

# Experimental Reorganization of the Cerebellar Cortex

## I. MORPHOLOGICAL EFFECTS OF ELIMINATION OF ALL MICRONEURONS WITH PROLONGED X-IRRADIATION STARTED AT BIRTH

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**ABSTRACT** The heads of Long-Evans rats were irradiated from birth with a schedule of repeated doses of low-level x-ray which prevented the acquisition of the postnatally-forming basket, stellate and granule cells. The absence of these microneurons led to several changes in the organization of the cerebellar cortex, studied at 30 days of age with light and electron microscopy. The somata of Purkinje cells were not strung out in a monolayer. Their primary dendrites were randomly oriented and arborized differently depending on the position of their somata in the depth of the cortex. Many Purkinje cell perisomatic processes, normally seen only in infants, were retained. Climbing and mossy fibers formed inconspicuous, symmetrical synapses with the somata and conspicuous, asymmetrical synapses with the perisomatic processes and dendritic thorns of Purkinje cells. Massive Purkinje cell dendrites with innumerable thorns made up much of the neuropil of the cortex. The dendritic thorns, in addition to forming true synapses, also established pseudosynapses with glial processes and synapses devoid of vesicles with the surface or the interior of Purkinje cell dendrites. The latter type of contact apparently led to autolysis of part of the dendrite, which was the only clear pathological phenomenon observed. These results indicated considerable autonomy in the growth of Purkinje cell dendrites, which arborized richly and sprouted innumerable small processes in the absence of parallel fibers, and formed postsynaptic dense membranes in contiguity with inappropriate processes, such as those of Purkinje cells or astrocytes.

In this series of experiments we have examined how a brain structure re-organizes itself when the normal course of its development is disturbed by various systematic manipulations. The purpose, of course, has been to gather information and make inferences about the organizational forces operating during normal development. The cerebellum was chosen as an ideal brain region for this task for the following reasons. Several of the cellular constituents of the cerebellum are formed after birth in altricial mammals, and synaptogenesis in the cerebellar cortex is a postnatal phenomenon. Therefore the development of this structure can be sufficiently disarranged after birth, when systematic manipulation becomes an easier experimental task than during embryonic

development. Such manipulations are further enhanced by the circumstance that the cerebellum is a superficially located and relatively segregated brain region, which make possible experimentation with its development without undue interference with other brain regions. Moreover, the cerebellar cortex, although a massive structure, has the same stereotyped organization throughout. It has a limited number of cell types, and only a few afferent and efferent pathways, which form a highly stratified, geometrically regular cytoarchitectonic framework. This, and the fact that a considerable body of data has recently been accumulated on the gross and fine morphology of the developing and mature cerebellum should make it somewhat easier, it was hoped, to interpret the

changes that are produced when its morphogenesis is disturbed.

In altricial mammals (Altman, '67) the interneurons, or microneurons (Altman and Das, '65) of the cerebellar cortex are formed after birth. The site of origin of these cells is the external granular layer (Lugaro, 1894; Athias, 1897; Cajal, '11), or external germinal layer (Altman, '72a), which sequentially gives rise to the basket cells of the lower molecular layer, the stellate cells of the upper molecular layer (Altman, '69) and to the Lugaro cells of the granular layer (Altman, '72c). The granule cells, which greatly outnumber all the other interneurons of the cerebellar cortex, are acquired throughout the entire period of postnatal neurogenesis, which lasts 21 days in the rat, with the bulk of them being formed during the second and third week after birth (Altman, '69; '72c). Also the maturation of the Purkinje cells, which are formed prenatally, begins after birth. Purkinje cells begin to form synapses with the axon terminals of basket and stellate cells, and with the parallel fibers of the granule cells, after these have commenced to differentiate (Larramendi, '69; Altman, '72b) and the interconnections of the cerebellar cortex are established over a period that outlasts the primary period of neurogenesis (Altman, '72b).

The following circumstances and experimental tools have made possible the systematic rearrangement of the morphogenesis of the cerebellar cortex. Multiplying cells are extremely radiosensitive (Hicks and D'Amato, '66) and if the developing cerebellum is exposed to a source of ionizing radiation, the cells of the external germinal layer are destroyed (Brunner, '20; Hicks, '58; Shofer et al., '64). Because mature (Hicks, '58) and even differentiating cells (Altman et al., '68b) of the brain are more radioresistant, it is possible to selectively eliminate the cells of the external germinal layer, without harming the already present immature cells of the cerebellar cortex and the maturing cells of the underlying medulla, through focal x-irradiation of the cerebellum with low-level x-ray (Altman et al., '67, '68a; Altman and Anderson, '71). The cells of the external germinal layer can be subtotally eliminated by exposure at birth to a single or two suc-

cessive daily doses of 150r or 200r x-ray. This does not do any visible harm to the Purkinje cells, as determined by light microscopic examination (Altman and Anderson, '71). If the same dose is delivered several days after birth, the cells that were already formed, including the migratory cells (Altman and Nicholson, '71), are spared. Studies of this type have revealed that a variable number of radio-resistant cells of the external germinal layer survive the exposure and these will reconstitute this matrix (Altman et al., '69a,b). The ultimate size of the population of interneurons is a function of the degree of recovery. Because the production of cells of the external germinal layer declines rapidly during the third week, the extent of recovery can be manipulated by prolonging for a variable number of days the exposure of the cerebellum to the same dose of x-ray, to prevent reconstitution, and if the recovery is delayed sufficiently to prevent recovery by the time the external germinal layer is naturally dissolved, a cerebellar cortex may be produced with repeated low doses which is devoid of all interneurons.

Given the knowledge of the chronology of the sequential differentiation of the cerebellar interneurons and having control over cell death and cell recovery during the limited period that the external germinal layer is present, it should be possible to "design and build" (Hicks and D'Amato, '61) a cerebellar cortex not only lacking in all interneurons but devoid of one or another selected kind of interneuron. The first illustration summarizes some of these possibilities (fig. 1), not taking into consideration possible morphogenetic forces which could interfere with such a rigid scheme of reorganization. Several of these schedules of irradiations have been attempted and in this first paper of the series we described the effects of irradiation with repeated doses of x-ray from birth on, which eliminates all the cells of the external germinal layer and prevents its regeneration.

#### MATERIALS AND METHODS

*Animals.* Pups of Long-Evans hooded rats that were bred in our laboratory for several years were used. At birth the size

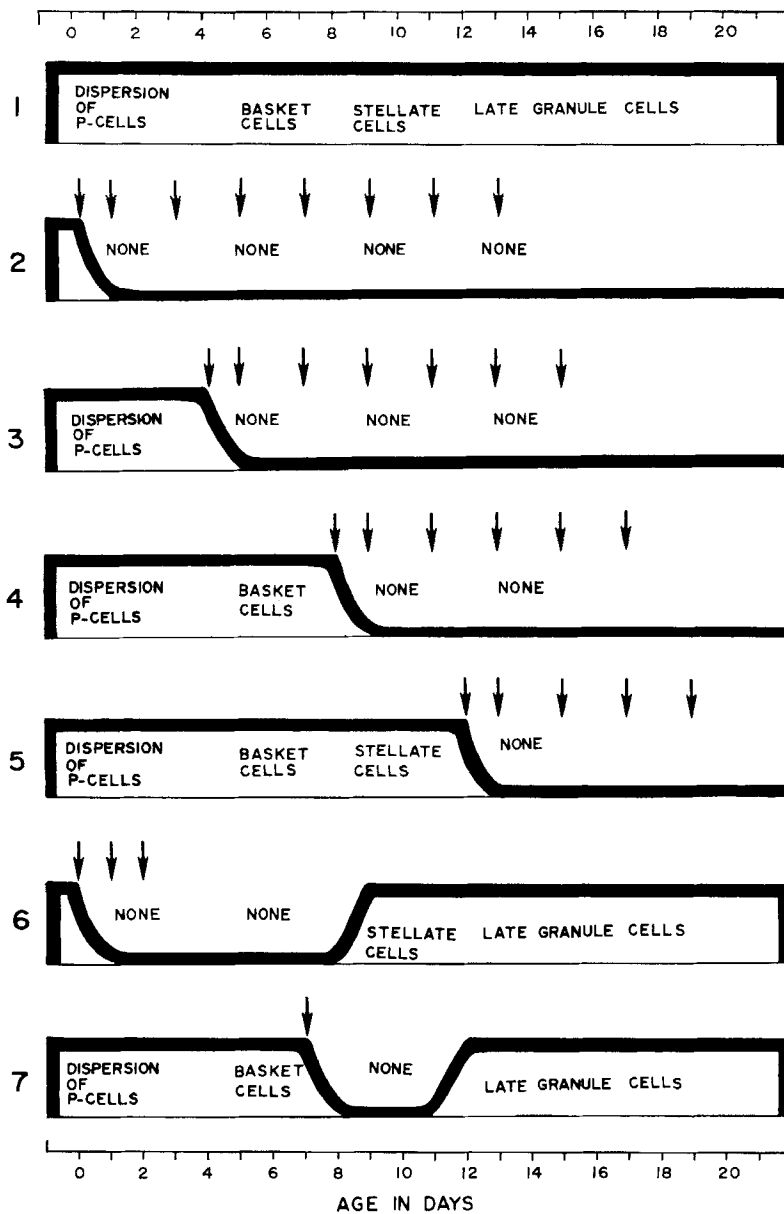


Fig. 1 Diagram illustrating the postulated consequences of different schedules of irradiation on cell acquisition in the cerebellar cortex. Thick line in top position indicates the presence (or regeneration) of the external germinal layer, in bottom position its destruction by the radiation doses delivered (arrows) on the days indicated. Row 1 shows schematically the sequential acquisition of different cell types in normal animals. Row 2 shows that with repeated doses of x-ray, which destroy and prevent regeneration of the external germinal layer, and indirectly the dispersion of Purkinje cells, the acquisition of interneurons could be altogether prevented (as described in this paper). By delaying the commencement of irradiation the dispersion of Purkinje cells may take place (row 3); the effects of this schedule are dealt with in the succeeding paper of this series (Altman and Anderson, '72). With other schedules the early-forming interneurons may be spared (rows 4-5). By delivering only a few doses (row 6) or a single one (row 7) the external germinal layer can regenerate and thus the late-forming interneurons may be selectively formed.

of each litter was adjusted to six pups per mother. The pups were removed daily from the breeding cages and moved to the radiation facilities. To ensure the acceptance of the young by their mothers when returned, the mothers were removed from the breeding cages before the pups were taken out and were placed into separate cages where they received supplementary food. The pups were consistently replaced in the breeding cages before their mothers were returned.

*Radiation procedure.* The radiation source was a 300 KV x-ray unit. Distance between the source and the animals was 36 cm and a 1 mm copper sheet was used for filtration. The pups were immobilized in 1 mm thick soft plastic tubing and were placed into lucite blocks which had holes of increasing diameter that could snugly accommodate the growing animals. At a right angle to the holes, two movable 16 mm thick lead shields permitted the changing of the beam width, and only the heads of the growing animals were exposed. A Victoreen 250 r probe was used for measuring exposure. The probe was placed in the lucite block and readings for each hole were taken before and after the irradiation sessions. The average error was  $\pm 1$  r. Exposure rate underneath the shielding was 2 r. In this manner, the heads of pups of an entire litter were irradiated simultaneously at an exposure rate of about 50 r/minute. On days 0 and 1 200 r was delivered, then the exposure was reduced to 150 r and restricted to every second day, on days 3, 5, 7, 9, 11 and 13, with a total of eight exposures.

*Tissue processing.* All the animals were killed at 30 days of age. Ten control and ten irradiated animals were perfused with 10% neutral formalin and their brains were embedded in Paraplast, cut sagittally at 6  $\mu$  and stained with cresyl violet and hematoxylin-eosin. Several sections from each animal were cut at 20  $\mu$  and prepared with Bodian's protargol-S method. The cerebella of two control and two irradiated animals were impregnated, following the Golgi-Cox method, and cut at 100  $\mu$ . The brains of four control and four irradiated animals were prepared for electron microscopy. These animals were perfused

with 6% buffered glutaraldehyde. After further fixation in the same solution, the cerebella were embedded in agar and cut sagittally into 235  $\mu$  thick blocks on a Sorval chopping microtome. These blocks were post-fixed in 1% osmium for one hour and embedded in Epon-Araldite. Thick and thin sections of the pyramis in the vermis were cut on a Porter-Blum MT-2 ultramicrotome. The thick sections were stained with azure B for light microscopy. The thin sections were stained with lead citrate and uranyl acetate and viewed and photographed with a Philips-300 electron microscope.

All the prepared cerebella were of satisfactory quality and were utilized for both quantitative and qualitative evaluation.

*Quantitative evaluation.* Matched, hematoxylin-eosin stained sections were used which were cut parasagittally in a plane corresponding to the lateral coordinate of 950  $\mu$  in König and Klippel's ('63) atlas of the rat brain. With a Zeiss macroprojector the entire vermis was projected at  $\times 65$  magnification, the pyramis separately at  $\times 220$ . The outline of the vermis and the boundaries of the different layers were drawn on paper and then traced with an Ott planimeter to obtain an estimate of the areas occupied by the molecular, granular and medullary layers. In the case of pyramis, a perpendicular line was drawn from the depth of the fissure through the medullary layer and all measurements and counts taken within these boundaries. All Purkinje cells and Golgi cells that had a visible nucleolus were counted at  $\times 1500$  magnification under oil immersion. The number of granule cells in the control animals was counted in ten random samples of  $49 \times 49 \mu$  in each lobe (or pyramis alone) from which the number of granule cells in the total vermis (or pyramis) was estimated; in the irradiated animals all the granule cells that were present were counted. In the molecular layer basket cells were identified as having nuclei about 7–11  $\mu$  in diameter and visible dendritic branchings. Stellate cells and glia could not be distinguished and were counted together. Granule cells in the molecular layer were counted in a separate category. Endothelial cells were not counted.

## RESULTS

*Light microscopic observations*

*Qualitative observations in Nissl-stained sections.* In all the irradiated animals the area of the vermis was drastically reduced at 30 days. This retardation could be attributed to the great sparsity of granule, basket and stellate cells. In most lobules (except the early-maturing nodulus and ventral uvula) a granular layer could not be identified, and the molecular layer was either altogether absent or formed a very thin band, virtually free of cell bodies (fig. 2A). Almost the entire cortex was composed of Purkinje cells, which were dispersed four to eight cells deep from the surface to its depth (fig. 2B). There was no apparent change in the size of the soma of Purkinje cells, some were misshapen, many others normal in appearance (fig. 2C), but with their apical dendritic poles randomly oriented (fig. 2B). This confirmed previous observations that irradiation from birth onward interferes with the dispersion of Purkinje cells (Altman et al., '69b) and the acquisition of the postnatally-formed interneurons (Altman and Anderson, '71) and, as a consequence, the developing cortex is not properly laminated. In the early-maturing nodulus and ventral uvula the few interneurons that were differentiating at birth were spared. There were never any signs of gliosis or of pathological vascularization. In the medullary layer, oligodendroglia cells were extremely scarce.

*Quantitative data.* Planimetric measurements of matched Nissl-stained sections showed a drastic reduction in the parasagittal area of the vermis, from a mean of 22.1 mm<sup>2</sup> in the controls to a mean of 3.0 mm<sup>2</sup> in the irradiated rats. There was a reduction (9%) in the total number of Purkinje cells, from 752 ( $\pm$  82) in the control sections to 682 ( $\pm$  49) in the experimentals; but this difference was not statistically significant. The results of cell counts in the pyramis are summarized in table 1. The reduction in Purkinje cells was relatively small, that in Golgi cells was appreciable. However, because of the dispersion of the Purkinje cells and the absence of cortical lamination, the distinction between Purkinje and Golgi cells was often

uncertain. No basket cells could be clearly identified, and very few granule cells, and many of the latter were situated in the rudimentary molecular layer.

