

THE VISUALIZATION OF CONCAVALIN A BINDING SITES IN PURKINJE CELL SOMATA AND DENDRITES OF RAT CEREBELLUM

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ABSTRACT

The localization of concanavalin A (Con A) binding sites in Purkinje cell somata and dendrites has been studied using a peroxidase labeling technique. In the somata, the nuclear, Golgi, and endoplasmic reticulum (ER) membranes are rich in Con A binding sites. The hypolemmal cisternae, which are continuous with the ER from the soma and throughout the dendritic tree of Purkinje cells, are also rich in Con A binding sites. Other cisternae seen in these dendrites do not bind detectable amounts of Con A. The results suggest that a cisternal system, rich in carbohydrate, may be continuous from the nuclear envelope to distal dendritic segments of Purkinje cells. Such a system could play a role in the movement of materials from Purkinje somata to dendrites.

The remarkable specificity of concanavalin A (Con A) in binding primarily α -D-mannose or α -D-glucose sugar residues (1, 14) is the basis for cytochemical stains using Con A coupled to ferritin (12) or horseradish peroxidase (3) to visualize the surface topography of sugars on cell surfaces. This "staining" may also be used to study the localization of specific sugar residues in the macromolecular constituents of organelles within the cell. In this paper we examine the binding of Con A to structures within the Purkinje cell of rat cerebellum using a Con A-peroxidase-labeling technique (2). Evidence is presented that elements of the endoplasmic reticulum (ER) are selectively rich in Con A binding sites, particularly the "hypolemmal cisternal system" which extends from the Purkinje cell soma to the distal portions of the dendritic tree (6, 13).

MATERIALS AND METHODS

Concanavalin A (Calbiochem, Grade A, San Diego, Calif.), horseradish peroxidase (Sigma Chemical Co., Type VI, St. Louis Mo.), glucose oxidase (Sigma Type II), α -methyl-D-mannoside (Sigma no. M-3752), α -D-glucose (Eastman Kodak, Co., Rochester, N. Y.), and 3,3'-diaminobenzidine (Sigma no. D-8126) were all used in the cytochemical procedures. Glutaraldehyde (8%) was purchased from Polysciences, Inc. (Warrington, Pa.).

Preparation of Tissue

Adult Sprague-Dawley rats (200–250 g) were anesthetized by intraperitoneal injection of 35% chloral hydrate (1 ml/kg body weight) and then perfused through the heart for 20 min with a fixative (10) containing freshly prepared 4% paraformaldehyde and 0.1% glutaraldehyde in 0.12 M Millonig's phosphate buffer, pH 7.2 (11), with 0.2% CaCl_2 added. After perfusion the cerebellum was removed and stored overnight at 4°C in 4% phos-

phate-buffered paraformaldehyde without glutaraldehyde. The fixed cerebellum was cut parasagittally into 75- μ m slices with a Sorvall TC-2 tissue sectioner (Ivan Sorvall, Inc., Newton, Conn.). The slices were collected in 0.12 M Millonig's buffer, and the best examples were selected under the dissecting microscope for cytochemical study.

Cytochemical Procedures

The method of Con A-peroxidase-labeling of carbohydrates was essentially as described by Bernhard and Avrameas (2). All steps were performed with constant gentle agitation at room temperature. The 75- μ m cerebellar slices were washed for 15 min in phosphate-buffered saline (PBS), pH 7.2. The slices were transferred to a solution of Con A (1 mg/ml) in PBS and incubated for 45 min. They were washed in 3 \times 30-min changes in PBS and placed in a solution of horseradish peroxidase (0.1 mg/ml) in PBS for 45 min followed by 3 \times 30-min washes in PBS. The slices were next placed in a solution containing 50 mg 3,3'-diaminobenzidine (DAB), 20 μ g glucose oxidase, and 0.045 g glucose (7) in 100 ml of PBS. The glucose-glucose oxidase generated the H₂O₂ needed in the peroxidase-DAB reaction (7). After 15 min, the slices were removed from this solution and washed for 30 min in PBS. The cerebellar folia were dissected into separate pieces before postfixation with 2% OsO₄ in 0.12 Millonig's buffer, pH 7.2. The tissue was dehydrated through an ethanol series and propylene oxide and flat embedded in Epon-Araldite. Ultrathin sections were cut parallel to the parasagittal face with a diamond knife, and superficial sections were examined in an Hitachi II B electron microscope both with and without prior staining in lead citrate (17) and uranyl acetate. Control sections were incubated in PBS alone followed by horseradish peroxidase or were incubated with Con A (1 mg/ml) in the presence of 0.2 M α -methyl-D-mannoside, an inhibitor of specific Con A binding to sugars (cf. 15). Some of the 75- μ m slices were processed for light microscopy in the following manner. After the cytochemical procedures, the slices were treated for 30 s with a solution containing 0.04% OsO₄ in PBS followed by dehydration through an ethanol and xylene series and were mounted on glass slides.

