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MEMBRANE STRUCTURE IN MAMMALIAN ASTROCYTES: A REVIEW OF FREEZE-FRACTURE STUDIES ON ADULT, DEVELOPING, REACTIVE AND CULTURED ASTROCYTES

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SUMMARY

The application of freeze-fracture techniques to studies of brain structure has led to the recognition of two unsuspected specializations of membrane structure, each distributed in a specific pattern across the surface of astrocytes. 'Assemblies' (aggregates of uniform, small particles packed in orthogonal array into rectangular or square aggregates) are found to characterize astrocytic plasma membranes apposed to blood vessels or to the cerebrospinal fluid at the surface of the brain. These particle aggregates are much less densely packed in astrocytic processes in brain parenchyma. Assemblies are not fixation artifacts, have been shown to extend to the true outer surface of the membrane, are remarkably labile in the setting of anoxia, and are at least in part protein. The function of assemblies is unknown, but their positioning suggests that they may have a role in the transport of some material into or out of the blood and cerebrospinal fluid compartments. A second specialization of intramembrane particle distribution, the polygonal particle junction, links astrocytic processes at the surface of the brain, and also links proximal, large caliber astrocytic processes in brain parenchyma. The function of this membrane specialization also is unknown, but it may subserve a mechanical role.

I. INTRODUCTION

Light-microscopic studies utilizing various heavy metal impregnation techniques led to the early realization that the morphology of astrocytic processes varies with the location of the astrocyte in the brain (Ramon y Cajal, 1913, 1916; Rio-Hortega, 1919, 1921). 'Protoplasmic' astrocytes, located in nuclear masses or cerebral cortex, tend to have radially arrayed processes distributed in a roughly spherical volume. 'Fibrous' astrocytes in the white matter tend to have fewer processes distributed in

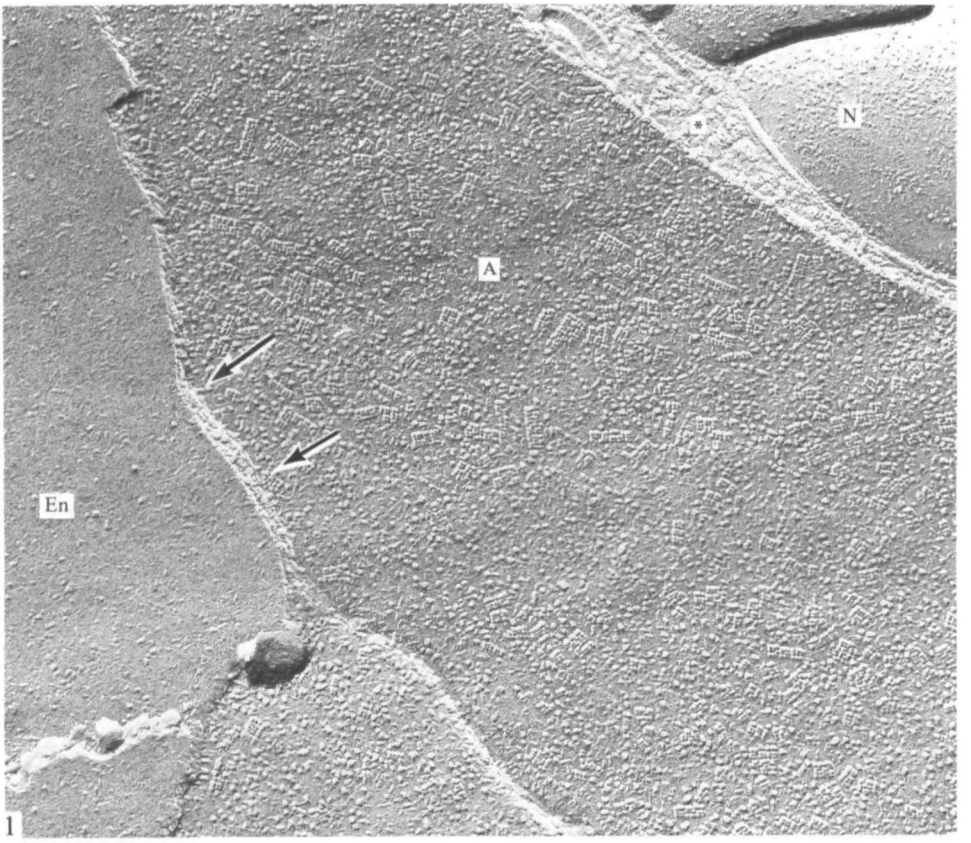
an ellipsoidal volume whose long axis parallels the course of adjacent myelinated axons (Kolliker, 1889–1902, and Andriezen, 1893, as cited by Ariens Kappers, Huber & Crosby, 1960). Electron-microscopic studies of thin-sectioned preparations were able to examine the distribution of very fine processes, too small for light-microscopic analysis, and established that astrocytic processes invest all blood vessels in brain parenchyma, form the surface of the brain (the glial limitans), and in consistent fashion surround certain neuronal elements. Astrocytes are linked by numerous gap junctions and desmosome-like puncta adhaerentia, but not tight junctions. The use of freeze-fracture techniques, which expose for high-resolution electron-microscopic study the internal organization of plasma membranes, has in recent years led to the recognition of two unsuspected membrane specializations unique to astrocytes in the central nervous system (Dermietzel, 1973, 1974; Landis & Reese, 1974; Nabeshima *et al.* 1975; Hanna, Hirano & Pappas, 1976; Brightman *et al.* 1978; Gulley, Landis & Reese, 1978; Anders & Brightman, 1979; Landis & Reese, 1981*a*). One of these specializations, which we have termed 'assemblies', is positioned at the interfaces between blood and brain and between cerebrospinal fluid and brain, and may be the morphological representation of a previously unappreciated astrocytic contribution to the maintenance of brain extracellular fluid composition.