*Observations in Bodian-stained sections.* For the proper evaluation of the changes seen in the irradiated cerebella, it is necessary to describe in detail the typical staining pattern obtained with Bodian's protargol-S staining procedure in the normal cerebellar cortex. Generally the color of the tissue is rusty and the cell nuclei are made conspicuously visible by their darker tone. The perikarya of Purkinje cells are often outlined and, depending on the quality of the preparation, the primary dendrites and their thicker, smooth branchlets may be delineated by their coppery hue. Against this somewhat variable background, an opaque, dark gray or black, metallic deposit is seen quite consistently on fine and coarse axons in a few selected zones of the cerebellar cortex (figs. 7-9). In the molecular layer three zones may be distinguished (fig. 7). In the upper third there are no impregnated fibers (except rarely along a capillary). In the middle molecular layer some very fine sagittally oriented horizontal fibers are seen, and a few vertically oriented ones. In material that is not strongly impregnated these fibers may not be visible. The highest concentration of thick and thin fibers are seen in the lower third of the molecular layer. The thick fibers are typically situated just above the somata of the Purkinje cells and they follow the contours of the cortex. Many can be traced over long distances, past many Purkinje cells; some make sharp turns along their course or even full loops. The thicker fibers give off finer collaterals and these may be entwined on the lower aspects of Purkinje cell primary dendrite and contribute to the rich basket-like plexus formed around the somata of Purkinje cells (fig. 8). The latter may end beneath the soma in the form of a pointed paint brush (fig. 9). Only a few impregnated fibers are seen in the granular layer, which tend to be of fine caliber and may be Purkinje cell axons (figs. 3, 9). In the folding region of the folia, where the fibers of the medullary layer are radially oriented, these fibers may be more numerous. In the medullary layer many fibers are impreg-

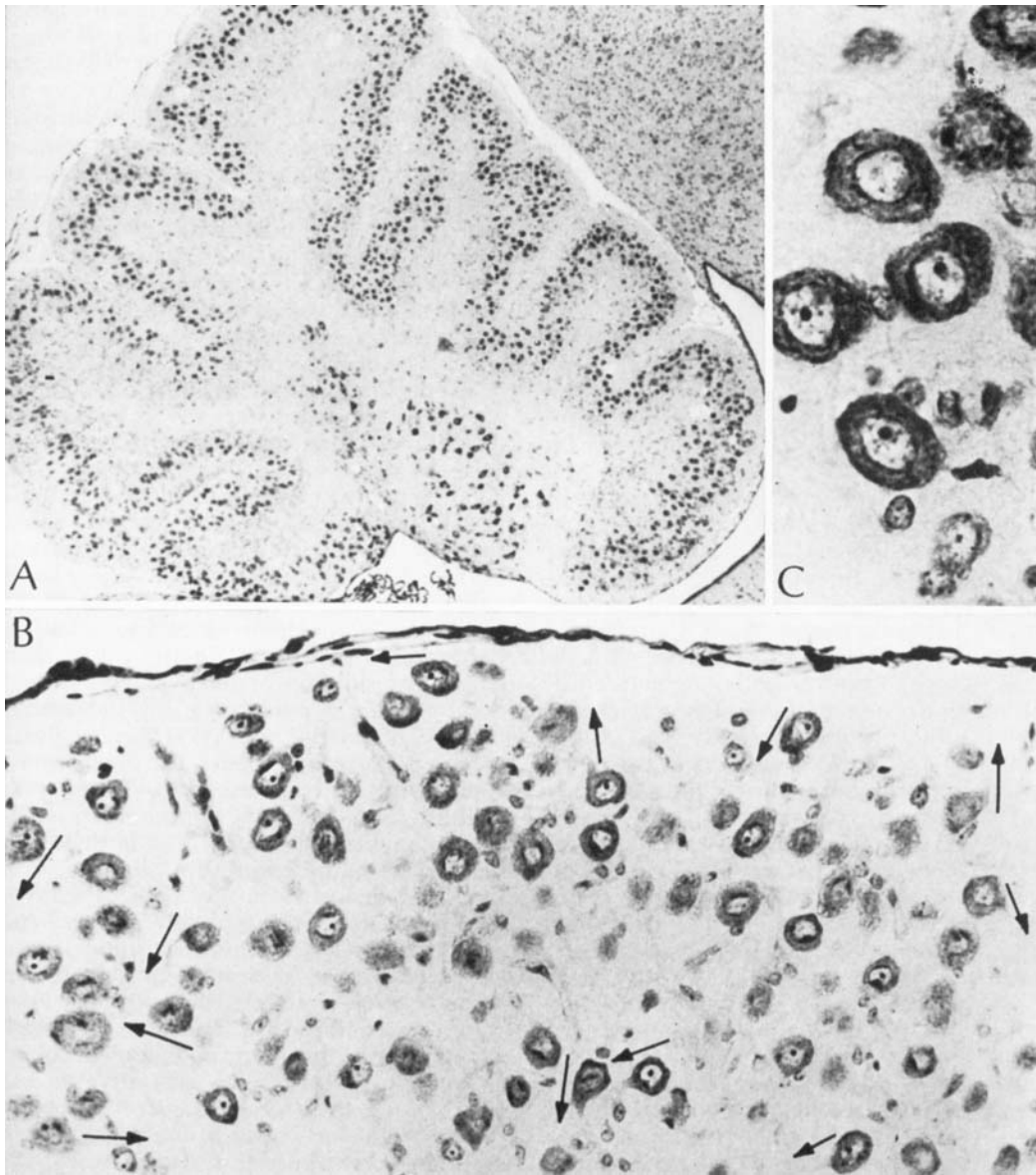


Fig. 2 A. Vermis of a rat that was irradiated repeatedly from birth onward. Hematoxylin-eosin,  $\times 40$ . B. Posterior vermis with the Purkinje cells scattered throughout the cortex. Arrows indicate the orientation of the apical poles of some of the Purkinje cells,  $\times 256$ . C. Purkinje cells in posterior vermis,  $\times 640$ .

nated (fig. 7); others particularly most of the coarse ones, are rusty in color, like the dendrites of Purkinje cells, and were not considered selectively impregnated.

The absence of impregnated axons in the upper molecular layer indicates that

the axons of stellate cells and parallel fibers are not reacting with this stain. The absence of vertically oriented fibers in the upper two thirds of the molecular layer indicates that the climbing fibers are not stained. The scarcity of fibers in the granu-

TABLE 1

*Changes produced in the pyramis by prolonged x-irradiation started at birth*

	Control	Irradiated	Reduction
	(N = 10)	(N = 9)	%
No. of Purkinje cells	66	60	9
No. of Golgi cells	39	16	59
No. of basket cells	176	0	100
No. of granule cells (total)	24,675	149	99
No. of cells in mol. layer	1,089	61	94
No. of granule cells in mol. layer	109	41	62

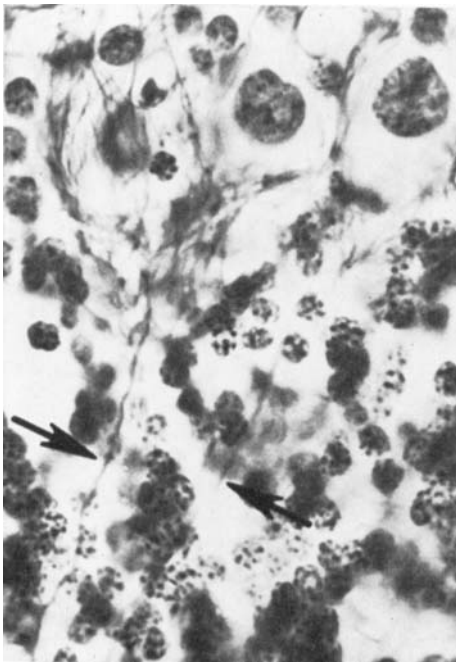


Fig. 3 Basket cell terminals around the somata of Purkinje cells and two stained fibers (arrows) that may be the axons of Purkinje cells. Bodian's protargol-S method,  $\times 818$ .

lar layer and in the glomeruli, furthermore, suggests that mossy fibers and their terminals are not reacting either. Accordingly, the fibers stained in the medullary layer cannot be climbing and mossy fibers but are presumably the axons of Purkinje cells. From this observation and from the prominent staining in the lower zone of the molecular layer and of baskets around the somata of Purkinje cells, it is concluded that in the normal cerebellar cortex the Bodian technique selectively impregnates the axons of basket cells and of Purkinje

cells (including, presumably, the recurrent collaterals of the latter).

The pattern of staining in the irradiated cerebella is different (figs. 10, 11). Two fiber bands are visible: a sagittally and horizontally oriented supraganglionic, smaller assembly of thick and thin fibers; and a much richer collection of radially oriented fibers in a circumferential position around the somata of Purkinje cells, which are distributed throughout the entire depth of the cortex. The fibers that form fine basket plexuses around the soma and paint brush endings at the tip of the Purkinje cells were not clearly seen in these animals. The radially oriented fibers that surround the Purkinje cells are interpreted as the axons and recurrent collaterals of Purkinje cells and their great density is partially attributed to the high packing density of Purkinje cell somata. The horizontal fibers are either also components of these recurrent collaterals or else belong to the few basket cells that may be scattered in the cortex.

*Observations in Golgi-impregnated sections.* The Purkinje cells that are distributed throughout the entire depth of the cortex of the irradiated animals were impregnated in large numbers. The apical poles (or initial portions of the primary dendrites) were randomly oriented and their dendritic branches assumed a great variety of shapes, apparently depending on the position of the soma within the cortex (superficial or deep) and on the orientation of the apical pole.

Purkinje cells whose somata were close to the surface of the cortex, and whose apical poles were directed upward, tended to have a dendritic tree resembling a weeping willow. Presumably this was due to the circumstance that the ramifying dendrites were deflected downward when

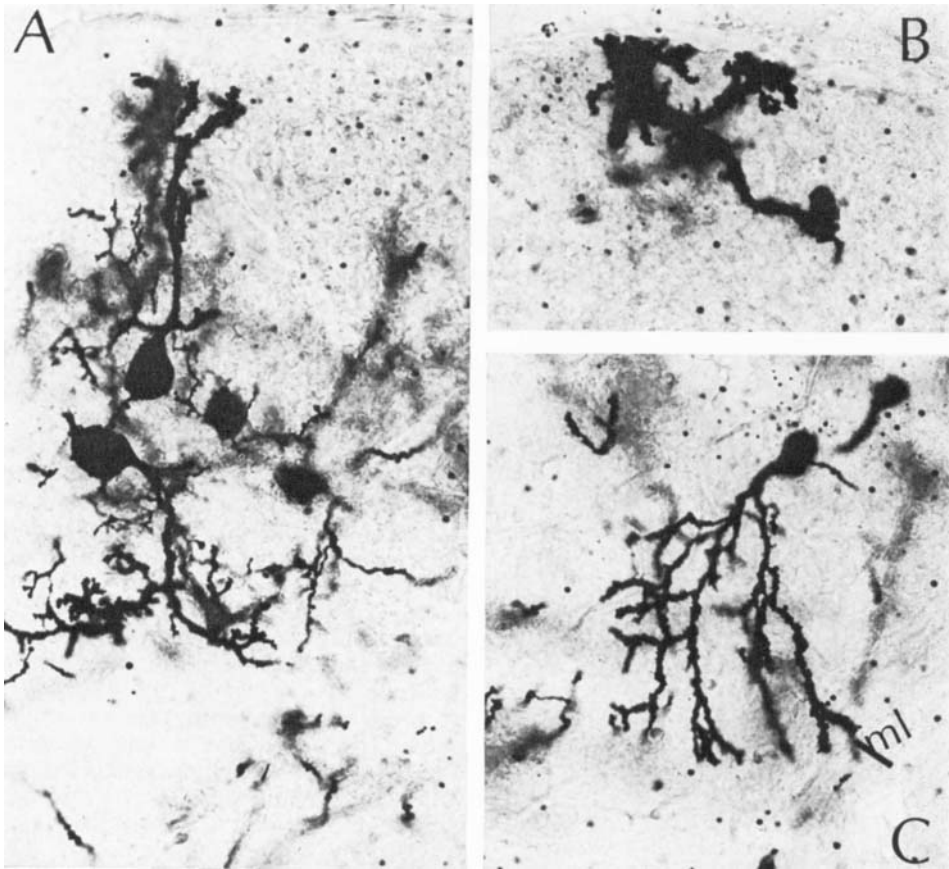


Fig. 4 A. Purkinje cells in the pyramis of an irradiated rat, situated in the intermediate zone of the cortex. The dendritic tree of one of the cells has grown toward the surface, that of the other downward. B. A weeping willow type Purkinje cell. Its soma is located near the surface of the cortex and the dendrites, after reaching the surface, are deflected downward. C. A Purkinje cell in the depth of the cortex with its dendrites penetrating into the medullary layer. Golgi-Cox,  $\times 256$ .

reaching the surface (figs. 4B, 5D, upper right). Purkinje cells in a similar position but with apical poles oriented sideways often had a long primary dendrite, coursing parallel to the surface, and with few branchlets (fig. 5D, upper center). Purkinje cells situated deeper in the cortex showed a great variety of dendritic branchings. Some had a few coarse dendrites directed upward (figs. 4A, 5A); others had sideways growing dendrites, which after a short course were deflected upwards (fig. 5C) or downwards (figs. 4A, 5D). All these dendrites had several features in common: there were many massive branches which had the characteristics of primary den-

drites; there were relatively fewer fine branchlets; the massive dendrites had innumerable long thorns on them; the dendrites were distributed in three dimensions (not planar). Very common was an additional type of Purkinje cell. This was directed downward and the dendritic branches, often thinner and more numerous than in the other Purkinje cells, reached and penetrated the medullary layer (figs. 4C, 5D, bottom right).

In addition to Purkinje cells, Golgi cells were also seen in appreciable numbers. The Golgi cells tended to have a very rich axonal plexus (fig. 6), comparable to that seen in normal animals. Climbing fibers



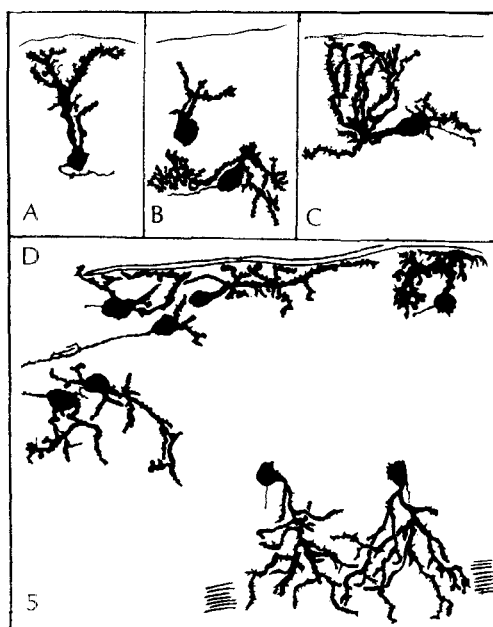


Fig. 5 Camera lucida tracings of Purkinje cells from the vermis of an irradiated rat. All the major types of Purkinje cells, as described in the text, are illustrated. The prominent thorns on the dendritic trunks are evident.



Fig. 6 Camera lucida tracing of a Golgi cell, together with a Purkinje cell whose dendritic tree has grown downward; from the vermis of an irradiated rat.

were identified, and what appeared to be mossy fibers could occasionally be traced to the surface. Impregnated granule cells, basket cells and stellate cells were not seen.

#### *Electron microscopic observations*

Light microscopic examination of the thick, epon-embedded sections showed the prevalence in the irradiated cortices of the somata of Purkinje cells and of bulky, closely packed Purkinje cell dendrites. Occasionally these dendrites were nearly half as wide as the Purkinje cell perikarya and were oriented randomly in all directions (figs. 12–14). Much of the cortical tissue was composed of these two cellular elements; the other constituents of the normal cerebellar cortex were less conspicuous or altogether absent.