RESULTS

Light microscopy of the 75- μ m slices treated with Con A-peroxidase shows reaction product around the nuclear border and within the cytoplasm of cerebellar Purkinje cells (Fig. 3). The cytoplasmic product seen at the periphery of the cell body continues into the distal portions of the dendritic tree (Fig. 3). This reaction is strongly inhibited by α -methyl-D-mannoside (Fig. 2) and is absent in those sections treated with horseradish peroxidase but not Con A (Fig. 1).

In the electron microscope preparations, cerebellar slices treated with Con A-peroxidase show electron-opaque product on the membranes of several organelles in the Purkinje cell somata (Figs. 4-7). The nuclear envelope is stained but the nuclear pores are not (Figs. 4, 6, and 7). Con A-positive cisternal profiles are seen to be continuous with the nuclear envelope (Figs. 4 and 6), and these cisternae may connect the perinuclear space with the ER (9). Both rough and smooth ER are stained on the cisternal side of the membrane throughout the cell body (Figs. 4, 5, and 7). At the periphery of the cell, the hypolemmal cisternae (13) are seen as discontinuous, stained profiles aligned with the plasma membrane, and these profiles are often continuous with the ER located in deeper portions of the somata (Fig. 4). The Golgi apparatus is usually stained with Con A-peroxidase (Figs. 4 and 7), whereas mitochondrial membranes are not (Figs. 4 and 7). Con A-peroxidase staining in the neuropil is seen mostly on plasma membranes (Figs. 4, 8, and 9). The luminal wall of blood vessels is strongly Con A-positive (Fig. 4).

When dendritic profiles are traced from the region of Purkinje somata to the molecular layer, the stained hypolemmal cisternae may be followed for relatively long distances (Figs. 8-10; also see reference 12). The localization of Con A reaction product within these cisternae is seen best in higher magnification micrographs (Fig. 10). The cisternae aligned beneath the dendritic plasma membrane are occasionally interrupted, presumably as they pass in and out of the plane of section (Figs. 8-10). Electron-opaque reaction product appears to be located preferentially on the membrane facing the cisternal space (Fig. 10). Large dendritic profiles in the Purkinje and molecular layers seen in transverse or oblique section also reveal stained hypolemmal cisternae (Figs. 4, 8, and 9). In some dendritic profiles, short segments of the hypolemmal cisternae are not stained (Figs. 8 and 9). This is probably due to failure of Con A or peroxidase to penetrate the slice evenly, since the relative incidence of such unstained areas increases as the block is sectioned deeper. Smooth ER profiles other than the hypolemmal cisternae are seen frequently in the Purkinje cell dendrites (Figs. 8 and 9), but these profiles are never observed to be stained with Con A-peroxidase except at those proximal dendritic portions which are continuous with the soma (Fig. 4).

Electron microscope examination of control tissues shows that the binding of Con A to