II. GENERAL CHARACTERISTICS OF ASTROCYTES IN THIN-SECTIONED ELECTRON MICROSCOPIC PREPARATIONS

The cytological features of astrocytes as visualized in thin-sectioned preparations have been extensively described (Schultz, Maynard & Pease, 1957; Mugnaini & Walberg, 1964; Maxwell & Kruger, 1965; Wendell-Smith, Blunt & Baldwin, 1966; Palay, 1966; Vaughn & Peters, 1967; Peters, Palay & Webster, 1976). Most large astrocytic processes contain intermediate filaments, distinct from those present in neurones both on the basis of appearance and on the basis of antigenicity. Glycogen and lipofuscin deposits also are present in astrocytic cytoplasm. The most interesting

Fig. 1. Assemblies on the protoplasmic fracture face of two astrocytic processes investing a capillary in cerebellar cortex. The plane of fracture has exposed the extracellular half of the abluminal membrane of two endothelial cells (En), cross-fractured the extracellular space occupied by the basal lamina (arrows), exposed the protoplasmic fracture face of the luminal membrane of two astrocytic processes (A), cross-fractured the astrocytic cytoplasm and astrocytic abluminal membrane (asterisk) to reveal fracture faces of neuronal elements (N) in the cerebellar parenchyma. Scattered across the astrocytic membranes are numerous 'assemblies', rectangular or square aggregates of uniform, small (5–7 nM) particles packed in orthogonal array. The assemblies vary in the number of constituent particles, and have no consistent orientation with respect to one another. No assemblies are present in endothelial or neuronal membranes. (79000 ×.)

Fig. 2. Extracellular half of an astrocytic membrane apposed to a capillary. The protoplasmic half of the abluminal capillary endothelial cell membrane is present at the lower left. Arrows indicate the cross-fractured basal lamina. Assemblies in the astrocytic membranes are represented on the extracellular half of the membrane (A-E) by a subtle array of pits, complementary to the particle aggregates which remain associated with the protoplasmic half of the membrane. Here, the plane of fracture cross-fractured the astrocytic cytoplasm (asterisk) to expose the protoplasmic half of the abluminal membrane (A-P), where it faces elements of the cerebellar parenchyma. There is a striking difference between the densely packed assemblies in the portion of the astrocytic membrane facing the blood vessel (A-E) and the complete absence of the assemblies in the membrane of the same process where it faces neuronal elements (A-P). (79000 ×.)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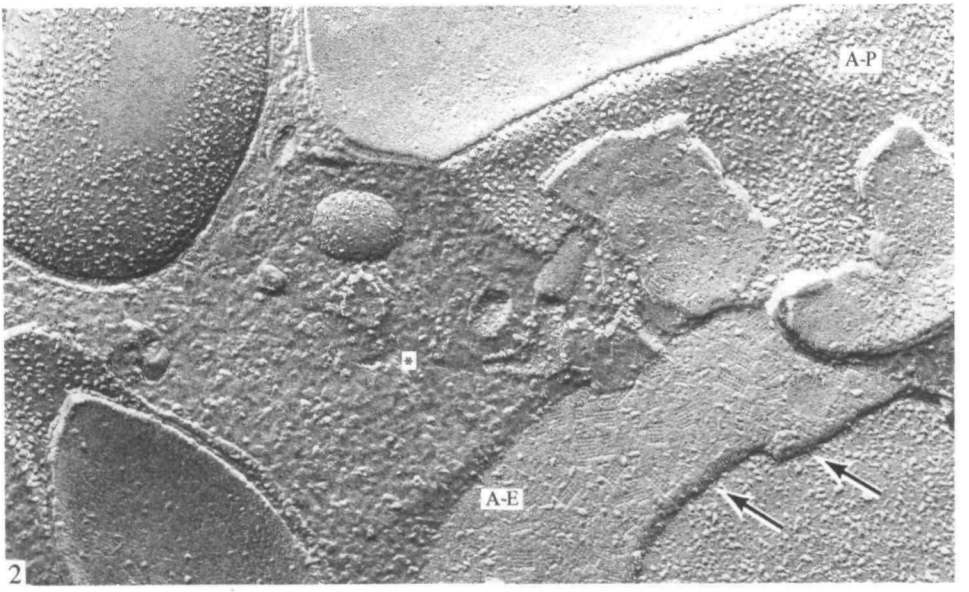
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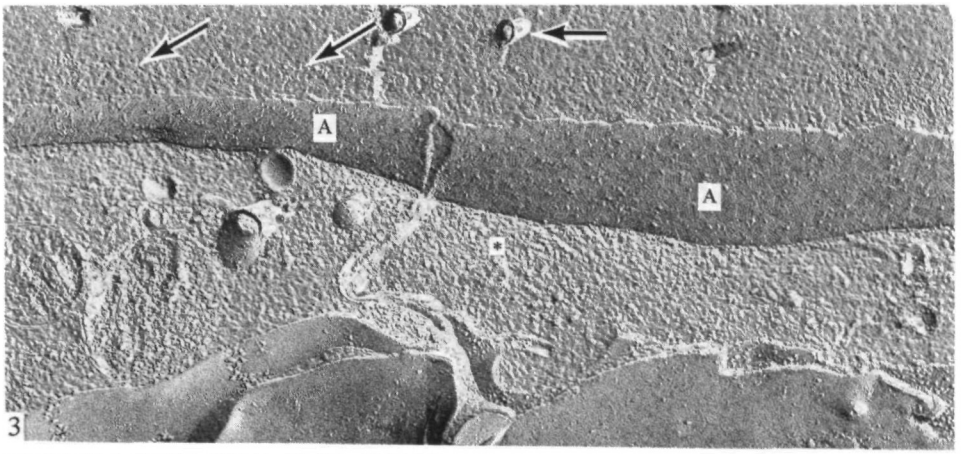