*The somata of Purkinje cells.* The cell body of many Purkinje cells had a regular appearance in terms of the shape of the nucleus and the perikaryon, and of the concentration and distribution of the various cell organelles. Many more, however, tended to have lobulated or distorted nuclei, a condition that was typically associated with the asymmetrical accumulation of cytoplasm rich in endoplasmic reticulum at one or several loci near the nucleus, producing a deep indentation at these sites (fig. 16). Often this indentation was near the apical pole of the cell. In many cells lysosomes accumulated in the cytoplasm and small chromatin clumps appeared in the nuclei of these cells. Obvious signs of pathological changes were not seen.

Boutons with synapses were present in appreciable numbers on the soma of Purkinje cells; their frequency was comparable to that seen in normal animals. In addition, boutons and synapses were also seen on perisomatic processes; the latter are no longer present in normal animals at 30 days (Altman, '72b).

Whereas in normal animals only one kind of synapse is ever seen on the soma of Purkinje cells, i.e., what is generally considered to be a basket cell synapse, many more types were seen at this site in the irradiated animals. In spite of the scarcity of basket cells in the irradiated cerebella, typical basket cell boutons were present in at least two of the animals. In these boutons, when cut longitudinally,

scattered small clusters of vesicles were seen in association with small patches of inconspicuous symmetrical synapses (fig. 17). Microtubules and neurofilaments were numerous in these boutons; they were visible also in transverse cuts through the bouton (fig. 18). A somewhat different type of bouton is shown in figure 19; this may either be that of a basket cell or, in view of the difference in the concentration of vesicles, that of a recurrent Purkinje cell collateral.

A typical basket cell bouton is seen forming synapses with two adjacent Purkinje cell somata in figure 20A. In this micrograph another type of bouton is also seen; it is richly filled with vesicles and forms a synapse with one (possibly both) of the Purkinje cells. A similar bouton is seen also in figure 20B on the soma of a Purkinje cell and adjacent to it there is a similar bouton which forms a conspicuous asymmetrical synapse with a dendrite. The latter bouton has the characteristics of a climbing fiber; perhaps in the cerebellar cortex deprived of its interneurons, climbing fibers form symmetrical synapses with the soma of Purkinje cells, in addition to forming asymmetrical synapses with dendrites (as they do under normal conditions). This possibility was borne out by additional observations, as illustrated in figures 21–22. This bouton forms a conspicuous asymmetrical synapse with a dendrite and an equally conspicuous but symmetrical synapse with the soma of a Purkinje cell. Figure 23 shows another bouton which has many of the characteristics of a mossy fiber terminal. It forms conspicuous asymmetrical synapses with three dendrites and less conspicuous symmetrical synapses with the soma of a Purkinje cell.

These observations lead to two conclusions. First, the perikaryon of the Purkinje cell, when not pre-empted by boutons and synapses of basket cells, as in normal conditions, may also form synapses with climbing and mossy fibers. Second, the synapses thus formed are always symmetrical, that is, the postsynaptic membrane of the Purkinje cell soma is not affected by the properties of the presynaptic elements.

In addition to this modification in the type of synapses formed by the somata

of Purkinje cells, perisomatic processes, which disappear in normal animals by the fifteenth day, were also seen on Purkinje cells in the 30 day old, irradiated animals. These perisomatic processes are strictly synaptic structures which form asymmetrical conspicuous synapses with climbing fibers (fig. 24) and possibly also with mossy fibers. The perisomatic processes are devoid of all the organelles seen in the soma, except for a few subsynaptic cisterns, and are considered to be specialized structures which can receive asymmetrical (excitatory) synapses, which cannot be formed directly on the soma.

*The dendrites of Purkinje cells.* Throughout the depth of the cerebellar cortex, as indicated by light microscopy (figs. 13, 14), massive dendritic trunks were present in high concentration, oriented randomly in all planes (figs. 25, 26). These trunks had many of the features of normal primary or secondary smooth dendrites, such as a high concentration of microtubules, cisterns and mitochondria. (The latter were usually surrounding the inner core of the dendrite.) On these dendritic trunks two types of boutons and synapses were present. One of these had the appearance of basket or stellate cell terminals, with inconspicuous, symmetrical synapses (figs. 27, 28). The other type, with conspicuous, asymmetrical synapses were considered to represent climbing fiber synapses. These synapses were rare; more commonly such fibers had attachment plaques on the smooth aspect of the dendrite and synapses on its thorns (fig. 29).

Unlike normal "smooth" dendrites, these massive trunks had few branchlets and were, instead, profusely studded with slender and long thorns or spines (fig. 30). These thorns, which resemble parallel fibers in cross section but lack microtubules, represent another prominent feature of the cerebellar cortex of animals irradiated from birth onward. They are considered to represent the spiny branchlets and spines of the normal dendrite and are specialized surfaces with a predilection to form synapses. The synapses formed were always of the conspicuous asymmetrical type. Thorn synapses were common with climbing fibers (figs. 31, 32) and possibly also with mossy fibers (fig. 33). More

curious, however, were the many contacts established with the processes of glia cells, including fibrous astrocytes (figs. 33–35). These junctions are considered pseudo-synapses because only the typical post-synaptic membrane was present in the dendritic thorn; there was no presynaptic dense membrane in the glial process.

Finally, Purkinje cell dendritic trunks also form abnormal, conspicuous and elongated "synapses" with the dendrites of Purkinje cells. These abnormal Purkinje cell dendro-dendritic contacts (which lack synaptic vesicles) occur either on the surface of large Purkinje cell dendrites (fig. 36) or penetrate their interior (fig. 37); they often alternate with apparently normal axodendritic synapses (fig. 37). It was not possible to determine whether these dendro-dendritic synapses are formed with other or the same Purkinje cell. A curious phenomenon was the autolysis of some of the large Purkinje cell dendritic trunks (fig. 38) which was apparently associated with the formation of abnormal dendro-dendritic synapses (fig. 39). The possible sequence of events leading to this degeneration is reconstructed in figures 40–43.

*Mossy fibers and climbing fibers.* Mossy fiber rosettes were seen in great numbers throughout the cerebellar cortex from its depth (fig. 45) to its surface (fig. 44). Typically, the large terminal had an appreciable accumulation of mitochondria in its core and was filled with densely packed vesicles; a few larger dense core vesicles were scattered among them. The relative concentration of these rosettes was higher in the irradiated (fig. 45) than normal cerebella, presumably due to the great reduction in the size of the cerebellar cortex. In the cerebella in which granule cells were absent altogether, these mossy fiber terminals formed synapses with several identified and unidentified cells and processes. Among these were presumed Golgi cell dendrites in the depth of cortex (fig. 45), Purkinje cell perisomatic processes and, particularly in the superficial part of the cortex, the thorns of Purkinje cell dendrites (figs. 33, 44). In the cortices in which a few granule cells were present the mossy terminals formed synapses and attachment plaques with the dendrites of

granule cells. The myelinated fibers seen throughout the cerebellar cortex of the irradiated animals were presumed to be the mossy fiber axons that reached to the surface of the cortex. The majority of these mossy fibers appeared normal, but a few showed signs of degeneration, and occasionally advanced autolysis.

The climbing fibers were as preponderant as the mossy fibers; they formed, as described earlier, innumerable asymmetrical, conspicuous synapses with the thorns of Purkinje cell dendritic trunks. Occasionally climbing fibers were seen that also had desmosome-type contacts with the smooth surface of the dendrites (fig. 29). The identity of other boutons with conspicuous asymmetrical synapses on the smooth surface of dendrites (fig. 27) could not be determined; presumably they were either climbing or mossy fiber terminals.

*Other cellular elements.* Occasional cell bodies near the surface of the cortex, with symmetrical and asymmetrical synapses, were presumed to be stellate or basket cells. They were often unusually large and in the depth of the cortex could not be distinguished from Golgi cells. Their massive dendrites displayed both symmetrical and asymmetrical synapses.

Similarly an occasional cell was seen throughout the cortex, with the characteristics of granule cells. In some irradiated cerebella few or no parallel fibers were present; in others there were several in the depth of the cortex. Curiously, these parallel fibers tended to have many more microtubules in cross section than parallel fibers in the normal cortex (up to a dozen against 3–4) and usually also had a few neurofilaments (fig. 46).

Although there were no signs of a gliosis in the irradiated cerebella, astrocytes were common in the cortex. Unlike in normal cerebella, their processes were rich in filaments (figs. 33, 35). Other types of glia cells were also seen. In the medullary layer many of the fibers were unmyelinated; others had unusually thin myelin sheaths (fig. 47).

#### DISCUSSION

Low-level irradiation of the cerebellum at birth destroys the cells of the external germinal layer and, if the irradiation is

repeated periodically to prevent regeneration, a cerebellar cortex develops essentially devoid of basket, stellate and granule cells. Only in the nodulus and ventral uvula are a few granule cells present, presumably because in these early-maturing lobules the differentiation of some of the cells has begun before birth. The elimination of the cortical interneurons is considered a direct effect of radiation. The specific aim of this study has been to examine the indirect effects of radiation, that is, the consequences of the absence of interneurons on the morphogenetic organization of the cerebellar cortex. The thesis of this discussion is that most (if not all) of the changes seen in the extant elements of the cortex (particularly in the structure and connections of Purkinje cells) are the results of the absence of interneurons (and consequent alterations in their morphogenetic interrelations) and are not due to direct damage produced by radiation in their biochemical organization and developmental potency. The evidence supporting this thesis, together with some of the counterindications, will be summarized at the end of this discussion.

The following major changes were seen in the cerebellar cortex devoid of micro-neurons:

(a) The Purkinje cells were not strung out in a monolayer parallel to the surface, but were scattered several-cell deep throughout the cortex. The following considerations may account for this: At birth the Purkinje cells are densely packed throughout the depth of the small cerebellum. The monolaminar dispersion of Purkinje cells parallel to the surface begins after birth (Addison, '11) and is not completed until the third to seventh day in different parts of the vermis (Altman, '69, '72b). The spatial requirement of this dispersion is an appreciable increase in the surface area of the cortex which allows accommodation of all these cells in a single row. The initial expansion in the surface area of the cerebellar cortex (Altman, '69, fig. 2), at a time when cell differentiation has not yet begun, can be attributed to the rapid proliferation of the cells of the external germinal layer which spread over a convoluting surface, maintaining throughout a proliferative zone with the constant

depth of about four to five cells (Altman, '72a). This expansion of the surface does not take place if the external germinal layer is destroyed by radiation and, as the space needed for dispersion does not become available, the Purkinje cells begin to grow and differentiate without dispersion.

(b) The apical poles and primary dendrites of Purkinje cells were not directed at a right angle to the surface but were randomly oriented. The motive force of the dispersion of Purkinje cells has not been clearly identified. At the time when the Purkinje cells begin to disperse, they start to develop their prominent apical cones, which are filled with "reticular" type of growth cytoplasm (Altman, '72b). This cytoplasm contains organelles which stream upward and provide material for the rapidly growing and richly arborizing Purkinje cell dendrites. When the external germinal layer is destroyed by radiation the apical cones, which normally point and grow toward the surface (Altman et al., '69b, fig. 2b), become randomly oriented (Altman et al., '69b, fig. 3b). The observation indicates that, whereas the growth of the apical cone of Purkinje cells is an autonomous event, the normal orientation of this growing structure depends on the presence and location of the external germinal layer. Conceivably the "attractive force" of the external germinal layer which makes the apical cones grow toward the surface also provides the motive power for the dispersion of Purkinje cells, which move radially upward until their progress is halted when they reach the bed of the earliest parallel fibers (Altman, '72a).

(c) In spite of the virtual absence of parallel fibers and the axons of stellate cells, the dendritic arbor of Purkinje cells was often well developed, indicating an autonomous factor in the growth of the dendritic system. However, the configurations assumed by the arborizing dendrites were consistently abnormal. The unusual shapes of the arborizing dendrites could be attributed to their autonomous growth, the random orientation of the primary dendrites, and the failure of Purkinje cells to disperse in a monolayer. For instance, the presence of the weeping willow type of Purkinje cells, previously observed in irradiated kittens by Shofer et al. ('64) and

Hámori ('69), can be explained by the necessary downward deflection of growing dendrites of cells located near the surface. Purkinje cells with weeping willow dendrites are particularly abundant in rats in which irradiation is started at four days (Altman and Anderson, submitted). Quite frequent were Purkinje cells with somata located deep in the cortex, apical poles directed downward, and arborizing dendrites spread through the medullary layer. Reference was made to this type of cell in a previous study (Altman and Anderson, '71, fig. 10). This phenomenon reflects again the autonomy of Purkinje cell dendritic growth which can take place in an abnormal location.

These abnormalities, which can be viewed as morphogenetic rather than pathological changes produced by irradiation, were observed and could be adequately examined with light microscopy. Many other changes were detected with electron microscopy or could be interpreted only with this method.

(d) On the somata of Purkinje cells, the normally transient perisomatic processes persisted in the mature cerebellum. Perisomatic processes appear in normal animals at about five days in the pyramis; their frequency is greatly reduced by the tenth day and they disappear thereafter. (Altman, '72b). These perisomatic processes are transient synaptic specializations which form conspicuous asymmetrical synapses with climbing fibers before the Purkinje cell primary dendrites are formed. Their disappearance is associated with the invasion of the smooth surface of the somata of Purkinje cells by basket cell terminals and the translocation of climbing fiber synapses to the thorns of outgrowing smooth dendrites (Larramendi, '69). In some of the irradiated cerebella it was surprising to see basket cell terminals on the somata of Purkinje cells; it was assumed that the few extant basket cells contacted more than their normal share of Purkinje cell somata. (The possibility that some of the "basket cell" terminals were those of the recurrent collaterals of Purkinje cell axons was suggested by the evaluation of the altered pattern of impregnation in the material stained with Bodian's technique). If it is assumed that due to the

scarcity of basket cells their terminals were greatly reduced, the survival of perisomatic contacts with synapses on them may be interpreted to be due to the diminished competition for synaptic sites on the soma by basket cell terminals.

(e) Even more surprising than the survival of perisomatic processes was the presence of climbing and mossy fiber terminals on the soma itself and the type of synapses they formed. In several instances typical climbing and mossy fiber terminals were identified which formed conspicuous, asymmetrical (excitatory?) synapses with various dendritic processes, as they normally do, and also inconspicuous, symmetrical (inhibitory?) synapses on the smooth surface of the Purkinje cell soma. This indicated that in the absence of basket cell terminals the Purkinje cell soma was ready to form synapses with terminals it does not normally react with, but that the type of contact established was dictated by the properties of the Purkinje cell soma, which formed with climbing and mossy fiber terminals thin postsynaptic dense membranes and not complex subsynaptic webs.

The impression was gained that the concentration of climbing and mossy fiber synapses was higher in the irradiated animals than in the controls, perhaps because these fibers were not affected by radiation whereas the cerebellar cortex as a whole was greatly reduced. That the climbing and mossy fibers are not harmed by exposure to a single very high dose of x-ray (2000 r) was previously reported by Hámori ('69). The major synaptic targets of the climbing fibers were the innumerable thorns of the massive dendritic trunks of Purkinje cells.

(f) The Purkinje cells developed a preponderance of massive trunks studded with thorns, but few if any spiny branchlets, and these dendritic thorns formed synapses with climbing and mossy fibers and "psuedosynapses" with glia cells. The lack of spiny branchlets was associated with the absence of granule cells, and it is suggested that the formation of spines is not an autonomous process but is dependent upon the presence of parallel fibers with which they form synapses. The great proliferation of thorns on the massive trunks could be partly attributed to the high concentration

of climbing fibers (Hámori, '69) but there was also a clear indication of another factor, namely, the proclivity of Purkinje cell dendrites to form synapses with any and all extant elements, indeed also with elements with which Purkinje cells do not form attachments under normal conditions. In the irradiated cerebellum Purkinje cell dendritic thorns formed "pseudo-synapses" with glia cells, with a typical complex subsynaptic web in the thorns but no presynaptic dense membrane in the glia, which must be structurally and functionally unsuited to form a true synapse.