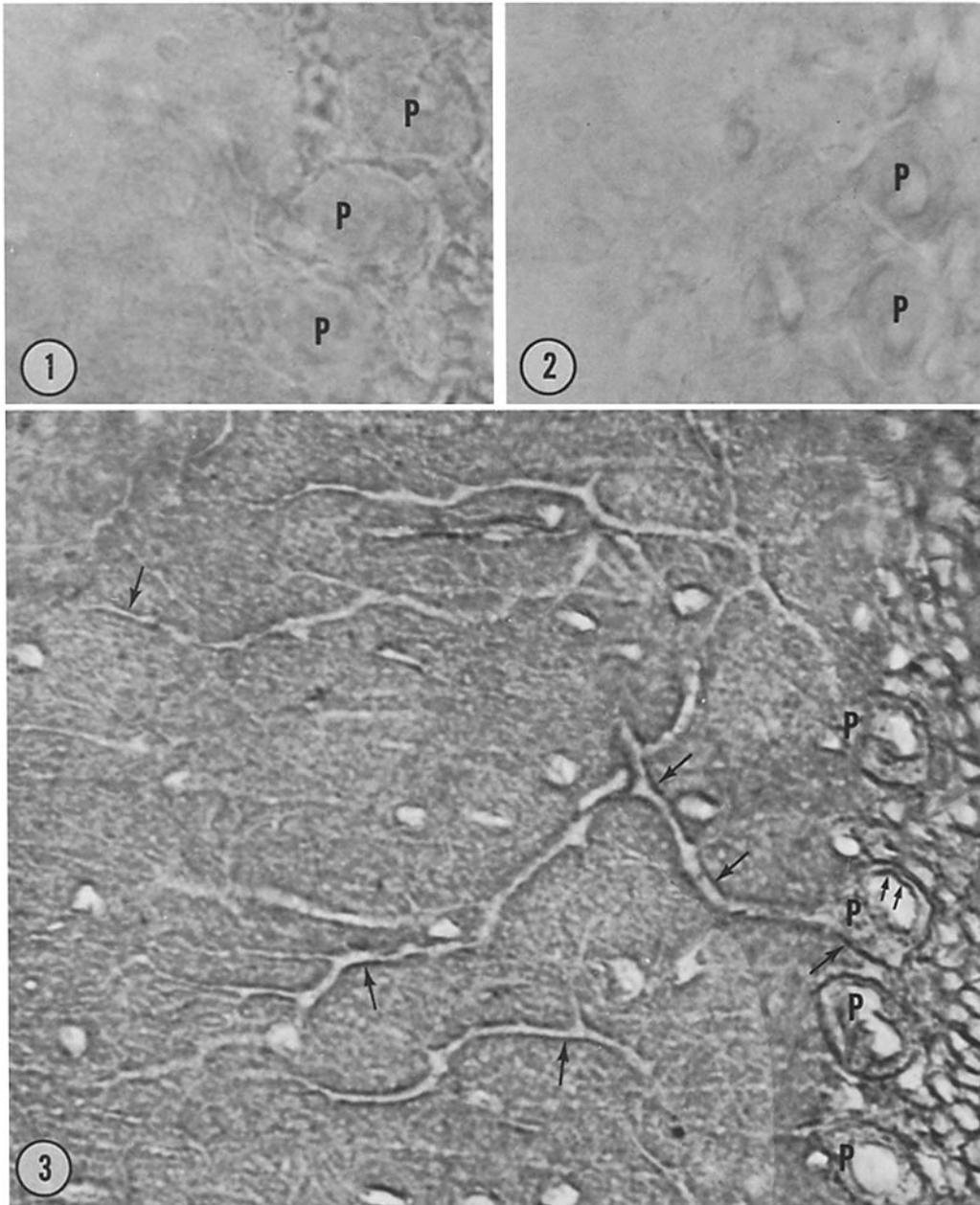


FIGURE 1 Light micrograph of a 75- μm slice of control tissue treated with horseradish peroxidase without prior incubation in Con A. No specific staining is seen. *P* = Purkinje cell. $\times 750$.

FIGURE 2 Light micrograph of a 75- μm slice of control tissue treated with Con A in the presence of 0.2 M α -methyl-mannoside. Only very light staining is seen. *P* = Purkinje cell. $\times 750$.

FIGURE 3 Light micrograph montage of portion of a 75- μm cerebellar slice treated with Con A-peroxidase (see Materials and Methods). Specific staining in Purkinje cells (*P*) is seen around the nuclear border (small arrows) and around the periphery (arrow) of the cell. This peripheral staining extends throughout the dendritic tree (arrows). $\times 750$.

