

# THE FINE STRUCTURE OF THE PURKINJE CELL

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## ABSTRACT

This paper describes the fine structure of the Purkinje cell of the rat cerebellum after fixation by perfusion with 1 per cent buffered osmium tetroxide. Structures described include a large Golgi apparatus, abundant Nissl substance, mitochondria, multivesicular bodies, osmiophilic granules, axodendritic and axosomatic synapses, the nucleus, the nucleolus, and the nucleolar body. A new and possibly unique relationship between mitochondria and subsurface cisterns is described. Possible functional correlations are discussed.

The large, uniform neurons, which are named after the Czech physiologist who first described them, form the sole output pathway of the cerebellar cortex and, as such, are an essential link in a complex feedback system which acts on the motor system of the body to regulate muscle tone and movement. The functional importance of these cells and their marked vulnerability to certain toxic conditions makes them of particular interest to the neurologist and neuropathologist.

Knowledge of the structure of these cells began with Purkinje who described them in a paper on the histology of the nervous system presented in Prague in 1837 (Fig. 1).

“Jedes dieser Körperchen ist mit dem stumpfen, rundlichen Ende nach Innen gegen die gelbe Substanz gekehrt, und zeigt in seinem Kolben deutlich sammt seinem Hofe den centralen Kern; das andere schwanzförmige Ende ist nach Aussen gerichtet, und verliert sich mit meist zweien Fortsätzen in der grauen Substanz bis nahe an die äussere Peripherie wo diese mit der Gefässhaut umspinnen ist”.<sup>1</sup>

Johannes Evangeliste Purkinje (39).

<sup>1</sup> “Each one of these bodies is turned with the blunt rounded end inward towards the yellow substance, and clearly shows in its body the nucleus as well as its halo; the other tail-shaped end is directed outward

In the century and a quarter since he described them, considerable detail has been added to knowledge of their structure. With Golgi's discovery of a method for selective impregnation of neurons by silver salts, in 1870, it became possible to trace the cell processes of individual cells for long distances (12). Using this technique, he observed and described the extensive dendritic ramifications of the Purkinje cell, and traced their axons and collaterals (13). In doing so, he elucidated many of the cellular relationships in the cerebellum. Ramón y Cajal, who refined Golgi's techniques and developed a number of his own, added much to our knowledge of the Purkinje cell and its relationships. He demonstrated the dendritic spines, the axosomatic, axodendritic and the axo-axonic synapses of the Purkinje cell, and the recurrent axon collaterals which synapse on adjacent Purkinje cells. He also discovered the basket cells and granule cell axons (40-43).

Franz Nissl (26) described the material, which bears his name, in a number of different nerve cells including the Purkinje cell in 1894, and over the following years its importance in regard to

and loses itself with usually two processes in the grey substance close to the periphery, where this is invested by the vascular membrane.”

cellular pathology was firmly established. Its equivalence with the granular endoplasmic reticulum of electron microscopy was demonstrated by Palay and Palade in a comparative light and electron microscopic study published in 1955 (36).

In 1898, Golgi, using a new staining method (14), described and illustrated a reticular network within the cytoplasm of the Purkinje cell of the

This view, however, has been questioned recently on the basis of studies on the Purkinje cells of owls (24); this point will be discussed later.

Cytoplasmic granules apparently distinct from both Nissl substance and Golgi apparatus have also been described in the Purkinje cell (2, 21, 47-49).

The numerous connections of the Purkinje cell were emphasized by Fox (10) who calculated

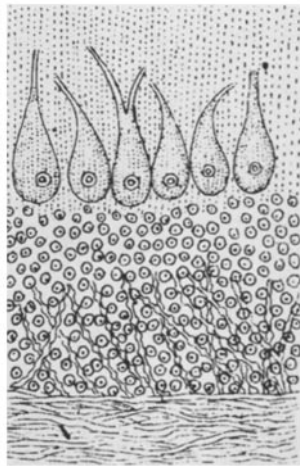


FIGURE 1

This plate is taken from Purkinje's description of the cerebellar cortex and shows, from top to bottom, the molecular layer, the Purkinje cell layer, the granule cell layer, and the white matter (39).



FIGURE 2

This drawing by Golgi illustrates the network which bears his name in the Purkinje cell of the owl, *Strix flammea* (14).

owl, *Strix flammea* (Fig. 2). This network, which could be seen only with special fixation and staining techniques, was considered by many to be an artifact (29). With the further application of the electron microscope to biological materials, the equivalence of the Golgi apparatus of light microscopy with the agranular reticulum of electron microscopy was demonstrated (3, 4).