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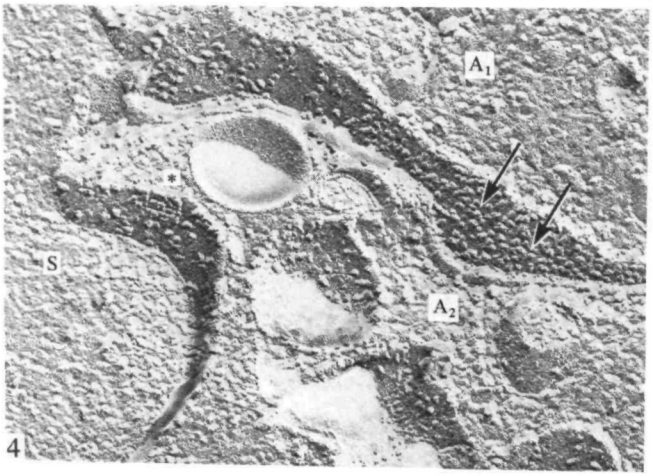

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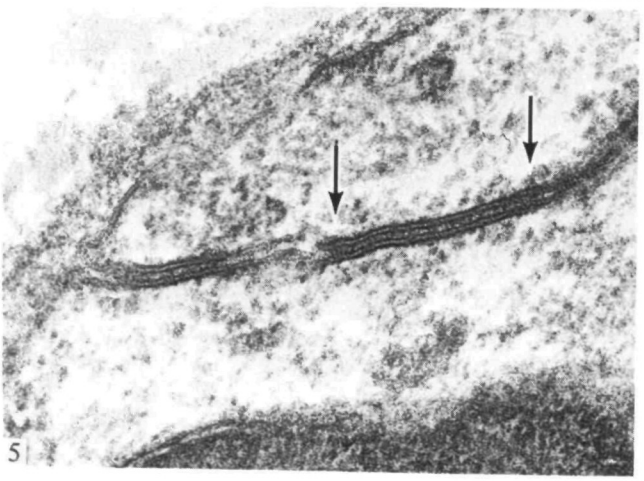

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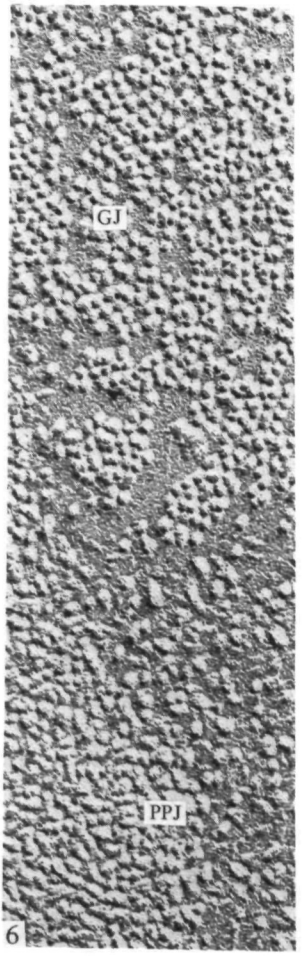

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feature of astrocytes examined with thin-section techniques is the extraordinary complexity of their distal processes. These processes do not randomly fill the space between neuronal elements, but are disposed around certain synapses and cell bodies. In the adult cerebellar cortex, for example, tenuous astrocytic processes entirely invest Purkinje cell bodies and dendritic arbors, extending to the finest branchlet spines contacting parallel fibre axons (Palay & Chan-Palay, 1974). Stellate cell bodies and dendrites frequently are positioned adjacent to large caliber Purkinje dendritic shafts, but are never completely invested. Occasionally one may observe a single parallel fibre axon bouton which forms synaptic junctions with a Purkinje cell spine and with a stellate cell dendrite; the contact with the spine will invariably be invested by astrocytic processes, while the stellate dendrite may not. Similarly, in the end bulbs of Held in the anteroventral cochlear nucleus there is a very characteristic pattern of astrocytic processes intercalated between the very large axonal bouton and the surface of the principal cell (Gulley *et al.* 1978). In general, as one examines cortical structures or nuclear masses, astrocytic processes are found in particular patterns specific to a given anatomical region.

While it may be useful to categorize astrocytic processes by the nature of the structure they invest, it is important to recognize that a single process may surround disparate structures. For example, one portion of a flattened astrocytic process may be adjacent to the basal lamina of a blood vessel, and the abluminal membrane of the same process may invest a neuronal structure. Astrocytic processes investing the surface of the cerebellar cortex frequently face blood vessels as they penetrate the cerebellar parenchyma from the subarachnoid space and also surround nearby parallel fibre-to-Purkinje spine synapses. Perhaps it is the fact that one process may be related to several different structures that led to the inaccurate perception that astrocytic processes simply filled space between neuronal elements and vascular structures. When, however, astrocytes are examined in freeze-fractured preparations they are found to have distinctly different membrane organization where they face various components of the brain parenchyma.