The formation of pseudosynapses indicates that the induction mechanisms of synaptogenesis resides not in the presynaptic site (in this case an incompetent glial process) but in the postsynaptic Purkinje cell, which displays an autonomous readiness ("hunger") for making synaptic contacts when proper functional junctions cannot be formed. That synaptogenic induction may originate in the Purkinje cell was suggested in a previous study of normal cerebellar neurogenesis (Altman, '71). At 10–15 days open coated vesicles were seen in great numbers in Purkinje cell dendrites opposite parallel fibers (which only occasionally showed them); their presence was interpreted to represent the initial step in synapse formation.

(g) The Purkinje cell dendritic thorns also formed "synapses" with Purkinje cell dendrites, either on their surface or penetrating into their core, which resulted in a club-ending enlargement of the dendritic trunk. It could not be determined whether these contacts were between thorns and trunks of the same or other Purkinje cells but in many instances these contacts (possibly when it was between elements of the same Purkinje cell) led to a pathological change, the autolysis of the dendrite with club ending. Neither the properties of these contacts, which cannot be true synapses as there are no vesicles on the "presynaptic" sites, nor the pathological changes produced by them are understood at present.

(h) In addition to these partially degenerating Purkinje dendrites, an occasional axon terminal showed a similar autolytic degeneration. Although this type of degeneration was not common in the 30 day old animals, it is conceivably a progressive pro-

cess. No other signs of pathological changes were seen in these animals; there was no gliosis, although fibrous astrocytic processes were more numerous and prominent than in nonirradiated animals. In view of the absence of signs of appreciable degeneration, it is surprising that there was some reduction (9%) in the number of Purkinje cells and an even more pronounced reduction in the number of Golgi cells. The clarification of these changes calls for an examination of irradiated cerebella at different stages of development and after prolonged survival periods.

#### ACKNOWLEDGMENTS

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*Note added in proof:* Two recent publications reported the presence of post-synaptic thickening in Purkinje cell dendritic thorns in cerebella devoid of granule cells. This was seen in cats following infection with feline panleukopenia virus (R. M. Herndon, G. Margolis and L. Kilham 1971 The synaptic organization of the malformed cerebellum induced by perinatal infection with the feline panleukopenia virus (PLV). II. The Purkinje cell and its afferents. *J. Neuropathol. Exp. Neurol.*, 30: 557-570) and in mice after injection with cycasin (A. Hirano, H. M. Dembitzer and M. Jones 1972 An electron microscopic study of cycasin-induced cerebellar alterations. *J. Neuropathol. Exp. Neurol.*, 31: 113-125).

## PLATE 1

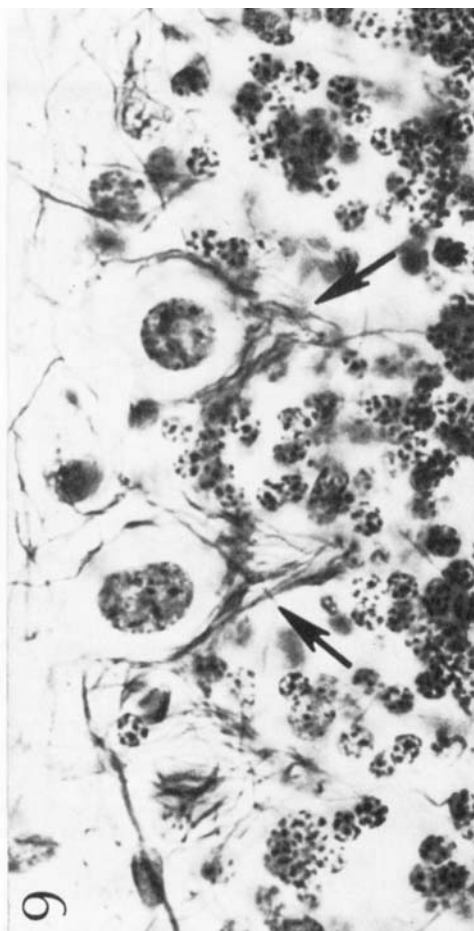
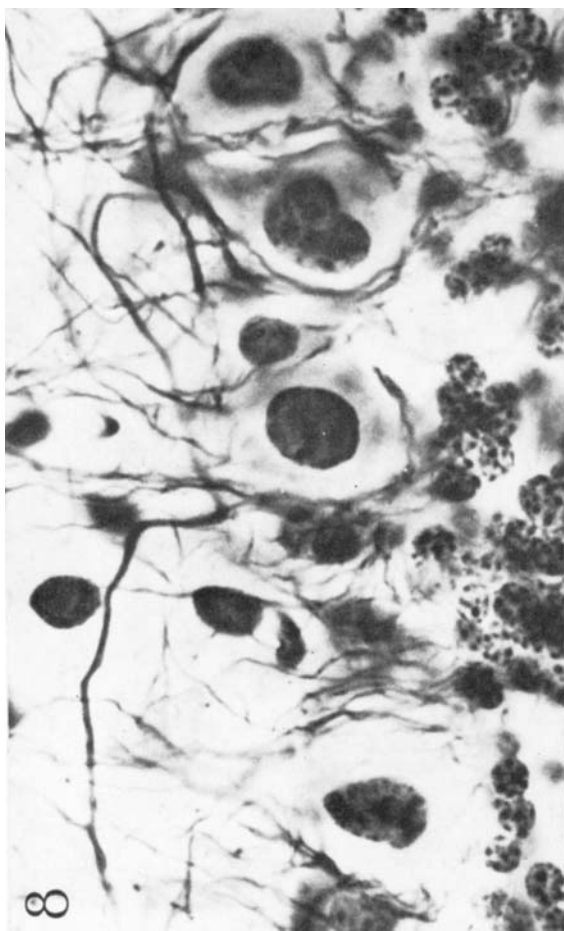
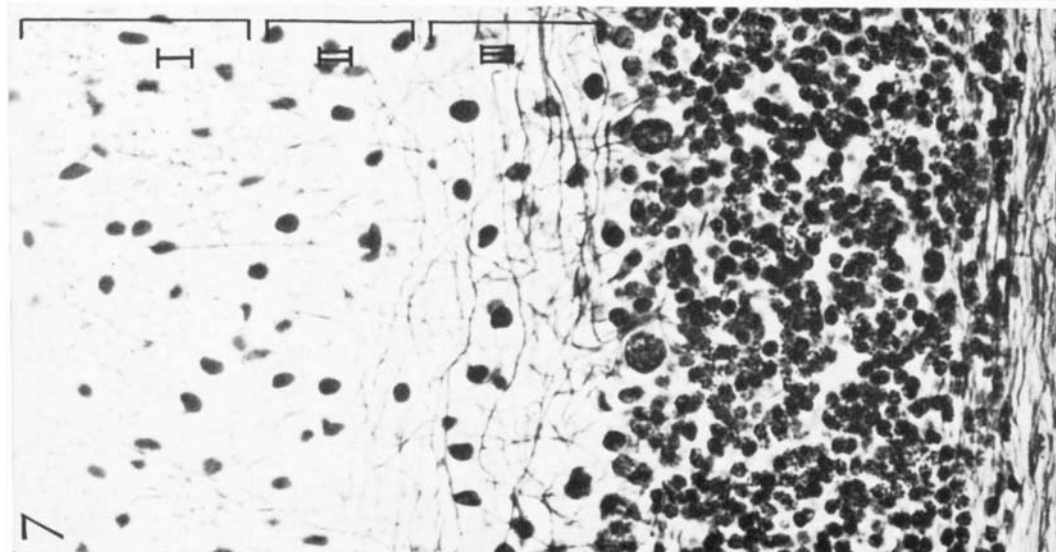
## EXPLANATION OF FIGURES

Staining pattern in the vermis of normal rats with Bodian's protargol-S method. Sagittal sections.

- 7 A portion of the declive. The molecular layer is divisible into three zones: I, an upper zone essentially devoid of impregnated fibers; II, the intermediate zone with a few, thin fibers; and III, the lower zone, which is rich in both thick and thin fibers. There are few impregnated fibers in the granular layer but many are seen in the medullary layer.  $\times 256$ .
- 8 Thick and thin impregnated fibers above the perikarya of Purkinje cells and fine fibers forming baskets around the perikarya.  $\times 1024$ .
- 9 Fine impregnated fibers forming a pointed paint brush pattern (arrows) below the perikarya of Purkinje cells. A Purkinje cell axon may also be impregnated,  $\times 818$ .



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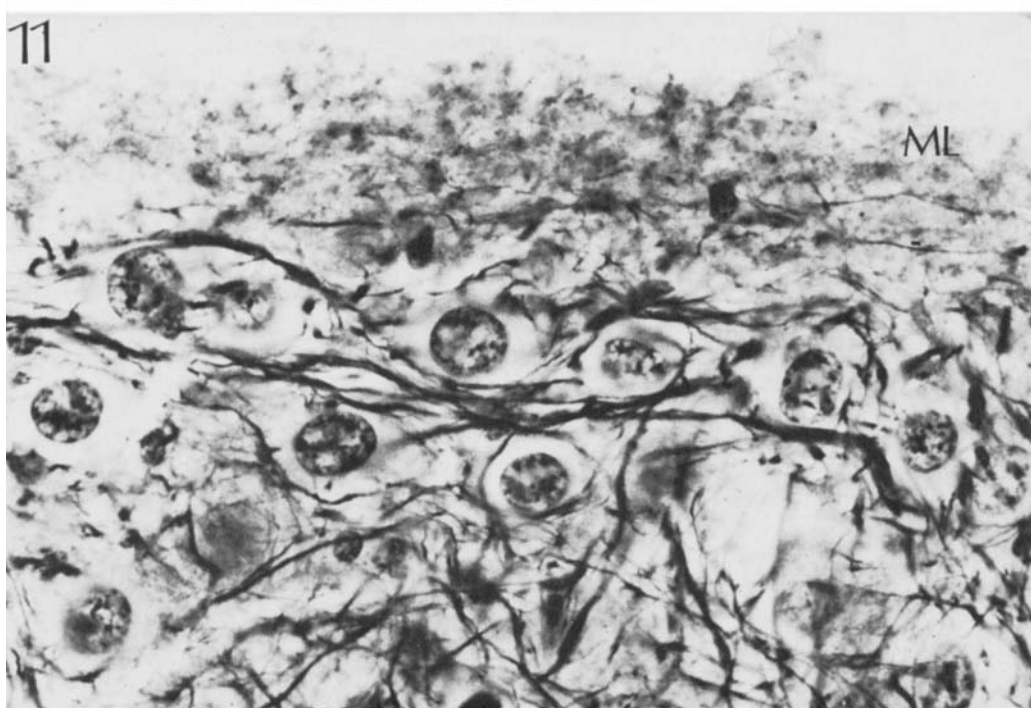
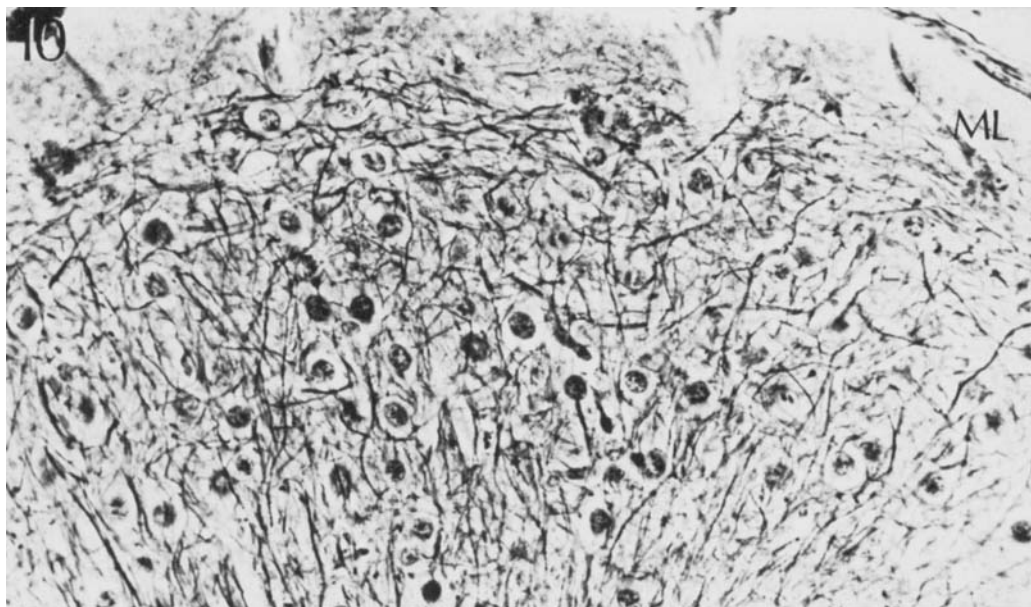


## PLATE 2

### EXPLANATION OF FIGURES

Staining pattern in the vermis of an irradiated rat with Bodian's protargol-S method. Sagittal sections.

- 10-11 There are few stained fibers in the narrow molecular layer (ML). Predominantly thick but also some thin fibers are seen in horizontal orientation and around the perikarya of the upper Purkinje cells. Deeper in the cortex more fibers are radially oriented. These are presumed to be axons of the aggregated Purkinje cells. Figure 10,  $\times 256$ ; figure 11,  $\times 640$ .

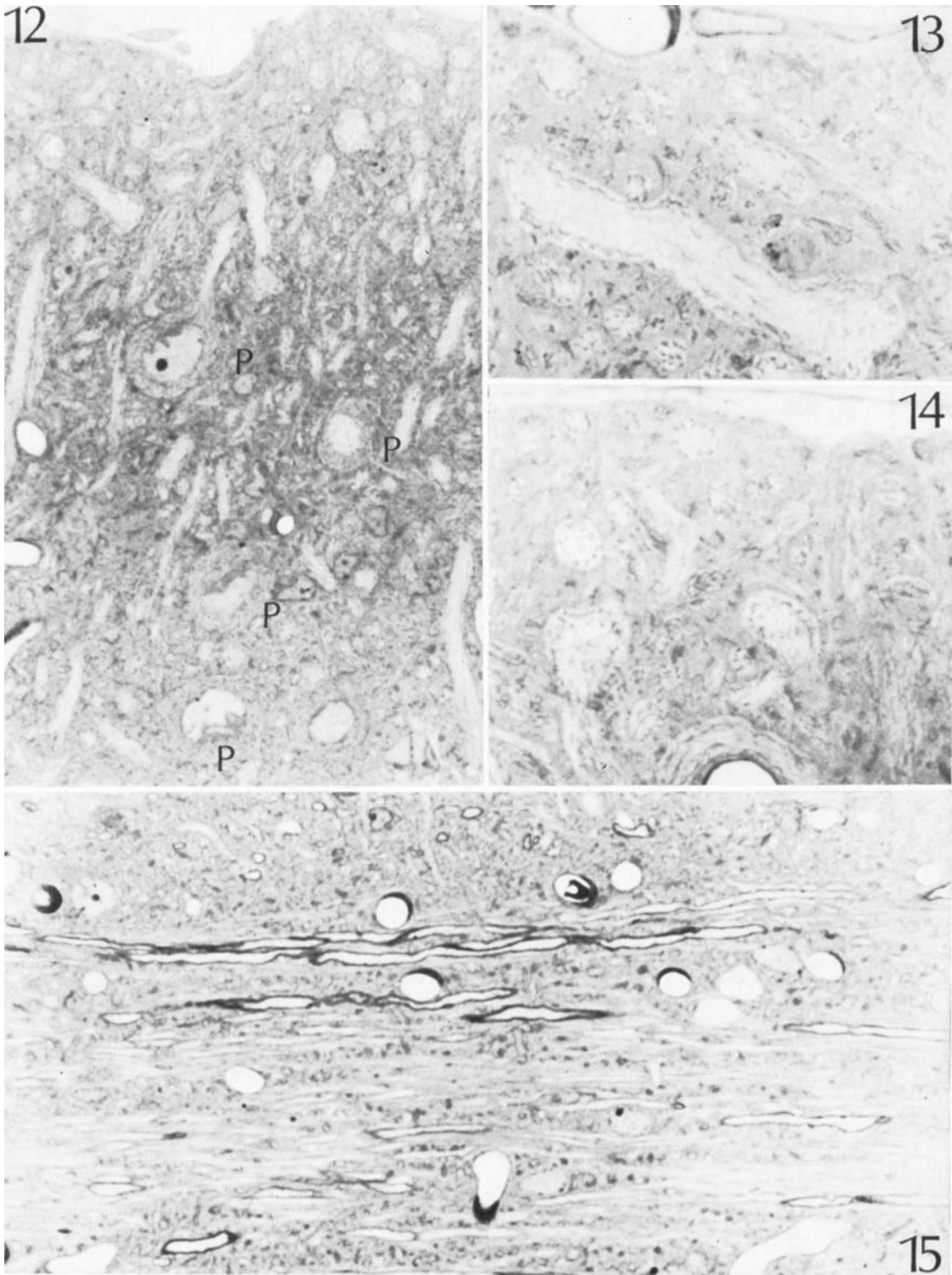


### PLATE 3

#### EXPLANATION OF FIGURES

The cerebellar cortex of an irradiated rat. "Thick" sagittal sections fixed with glutaraldehyde and osmium, and stained with azure B.

