Experimental Reorganization of the Cerebellar Cortex

II. EFFECTS OF ELIMINATION OF MOST MICRONEURONS WITH PROLONGED X-IRRADIATION STARTED AT FOUR DAYS

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ABSTRACT The heads of Long-Evans rats were irradiated from the fourth day after birth with a schedule of repeated doses of low-level x-ray which essentially prevented the formation of basket, stellate and granule cells in all except the earliest-forming lobules (nodulus and uvula). The morphogenic and synaptogenic effects of this treatment were examined with light and electron microscopy in 30 day old animals, with particular attention paid to the pyramis. Although when irradiation was started the Purkinje cells formed a monolayer and had upward oriented apical poles, they became scattered later and had randomly oriented dendrites. This secondary disorientation was attributed to insufficient space available in the arrested cerebellum for the rapidly expanding Purkinje cells. Although basket cell terminals were scarce, basket cell-like terminals were formed on the somata of Purkinje cells, apparently by recurrent axon collaterals of these cells. The most common synapses with the thorns of Purkinje dendrites were formed by climbing fibers but other elements, including glial processes, were also in contact with postsynaptic loci on the thorns. Many mossy fiber terminals reached the surface. Where parallel fibers were present they were often thicker than in unirradiated animals and contained neurofilaments. No pathological changes were seen in these cerebella, with the possible exception of excessive lobulation of the nuclei of many Purkinje cells.

In the first paper of this series (Altman and Anderson, '72) we described the morphological effects of irradiation of the cerebellum of rats from birth onward. A schedule of x-irradiation was used that prevented the dispersion of Purkinje cells and eliminated all the cells of the proliferating external germinal layer that give rise to the postnatally-forming basket, stellate, granule and Lugaro cells. One of the aims of these experiments has been to produce a cerebellar cortex devoid of microneurons and to examine the deficits produced by their absence with physiological and behavioral techniques. Because irradiation from birth on interferes with the dispersion of Purkinje cells, whereas differentiation of the cells of the external germinal layer does not start until the end of the first week (Altman, '69, '72a), it appeared desirable to start the irradiations after the dispersion of the Purkinje cells and before the onset of the differentiation of microneurons (Altman and Anderson, '72, fig. 1, row 3). A recent behavioral study has indeed revealed that the consequences of irradiation of the cerebellum from birth on are much more severe (Altman, Anderson and Strop, '71) than when irradiation is started at four days (Anderson and Altman, '72). In this paper we have examined the morphological effects of irradiation started at four days with a schedule that prevents regeneration; in subsequent papers the re-organization of the cerebellar cortex will be described following an irradiation schedule that permits partial regeneration of the external germinal layer (Altman, '73b).

MATERIALS AND METHODS

The maintenance of animals and the

radiation procedure were described in detail in the previous paper of this series (Altman and Anderson, '72). In the present study cerebella from rats were examined whose heads were exposed to 200 r on days 4 and 5 and then, to prevent regeneration, 150 r was delivered on days 7, 9, 11, 13 and 15. The animals were killed at 30 days. The processing of tissue for light microscopy, the quantitative histological procedures used for evaluation of this material, and the preparation of tissue for electron microscopy were described in the previous paper. A detailed examination was made of ten irradiated and ten control cerebella stained with cresyl violet, hematoxylin and eosin, and Bodian's protargol-S method; two irradiated cerebella were impregnated with the Golgi-Cox procedure; four irradiated cerebella (pyramis) were prepared for and examined with electron microscopy.

RESULTS

Light microscopic observations

Qualitative observations in Nissl-stained sections. Although the majority of Purkinje cells form a monolayer in the vermis of four day old rats, in the cerebellum of 30 day old animals in which cerebellar irradiation was begun on the fourth day the Purkinje cells were distributed one to three cells deep in the nodulus and uvula and were even more densely packed in the other lobules (fig. 1A). In the nodulus most of the Purkinje cells had their apical dendritic poles oriented toward the surface (fig. 1C); the orientation of dendritic poles was random in the other lobules (fig. 1B, D).

The molecular layer was thin but recognizable in the nodulus and uvula, where there were a few larger cells interpreted as hypertrophied basket cells (fig. 1C). In these early-maturing lobules there was also a moderate concentration of granule cells in the granular layer. But in the other lobules of the vermis the molecular layer was thin and devoid of cells, without a recognizable granular layer and only a few scattered granule cells.

Quantitative data. Planimetric measurements of matched parasagittal sections (right and left) from ten irradiated animals gave an areal reduction in the vermis from 22.1 mm^2 in the controls to 4.5 mm^2. There was a small (8.6%), nonsignificant reduction in Purkinje cells. The results of cell counts in the pyramis are summarized in table 1, indicating an overall, subtotal elimination of granule cells in the granular layer and of basket cells in the molecular layer.

Observations in Bodian-stained sections. The pattern of staining in the cerebellum of normal animals was described in detail in the previous paper (Altman and Anderson, '72). Briefly, in the molecular layer impregnated fibers were restricted to the lower one-half to one-third of the layer. These horizontally and sagittally oriented fine and coarse fibers were interpreted as the axons of basket cells; they had pointed brush-ending type of "basket" terminals around the somata of Purkinje cells (fig. 2A). There were few impregnated fibers in the granular layer but a high concentration in the medullary layer. The latter were interpreted as Purkinje cell axons, the former as the initial parts of the axons and their collaterals.

In the animals irradiated from the fourth day onward, as in those exposed from birth (Altman and Anderson, '72) a rich supraganglionic plexus of impregnated fibers was present throughout the thin molecular layer, irrespective of the number of basket cells or their virtual absence. These fibers were often continuous with the numerous randomly crisscrossing fibers among the deeply packed somata of Purkinje cells and with the fibers of the medullary layer. However, pointed brush-ending basket terminals around the somata of Purkinje cells were rare or altogether absent (fig. 2B). These impregnated fibers are interpreted to be axons and recurrent collaterals of Purkinje cells. Their high concentration among the somata of Purkinje cells is partly attributed to the higher concentration of Purkinje cells in the irradiated cortex but more importantly to a compensatory increase of Purkinje cell collaterals due to the scarcity or absence of basket cells.

Observations in Golgi-impregnated sections. Purkinje cells were impregnated in large numbers throughout the vermis (figs. 3–4). Somata close to the surface tended

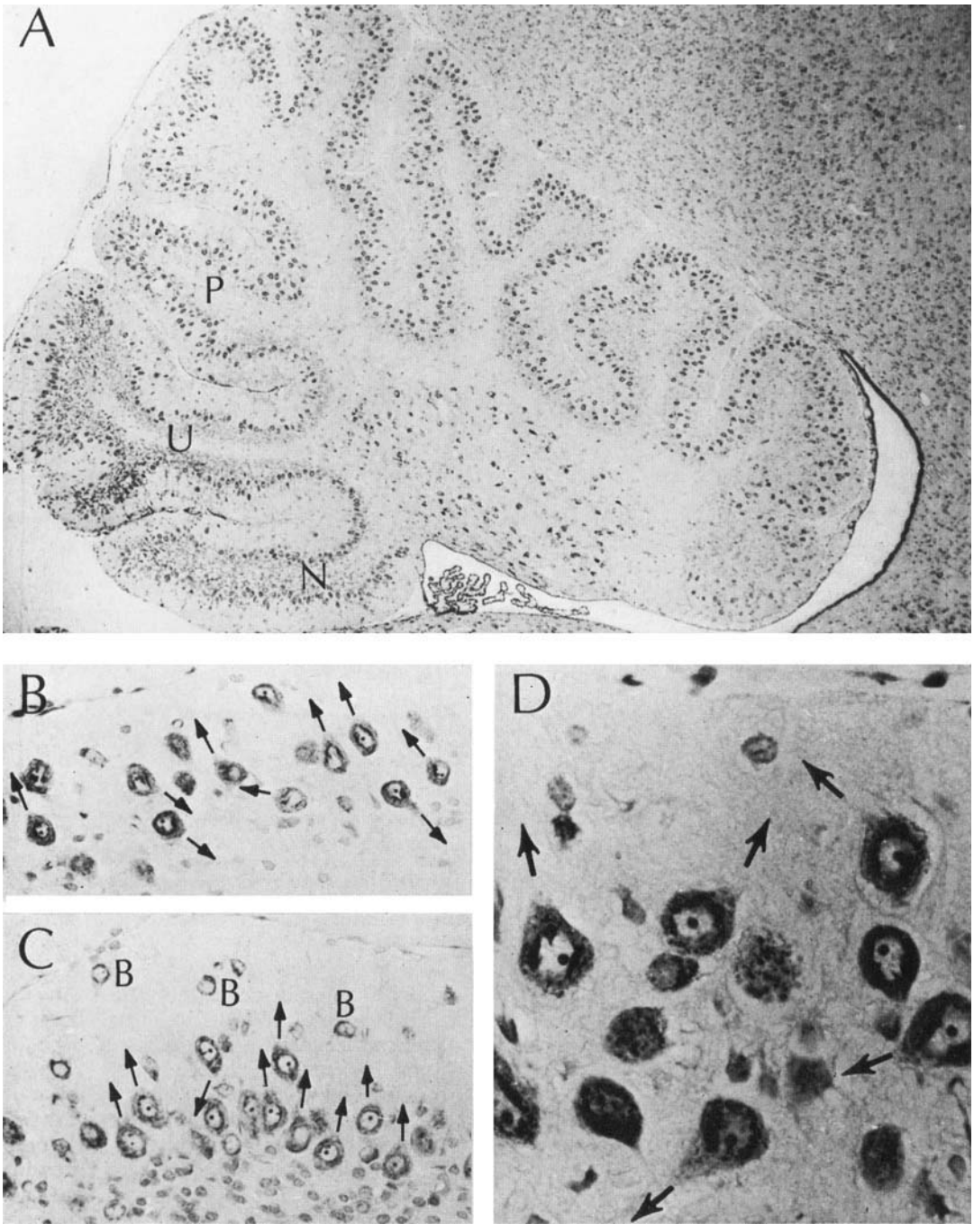

Fig. 1 (A) Parasagittal section of irradiated vermis. N, nodulus; P, pyramis; U, uvula. × 40. (B) Pyramis. Arrows indicate random orientation of Purkinje cell primary dendrites, × 256. (C) Nodulus. Orientation of Purkinje cell dendrites more regular. B, basket cells. × 256. (D) Tuber, × 640. Compare this figure with Altman and Anderson ('72), figure 2.