Fig. 3. Astrocytic processes comprising the glial limitans of cerebellar cortex. Collagen fibres (horizontal arrow) are present in the subarachnoid space and the basal lamina is evident as extremely subtle fibrils (oblique arrows). The plane of fracture has exposed the extracellular membrane half of two astrocytic processes (A) bordering the subarachnoid space, each marked by the imprints of many assemblies. The asterisk indicates the cross-fractured astrocytic cytoplasm. (58000 ×.)

Fig. 4. Two astrocytic processes (A_1 and A_2) of the cerebellar glial limitans, adjacent the subarachnoid space (S). The plane of fracture has exposed particles of a polygonal particle junction (arrows) on the protoplasmic membrane half of the upper astrocyte (A_1), where it is apposed to the lateral margin of a second astrocytic process (A_2). Note the assembly (below the asterisk) on the protoplasmic half of the membrane bordering the subarachnoid space. (108000 ×.)

Fig. 5. Polygonal particle junction, as visualized in a thin-section after tannic-acid mordant. In the region between the arrows, the membranes are parallel, separated by uniform space, and have electron-dense material interposed in the cleft. (108000 ×.)

Fig. 6. Particles of a gap junction (GJ) and polygonal particle junction (PPJ) on the protoplasmic membrane fracture face of a proximal, large-calibre process of a Bergmann astrocyte in the lower molecular layer. The gap junction particles appear hemispheric and organized into hexagonal array where densely packed. Particles of the polygonal particle junction are more irregular, usually elongate, and sometimes aligned in chains. (160000 ×.)

III. MEMBRANE STRUCTURE IN FREEZE-FRACTURED ADULT BRAIN

When freeze-fractured, adult astrocytic membranes are found to contain rectangular or square aggregates of small, uniform, particles packed with orthogonal symmetry on the protoplasmic fracture face (Figs. 1, 2). Where there are many such aggregates, the average number of constituent particles is greater than in regions where there are fewer aggregates. This feature of the membrane specialization led us to name them 'assemblies' provisionally, until the function could be determined and a more precise name devised. The number of assemblies per unit area in a given region of astrocytic membrane depends on the nature of the processes immediately apposed to that region of the astrocytic membrane. Astrocytic membranes facing blood vessels invariably contain many assemblies, while the abluminal membranes of the same astrocytic processes contain very few assemblies where they face neuronal structures (Fig. 2). Astrocytic processes next to the cerebrospinal fluid at the surface of the brain contain many assemblies (Figs. 3, 4), but fewer than astrocytic membranes next to blood vessels. Virtually no assemblies are present on astrocytic perikaryonal plasmalemma. The demarcation between an assembly-rich region and one with few assemblies can be very sharp, even though there is no specialization of intramembrane structure or of the cytoplasm which appears to constrain the distribution of assemblies.

We have utilized rapid freezing techniques (which circumvent chemical fixation and cryoprotection) to prepare cerebellar and cerebral cortex for freeze-fracture, and have determined that the assemblies are not simply artifacts of tissue preparation (Nabeshima *et al.* 1975; Reese & Landis, 1974; Landis & Reese, 1974*b*, 1981*a*). Moreover, assemblies were found to be uniquely labile. In tissue frozen at various intervals after circulatory arrest, assemblies appear to clump and subsequently disappear. This alteration in the morphology of the particles is evident as early as 35 s after circulatory arrest, and becomes progressively more complete in the first 15 min. The same dissolution of structure can be demonstrated in brain slices incubated in a nitrogen atmosphere or in the presence of dinitrophenol. The morphology of other intramembrane particle specializations, including gap junctions and synaptic junctions, is unchanged over the same intervals (Landis & Reese, 1979; Landis & Reese, 1981*a*).

Mammalian astrocytes have a second class of membrane specialization revealed by freeze-fracture techniques, which we have provisionally termed 'polygonal particle junctions' (Landis & Reese, 1981*b*). The membrane specialization is made up of densely packed, large irregular particles associated with the protoplasmic half of the membrane (Figs. 4–6). There is no orthogonal or hexagonal ordering to the particle packing. In every instance examined, an astrocytic membrane containing this specialization invariably faces another astrocytic process with the same specialization. It is not presently certain whether the protein represented by the intramembrane particle spans the extracellular space to insert in both membranes. This polygonal particle junction occurs in fairly predictable regions. The junction is nearly always present on the lateral surfaces of processes making up the glial limitans (Nabeshima *et al.* 1975), and is also commonly encountered on the large vertical shafts of Bergmann astrocytes in the cerebellar cortex. They are not a common

feature of the processes investing vascular structures, but occasionally can be identified where large, presumably proximal, astrocytic processes contribute to a vascular sheath. When comparable regions are examined in thin-sectioned electron microscopic preparations, there is no evidence of membrane or cytoplasmic specialization. The contour of the membrane, however, is quite parallel to the apposed astrocytic membrane and the two are separated by an extremely uniform extracellular cleft. When tannic acid is used as a mordant prior to *en bloc* staining with uranyl acetate, one can visualize a subtle electron density associated with the outer dense line of the astrocytic unit membrane in the regions where the junctional specialization is known to occur (Fig. 5). This polygonal particle junction is clearly different in both thin-sectioned and freeze-fractured preparations from gap junctions, but gap junctions are very frequently encountered adjacent to or intermingled with the polygonal particle junction (Fig. 6). Studies employing rapid freezing prior to freeze-fracture have confirmed that the junction is not an artifact of fixation. Its function is unknown, but the position of the junction suggests that it may have a mechanical role.