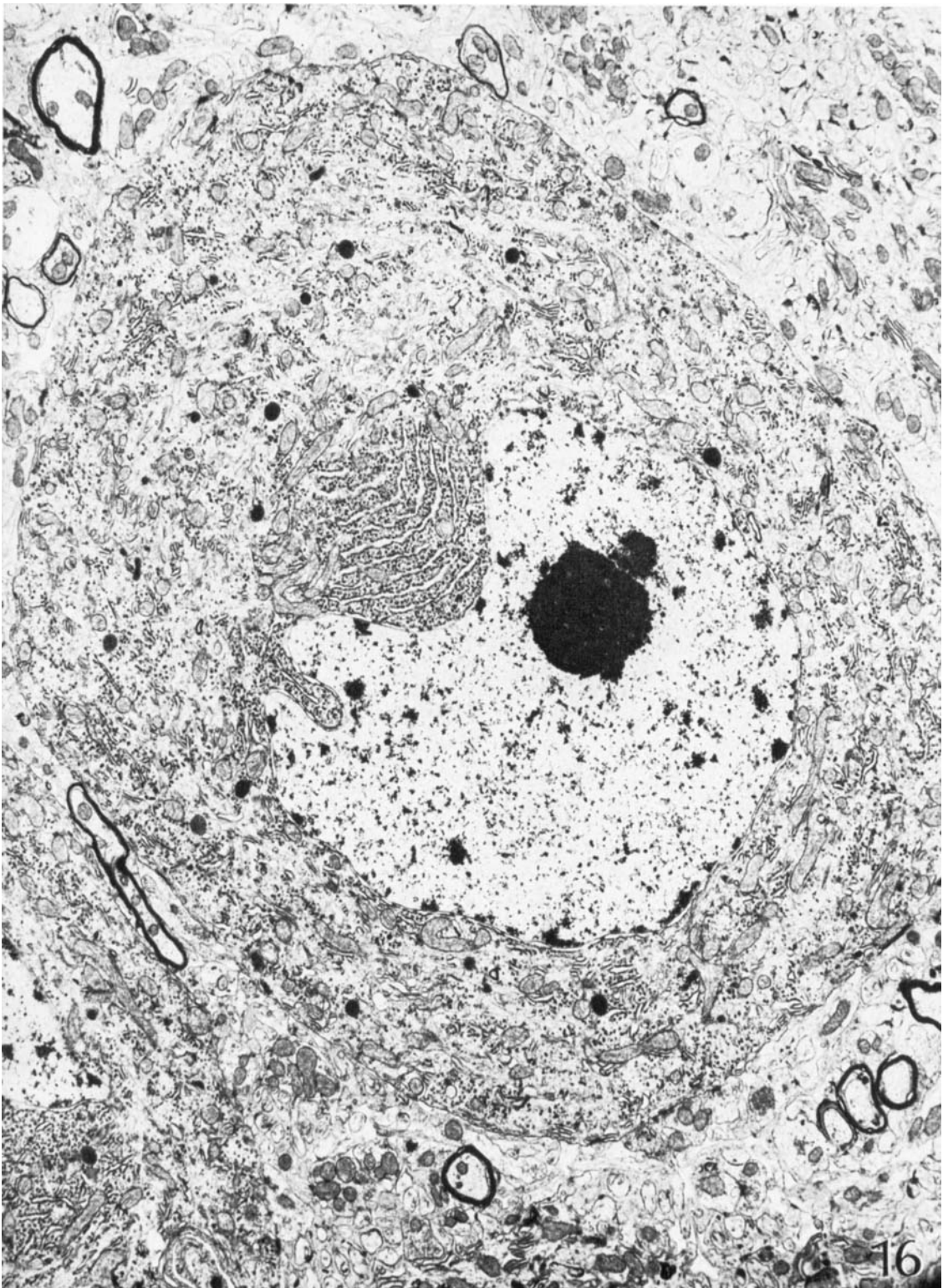
- 12 Upper zone of cortex with a few Purkinje cell perikarya (P) and several thick dendrites cut in different planes.  $\times 640$ .
- 13-14 Purkinje cell dendritic trunks near the surface of the cortex. Mitochondria are visible in peripheral position in the dendrites. (Compare with fig. 25.) Oil immersion,  $\times 1600$ .
- 15 Myelinated and unmyelinated axons in the medullary layer. (Compare with fig. 47.)  $\times 640$ .



#### PLATE 4

##### EXPLANATION OF FIGURE

- 16 The soma of a Purkinje cell from the pyramis of an irradiated rat. Note the accumulation of granular endoplasmic reticulum at one pole producing the nuclear indentation. This was commonly seen in the irradiated animals. The high concentration of lysosomes in the cytoplasm and small chromatin clumps in the nucleus often characterized the soma of Purkinje cells in irradiated animals.  $\times 7,752$ .



## PLATE 5

### EXPLANATION OF FIGURES

Examples of inconspicuous, symmetrical synapses on the somata of Purkinje cells in the pyramis of an irradiated rat.

- 17 An extremely long basket terminal on the lateral aspect of a Purkinje cell perikaryon (PC). Small clutches of synaptic vesicles are seen intermittently (arrows) forming *en passant* synapses. CV, coated vesicle; PF, parallel fibers,  $\times 13,680$ .
- 18 A basket terminal cut in cross section. Two inconspicuous, asymmetrical synapses (arrows) are seen. Neurofilaments (NF) are evident.  $\times 25,080$ .
- 19 This bouton with larger clutches of vesicles and somewhat more pronounced, but symmetrical synapses may be that of a Purkinje axon recurrent collateral. This identification is uncertain.  $\times 41,040$ .

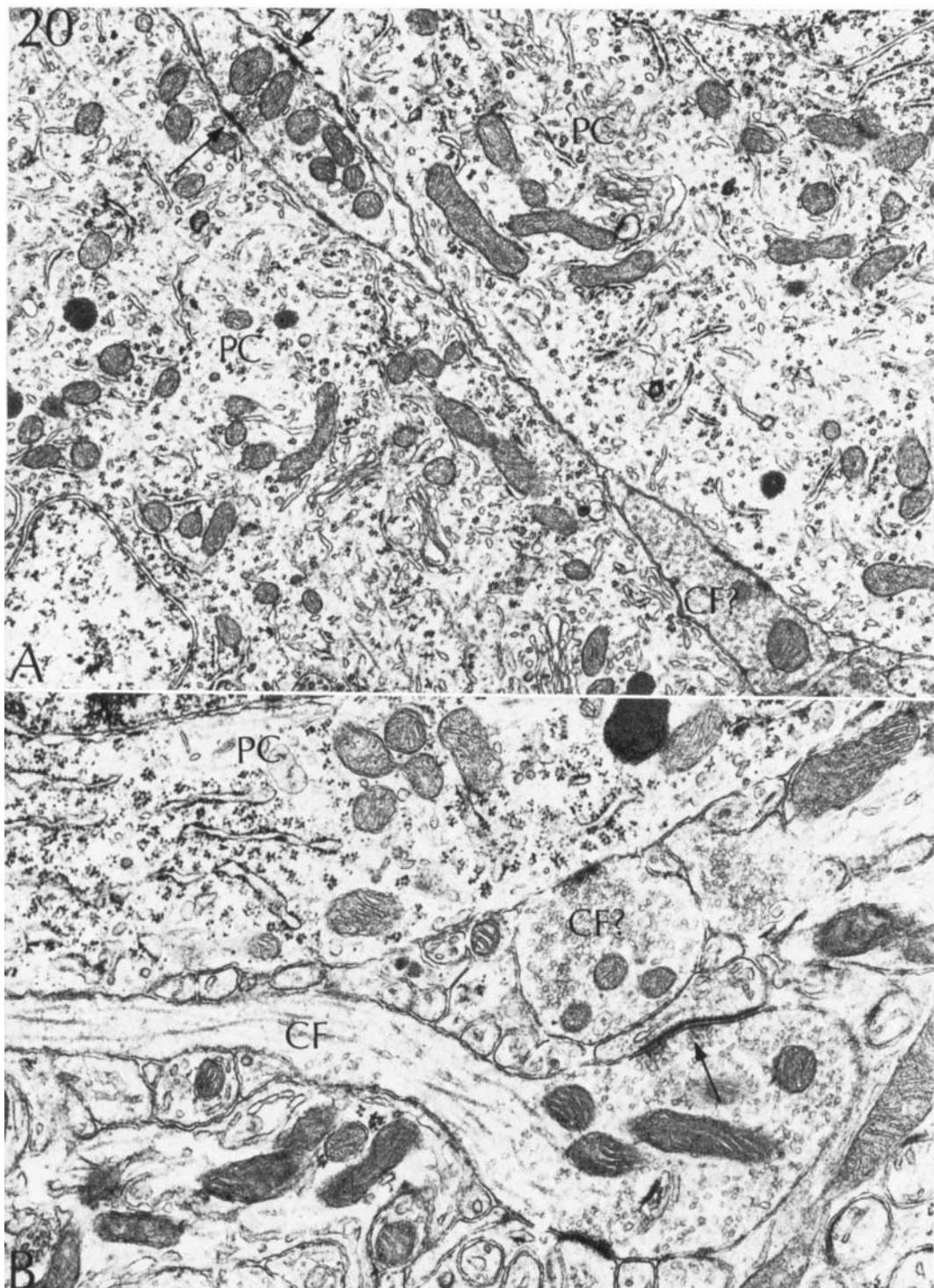




## PLATE 6

### EXPLANATION OF FIGURES

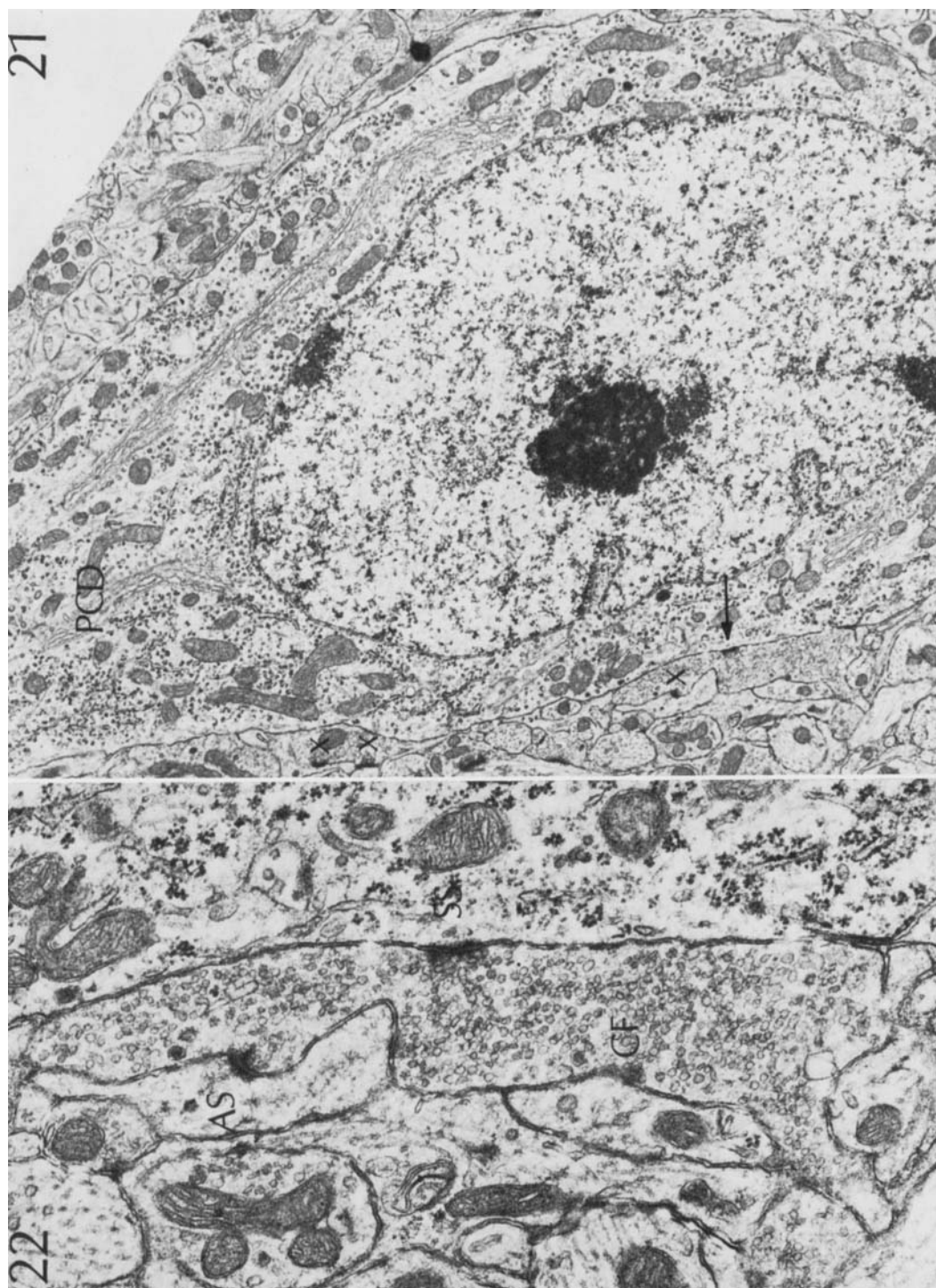
- 20 A. Presumed basket cell bouton forming synapses (arrows) with the perikarya of two Purkinje cells (PC). Another bouton, with dense accumulation of synaptic vesicles forms a synapse with at least one of the Purkinje cells (CF?). Suggestive evidence that the latter type of bouton, although it forms a symmetrical (inhibitory?) synapse may be that of a climbing fiber is marshalled in figures 20, 21 and 22.  $\times 16,188$ .
- B. On this Purkinje cell soma (PC) there is a bouton with densely packed vesicles (CF?) and a symmetrical synapse, which is reminiscent of the suspected climbing fiber bouton shown in figure 19. In addition there is an axon (CF) with a terminal bouton with similar accumulation of densely packed vesicles and with the typical conspicuous, asymmetrical synapse (arrow) of climbing fibers. That such a terminal may have asymmetrical synapses with dendrites and symmetrical synapses with the somata of Purkinje cells is shown in figures 21-22.  $\times 25,080$ .