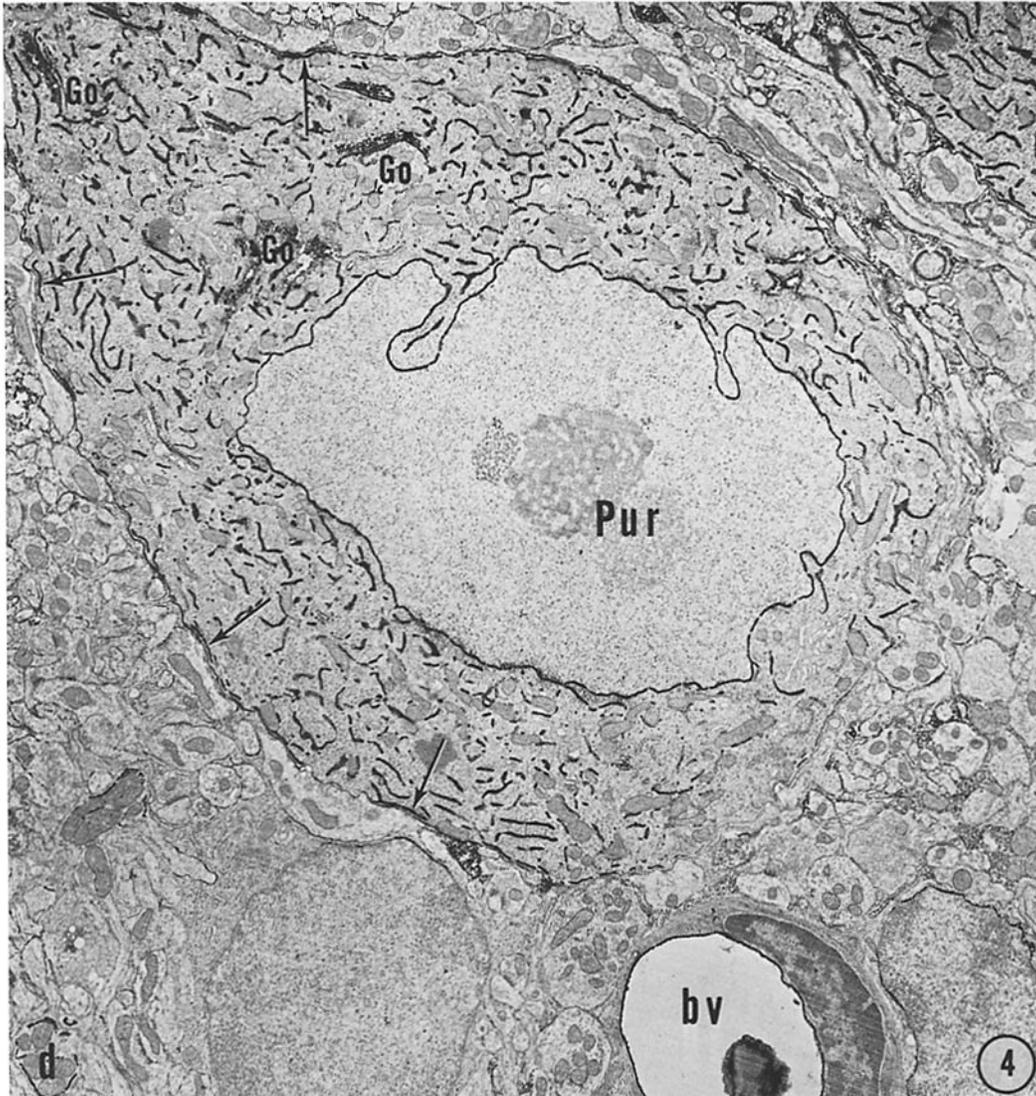


FIGURE 4 Electron micrograph of one Purkinje cell (*Pur*) soma and portion of another (upper right hand corner) treated with Con A-peroxidase. The nuclear envelope, Golgi (*Go*) and ER membranes are stained. At the periphery of the cell the hypolemmal cisternae (arrows) adjacent to the plasma membrane are stained. Stained cisternal profiles leave the nuclear envelope and approach the ER cisternae. At the cell periphery, cisternae of the ER merge with the hypolemmal cisternae. Cross sections of some dendrites (*d*) in the field show stained hypolemmal cisternae. The luminal wall of a blood vessel (*bv*) is stained. $\times 6,300$.

Purkinje cells are nearly absent in the presence of α -methyl-D-mannoside, and no specific staining is observed when the tissue is treated with peroxidase but not Con A (Fig. 11).