TABLE 1

Changes produced in the pyramis by prolonged x-irradiation started at four days of age

	Control	Irradiated	Reduction
			%
No. of Purkinje cells	66	56	16
No. of Golgi cells	39	28	30
No. of basket cells	176	12	93
No. of granule cells (total)	24,675	371	99
No. of cells in mol. layer	1,089	87	92
No. of granule cells in mol. layer	109	47	57

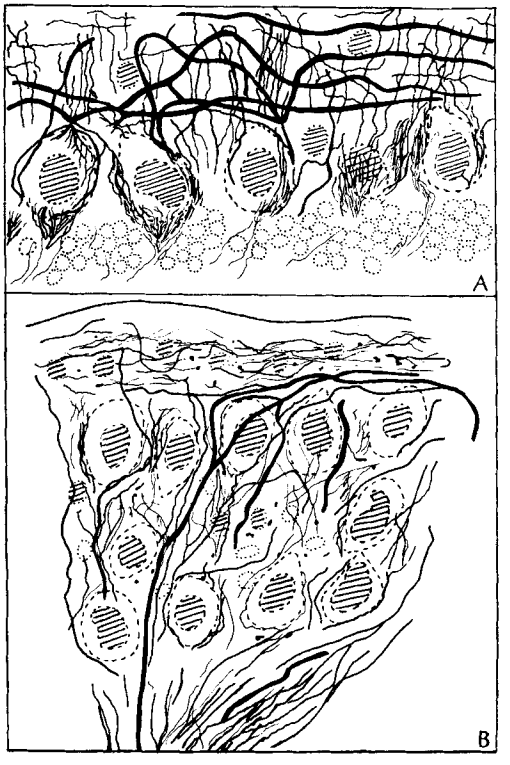

Fig. 2 Drawings of the distribution of fibers impregnated with the Bodian technique. (A) A row of Purkinje cells, part of upper granular layer and of lower molecular layer in a normal animal. Upper molecular layer (not shown) is devoid of impregnated fibers. (B) The cortex of an irradiated animal. Note high concentration of impregnated fibers among the Purkinje cells some of which can be traced from the medullary layer into the molecular layer. Note also absence of pointed-brush endings at the basal aspect of Purkinje cells.

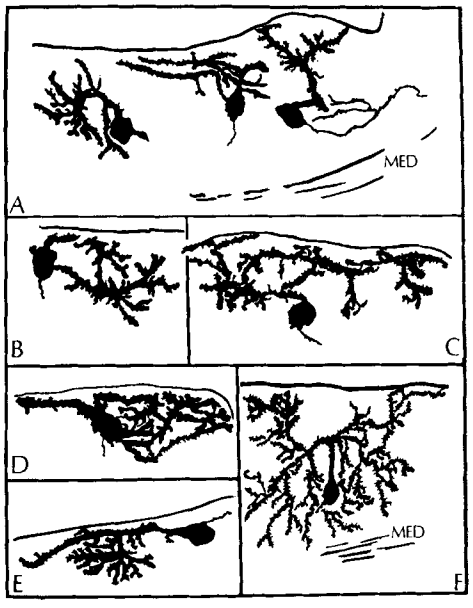

Fig. 3 Camera lucida tracings of Purkinje cells from different regions of the irradiated vermis. Note scarcity of fine branchlets and preponderance of thorns on massive dendrites. All these Purkinje cells have dendrites terminating above the medullary layer (MED).

to have upward or sideways growing dendrites with branchlets often deflected downward near the surface (fig. 3); those situated deeper had downward growing dendrites which arborized freely in the medullary layer (fig. 4A). The dendrites displayed several abnormalities (fig. 3). Typically several, nonplanar massive branches were seen richly studded with dendritic thorns; spiny branchlets were few or altogether absent. Many Purkinje cells had two "primary" dendrites, one growing upward, the other downward (fig. 4A). Basket cells or granule cells were not seen; Golgi cells were frequent (fig. 4B) as were Bergmann glia cells (fig. 4C). Climbing fibers were seen but mossy fibers could not be identified.

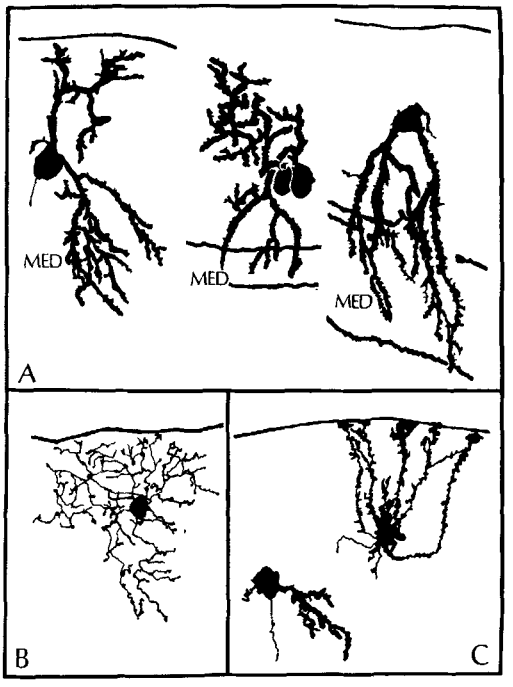

Fig. 4 (A) Purkinje cells with dendrites directed downward and penetrating the medullary layer. (B) Golgi cell. (C) Bergmann glia cell. MED, medullary layer.

Electron microscopic observations

The somata of Purkinje cells. The nucleus and perikaryon of many Purkinje cells appeared normal but a high proportion had lobulated nuclei with scattered, coarse chromatin clumps in the nucleoplasm. The lobulation was consistently caused by the invagination of cytoplasm rich in free and bound ribosomes (fig. 5). The cytoplasm was normal, rich in granular endoplasmic reticulum, and well supplied with Golgi apparatus, mitochondria, lysosomes, closed coated vesicles, and an occasional multivesicular body. Open coated vesicles were seen on the plasmalemma of the soma opposite cell processes, such as basket cell-like boutons and glial processes.

Elongated axon terminals with small clusters of synaptic vesicles formed inconspicuous symmetrical synapses (fig. 5, inset C) with the somata of Purkinje cells. Inconspicuous symmetrical synapses were also formed with the soma by boutons that had a higher concentration of synaptic vesicles and displayed characteristics of climbing fiber terminals. This type of bouton also formed conspicuous, asymmetrical synapses with thorns of the primary dendrite (fig. 5, inset B). Dendritic thorns also received boutons of somewhat different characteristics (fig. 5, inset A). Where such terminals (possibly of mossy fibers) formed synapses with the thorns, the smooth surface of the dendrite occasionally had desmosoid junctions with the bouton (fig. 5, inset A; fig. 6B).

Figure 7 illustrates a basket cell-like terminal issuing from a myelinated axon on the primary dendrite of a Purkinje cell. Since basket axons are unmyelinated, some of the terminals classified in normal animals as those of basket cells (possibly most of them) may derive from Purkinje cell axon recurrent collaterals (Altman and Anderson, '72).

The dendrites of Purkinje cells. The massive dendritic trunks seen in Golgi material were also found in high concentration with electron microscopy. Some reached the surface (fig. 8) where they became deflected or were oriented in various random planes. These dendritic trunks were richly supplied with mitochondira preferentially situated near the plasmalemma. There were two kinds of boutons on these dendrites (fig. 9), an oligovesicular type with an inconspicuous symmetrical synapse and a polyvesicular type with a more conspicuous asymmetrical synapse. The former is interpreted as the terminal of a Purkinje axon collateral or of an occasional basket cell, the latter as that of a climbing fiber. Occasionally several such presumed climbing fiber boutons were seen at a single site (fig. 12) but more prevalent were climbing fiber synapses with presumed thorns of Purkinje cells (figs. 10–11, 14). A single bouton often made synaptic contacts with many thorns and the density of such climbing fiber synapses was extremely high in many regions of the thin molecular layer. Less frequently than in animals irradiated from birth onward (Altman and Anderson, '72) Purkinje cell dendritic thorns formed pseudosynapses with glial processes (figs. 13–14). These dendritic thorns also displayed typical conspicuous junctional membrane thickening op-

posite boutons from basket cells or recurrent collaterals of Purkinje cell axons that formed inconspicuous symmetrical synapses with the smooth Purkinje cell dendrites (fig. 15). These observations indicated considerable nonspecificity on the part of Purkinje cell dendritic thorns, having postsynaptic membrane thickening opposite other processes than the terminals of climbing fibers. Significantly, the type of postsynaptic membrane thickening was always of the conspicuous type.

In addition to the many closed coated vesicles seen in the somata of Purkinje cells in association with the Golgi apparatus (fig. 5), open coated vesicles were present in high numbers in the 30 day old irradiated animals (figs. 5, 16), frequently opposite synaptic boutons, occasional parallel fibers and, rarely, glial processes. These observations suggested that synaptogenesis was still in progress at this age in the irradiated animals.

Basket cells. In agreement with the light microscopic observations, an occasional neuron was encountered in the molecular layer. The nuclei stained lightly, the cytoplasm was rich in free and bound ribosomes, mitochondria and Golgi apparatus, and the dendrites tended to be thick (fig. 17). Although these cells were much larger than normal basket cells, they were classified as such.