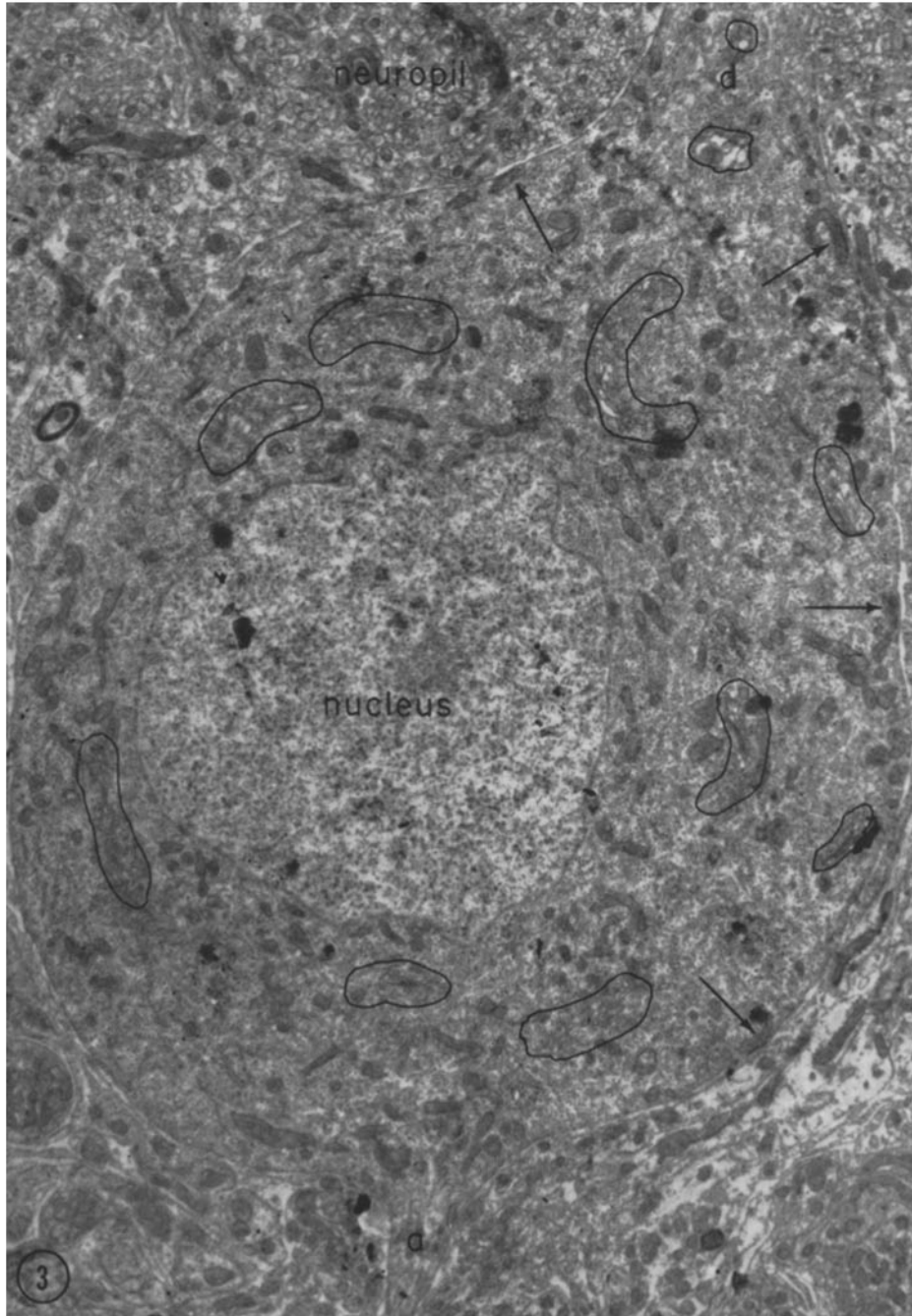
that there are about 61,000 dendritic spines on a single dendrite and that there are from 223,000 to 297,000 parallel fibres passing through the dendritic spread of a single Purkinje cell. More recent, electron microscopic observations by Fox (11) and by Gray (18) have confirmed the synaptic nature of the dendritic spines and elucidated their fine structure.

Many further observations on the fine structure of nerve cells, including Purkinje cells, have been

FIGURE 3

In this low power micrograph of a Purkinje cell, the dendrite (*d*) is readily seen at the upper right. The origin of the axon (*a*), cut at an angle, is seen at the bottom. The agranular membranes and vesicles of the Golgi apparatus (outlined) can be seen around the nucleus and in the base of the dendrite (compare with Fig. 2). Mitochondria and dense granules can be seen scattered throughout the cytoplasm along with numerous elements of granular endoplasmic reticulum. Several mitochondria in close association with subsurface cisterns are also seen (arrows).

A small pericellular space can be seen in some areas. This is artifact caused by shrinkage and is not a true pericellular space.  $\times 6,400$ .



made (1, 7, 9, 23, 30, 33); however, an adequate electron microscopic study of the Purkinje cell was not possible until the advent of fixation by perfusion with solutions of osmium tetroxide (37). The object of this paper is to give a systematic account of the Purkinje cell of the rat as it appears in the electron microscope after perfusion fixation with buffered osmium tetroxide. This will provide a baseline for the study of experimentally induced changes and also may provide some basis for correlation of structure with function.