IV. ACQUISITION OF ASTROCYTIC MEMBRANE SPECIALIZATIONS DURING POSTNATAL CEREBELLAR DEVELOPMENT

During the first two postnatal weeks in the mouse, precursors of granule cell neurons proliferate on the surface of the cerebellar cortex, giving rise to postmitotic cells which differentiate through bipolar and tripolar configurations before the nucleus and perikaryonal organelles migrate to mature positions in the internal granular layer (Ramon y Cajal, 1911; Mugnaini & Forstronen, 1967; Mugnaini, 1969). As the nucleus and perikaryonal organelles of the granule cell neurone migrate down through their radial process toward the internal granular layer, the vertical process is invariably aligned along a process of Bergmann astrocytes which extends from the level of the glial cell body in the Purkinje lamina to insert among the proliferating granule cell precursors. This juxtaposition has given rise to the idea that the astrocytic process might guide the radial process of the granule cell and subsequently the migrating nucleus across the terrain of the nascent molecular layer (Rakic, 1971). The same vertical processes of Bergmann cells also give rise to the flattened processes which constitute the cerebellar glial limitans. Early during postnatal development, the surface of the cerebellar cortex is occupied by the proliferating precursors of granule cell neurones, but over the first two postnatal weeks astrocytic processes extend from the cell bodies in the Purkinje cell lamina through the proliferating and differentiating neurones to reach the basal lamina at the cerebellar surface, and subsequently spread umbrella-like to cover the cerebellum.

In freeze-fractured developing cerebellar cortex, the proliferating precursors of granule cell neurones do not have assemblies in their membranes (Fig. 7). This suggests that simply being adjacent to the cerebrospinal fluid in the subarachnoid space is not sufficient to cause a cell to elaborate assemblies. As astrocytic processes reach the cerebellar surface and spread over adjacent proliferating cells, the membrane facing the cerebrospinal fluid acquires assemblies. The number of assemblies per unit area of glial limitans membranes increases as an average over the first two

postnatal weeks. The number of assemblies in adjacent astrocytic processes at any one age may be quite different, and it appears that differentiation of astrocytic processes is somewhat asynchronous. As in the adult, developing astrocytic processes facing blood vessels in the subarachnoid space may have more assemblies than astrocytic processes with no nearby vascular elements.

Freeze-fracture reveals bundles of granule cell axons traversing the molecular layer from external granular layer to the internal granular layer. These are often intertwined, and in older animals may have en passant boutons. In deeper molecular layer, the calibre of the proximal portion of astrocytic processes permits reliable identification. Often in superficial molecular layer it is possible to identify the astrocytic processes by their continuity with the glial limitans. Such criteria allow one to identify processes without considering intramembrane particle morphology. In developing cerebellar cortex in which the vertical components of the T-shaped parallel fibres and the apposed astrocytic processes may be distinguished, we have not been able to discern any specialization of intramembrane structure which might be a correlate of astrocytic guiding function in either glial or neuronal processes. This corroborates experience in thin-sectioned preparations, in which one cannot find evidence of junction formation between the migrating granule cell neuronal perikaryon and the presumably guiding vertical processes of the Bergmann astrocytes.

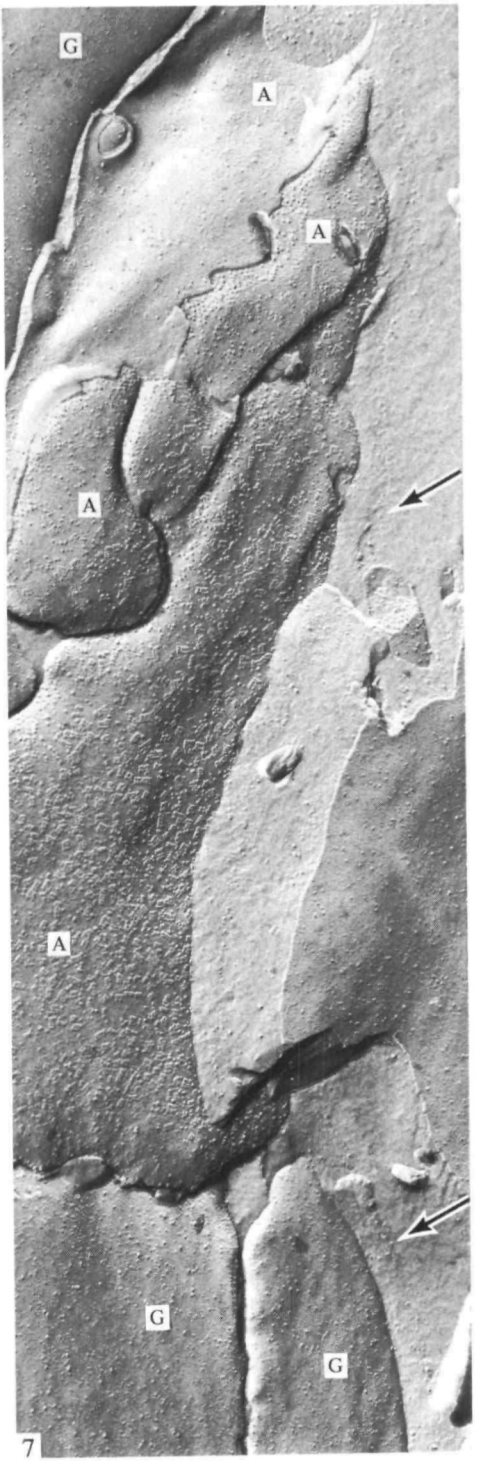
A preliminary analysis of glial:vasculature relationships in developing cerebellar cortex indicates that the glial investment of parenchymal blood vessels is complete by postnatal day 10. During the first two postnatal weeks, the number of assemblies varies markedly in adjacent perivascular glial processes, but most glial processes have acquired numbers in the adult range by 14 days.