On the somata and dendrites of these large basket cells there were many synapses (fig. 17–19). Frequent on the somata were medium-sized polyvesicular boutons with an occasional dense core vesicle which formed inconspicuous, symmetrical synapses (fig. 17B; 18). This class of boutons also formed conspicuous desmosoid junctions with the soma (figs. 17B; 18) and junctions that could be interpreted as asymmetrical synapses (fig. 19). The identity of these synapses could not be established; possibly they were those of the recurrent collaterals of Purkinje cells. Smaller boutons formed conspicuous asymmetrical synapses on the soma (fig. 17B) and dendrites (fig. 17A); these were presumably the synapses of the relatively rare parallel fibers.

Granule cells and parallel fibers. The scattered granule cells beneath the zone of Purkinje cells, too few in the pyramis to form a true granular layer, were associated with mossy fiber rosettes (fig. 20). Processes assumed to be the dendrites of granule cells had extended serrated contacts with these mossy terminals with conspicuous asymmetrical synapses (fig. 21). The same dendritic processes formed synapses or desmosoid junctions with more than one rosette (fig. 20). Granule cells were also scattered in the molecular layer, where mossy rosettes formed synaptic junctions with processes that had the characteristics of granule cell dendrites (fig. 22). Rarely, parallel fiber dendrites formed desmosoid junctions with each other in the molecular layer as they do in normal animals in the granular layer. The other contacts of mossy fibers were with presumed dendritic thorns of Purkinje cells (fig. 23) and unidentified processes.

Parallel fibers were present in small numbers in the molecular layer, either singly or in bundles composed of a few fibers (figs. 8, 16). Most of them had the average number of microtubules (3–6) in cross section; others had a higher concentration of microtubules and also several neurofilaments (fig. 24) which are not seen in normal animals. Parallel fibers had synapses with Purkinje cell dendrites and occasional basket cells (fig. 17).

Other cellular elements. Myelinated fibers were seen in high concentration in the medullary layer, but in decreasing frequency among the granule (fig. 20) and Purkinje cells (fig. 5) and in the molecular layer (fig. 9). An occasional myelinated fiber reached the surface of the cortex. The myelinated fibers traversing the molecular layer were considered mossy fibers.

Astrocytes were seen throughout the cortex and long fibrous astrocytic processes were frequent, vertically oriented through the molecular layer or horizontally near the surface (figs. 8, 22). A few cells with darkly staining nuclei and dark cytoplasm containing a rich accumulation of granular endoplasm reticulum were considered microglia.

DISCUSSION

In rats the Purkinje cells become dispersed over the surface of the cerebellar cortex during the first few days after birth

(Addison, '11; Altman, '72b), while the differentiation of the cells of the external germinal layer does not begin until the end of the first week (Altman, '72a). Therefore, by starting x-irradiation several days after birth, and not interfering with its growth during that period, a cerebellar cortex should develop which, though devoid of microneurons (except in early-maturing regions), will still be quasi-normal in structural organization with its Purkinje cells strung out in a monolayer. This expected result was not obtained. Even in the early-maturing nodulus (Altman, '69), where a thin molecular layer has developed with basket cells and a granular layer is present in the animals irradiated from the fourth day onward, the Purkinje cells were not dispersed in a monolayer but were scattered several cells deep. The cell-depth of the Purkinje cells was still higher in later-forming lobules, such as the pyramis.

The following considerations may explain this result. A precondition of the monolaminar dispersion of Purkinje cells during the first few days after birth is the rapid expansion of the surface area of the cerebellar cortex which provides space for the accommodation of the large number of Purkinje cells which at birth are distributed throughout the depth of the cortex. We attributed (Altman and Anderson, '72) the rapid expansion of the cortical surface during this period to the great increase in the cell population of the proliferative zone of the external germinal layer. This zone is composed of a sheet of cells of constant depth (Altman, '72a). The accommodation of this growing sheet requires the expansion and folding of the surface of the cortex before cortical cell differentiation has begun and when relatively little volumetric growth in the cerebellum has occurred. If the external germinal layer is destroyed by x-irradiation this expansion of the cortical surface does not take place, which accounts for the failure in the dispersion of Purkinje cells when irradiation of the cerebellum is started at birth (Altman and Anderson, '72).

The results of the present study indicate that even when irradiation is delayed until the fourth day, during which period the Purkinje cells become distributed in a monolayer and the initial growth of the cortex is not hindered, the Purkinje cells again become disarranged when radiation is begun. Presumably the early expansion of the cortical surface is not sufficient for the accommodation of Purkinje cells whose somata, apical cones and dendrites grow at a normal (possibly accelerated) rate in the irradiated animals (Altman and Anderson, '71, '72). This explanation implies that the continuing expansion of the surface depends on the growth of the external granular layer and possibly later on that of the molecular layer. It was interesting to note that in the early-maturing nodulus (and in part of uvula) the Purkinje cells, although not lined up in single file, had with few exceptions their apical poles directed toward the surface, whereas in the late-maturing pyramis and other lobules the apical cones were randomly oriented. It is assumed that there is a competition among the nonoverlapping dendritic domains of Purkinje cells such that the greater the crowding of Purkinje cells and the less lateral space available in the sagittal plane the fewer cells can grow in the preferred upward direction. This random orientation of Purkinje cell dendritic arbors was well illustrated by the Golgi material.

A ventrodorsal gradient was observed in the concentration of granule cells from the nodulus upward in agreement with the established normal gradient of cortical maturation (Altman, '69). A thin molecular layer was present in the nodulus, with basket cells one to two rows deep. In the pyramis (the lobule examined with electron microscopy) the molecular layer was much thinner with only scattered basket cells. In the late-maturing tuber and the folium vermis there were essentially no basket or granule cells. Wherever a few basket cells were present, they tended to be large, possibly because they had more extensive processes and more terminals than basket cells in normal animals. However, many of the basket cell-like terminals on the somata of Purkinje cells were apparently terminals of recurrent collaterals of Purkinje cell axons. This identity was indicated, first, by the impregnation pattern obtained with the Bodian technique, which showed in the medullary layer of irradiated cerebella a high concentration

of impregnated fibers. These fibers could be traced through the scattered Purkinje cells to the molecular layer but the typical pointed brush-ending terminals of basket cells were rare or absent. Second, a typical *en passant* basket cell type terminal on the soma of a Purkinje cell was found to be the ending of a myelinated axon and, accordingly, could not be that of a basket cell.

These observations suggest that if during development there is a scarcity of basket cell terminals, the synaptic sites on the soma of a Purkinje cell are occupied by other cell processes. The recurrent collaterals of Purkinje cells, like the basket cell terminals, are believed to inhibit the somata of Purkinje cells (Eccles et al., '67) and there is evidence that both basket cells and Purkinje cells use GABA as a transmitter (Kuriyama et al., '65; Obata and Takeda, '69; Woodward et al., '71; Curtis and Felix, '71). Therefore substitution of Purkinje axon collateral terminals in place of basket cell terminals would not represent a radical structural and functional reorganization. Indeed, some of the basket cell type terminals on the somata of Purkinje cells in normal animals might be Purkinje collateral terminals. However, the reorganization must be drastic when normally excitatory terminals, such as those of mossy fibers, establish synapses with the somata of Purkinje cells. Evidence for such presynaptic terminals was obtained in the previous study (Altman and Anderson, '72). Interestingly, the postsynaptic site has the characteristics of an inhibitory contact (inconspicuous dense membrane). The functional properties of such contacts are difficult to envisage.

Perhaps the greatest degree of nonspecific synaptic receptiveness was displayed by the Purkinje cell thorns. In addition to the many synapses with climbing fibers, their normal contacts, the supernumerary thorns had synapses with mossy fibers and pseudosynapses with glial processes. (These effects were recently observed following cycasin-induced interference with granule cell formation; Hirano, Dembitzer and Jones, '72). The facts that Purkinje cell somata and dendritic thorns form junctions with other than their normal affiliates (indeed pseudosynapses with nonneuronal elements), and that the type of membrane density in the postsynaptic site of the Purkinje cell is not altered by the different associations, indicate the dominant role played by Purkinje cells in synaptogenesis. The autonomously growing Purkinje cell hungers, as it were, for synaptic contacts and its endogenously developing receptive sites establish contacts with other than normal elements if those are not available. Possibly there is a preference hierarchy: if climbing fibers are not available, contacts are made with mossy fibers, and if necessary with glial processes. The number of contacts with glia was much smaller in these animals than in the animals irradiated from birth onward (Altman and Anderson, '72) and there was no sign of dendrodendritic contacts of Purkinje cells, possibly the least preferred association and one which apparently causes the autolysis of the Purkinje cell dendrite (Altman and Anderson, '72). This preference hierarchy, rather than strict specificity, and an orderly chronological sequence in the maturation of different types of terminals (Altman, '72b, '73a) may be the mechanisms ensuring the establishment of correct synaptic contacts under normal conditions.

Generally, the morphological alterations seen in the cerebellar cortex of rats irradiated from the fourth day onward resemble but are less severe than when radiation is started at birth (Altman and Anderson, '72). The overall reduction in basket and granule cells was lower, mainly because of their presence in early-maturing lobules. If the Purkinje cell dendritic thorns were less numerous than in the previous group, this may have been due to the presence of some parallel fibers. It was noted that these parallel fibers were often different than in normal animals; they were thicker, had a higher than normal complement of microtubules, and also had neurofilaments. Many mossy terminals reached the surface of the cortex, suggesting that they tend to grow upward until synaptic contacts are established with granule cell dendrites (Altman, '73a). Unlike in normal animals, in irradiated animals closed and open coated vesicles were frequent in the somata and dendrites of Purkinje cells at 30 days of age, indicating prolongation of synapto-

genic activity. Because of the paucity of interneurons much of this activity may have been abortive. In these cerebella there were no pathological changes, except for lobulation of the nuclei of Purkinje cells. This is seen sometimes in normal animals and may be associated in the irradiated cerebella with the twisted shapes of the Purkinje cell perikarya and dendrites.