#### MATERIALS AND METHODS

Rats weighing from 200 to 300 grams were anesthetized with intraperitoneal pentobarbital. Fixation was carried out by the perfusion method of Palay (37) with the minor modification that the cannula was placed in the descending aorta pointing in a cranial direction and the proximal aorta was ligated. When fixation was successful, the entire cerebrum and cerebellum were blackened save for a few small areas. The fixative used was acetate veronal buffered 1 per cent osmium tetroxide with salts added, as described by Zetterqvist (52), and calcium chloride added to bring the solution to a final calcium concentration of 5.4 mg of calcium per ml of solution. We have found fixation with this method superior to immersion fixation and also to fixation by perfusion with cold solutions of osmium tetroxide. Observations were made on four rats perfused in this fashion along with some observations made on tissue fixed by immersion and tissue fixed by perfusion with buffered 2 per cent permanganate solution. The latter perfusion method, which was performed on five rats, was found to be less satisfactory and technically more difficult than perfusion with solutions of osmium tetroxide. Forty minutes after the start of the perfusion, the cerebellum was removed and blocks were cut in a manner which allowed for embedding in the desired plane. The blocks were then dehydrated through a graded series of ethyl alcohol followed by propylene oxide and embedded in Epon 812 (22). After polymerization the blocks were trimmed and sectioned on a Porter-Blum microtome with a glass knife. The sections were picked up on copper grids coated with formvar and carbon, stained with alkaline lead acetate (38, 50), and examined with an RCA EMU 3E electron microscope. Adjacent thick sections were examined with the phase contrast microscope.

#### OBSERVATIONS

In the electron microscope, as in light microscopy, the Purkinje cells are large cells with a diameter of 25 to 40 microns (Fig. 3) situated between the

granule cell layer and the molecular layer of the cerebellum. Their dendrites arborize in a plane at right angles to the cerebellar folia, and the tertiary dendrites are studded with dendritic spines. Unlike the arrangement in many neurons, the Purkinje cell axon takes off directly from the cell body with no axon hillock. In the electron microscope, properly oriented sections show the Purkinje cell dendrites as they divide and redivide in the molecular layer (Fig. 5). Numerous mitochondria and elements of granular endoplasmic reticulum can be seen in the peripheral portion of the larger dendrites, and dendritic canaliculi (17, 33, 37) are regularly arranged throughout the dendrites. The tertiary branches of the dendrites contain few canaliculi and the mitochondria are elongated with their cristae usually running parallel to their long axis. These tertiary branches are studded with small projections which usually make one synaptic connection and can readily be seen to be equivalent to the dendritic spines seen with the light microscope.

The initial segment of Purkinje cell axon is unmyelinated but the remainder of the axon is heavily myelinated. Within the axon are a moderate number of canaliculi, mitochondria, and scattered vesicles ranging in size from about 50 to 150  $\mu$ .

Three types of synapse are seen on the Purkinje cell. The first type (type 1 of Gray) is the axodendritic synapse (Fig. 6) which is composed of a terminal bouton and a dendritic spine. This bouton consists of a terminal axonal enlargement containing one or more mitochondria and synaptic vesicles. The pre- and postsynaptic membranes are separated by about 300 Angstroms and within the synaptic cleft is a thin dense line. The postsynaptic membrane, unlike that in the axosomatic synapse, is usually considerably thickened relative to other portions of the Purkinje cell membrane. The spine apparatus (which is frequently seen in dendritic spines in the cerebral cortex (16, 17) has not been found in the spines of the Purkinje cell. The second type (type 2 of Gray) (16-19) is the axosomatic synapse (Fig. 7) which consists, as in the case of the axodendritic synapse, of a terminal expansion or bouton containing numerous synaptic vesicles and usually one or more mitochondria. One side of this bouton is closely applied to the cell body, and the synaptic vesicles are more densely packed along this region of close contact. The pre- and postsynaptic mem-