It has been extremely difficult to examine in quantitative fashion the acquisition of gap junctions by astrocytic processes. Preliminary studies, however, suggest that the number of gap junctions linking processes of the glial limitans does not reach adult values until the third or fourth postnatal weeks – after virtually all cells of the external granular layer have migrated away.

V. ASTROCYTE MEMBRANE STRUCTURE IN WEAVER AND STAGGERER MUTANT MICE

In mice homozygous for the recessive weaver (*wv*) gene, Purkinje cells and granule cell precursors appear to arise at normal times and reach normal positions during embryonic development, but during postnatal development the cells of the proliferating external granular layer fail to differentiate into bipolar and tripolar configurations (Rakic & Sidman, 1973*a*, *b*; Sotelo, 1975). Virtually no granule cells migrate to mature positions in the internal granular layer. Instead, postmitotic granule cell neurones seem to degenerate in the external granular layer. It had been

Fig. 7. Astrocytic processes (A) appearing on the surface of normal cerebellar cortex of postnatal day 9. The cerebellar parenchyma is at the left, and the subarachnoid space is to the right. Three cells of the proliferating granule cell precursor population (G) are also present at the surface adjacent to the cerebrospinal fluid, but have no assemblies. The number of assemblies per unit area varies in the different astrocytic processes at this age; the largest process here contains 180 assemblies μm^{-2}. The basal lamina (arrows) is barely visible in cross-fracture subarachnoid space. (48000 ×.)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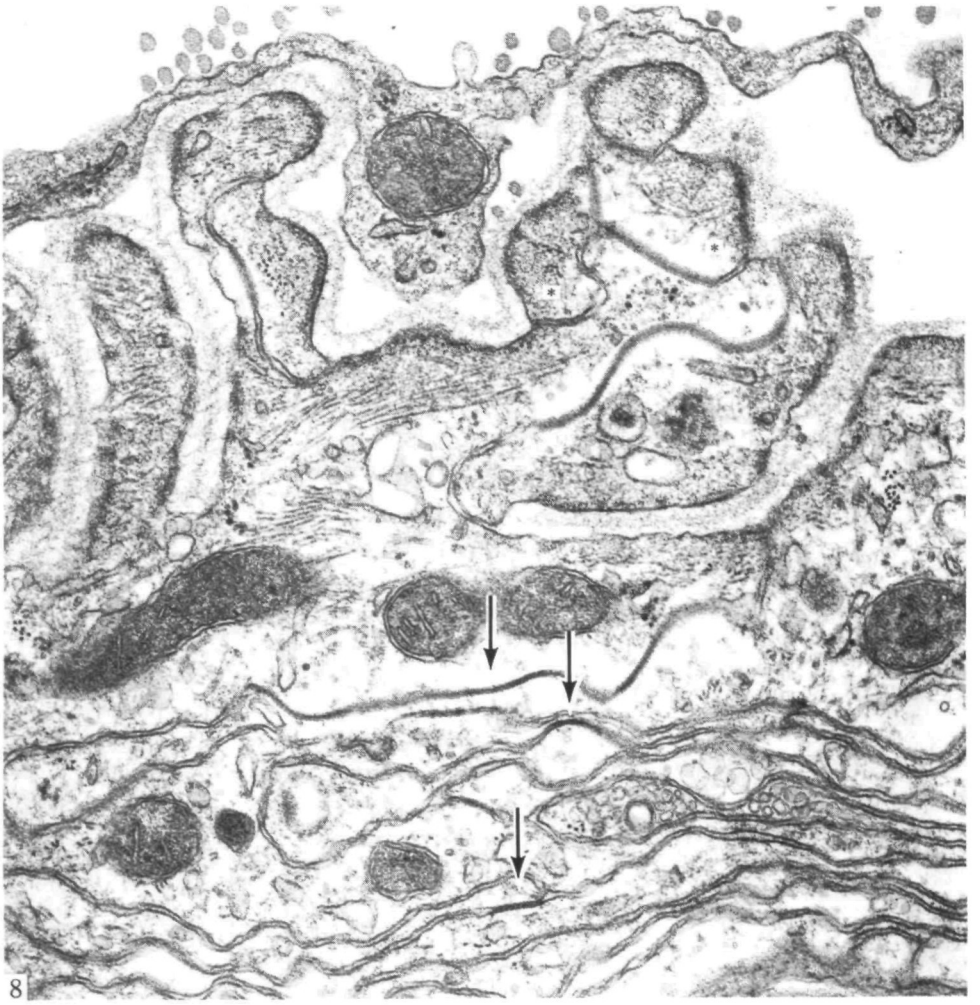
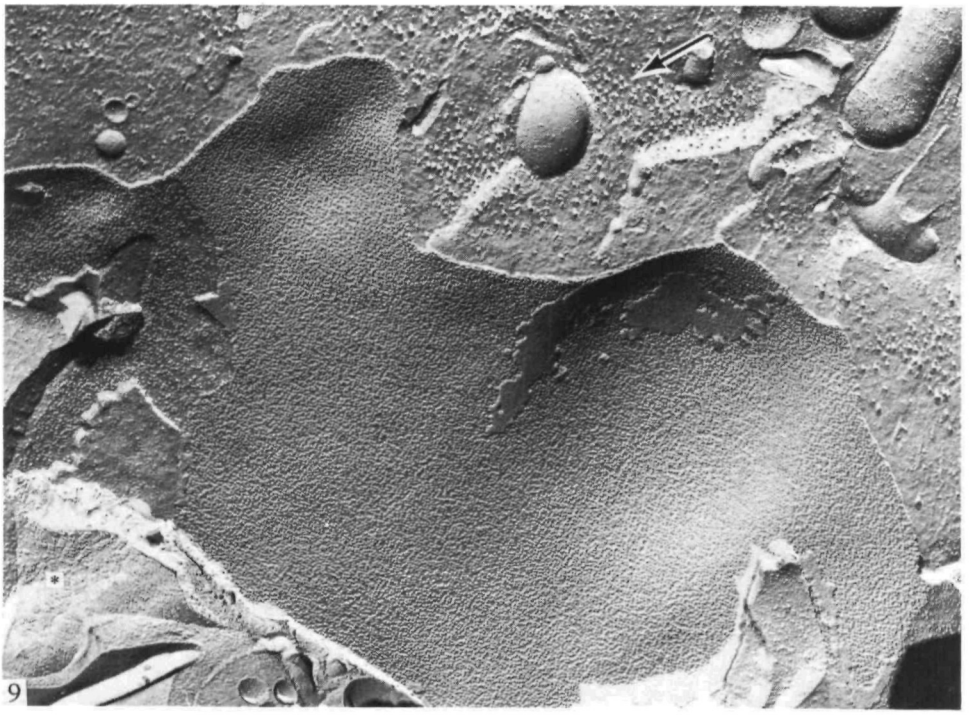
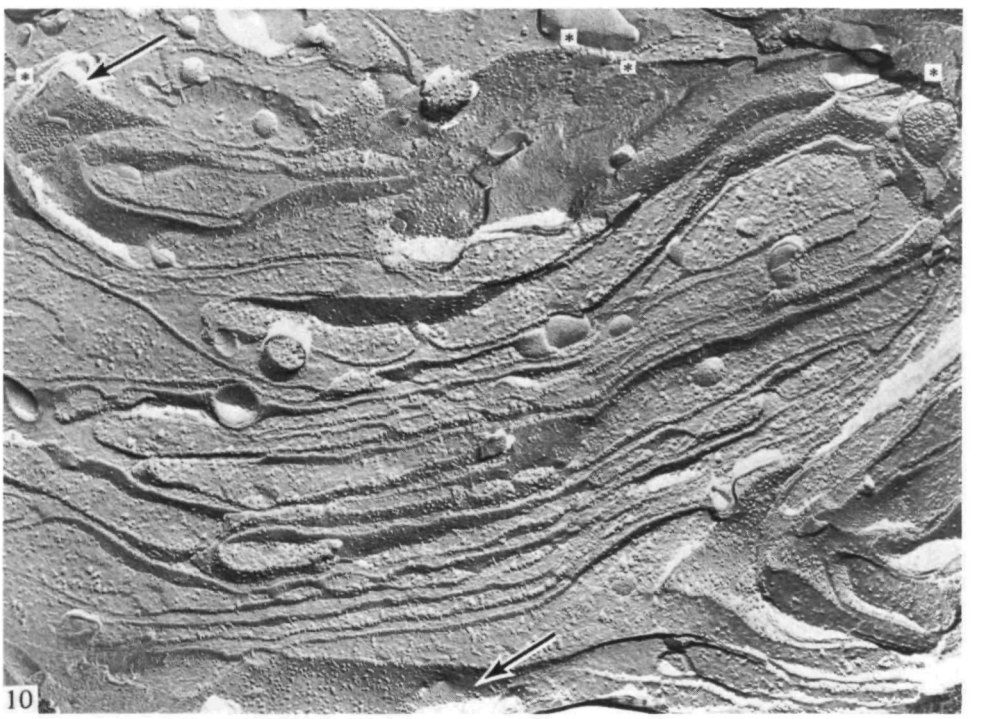
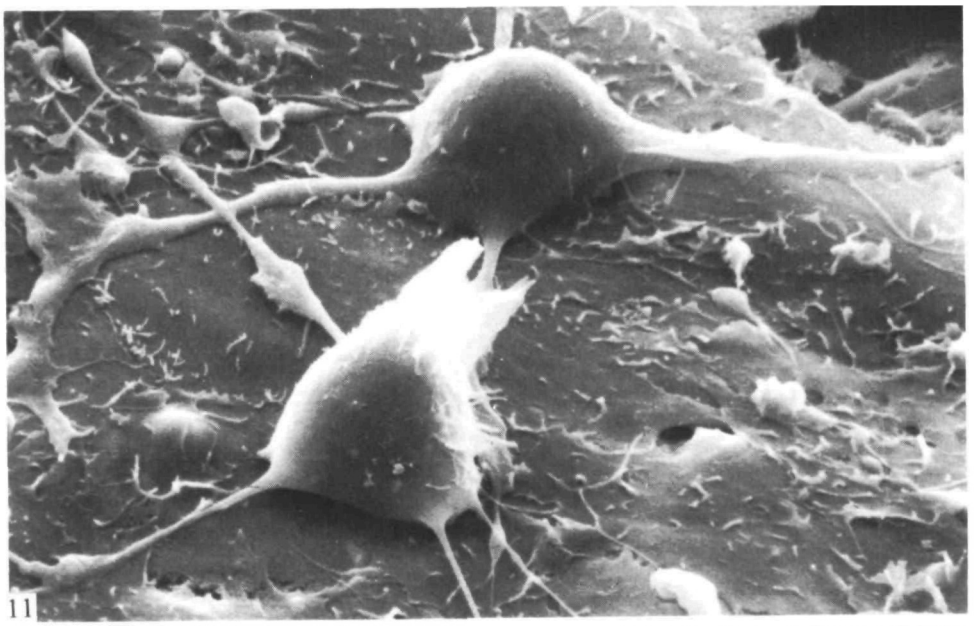