## PLATE 7

## EXPLANATION OF FIGURES

- 21 A long bouton with densely packed vesicles is seen along the soma of this Purkinje cell and its primary dendrite (PCD). It forms asymmetrical synapses with dendritic processes (Xs) and a marked but symmetrical synapse with the soma of the Purkinje cell (arrow).  $\times 9,120$ .
- 22 Enlargement of the bouton, interpreted as that of a climbing fiber (CF), which forms an asymmetrical synapse (AS) with a dendrite and a symmetrical synapse (SS) with the soma of the Purkinje cell. Apparently the soma of the Purkinje cell can form only an "inconspicuous" postsynaptic dense membrane  $\times 31,920$ .



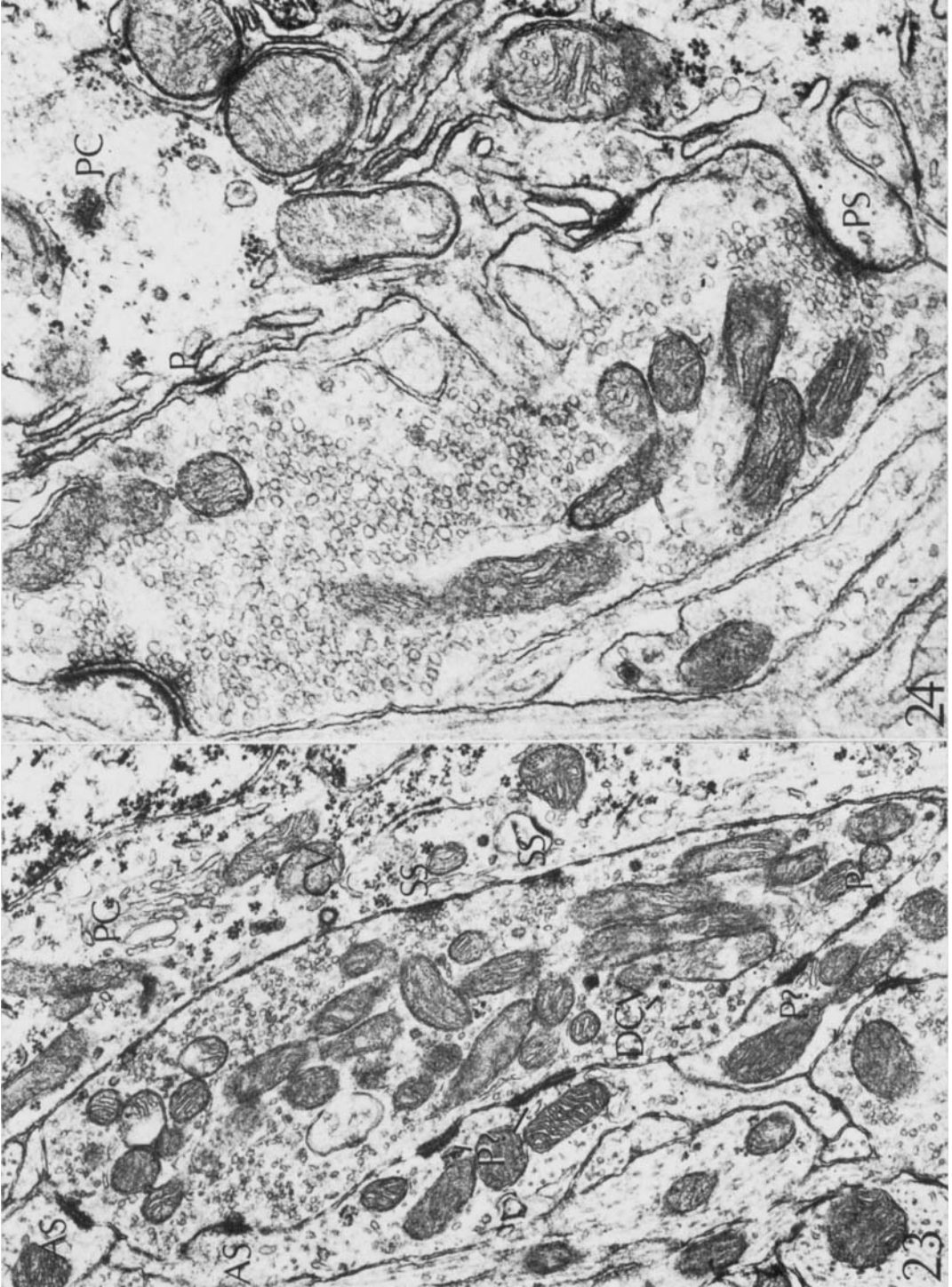
## PLATE 8

## EXPLANATION OF FIGURES

- 23 The high concentration of dense core vesicles (DCV) and the many conspicuous, asymmetrical synapses (AS), and possibly attachment plaques (P) that this bouton forms with several dendrites indicates that this is a mossy fiber terminal. This bouton also forms inconspicuous symmetrical synapses (SS) with the soma of a Purkinje cell (PC). CV, coated vesicle.  $\times 25,080$ .
- 24 This bouton, which is identified as that of a climbing fiber, forms a conspicuous, asymmetrical synapse with an identified perisomatic process (PS) of a Purkinje cell and another one which may be a perisomatic process or a dendritic thorn (upper left corner). Small attachment plaques (P) are also formed with the soma of the Purkinje cell (PC).  $\times 41,040$ .



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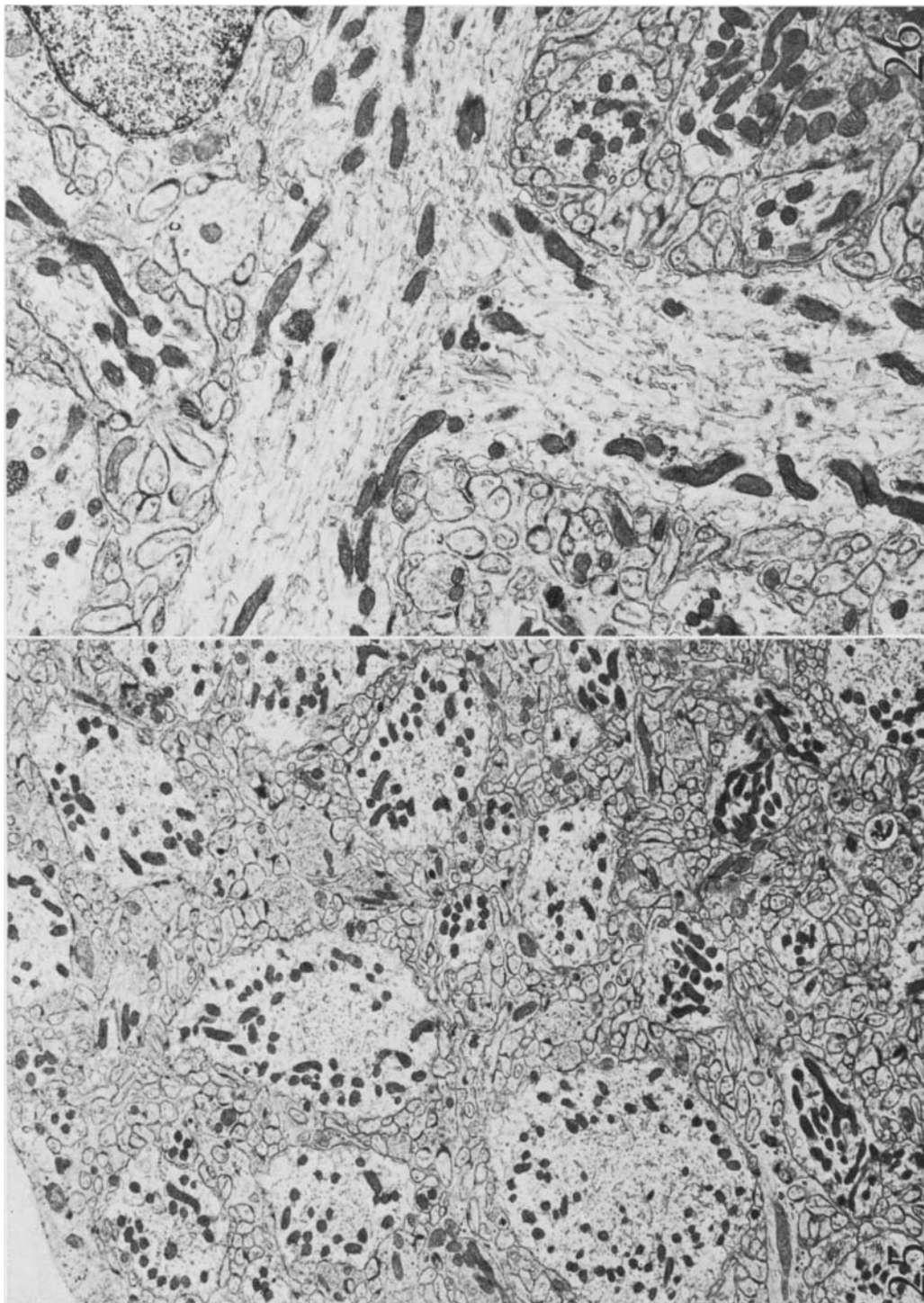
## PLATE 9

## EXPLANATION OF FIGURES

- 25 Large dendrites, cut in cross section near the surface of the cortex. These trunks are apparently oriented horizontally to the surface and must belong to the type of Purkinje cells illustrated in figure 5 either with parallel or descending (weeping willow) dendrites. Note the peripheral distribution of mitochondria, which is characteristic of mature dendrites (Altman, '72b). The round and elongated processes among the massive dendritic trunks are not parallel fibers (which are absent or extremely scarce) but the thorns of the Purkinje cell dendrites (see below).  $\times 6,384$ .
- 26 A single large bifurcating dendritic trunk from near the surface of the cortex.  $\times 11,172$ .



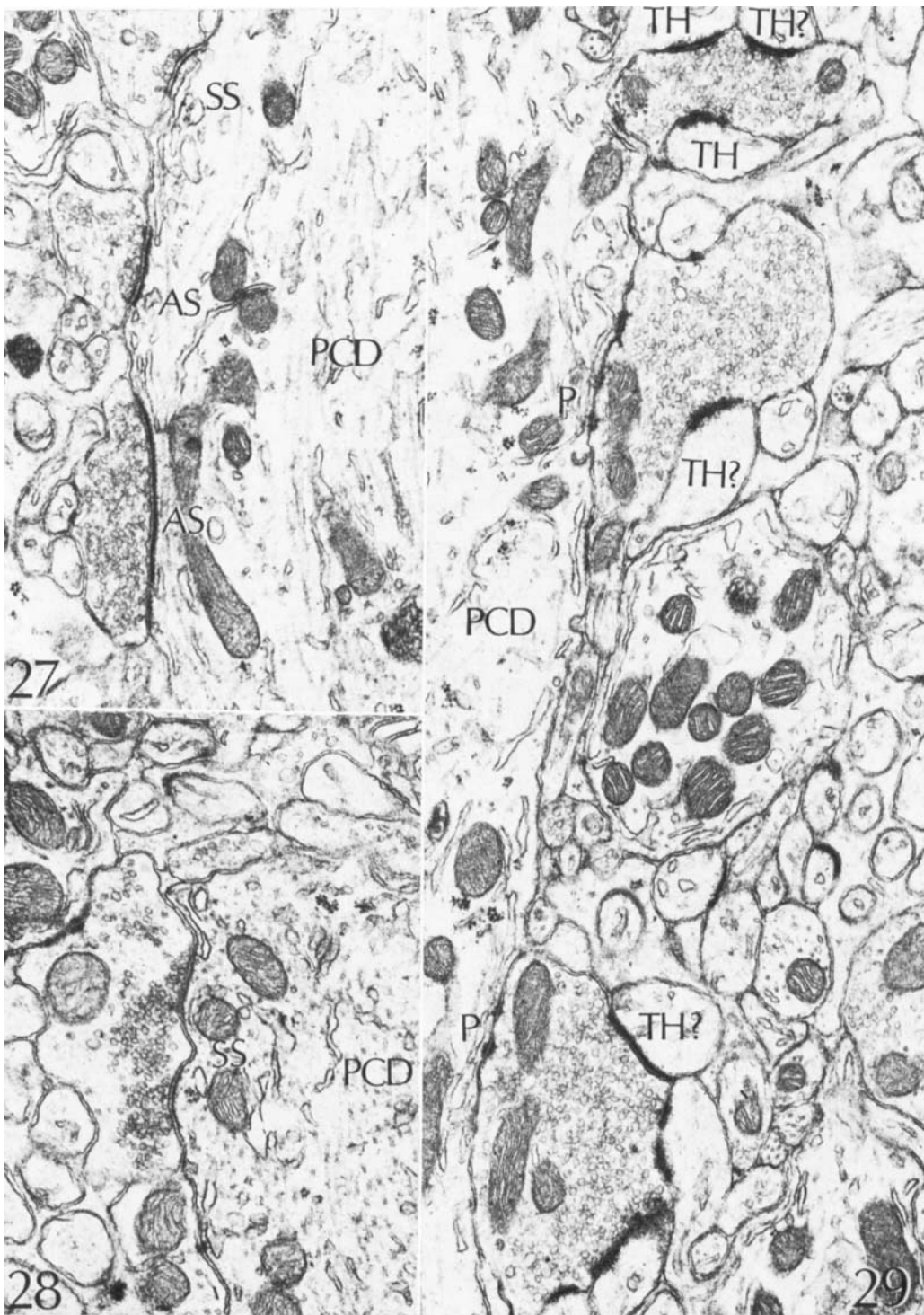
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## PLATE 10