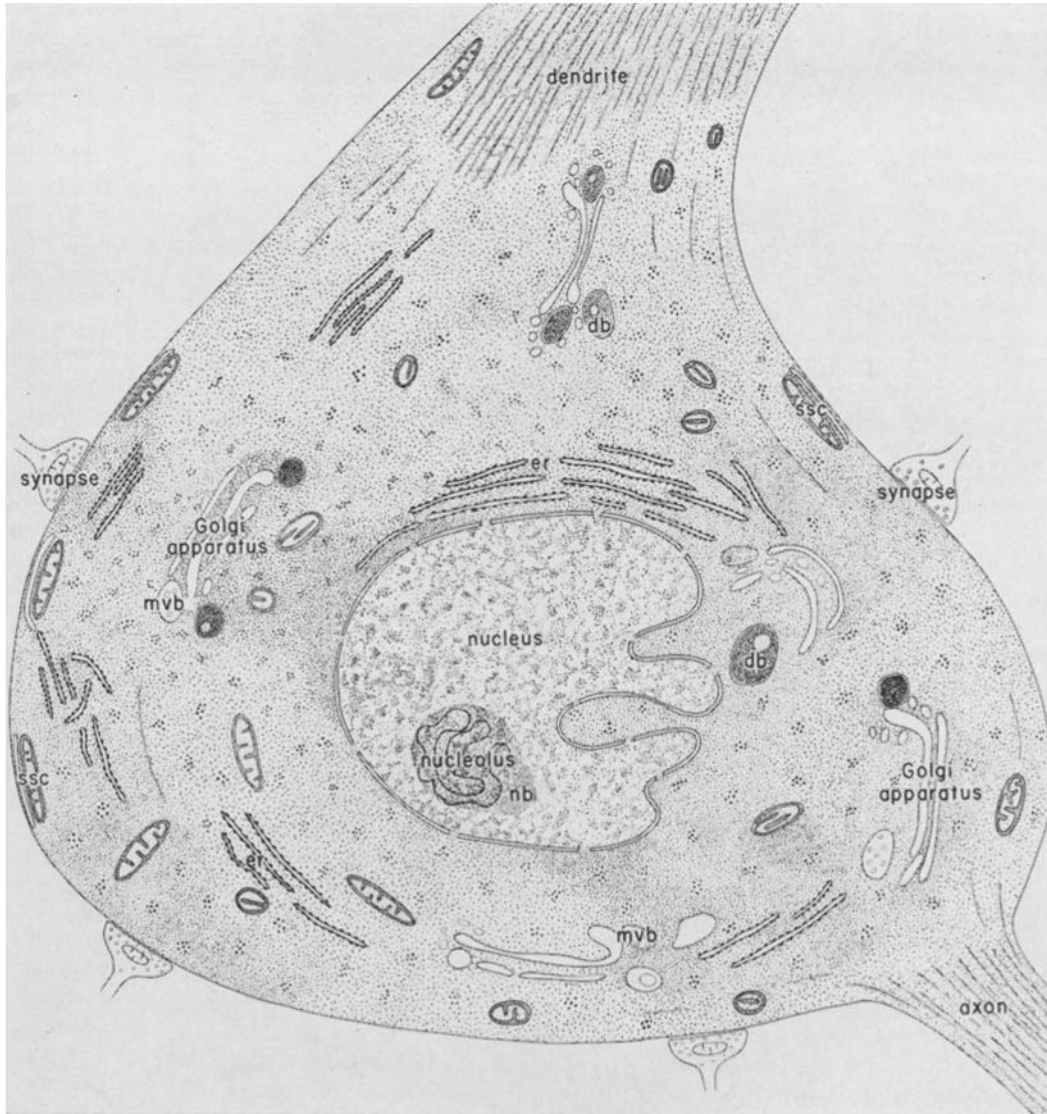


FIGURE 4

This composite drawing of a Purkinje cell illustrates many of the structures described in this paper. The axon is seen at the lower right and the dendrite is at the top. Also shown are axosomatic synapses, subsurface cisterns (*ssc*) with associated mitochondria, the Golgi apparatus, multivesicular bodies (*mvb*), endoplasmic reticulum (*er*), and dense bodies (*db*). In the nucleus, the nucleolus and nucleolar body (*nb*) are easily seen. Neural tubules can be seen concentrated in the dendrite and axon. Drawing by Miss E. Swezey.

branes are separated by a space of 180 to 200 Angstroms. The structure of the axo-axonic synapse has not been worked out in detail. These latter synapses are located on the proximal, unmyelinated, portion of the Purkinje cell axon.

Beneath the cell membrane of the body of the cell, narrow membrane-bounded cisterns similar to those described by Rosenbluth in cells of the acoustic ganglion are frequently seen (45, 46). Each subsurface cistern has, in most instances, a

mitochondrion closely applied to its inner surface (Fig. 8). In most instances, the mitochondrion appears to cover virtually the entire inner surface of the cistern. Subsurface cisterns without associated mitochondria were also occasionally seen in these cells. In some instances, there appeared to be continuity between the subsurface cisterns and the endoplasmic reticulum. In a number of instances, there appeared to be one or two ring-like structures, possibly vesicles or, more likely, cross-sectioned tubules, at one end of a subsurface cistern. The subsurface cisterns do not appear to bear any constant relationship with any extracellular structure.

Scattered throughout the cytoplasm and in the major dendritic branches, the roughly parallel membranes of the granular endoplasmic reticulum can be seen. These membranes have, on their outer surfaces, numerous 150 Angstrom dense ribonucleoprotein granules (31, 32). Additional unattached RNP granules appear scattered throughout the cytoplasm. Parallel arrays of these granular membranes are common only in the region of the nucleus, the membranes found in other parts of the cell being usually irregularly scattered (Figs. 4, 5, 9, and 10).

Located in the cytoplasm about the nucleus and in the region of the base of the dendrite, numerous arrays of closely packed agranular membranes with associated membrane-bounded spaces and vacuoles are seen (Figs. 3, 4, and 9). These structures usually are arranged in a semilunar or circular pattern and have been shown by Dalton and Felix (3, 4) to be the Golgi apparatus of light microscopy. Within some of the Golgi vesicles, dense granular material is occasionally seen. The Golgi network is considerably more extensive than that in cells from most other tissues.

Small, round or oval multivesicular bodies are commonly present and are usually seen in close association with the Golgi apparatus. Some of these multivesicular bodies contain varying amounts of dense granular material and occasion-

ally a myelin figure. In some bodies the dense granular material almost completely obscures the vesicles, and these examples appear to be a stage in the development of the dense osmiophilic bodies which are present in all these cells (Figs. 4, 8, and 9). These dense bodies are bounded by a distinct membrane, and the larger ones often contain one or more vacuoles. The vacuoles appear clear in unstained and in lead-stained material, but are moderately electron opaque in phosphotungstic acid-stained material.