DISCUSSION

The results presented in this paper show that the nuclear, Golgi, and ER membranes in Purkinje

cell somata are rich in Con A binding sites. Hirano et al. (5) have shown that fragments of ER in a myeloma cell homogenate bind a Con A-ferritin conjugate on the membrane face away from attached ribosomes. Our results also suggest that the Con A binding sites are located on the cisternal rather than the cytoplasmic side of the ER. If the macromolecules being stained are glycoproteins,

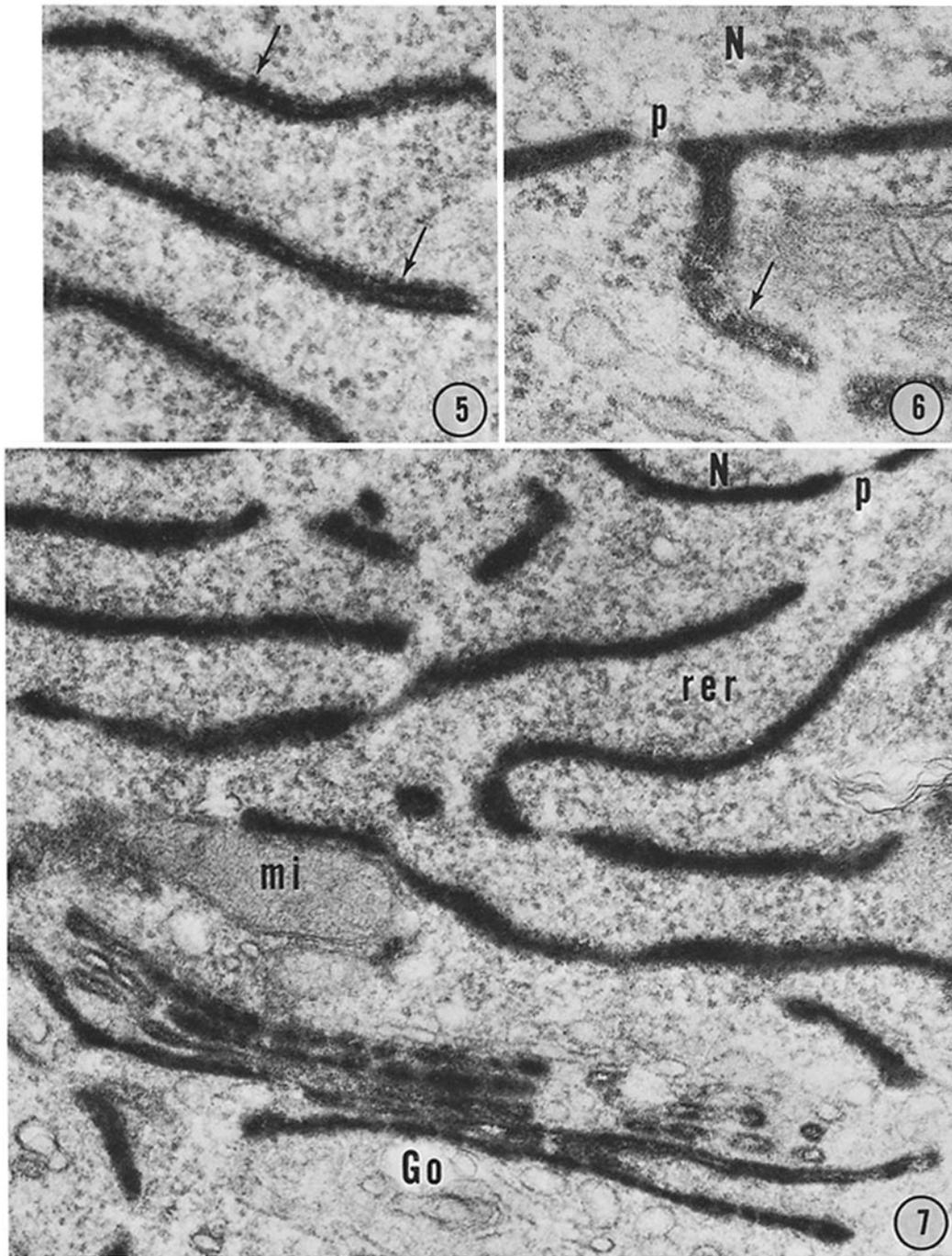


FIGURE 5 An electron micrograph showing Purkinje cell ER profiles stained on the side of the membranes toward the cisternal space and away from the side with attached ribosomes (arrows). $\times 71,400$.

FIGURE 6 Electron micrograph showing label in the perinuclear space and a cisternal profile (arrow) continuous with the nuclear envelope. The nuclear pore (*p*) is not stained. *N* = nucleus. $\times 137,800$.

FIGURE 7 Electron micrograph showing label in the perinuclear space, Golgi (*Go*), and ER (*rer*) membranes. *N* = nucleus, *p* = nuclear pore, *mi* = mitochondrion. $\times 63,600$.