### EXPLANATION OF FIGURES

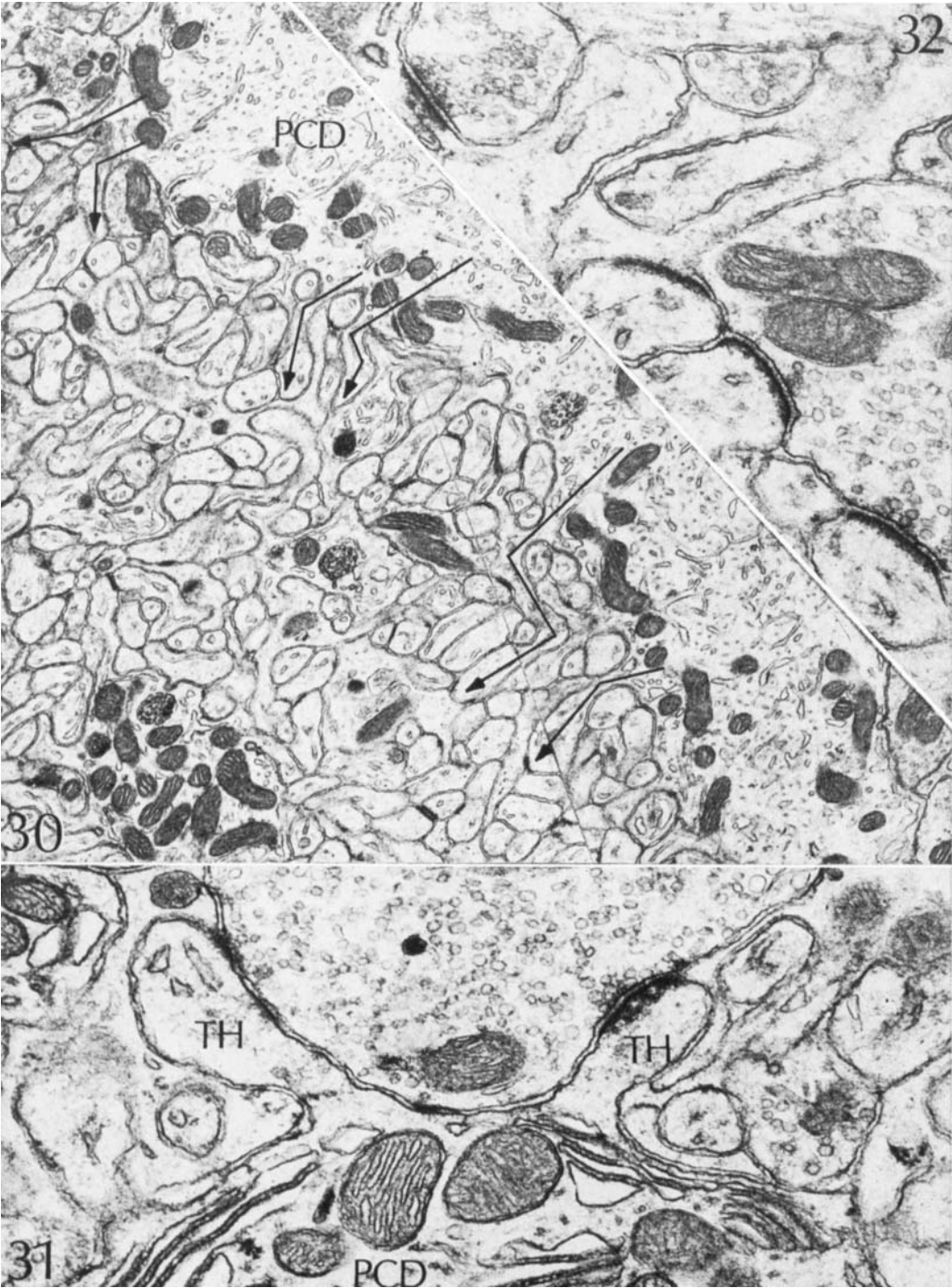
- 27 Large Purkinje cell dendrite (PCD) with a bouton forming an inconspicuous symmetrical synapse (SS) and two others forming conspicuous, asymmetrical synapses (AS). The former is presumed to be a basket or stellate cell synapse; the latter are presumably climbing fiber synapses.  $\times 25,080$ .
- 28 Large Purkinje cell dendrite (PCD) with basket or stellate cell type bouton and synapse (SS).  $\times 25,080$ .
- 29 Large Purkinje cell dendrite (PCD) with three climbing fiber boutons having attachment plaques (P) with its smooth surface and conspicuous, asymmetrical synapses with its evident (TH) and presumed thorns (TH?).  $\times 25,080$ .



**PLATE 11**

**EXPLANATION OF FIGURES**

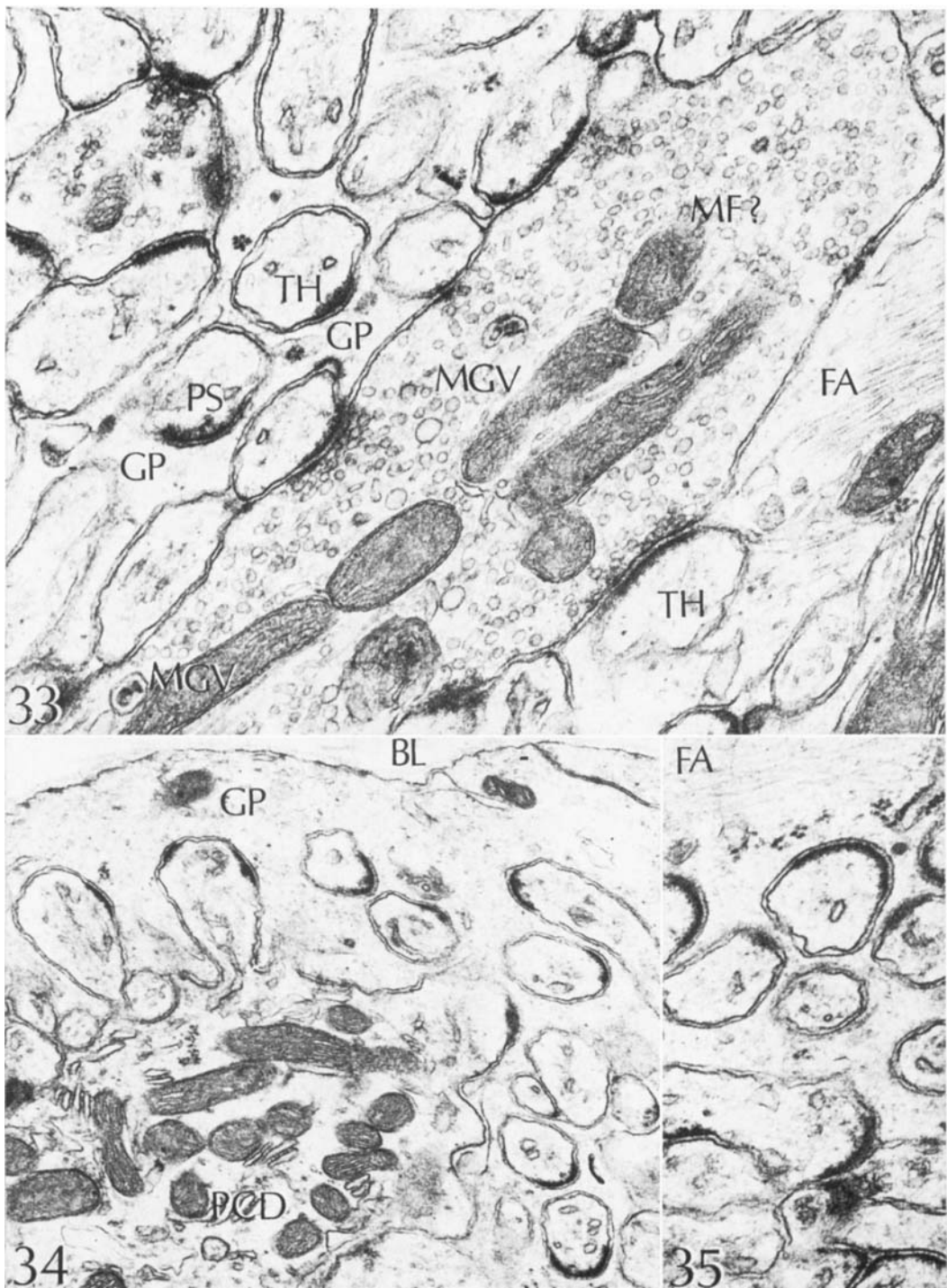
- 30** Large Purkinje cell dendrite (PCD) with dendritic thorns issuing from it (some indicated by arrows) and surrounded by a mass of such thorns. These dendritic outgrowths constitute a substantial part of the tissue of the cerebellar cortex in these irradiated animals.  $\times 16,188$ .
- 31-32** Synapses formed by the dendritic thorns (TH). The synapses are always of the conspicuous asymmetrical kind, that is, there is a rich subsynaptic web in the thorn.  $\times 41,040$ .



## PLATE 12

### EXPLANATION OF FIGURES

- 33 The presence of apparently multiplying granular vesicles (MGV) suggests (Gray, '61; Altman, '71) that this may be a mossy fiber rosette (MF?). The presumed Purkinje cell dendritic thorns (TH), in addition to establishing synapses with boutons also form pseudo-synapses (PS) with glial processes (GP). Typically, there is only a postsynaptic membrane, with the characteristic thick subsynaptic web of Purkinje cell thorns and spines. FA, fibrous astrocytic process.  $\times 41,040$ .
- 34 The thorns of a Purkinje cell dendrite (PCD) form pseudo-synapses near the surface of the cortex (BL, basal lamina) with a glial process (GP) which is presumably the endfoot of a Bergmann astrocyte.  $\times 25,080$ .
- 35 Dendritic thorns forming pseudosynapses with fibrous astrocytic processes (FA).  $\times 25,080$ .

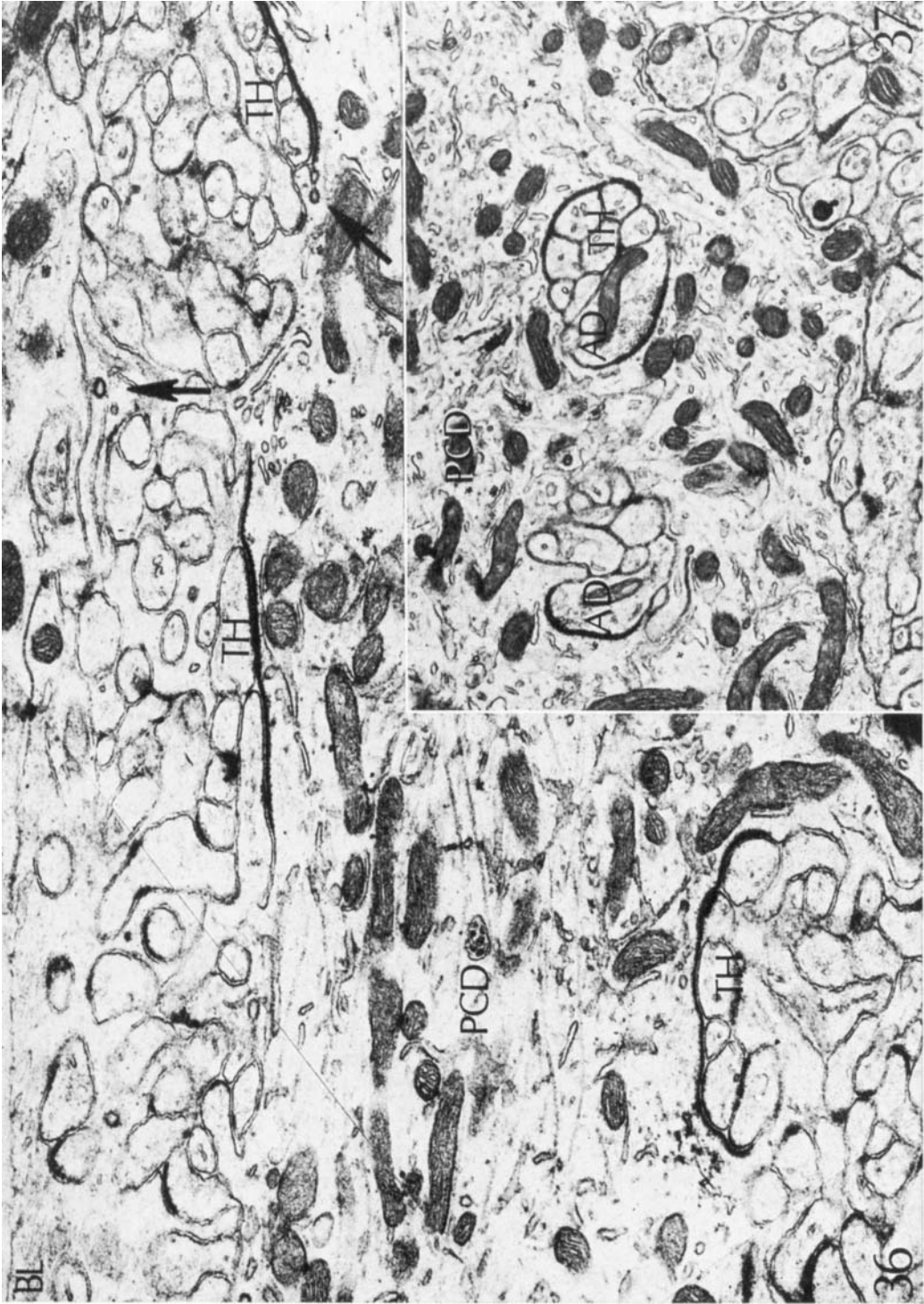


## PLATE 13

## EXPLANATION OF FIGURES

- 36 Large Purkinje cell dendrite (PCD) oriented parallel to the surface of the cortex (BL, basal lamina). Presumed dendritic thorns (TH) of other or same Purkinje cell form dendro-dendritic "synapses" on the smooth surface of the dendritic trunk. Vesicles are lacking in the dendritic thorns but there is a presynaptic thickening. These abnormal synapses tend to be quite long and are often associated with coated vesicles (arrows).  $\times 25,080$ .
- 37 Large Purkinje cell dendrite (PCD) with dendritic thorns (TH) and axodendritic terminals (AD) penetrating and forming synapses in its interior.  $\times 16,188$ .