The mitochondria in the cell body are similar to those seen in other neurons (5, 20). They are generally sausage-shaped and in most instances the cristae run at right angles to the long axis. Occasional irregular and branched forms are seen. The mitochondria in the smaller dendritic branches, on the other hand, tend to be more elongated and their cristae usually run parallel to the long axis of the mitochondrion. These elongated mitochondria are often helpful in identifying the smaller dendritic branches of the Purkinje cell.

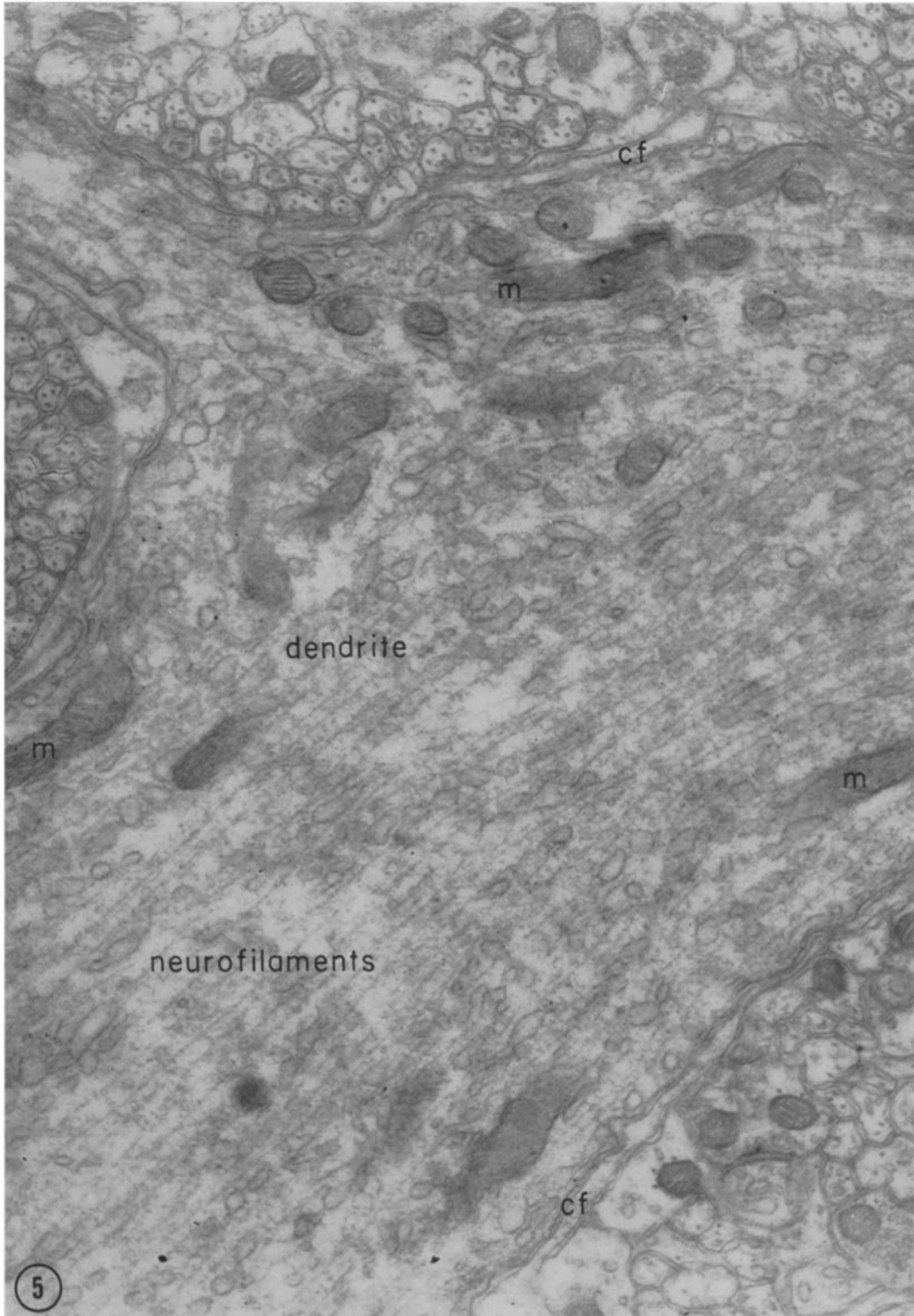
The nucleus has a roughly circular outline with numerous indentations and infoldings of the nuclear membrane which consists of two electron opaque parallel membranes separated by a narrow but variable space of low electron opacity. The nuclear membrane can be seen to be perforated at frequent intervals by nuclear pores (51). The nuclear material in well fixed specimens is evenly distributed, finely dispersed, and granular. In inadequately fixed specimens it is clumped with clear areas between the clumps.

The nucleolus (Fig. 10) appears as an aggregation of closely packed, very dense granules of variable size. At one side of the nucleolus is the nucleolar body which has a different pattern of granularity. In permanganate-fixed material, the dense granular material in the nucleolus is not fixed and there remains a faintly stained background against which a number of fine dense filaments may be seen, but the nucleolar body is unchanged in appearance.