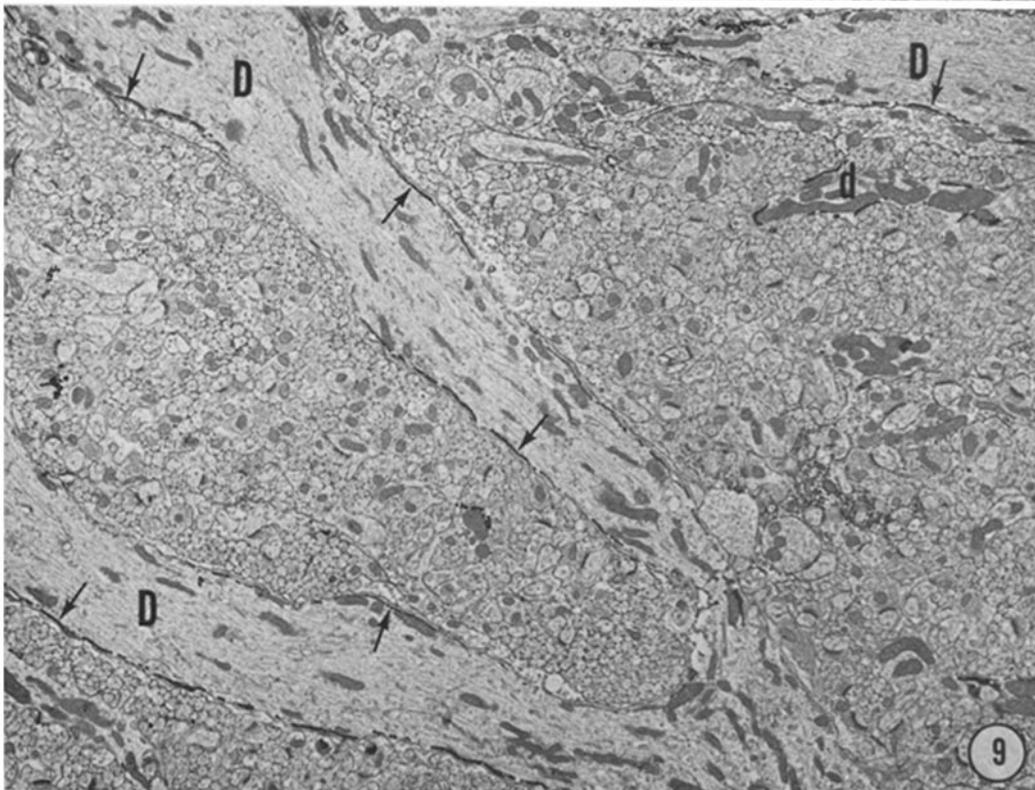
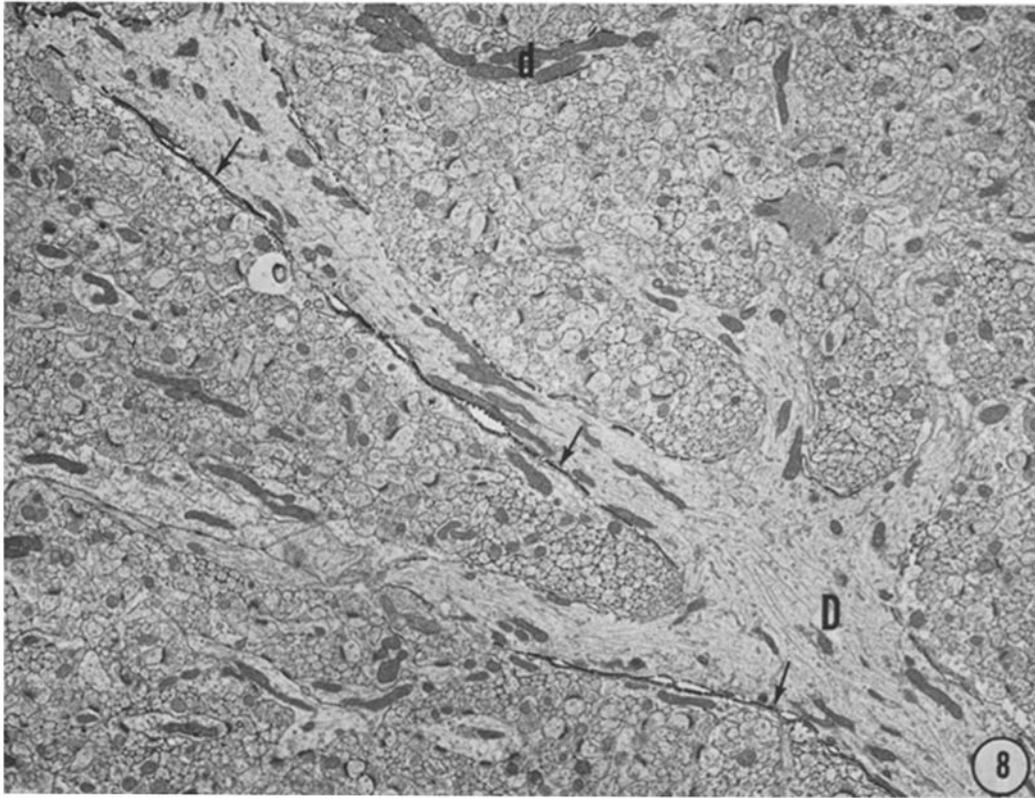