ACKNOWLEDGMENTS

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PLATE 1

EXPLANATION OF FIGURE

5 Part of the soma and primary dendrite of a Purkinje cell from an irradiated rat. Note cytoplasmic invaginations into nucleus (in) which are filled with free or membrane bound ribosome particles. Closed coated vesicles (ccv) are common in association with the Golgi apparatus and open coated vesicles (ocv) are seen in apposition to a basket cell-like terminal (B1). The latter is characterized by a small concentration of synaptic vesicles and inconspicuous, symmetrical synapses (inset C). Other type of boutons (B2) which have a higher concentration of vesicles and show some of the characteristics of climbing fiber terminals but form inconspicuous, symmetrical synapses are common. On a thorn issuing from the primary dendrite a similar bouton (B3; inset B) forms a conspicuous, asymmetrical synapse. Dendritic thorns form conspicuous, asymmetrical synapses with still another type of bouton (B4; inset A). This type of bouton forms desmosoid contacts (ds) with the smooth surface of the dendrite. Magnifications: major micrograph, × 11,172; inset A, × 41,040; insets B and C, × 16,188.

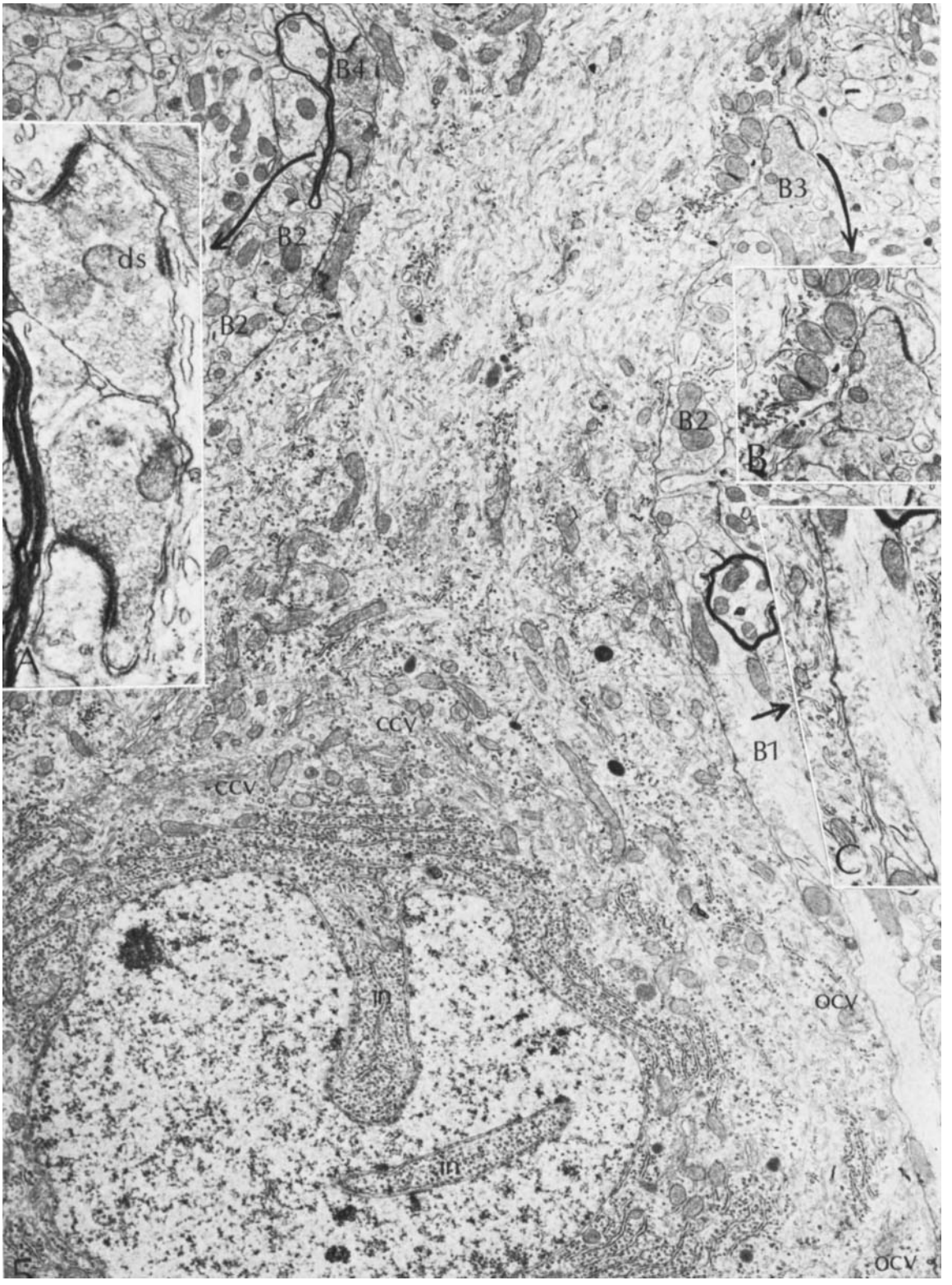

B4
B3
B2
B2
ds
B2
B
A
B1
C
CCV
CCV
m
m
OCV
OCV

PLATE 2

EXPLANATION OF FIGURE

6 (A) Two boutons on the soma of a Purkinje cell in the vicinity of a *massive dendritic process* (DP). The upper bouton (enlarged in B, top) has a desmosoid junction or possibly conspicuous, asymmetrical synapse, the lower bouton (B, bottom) has smaller vesicles and an ambiguous synapse. These type of boutons do not form synapses with the soma of Purkinje cell in unirradiated animals and their identity (climbing or mossy fibers?) is uncertain. A, × 16,188; B, × 41,050.

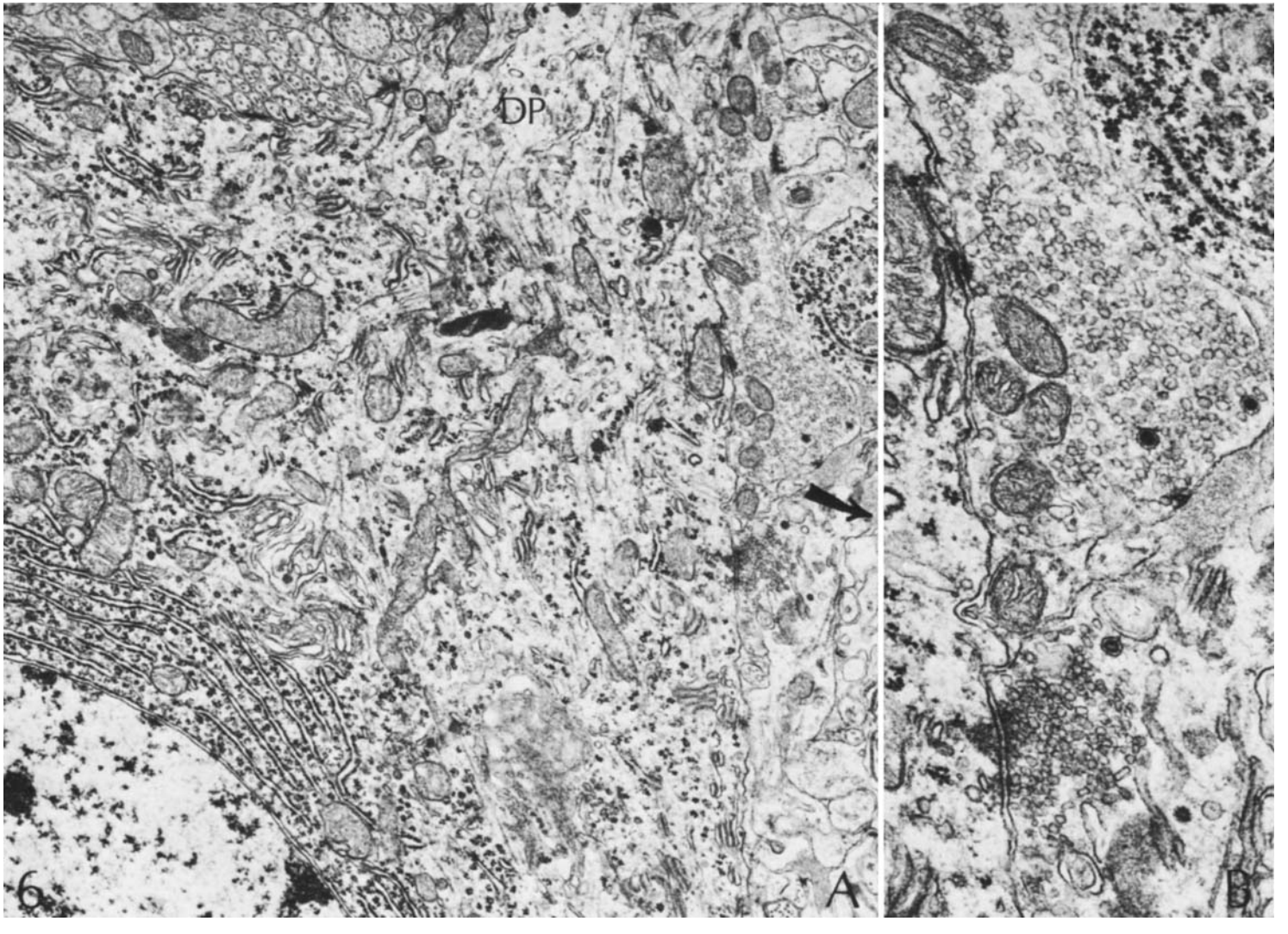

DP
6
A
B

PLATE 3

EXPLANATION OF FIGURE

7 Elongated terminal of a myelinated axon making multiple synaptic contacts with the primary dendrite of a Purkinje cell. The inconspicuous, symmetrical synapses with small clusters of vesicles are like those of basket cell terminals, but basket cell axon are unmyelinated. On the basis of supplementary evidence with the Bodian staining technique (Altman and Anderson, '72) which indicates that the impregnated fibers in the lower molecular layer and around the soma of Purkinje cells are not mossy or climbing fibers, these terminals taking the position of basket cells are identified as the terminals of Purkinje cell axon recurrent collaterals. × 25,080.