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#### FIGURE 5

This micrograph shows a secondary dendrite of a Purkinje cell at a point of branching. The dendritic canaliculi can be seen running a parallel course from lower left to upper right. A few elements of endoplasmic reticulum and mitochondria (*m*) can be seen at the periphery of the dendrite. Closely applied to the dendrite, on either side, climbing fibers can be seen (*cf*). Neuropil is readily distinguished at the lower right and upper left.  $\times 18,000$ .



## DISCUSSION

The multivesicular bodies and dense granules illustrated in this paper are similar to those described in the hypothalamus of goldfish (34, 35), mice (25), and chickens (8). The presence of transition forms from the multivesicular body to the dense granules has been thoroughly described and illustrated by Palay (34) in preoptic neurons of the goldfish, and these same transition forms are seen in the rat Purkinje cell. Enzyme studies carried on at the electron microscope level on similar dense granules in cells of the spinal cord reveal acid hydrolase activity in these granules (28) and, as a result of these studies, Novikoff considers them to be lysosomes.

Chouinard (2) has described two types of granules seen in the Purkinje cell of the cat with light microscopy. The first type has the histochemical characteristics of a neutral mucopolysaccharide and is diffusely distributed throughout the body of the cell. The second type is lipofuscin and is found mainly in clumps about the base of the dendrite and in the major dendritic branches. The size and distribution of the granules in our material seem to correspond with those of type 1, and lipofuscin has not been seen in our material prepared for light microscopy. This is probably due to the young age of the animals used both for light and electron microscopic studies. The elucidation of the true nature of these granules and their relationship to lysosomes, neutral mucopolysaccharide granules, or lipofuscin granules awaits further study.

Malhotra and Meek (24) in their study of the Purkinje cells of owls expressed the opinion that there is too little agranular reticulum in the Purkinje cell for it to be the equivalent of the Golgi

apparatus of light microscopy. Their view, however, is based on immersion-fixed material in which we consider the granular endoplasmic reticulum to be dilated at the expense of other intracellular spaces. In immersion-fixed material, many of the elements of agranular reticulum are not recognizable. Fig. 3 is rather typical of the Purkinje cell seen after perfusion fixation; the agranular reticulum conforms in extent and distribution with the Golgi apparatus of light microscopy (compare Figs. 2 and 3).

The difference in appearance of the nucleolus in permanganate-fixed material is due to the permanganate which does not fix the nucleolar RNA as does osmium tetroxide. The remaining dense filamentous material in the nucleolus after permanganate fixation may represent the nucleolonema (6, 51).

The interesting association of mitochondria and subsurface cisterns suggests a number of possible functions. An arrangement of this sort might be very useful in supplying high energy phosphates such as ATP, produced by the mitochondria, to a structure which might be involved in the active transport of metabolites into or out of the cell. The close association and apparent continuity of these structures with the granular endoplasmic reticulum suggests the possibility that these structures control the movement of material into and out of the channels of the endoplasmic reticulum.

Investigations carried out on living tissue cultures have suggested to some investigators that mitochondria migrate from the region of the nucleus to the periphery and back, and that mitochondria might make use of an arrangement such as the subsurface cistern to pick up metabo-

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FIGURE 6

Near the center of this micrograph is an axodendritic synapse (circled). The terminal axonal enlargement, which is above, contains numerous synaptic vesicles, and a mitochondrion is readily seen. Between the presynaptic and postsynaptic membranes, a thin dense line (arrow) can be seen. At the left is a secondary branch of a Purkinje cell dendrite which contains a mitochondrion at *m*. The origin of the dendritic spine making synaptic contact is out of the plane of section.  $\times 42,000$ .

FIGURE 7

This micrograph shows an axosomatic synapse (circled). Near the center of the figure is the enlarged synaptic terminal (probably of a basket cell) containing two mitochondria and numerous synaptic vesicles clustered about the presynaptic membrane. To the left is the Purkinje cell cytoplasm. At the far left, elements of the Golgi apparatus (*G*) are seen. Mitochondrion, *m*; synaptic vesicles, *sv*.  $\times 40,000$ .





lites at the periphery before migrating back to the nuclear region (27). The absence of intermediary forms may be considered to be evidence against the interpretation that they are a stage in the formation of mitochondria from the cell wall (44). It seems likely that these cisternae are involved in the transport of some material into or out of the Purkinje cell, but both the material transported and the mechanism of transport remain unclear.

At present, it is not possible to distinguish excitatory from inhibitory synapses on a morphologic basis. The evidence for the existence of two functionally different synapses is well documented physiologically (15) and it seems reasonable to look for anatomical differences to correlate with this. In the present study, we have confirmed the

previous observations of Gray (18) who found that cerebellar axosomatic and axodendritic synapses differ in structure, the axodendritic synapse demonstrating an additional dense line between appositional surfaces of the cell membranes of the synapse. It is tempting to speculate that the presence of such a morphologic difference might be correlated with a functional difference.

Dr. Herndon is a United States Public Health Service postdoctoral Fellow at the Montreal Neurological Institute.