FIGURE 8 Electron micrograph showing longitudinal (*D*) and oblique (*d*) sections of Purkinje cell dendrites in the molecular layer of cerebellar cortex. The hypolemmal cisternae (arrows) are stained; other smooth ER profiles in the dendrite are not stained. $\times 5,400$.

FIGURE 9 Another field in the molecular layer showing large (*D*) and small (*d*) dendritic profiles with stained hypolemmal cisternae (arrow). $\times 5,400$.

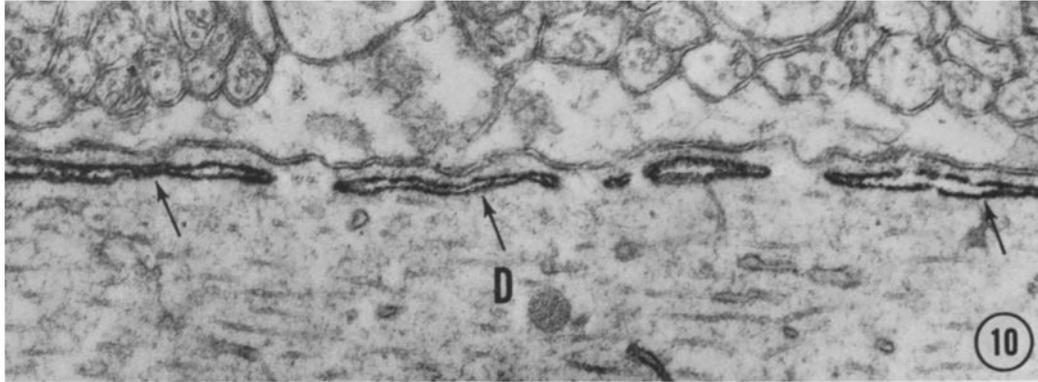


FIGURE 10 Higher magnification of the stained hypolemmal cisternae (arrows) in a Purkinje cell dendrite (D). $\times 39,700$.

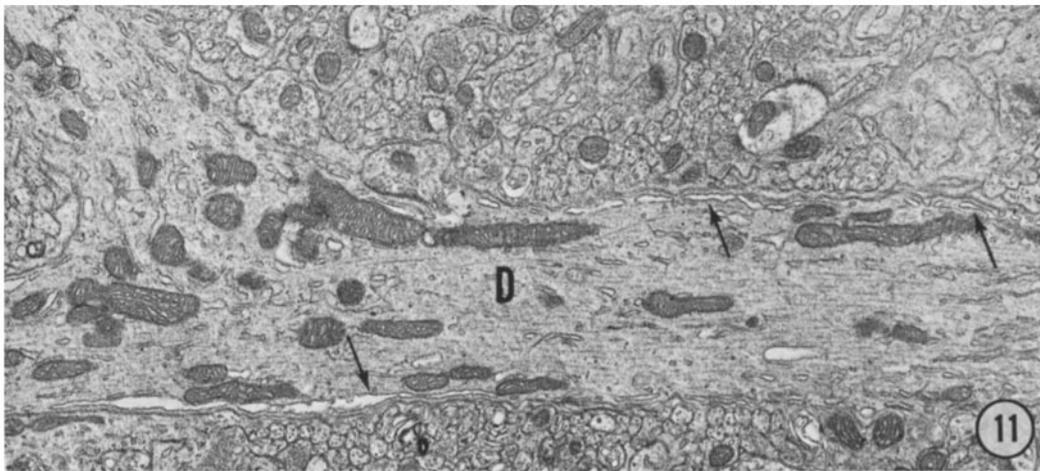


FIGURE 11 Electron micrograph of a Purkinje cell dendrite (D) of control tissue treated with peroxidase, but not Con A. The hypolemmal cisternae (arrows) are not labeled. $\times 10,900$.