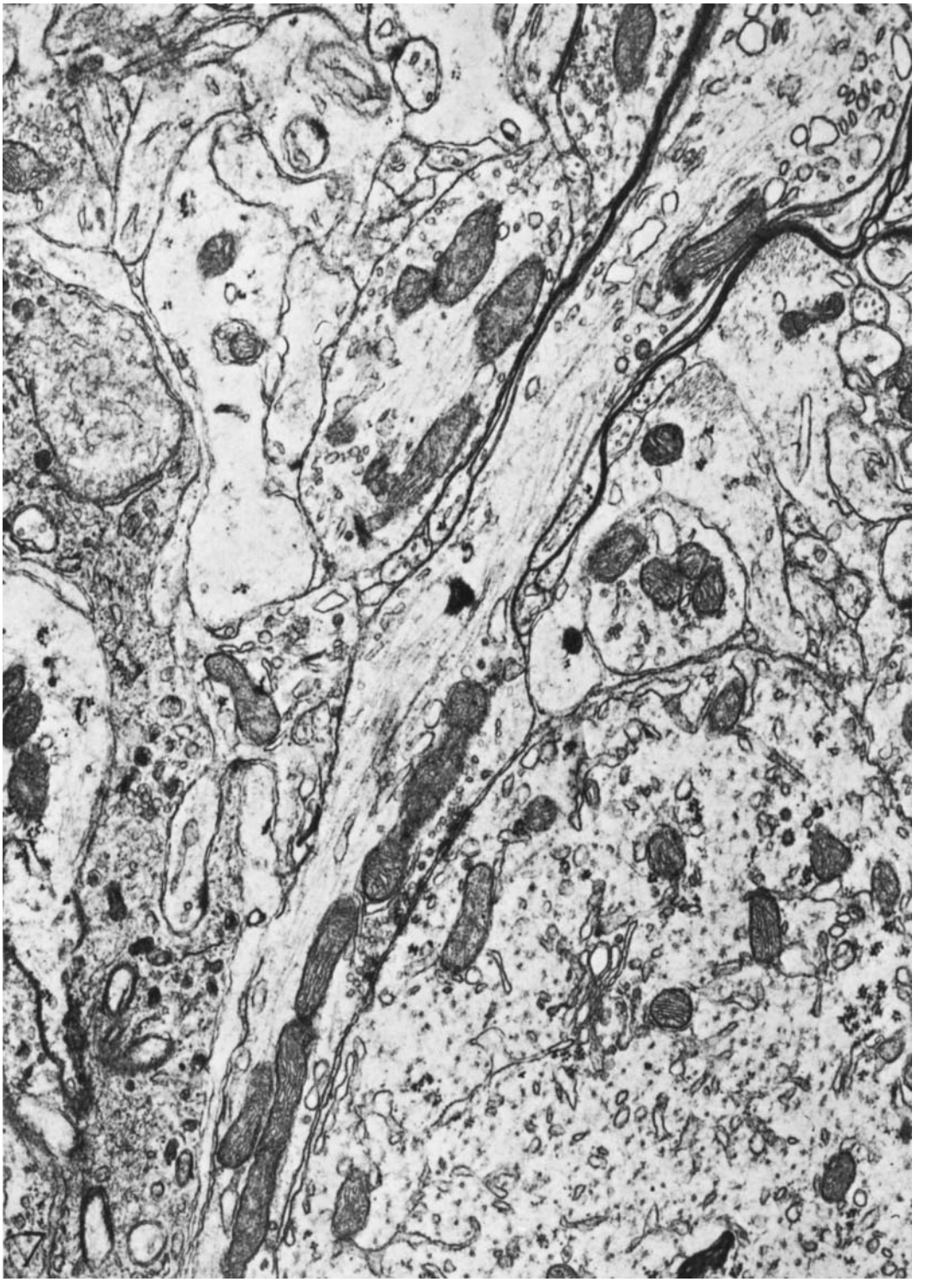


PLATE 4

EXPLANATION OF FIGURES

8 Massive Purkinje cell dendrite (PD) that reaches the surface of the cortex (AP, astrocytic process). Many boutons (B) contact the dendrite and in this region there are also many parallel fibers (pf) × 13,680.

9 Another massive Purkinje cell dendrite with numerous boutons (B). Inset shows two of the upper boutons enlarged. One is oligovesicular with inconspicuous, symmetrical synapse, presumably that of a basket cell or Purkinje cell axon recurrent collateral (bottom). The other (top) is a polyvesicular bouton with a conspicuous, asymmetrical synapse, presumably that of a climbing fiber. The latter are relatively rare on the smooth surface of the dendrite. × 9,120.

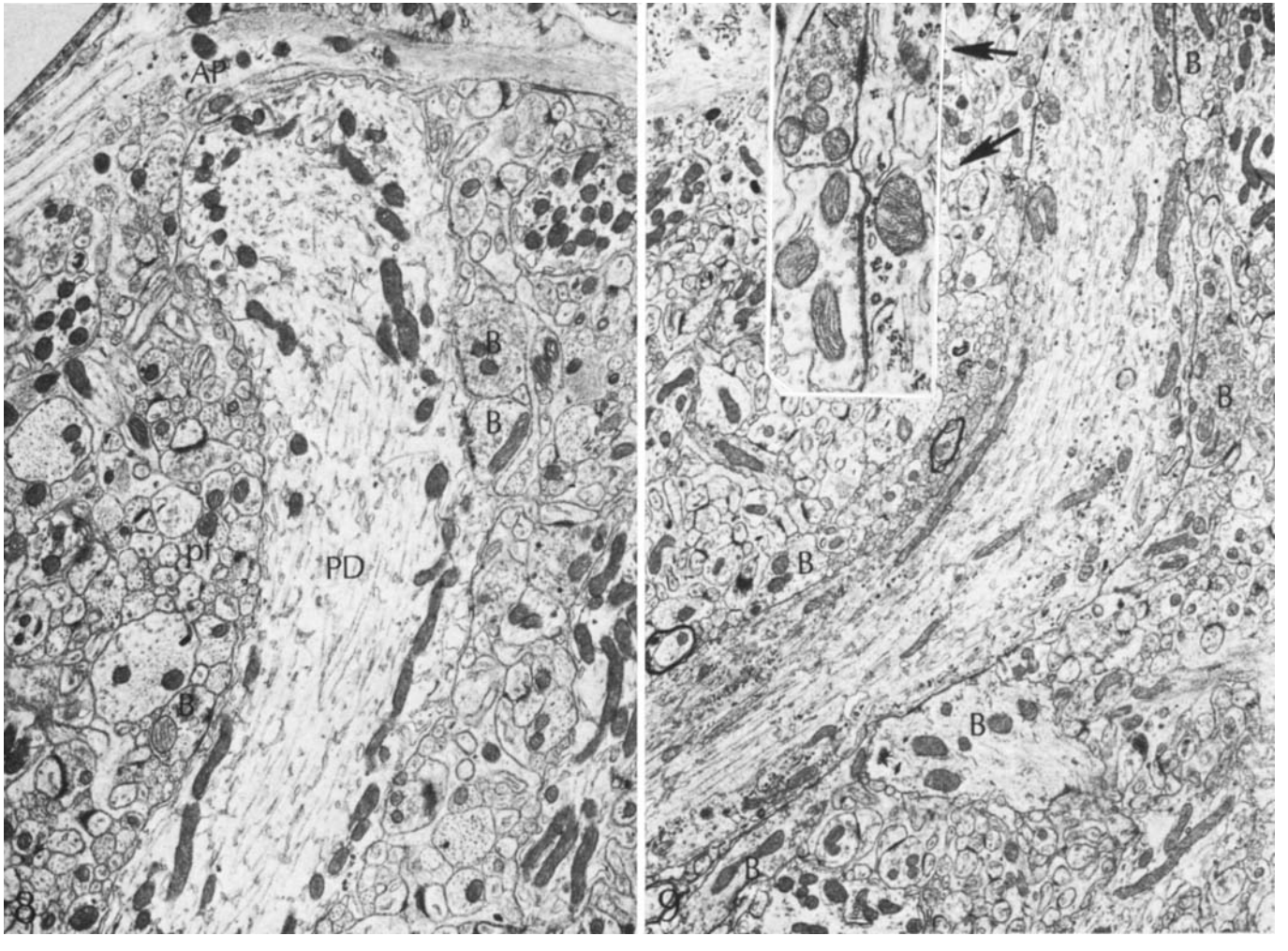
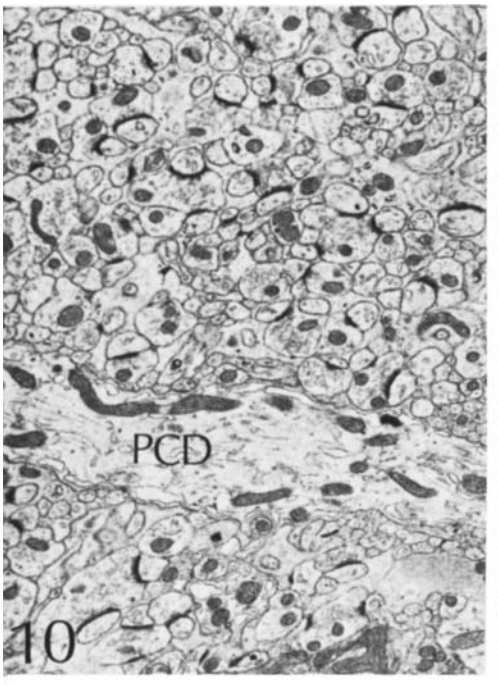