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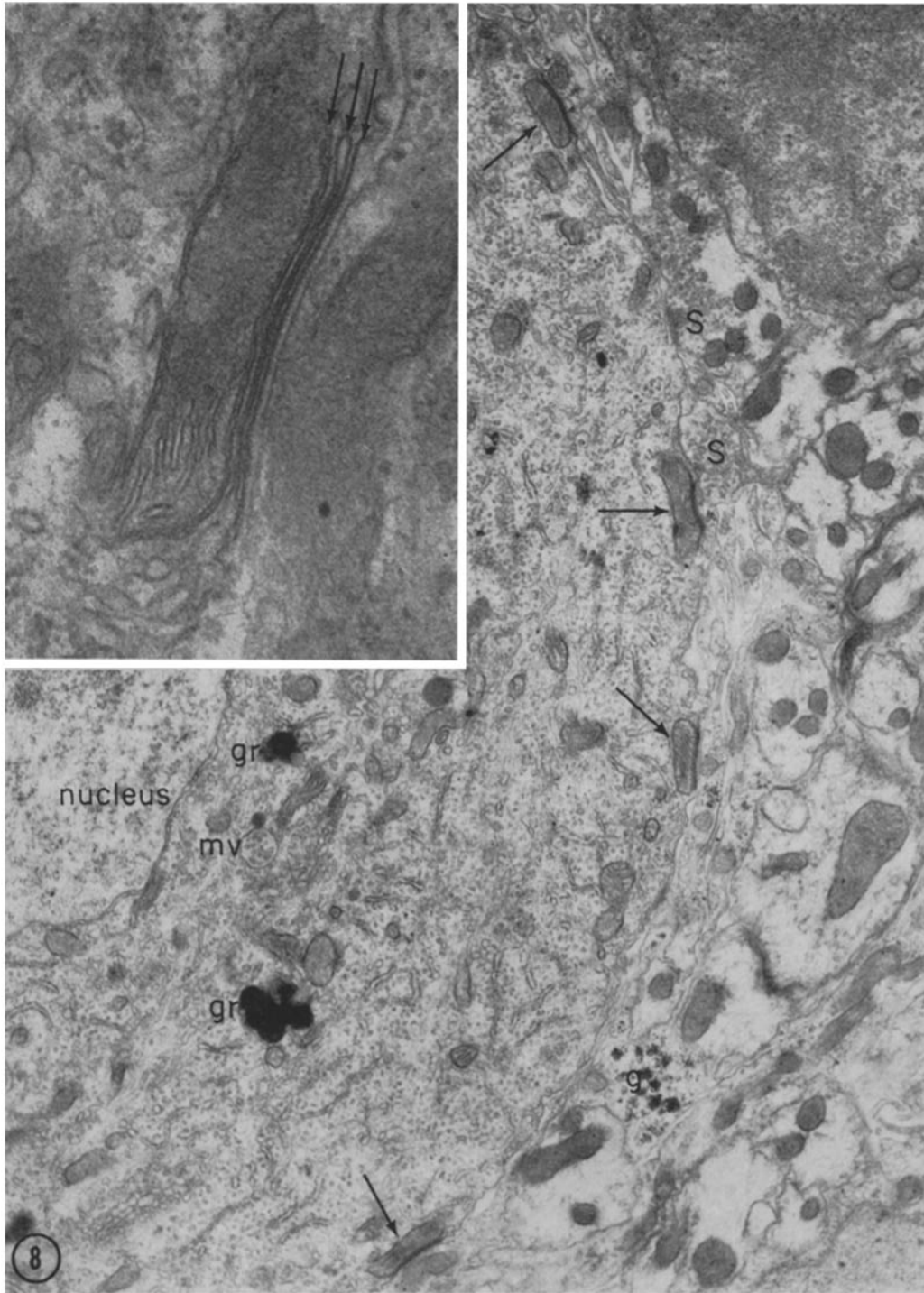
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#### FIGURE 8