then the intensity of staining suggests that the membranes of the ER are relatively rich in mannose- or glucose-containing "glycoproteins," or that the mannose or glucose in these glycoproteins is in a particularly favorable position in the oligosaccharide chain to bind Con A.

The nuclear envelope and perikaryal ER of cell types in the cerebellum other than Purkinje cells are stained by this method, but only in an irregular manner. While this might reflect differences in the chemical composition of the ER of these cell types, we believe that the Con A or peroxidase itself penetrates the larger Purkinje cells more easily than the smaller basket, stellate, granule, and neuroglial cells because the Purkinje cells are more

likely to be sliced open by the tissue chopper. The uneven staining of the plasma membrane of Purkinje cells may be due also to uneven penetration of the Con A or peroxidase into the neuropil.

Con A-peroxidase-stained profiles of the ER cisternae in Purkinje cell somata merge into the hypolemmal cisternae which continue to be stained throughout most of the extensive dendritic tree. In general, the perinuclear space, the rough and smooth ER, and the Golgi complex of eucaryotic cells are thought to be in continuity with each other (9), and, at least in the Purkinje cell, this probable continuity would appear to extend great distances from the cell body via the hypolemmal system of cisternae (13; see Results).

It has been noted (6) that a system such as the hypolemmal cisternae could play a role in the process by which materials synthesized in the perikarya of neurons are transported to distal regions of their processes. Most of the interest in this area has focused on the movement of macromolecules from the soma to axon terminals (cf. 8); however, there is as yet little direct evidence that this transport takes place via one or another of the various organelles seen in axon profiles. One fine structural study (16) shows that the perikaryal ER and a reticulum in the axons of cultured chick embryonic neurons contain several common enzyme activities. Another cytochemical study (4) shows the enzyme thiamine pyrophosphatase within the Golgi and ER elements of neuronal cell bodies in the pontine reticular formation. This enzyme is also seen in smooth ER profiles in axons coming from these cell bodies, as well as in synaptic vesicles in the terminals. The suggestion is made that the enzyme may be synthesized in the cell body and transported within a smooth ER system in the axon which may give rise to vesicles in the terminal (4). Similar in concept to this interpretation, the present results suggest that the hypolemmal cisternae are probably part of a specialized cisternal system which is continuous from the Purkinje cell body throughout the dendritic tree.

We assume in this discussion that the Con A is staining glycoproteins that are structural components of the cisternal membranes. This assumption is attractive because glycoproteins are thought to be involved in the "recognition" of molecules, primarily on cell surface membranes (cf. 18). A similar recognition capacity on cisternal membranes could facilitate the selection of which molecules synthesized in a cell body would be moved along the hypolemmal cisternae into the dendrites. A second reason why glycoproteins would be appropriate molecular species lining a transport system is that, because of their anionic residues (cf. 18), they could serve as a cationic exchange "column", thus providing a mechanism for transport. At least one other possible explanation for the Con A staining of these cisternae which must be considered is that the glycoproteins being stained are themselves being moved through the cisternae. Although the stain is usually intimately associated with the cisternal membrane in the Purkinje dendrites, there is some buildup of stain in the cisternal space (Fig. 10). If this is not due to an artifactual enhancement of the reaction of

peroxidase on DAB, it might represent Con A binding sites on macromolecules within the cisternal space. The presence of such a pool of glycoproteins in the hypolemmal cisternae could serve as a reservoir for renewal of plasma membrane glycoproteins. The close association of the cisternae with the plasmalemma would greatly facilitate such an exchange.

Although the suggestions made in this discussion and in previous work (6) await proof, we believe them to be reasonable and likely hypotheses regarding a possible function of the hypolemmal cisternae. It will be of interest to see if the staining procedures used in this paper might detect other forms of a continuous system in the dendrites and axons of other neurons.

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