AP
B
B
pf
PD
B
B
B
B
B
B
B
B

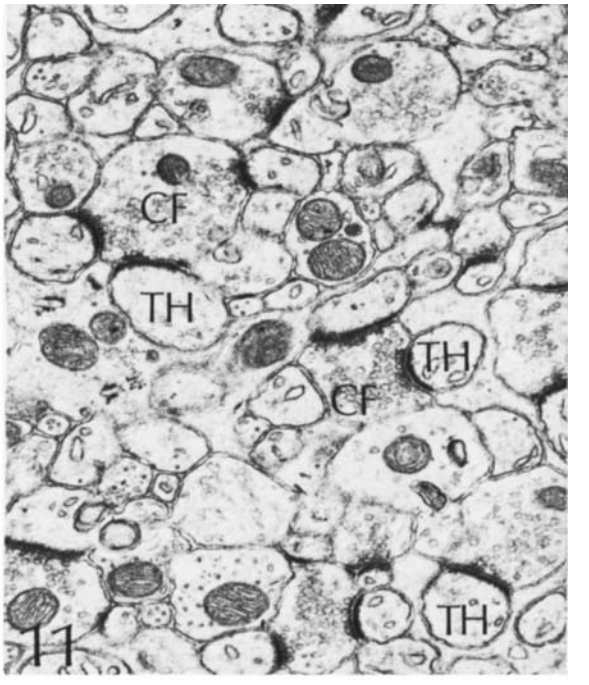
PLATE 5

EXPLANATION OF FIGURES

10–11 Climbing fiber boutons (CF) with synapses on presumed thorns (TH) of Purkinje cell dendrites (PCD). Continuity of the thorns with the Purkinje cell dendrite is not seen here. (See Altman and Anderson, '72, figs. 30, 31, 34, 36, and figs. 13, 14 below.) Some parallel fibers are also visible. A, × 9,120; B, × 25,080.

12 Presumed climbing fiber boutons surrounding a Purkinje cell dendritic branchlet (PCD). × 25,080.


PCD
10


CF
TH
TH
CF
TH
11

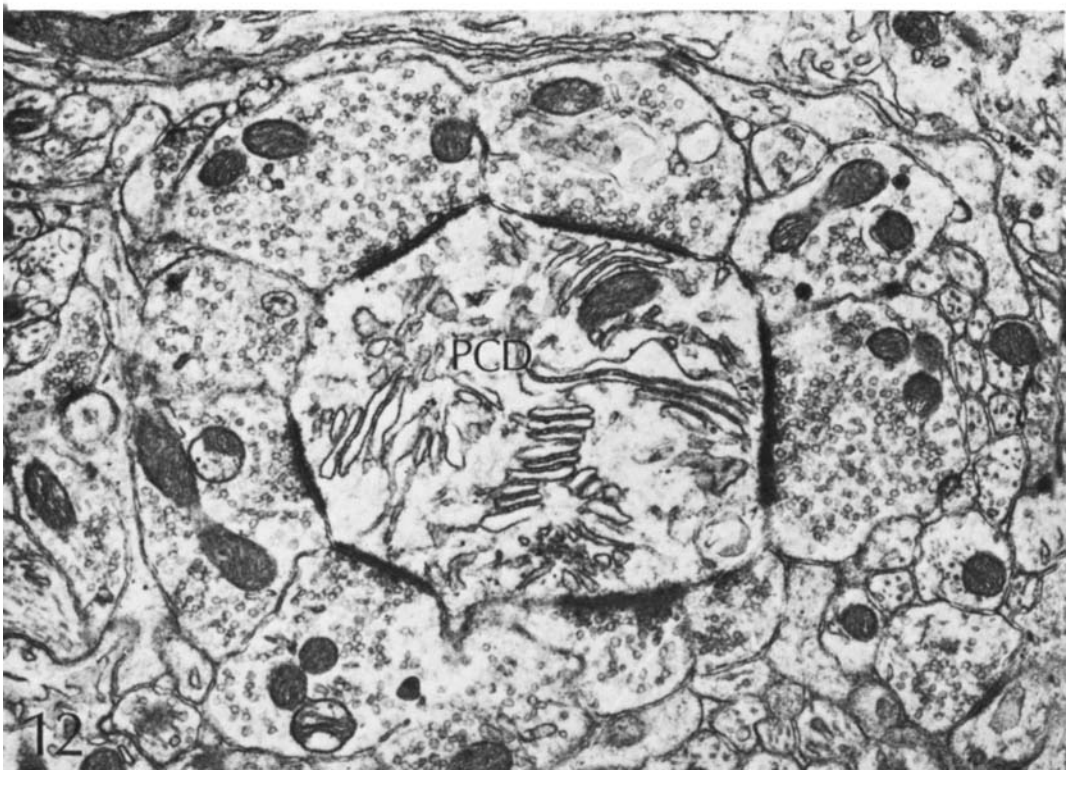

PCD
12

PLATE 6

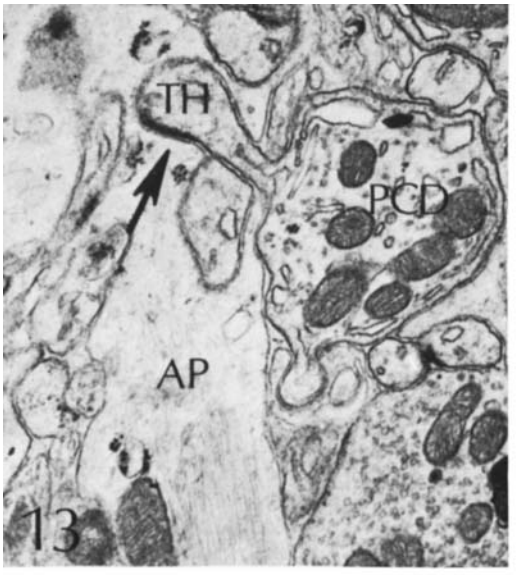
EXPLANATION OF FIGURES

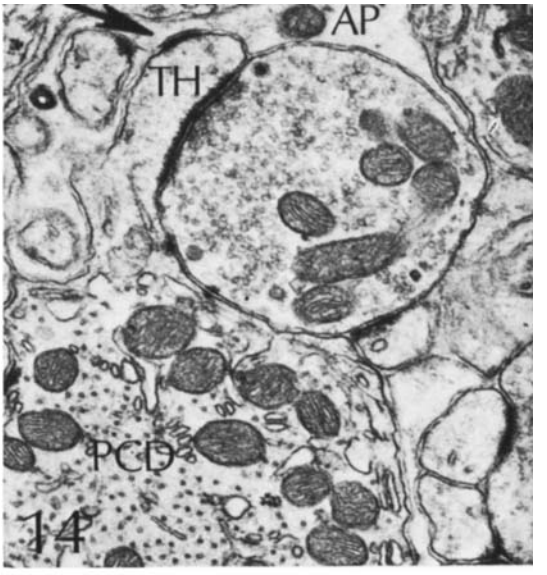
13 The thorn (TH) of a Purkinje cell dendrite (PCD) forming a pseudosynapse (postsynaptic membrane thickening at arrow) with a fibrous astrocytic process (AP). × 25,080.

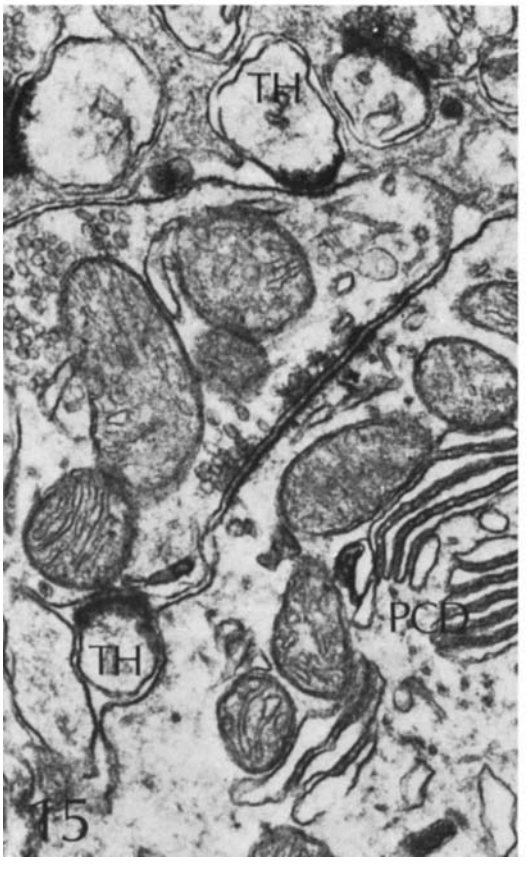
14 The thorn of a Purkinje cell dendrite forming a conspicuous, asymmetrical synapse with an unidentified bouton, perhaps a climbing fiber (center). The same thorn also forms a pseudo-synapse with an astrocytic process (arrow). × 25,080.

15 Bouton with inconspicuous, asymmetrical synapse on the smooth surface of a Purkinje cell dendrite. The membrane thickening on the thorns is of the conspicuous type. × 21,040.

16 A Purkinje cell dendrite with open coated vesicles (arrows) opposite parallel fibers (pf) and a bouton (upper right). × 41,040.