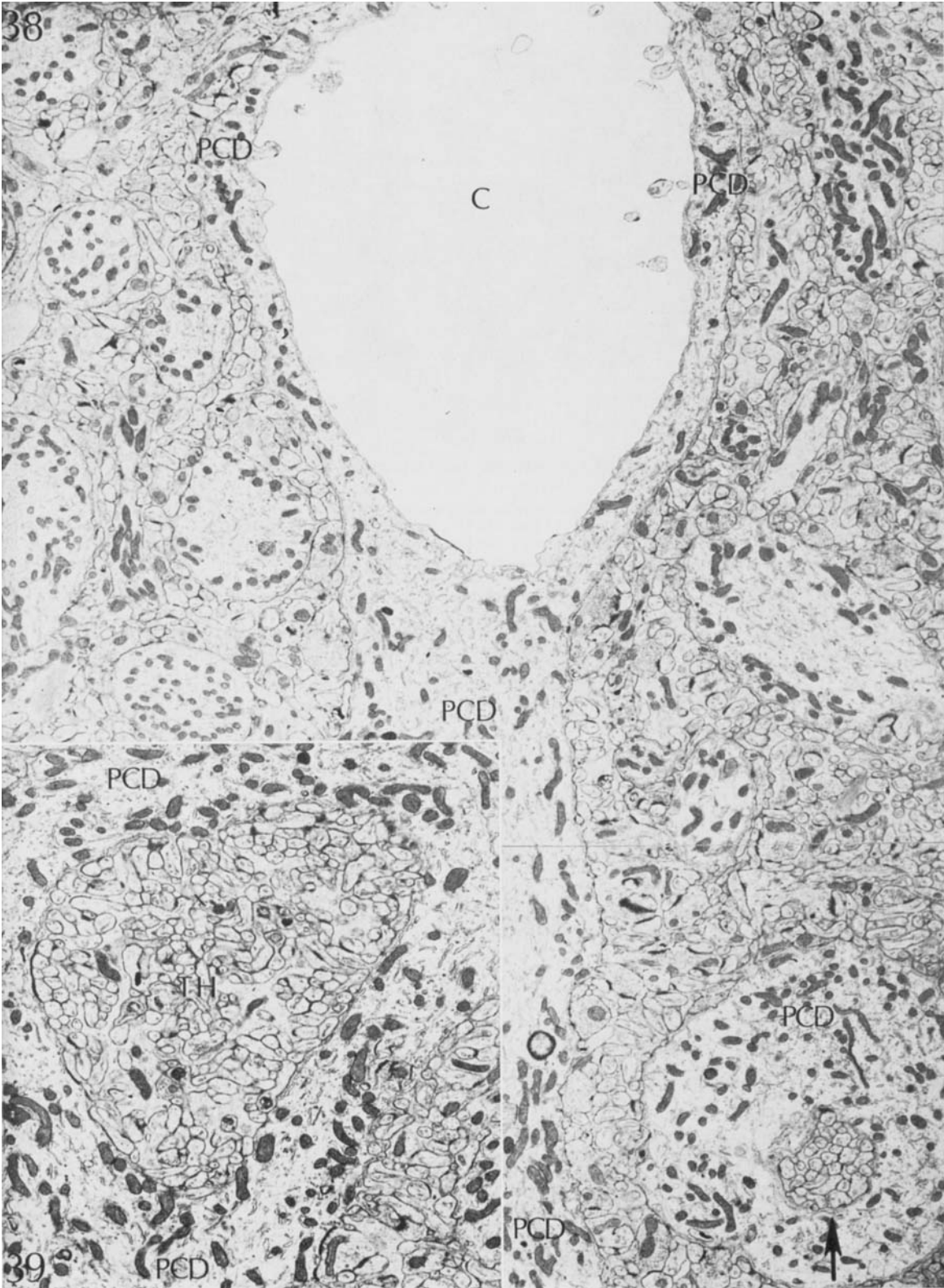




## PLATE 14

### EXPLANATION OF FIGURES

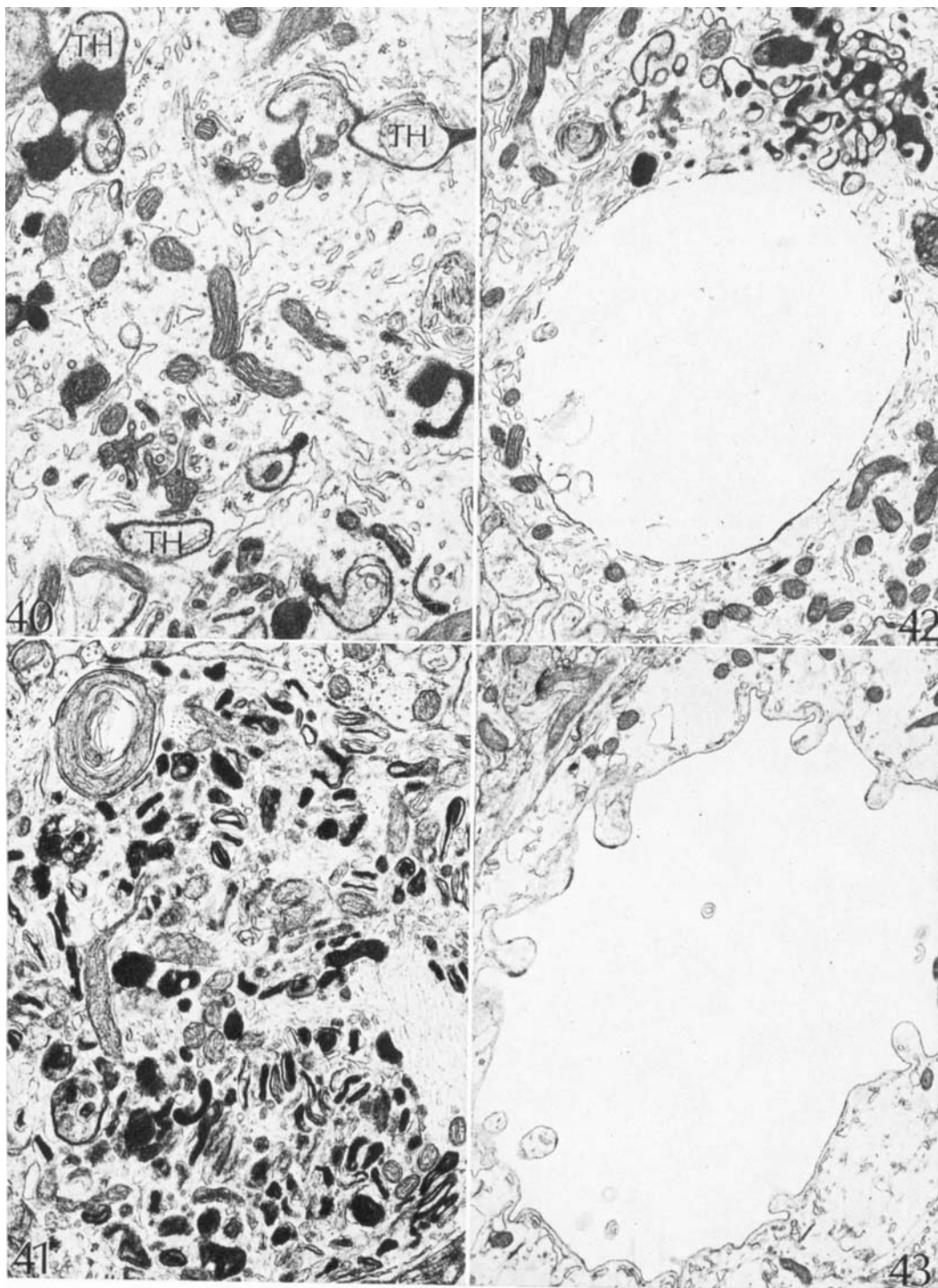
- 38 A large Purkinje cell dendrite (PCD) near the surface of the cortex with a club-like ending and a cavity (C) in its interior. Another Purkinje cell dendrite in this micrograph has presumed dendritic thorns in its interior (lower right corner, arrow).  $\times 7,752$ .
- 39 Another Purkinje cell dendrite (PCD) with club ending. The interior of this is occupied by presumed dendritic thorns (TH) of Purkinje cells.  $\times 7,752$ .



## PLATE 15

### EXPLANATION OF FIGURES

- 40-43 This series of micrographs suggests the possible sequence in the autolysis of Purkinje cell dendrites. The expansion of subsynaptic densities associated with dendritic thorns (TH) may be seen in figure 40. The degenerative process may then extend to other organelles, as indicated by the darkened mitochondria in figure 41. The degenerated organelles lead to gradual autolysis of the interior of dendrite, as indicated by figure 42 and the cavity enlarges until all the degenerated material is eliminated (fig. 43). Figures 40-41,  $\times 25,080$ ; figure 42,  $\times 16,188$ ; figure 43,  $\times 13,680$ .



## PLATE 16

### EXPLANATION OF FIGURES

- 44 Surface of the cerebellar cortex (BL, basal lamina) with large Purkinje cell dendrites (PCD) oriented parallel to the surface in the coronal plane, with glial processes, including fibrous astrocytes (FA) adjacent to the surface. A large mossy fiber rosette (MF) is seen forming synapses with processes interpreted to be Purkinje cell dendritic thorns (TH).  $\times 25,080$ .
- 45 Mossy fiber rosettes (MF) in high concentration in the deeper portion of the cerebellar cortex. The identity of the processes with which they form synapses (possibly Golgi cell dendrites) could not be established. PC, perikaryon of Purkinje cell.  $\times 16,188$ .

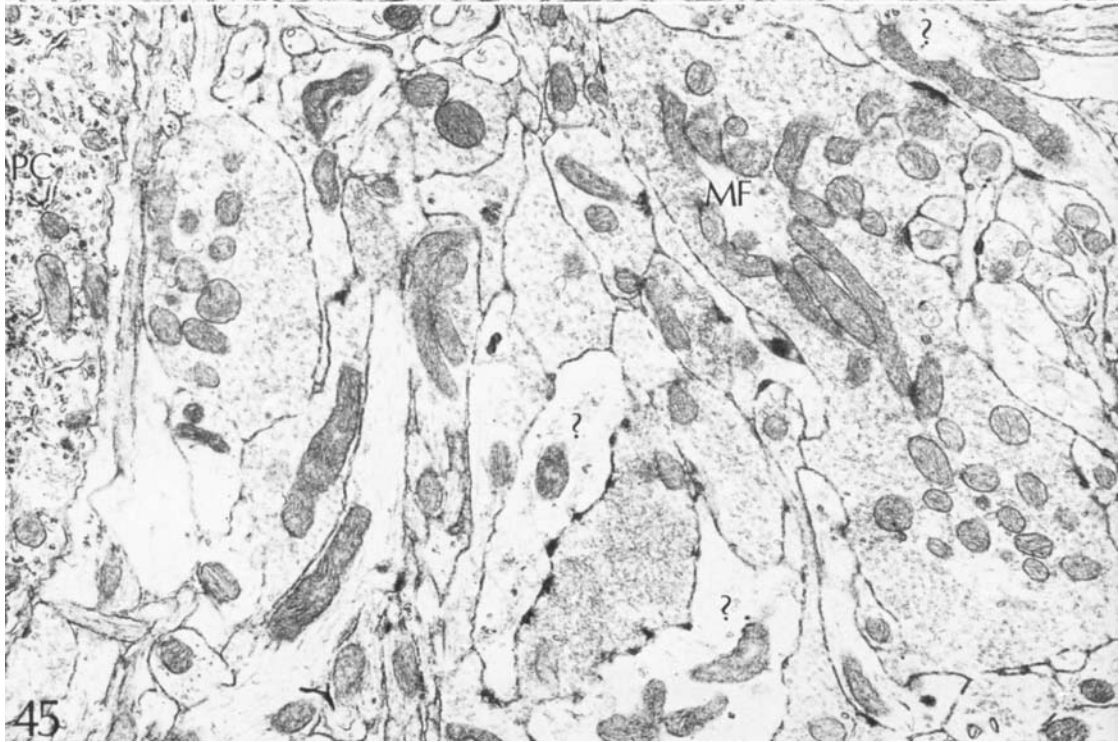
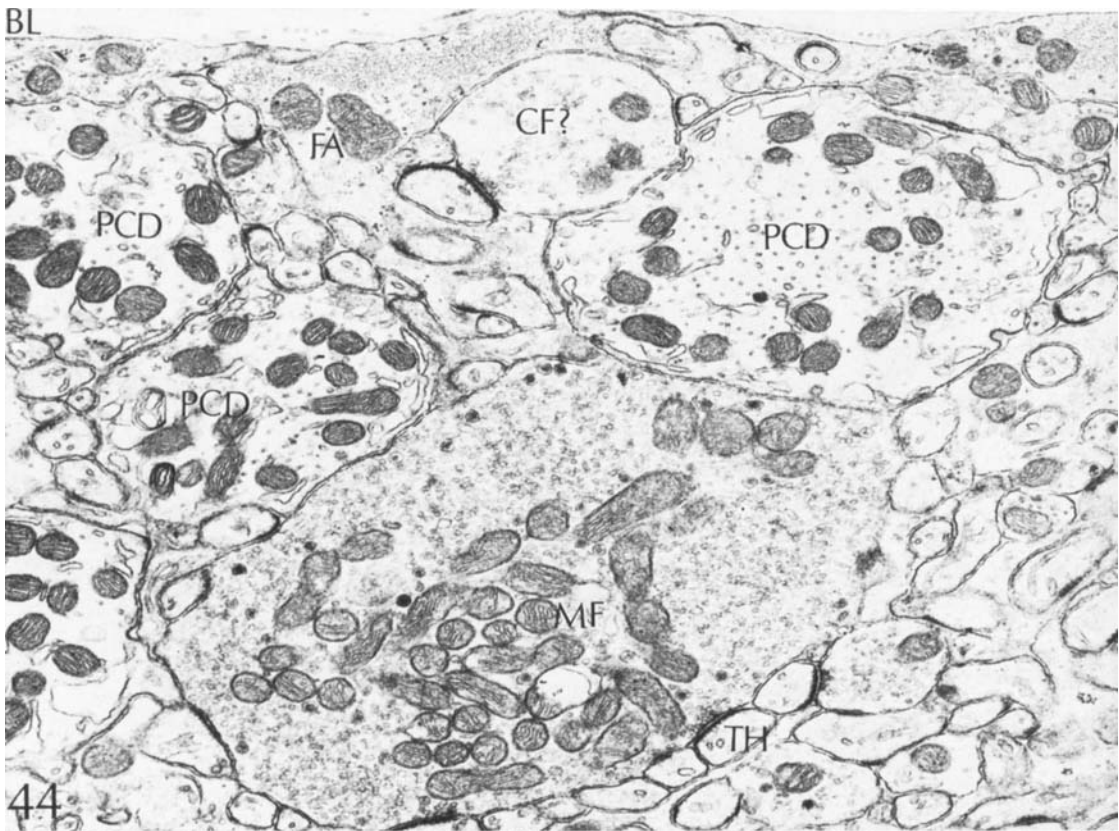
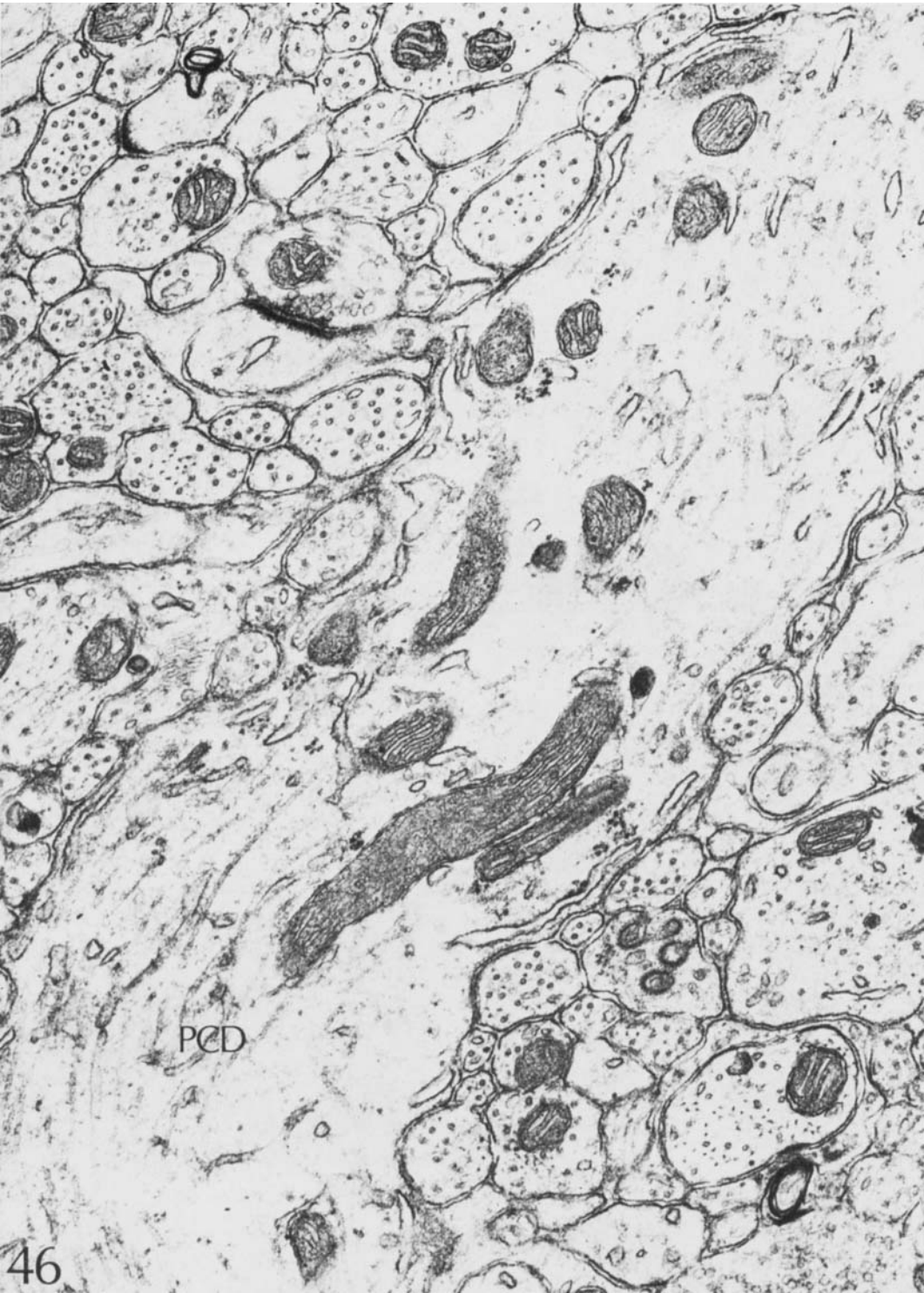


PLATE 17

EXPLANATION OF FIGURE

- 46 A Purkinje cell dendrite (PCD) surrounded by parallel fibers. These parallel fibers are rare in rats whose cerebellum was irradiated from birth onward and are peculiar in having a higher than normal concentration of microtubules. They also contain neurofilaments, which are not seen in parallel fibers in normal animals.  $\times 41,040$ .





**PLATE 18**

**EXPLANATION OF FIGURE**

- 47 The medullary layer in pyramis of an irradiated rat. Note the high portion of unmyelinated axons and the very thin myelin sheath of others.  $\times 9,120$ .

