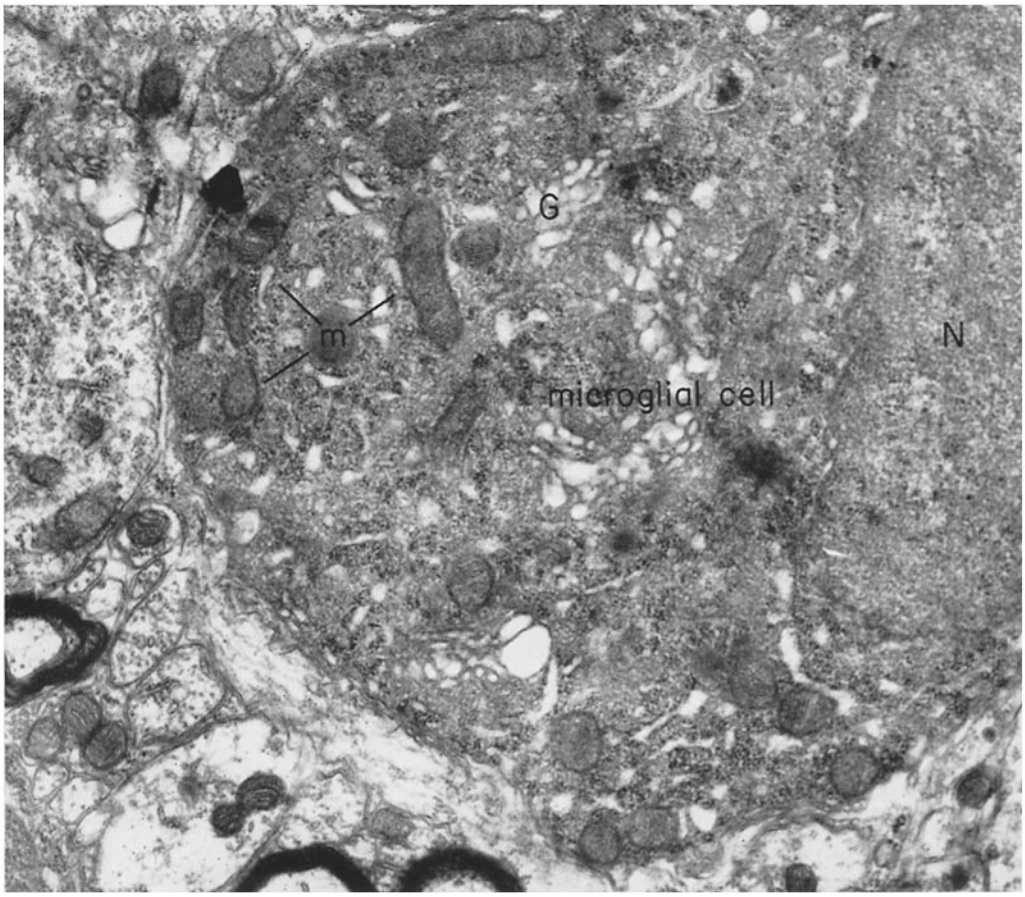

FIGURE 13. This micrograph of a microglial cell shows the elongate nucleus (*N*) and very dense cytoplasm. The mitochondria (arrows) are smaller than those in the oligodendrocytes and are often difficult to see against the dense cytoplasmic background. The well developed Golgi complex (*G*) with many vacuoles occupies a large proportion of the cytoplasm. × 19,000.

FIGURE 14. This drawing of a granule cell illustrates the Golgi apparatus (*G*), the rather sparse endoplasmic reticulum (*er*), mitochondria (*m*), and a dense body (*d*). A nucleolus (*n*) is shown in the nucleus. The relatively sparse cytoplasm is apparent.

FIGURE 15. This drawing illustrates a Bergmann astrocyte which has only a thin rim of cytoplasm on one side of the nucleus and a relatively large cytoplasmic mass on the other. RNP particles, dense bodies (*d*), mitochondria, the Golgi apparatus (*G*), and a few tubular elements of the granular endoplasmic reticulum are illustrated. At the left are the bases of two processes.