TH
PCD
AP
13


AP
TH
PCD
14


TH
PCD
TH
15

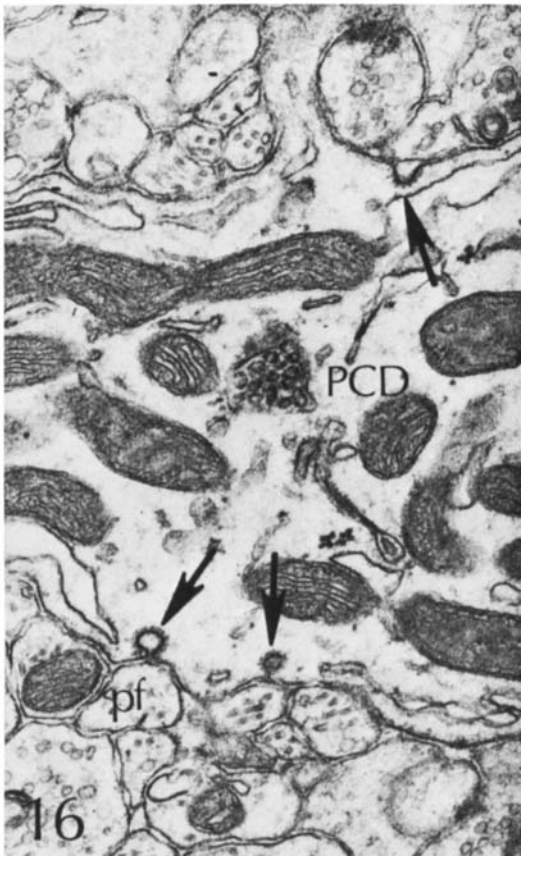

PCD
pf
16

PLATE 7

EXPLANATION OF FIGURES

17 This cell, which is presumed to be a basket cell, is situated near a Purkinje cell and has a massive dendrite (BD). Several synapses are seen on the dendrite and some of these are shown enlarged in inset A with parallel fibers (pf). The latter type of synapses is also present on the soma (inset B) with conspicuous, asymmetrical synapses. Also prevalent on the soma, and the dendrite, are boutons with inconspicuous symmetrical synapses (B1) and one of these (B2) also has a desmosoid junction (dj). × 9,120; insets A and B, × 41,040.

18 Another presumed basket cell with a bouton that forms an inconspicuous symmetrical synapse as well as a desmosoid junction, as shown in inset. × 13,680; inset, × 41,040.

19 On this basket cell there is a bouton that forms an asymmetrical and perhaps a symmetrical synapse (inset). × 13,680; inset, × 41,040.

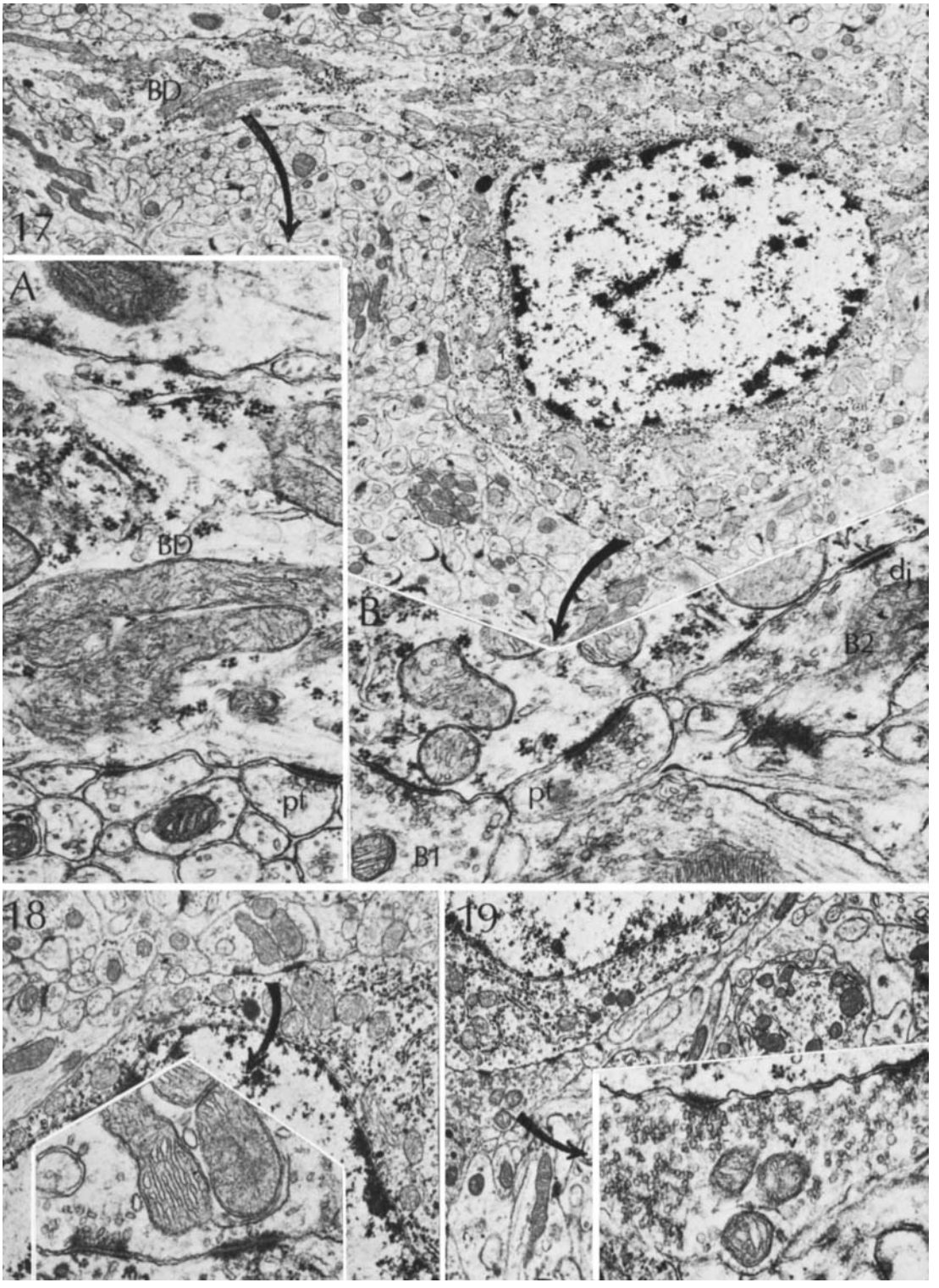

17
BD
A
BD
pt
B
di
B2
pt
B1
18
19

PLATE 8

EXPLANATION OF FIGURES

20 Scattered granule cells (GC) beneath the zone of Purkinje cells. Several mossy fiber rosettes (RO) are in their vicinity. One dendritic process may have contacts with more than one rosette (double arrows). × 11,172.

21 The extensive synaptic contacts made by the abundant mossy fiber rosettes with the scarce presumed granule cell dendrites (GD?) are typical as is the serrated interdigitation. × 31,920.

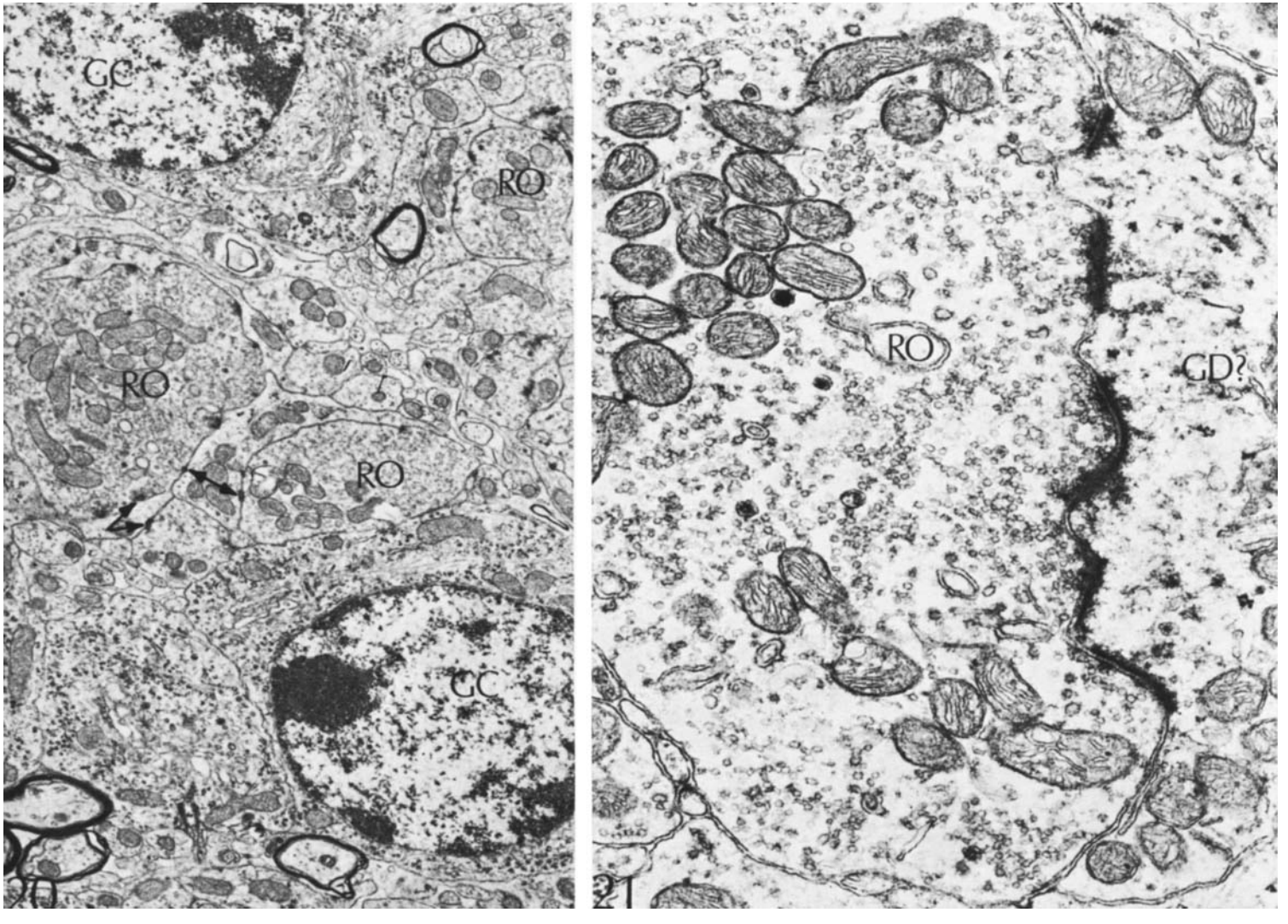
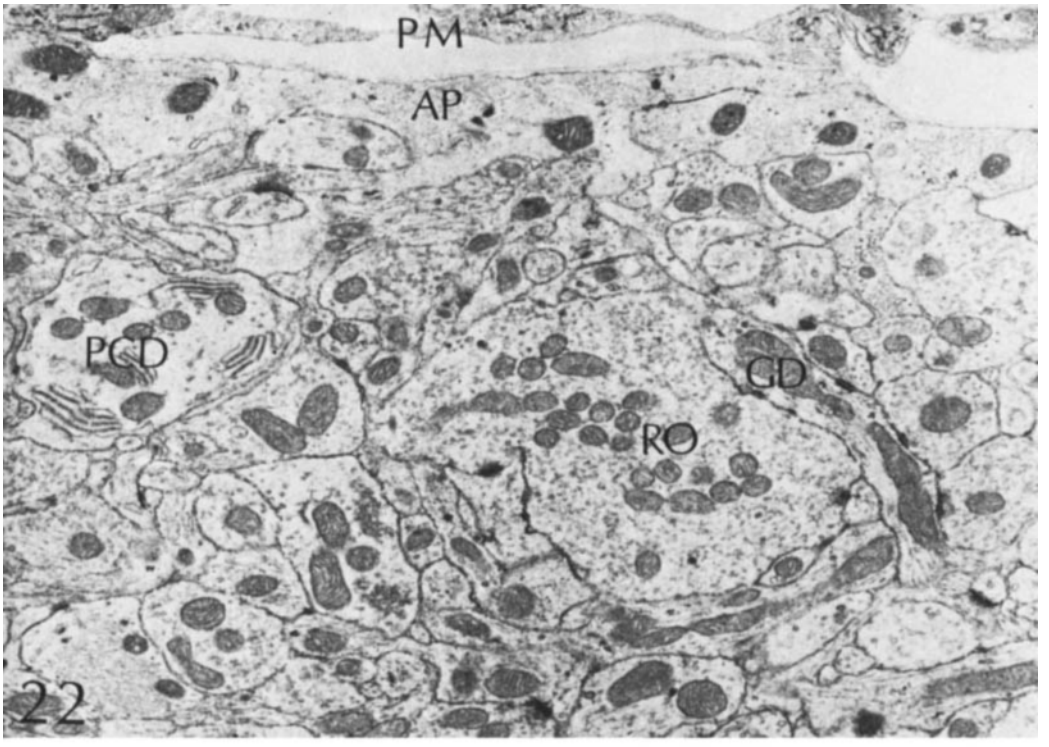