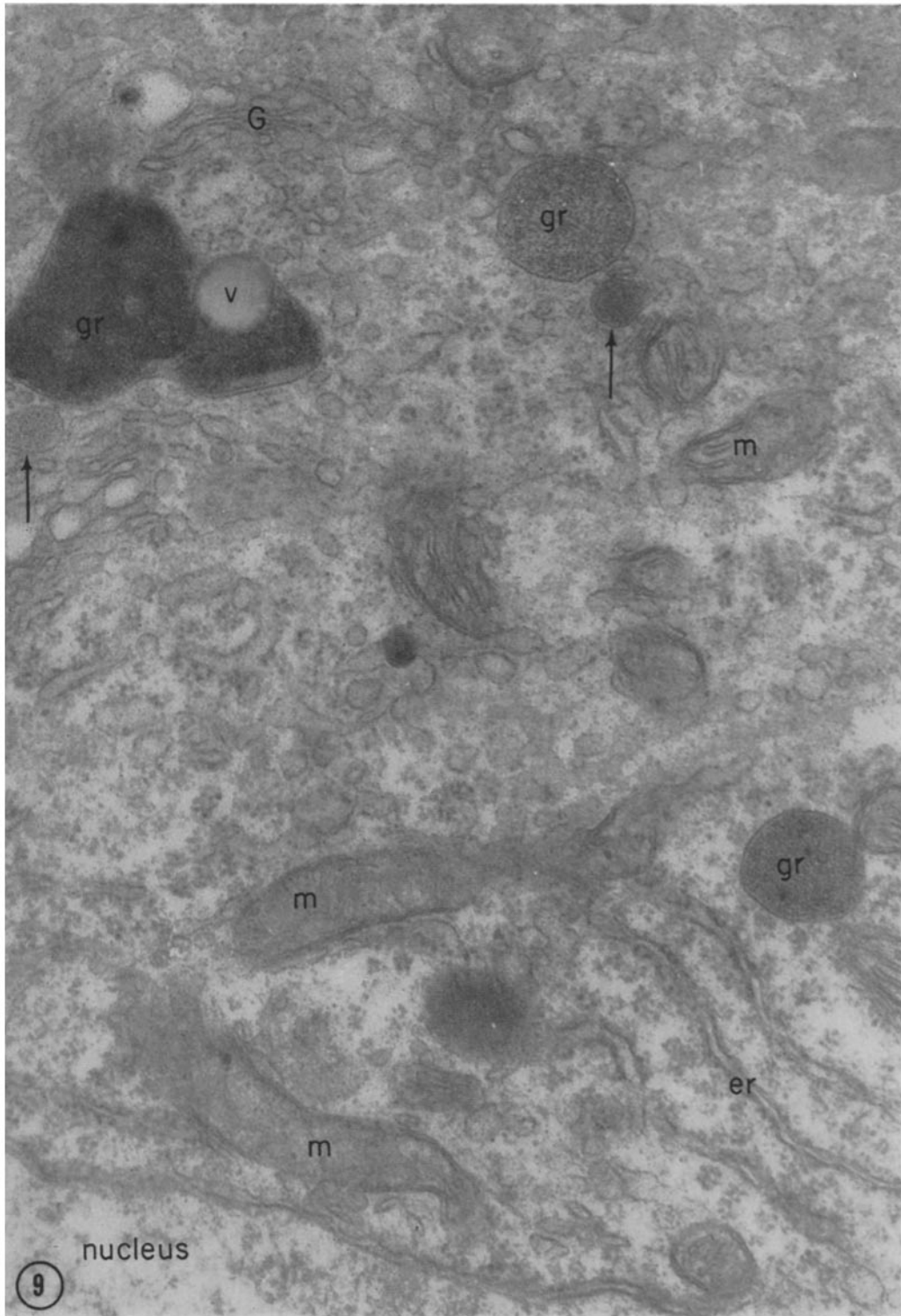
In this micrograph of a Purkinje cell, four mitochondria can be seen in close apposition to subsurface cisterns (arrows). A multivesicular body (*mv*) and dense granules (*gr*) can be readily distinguished. The nucleus and nuclear membrane can be seen at the left ( $\times 10,500$ ). Glycogen (*g*) can be seen in a basket fiber at lower right. *Inset* at the upper left is a higher power ( $\times 53,000$ ) view of a mitochondrion in close apposition to a subsurface cistern. The arrow on the left indicates the mitochondrial membranes. The middle arrow points to the loop at the end of the subsurface cistern, and the arrow on the right indicates the cell surface membrane. Just above the subsurface cistern an indentation in the cell membrane is seen, and just to the left of this, partially covered by the arrows, is a ring structure, possibly a vesicle or cross-section of a tubule.



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FIGURE 9

In this micrograph of Purkinje cell cytoplasm, the nuclear membrane can be seen at the lower left. Granular endoplasmic reticulum (*er*), RNP granules, mitochondria (*m*) and Golgi apparatus (*G*) are easily seen. Three dense granules of varying density and size are apparent (*gr*). The largest granule contains a vacuole (*v*). Within the vesicles of the Golgi apparatus (arrows) some dense granular material can be seen.  $\times 34,500$ .



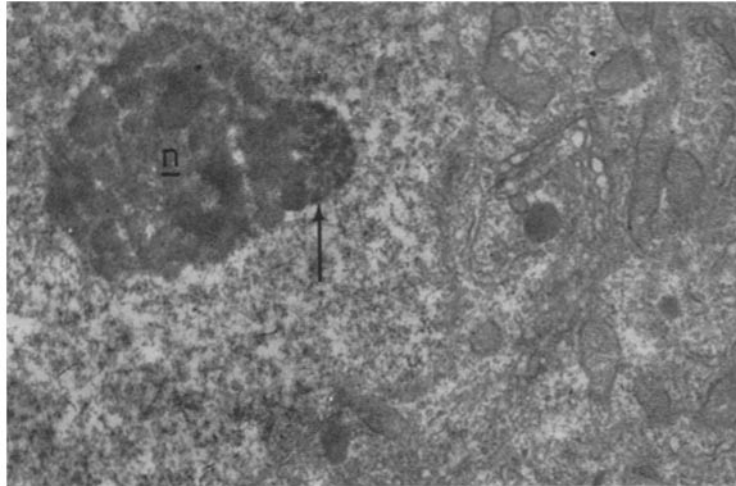


FIGURE 10

This electron micrograph demonstrates the nucleolus (*n*) and the nucleolar body (arrow). Elements of the Golgi network, a dense body, and mitochondria are present also.  $\times 13,000$ .

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