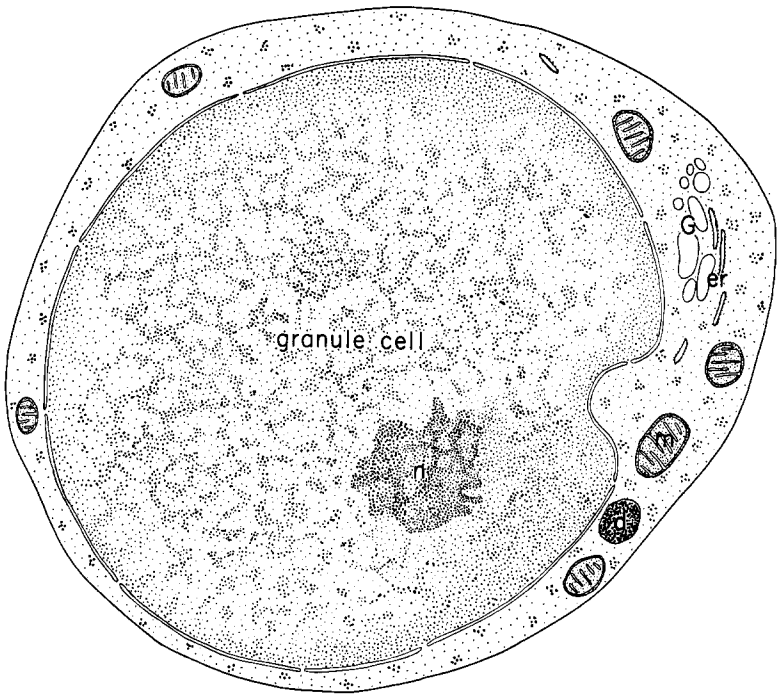

FIGURE 14

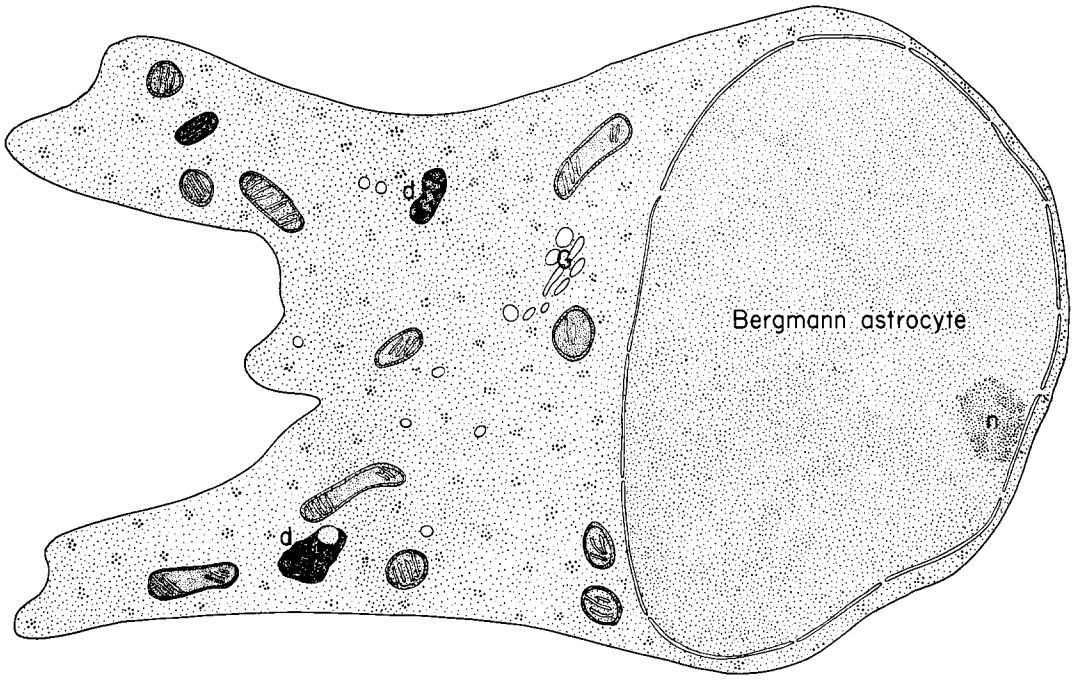

FIGURE 15

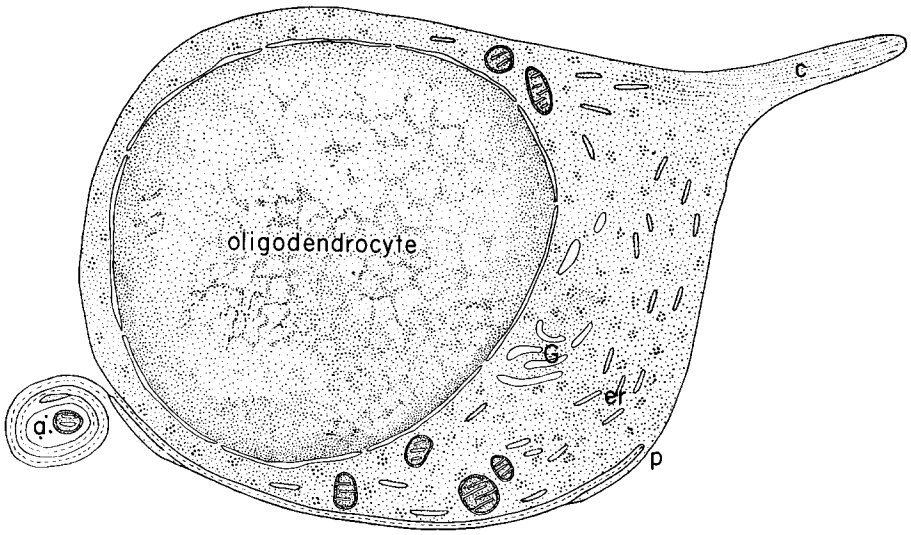

FIGURE 16. This drawing shows what the author believes to be the relationship between the oligodendrocyte and myelin. The oligodendrocyte cytoplasm contains numerous RNP particles, an extensive granular endoplasmic reticulum (*er*), and a prominent Golgi apparatus (*G*). At the bottom, a thin process (*p*) is seen arising from the cell body and running along the plasma membrane. Over a large portion of the length of this process, the two membranes are shown as being fused, as apparently occurs in many instances. The interperiod line shown between the process and the plasma membrane has been seen on several occasions but is not invariably present. At the lower left, the process spirals around an axon (*a*) to form a myelin sheath. A larger cell process containing canaliculi (*c*) is seen at the upper right.

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