GC
RO
RO
RO
GC
RO
GD?

PLATE 9

EXPLANATION OF FIGURES

22 Mossy fiber rosette (RO) close to the surface of the cortex (AP, astrocytic process of Bergmann glia; PM, pial membrane). What is probably a granule cell dendrite (GD) forms a synapse with the rosette and desmosoid junctions with other processes. PCD, Purkinje cell dendrite. × 13,680.

23 Mossy fiber rosette forming synapses and desmosoid junctions near the surface of the cortex with presumed granule cell dendrites (note presence of mitochondria, which are not seen in thorns). × 25,080.


PM
AP
PCD
GD
RO
22

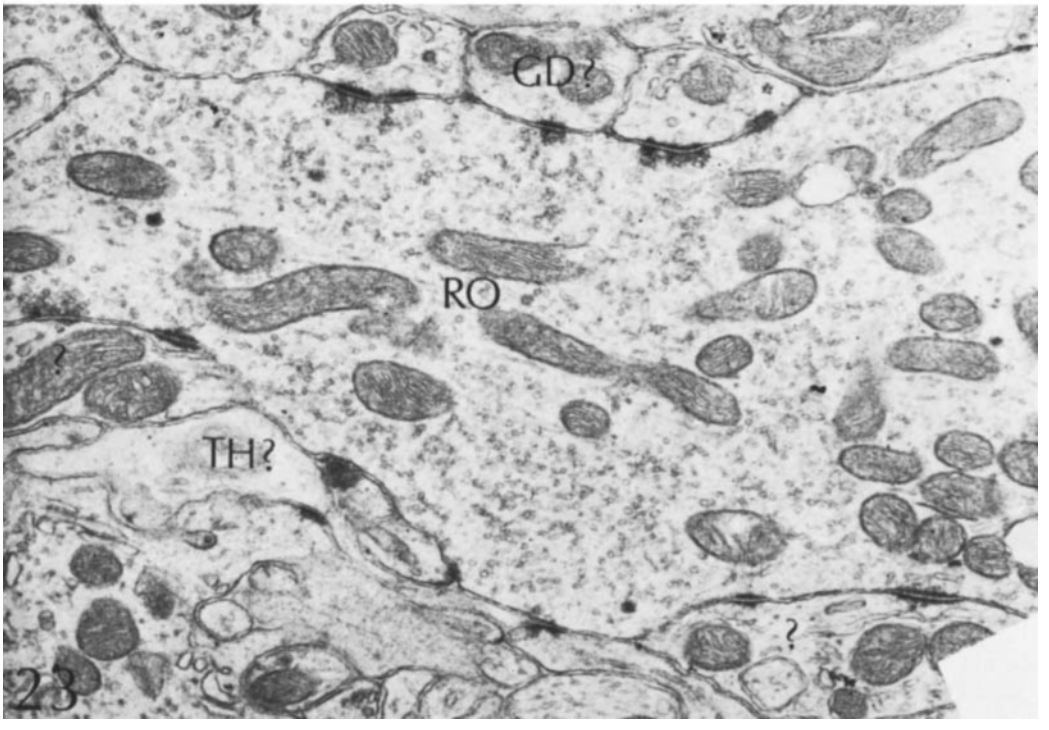
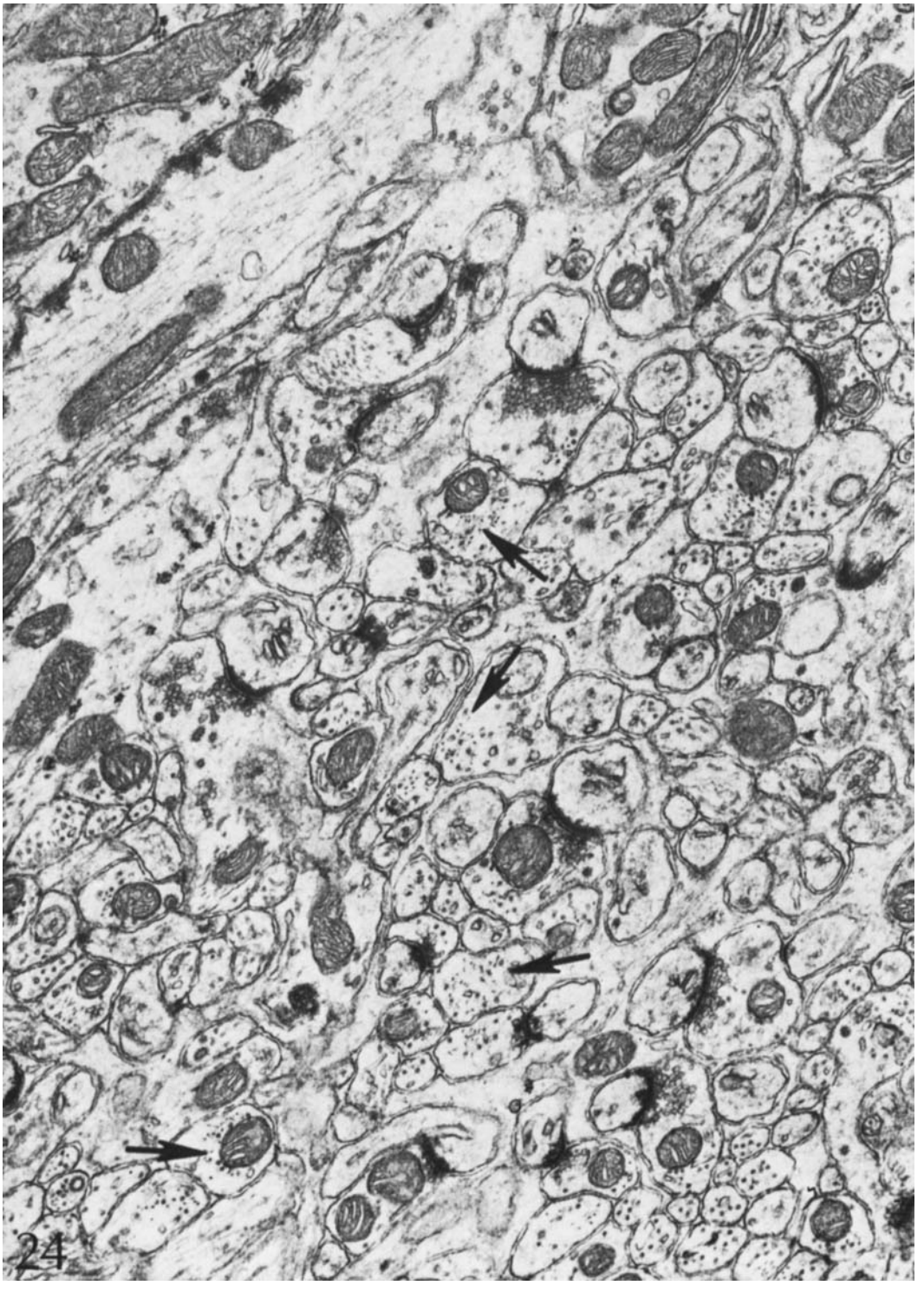

GD?
RO
?
TH?
?
23

PLATE 10

EXPLANATION OF FIGURE

24 Parallel fibers tend to be of higher caliber and may have more microtubules than in normal animals and they also contain neurofilaments (arrows) which are not seen in the unirradiated cerebella. × 25,080.


24