Fig. 8. Astrocytic processes comprising the cerebellar glial limitans in a staggerer mouse. The cerebellar surface is thrown into folds by the loss of volume which accompanies the granule cell degeneration. The horizontal lamellae of the astrocytic processes here are in a region normally occupied by parallel fibres. The cytoplasm of the astrocytic processes contain some glial filaments. Arrows indicate gap junctions (more are present, but obliquely sectioned) and the sites of two probable polygonal particle junctions are below the asterisks (this specimen was not treated with tannic acid, and the densities associated with the polygonal particle junctions are difficult to recognize). (42000×.)





Figs. 9 and 10. For legend see p. 41.



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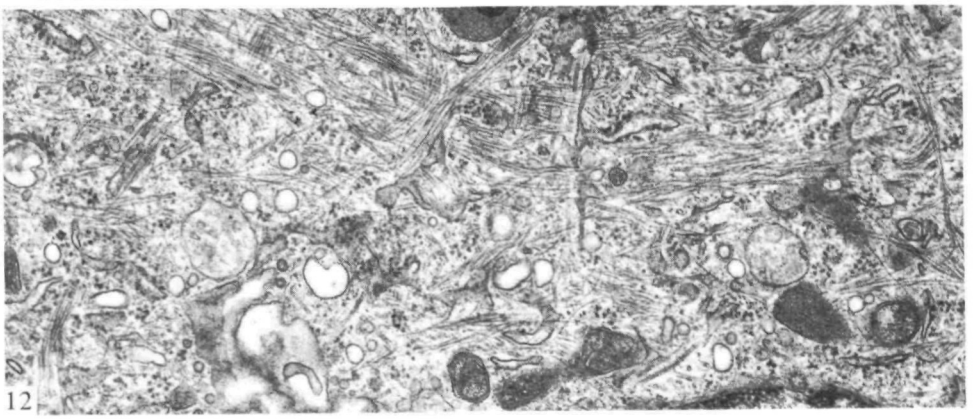

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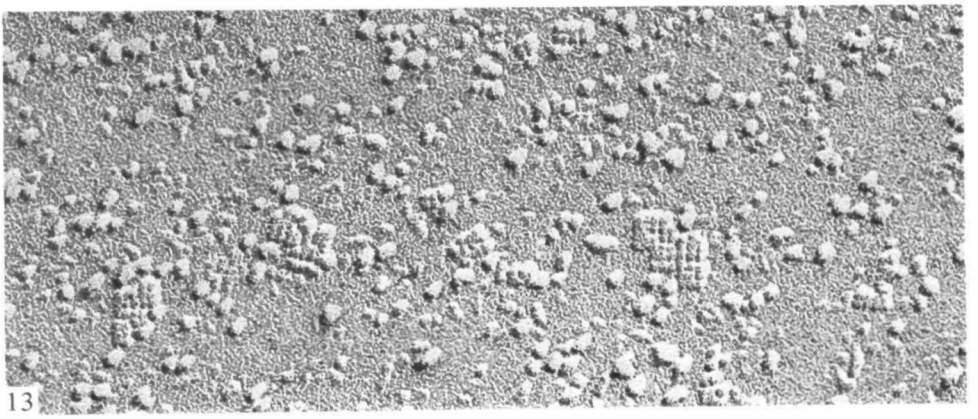

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suggested that the neuronal death was caused by some failure in the developmental interaction by which astrocytic processes guide migrating granule cells (Rakic & Sidman, 1973*c*).

When developing weaver mice were examined with freeze-fracture techniques at several ages during postnatal development, it proved impossible to clearly identify any abnormality of intramembrane particle structure (Hanna *et al.* 1976; Landis & Reese, 1977). In particular, perivascular and glial limitans processes acquired assemblies in roughly normal sequence and numbers. As in the normal, there did not appear to be any specialization of intramembrane structure where vertical glial processes were apposed to cells in the external granular layer. Astrocytic processes investing Purkinje cell dendrites in the molecular layer were indistinguishable from normal. In adult weaver mice, however, the gap junctions between adjacent processes of the glial limitans were very extensive, containing more particles per junction than ever observed in normal (Fig. 9).

Mice homozygous for the staggerer (*sg*) gene also manifest granule cell death during postnatal development, but in this instance the granule cell neurones manage to migrate to normal positions in the internal granular layer and to establish synaptic junctions with mossy fibres prior to cell death (Landis & Sidman, 1978). The normally differentiating granule cells leave many parallel fibres in the growing molecular layer during the first two postnatal weeks, but these morphologically normal axons never establish synaptic junctions on their principal target, the Purkinje cell dendritic spines. Purkinje cells, especially as visualized in golgi impregnations, are unique in this mutant in that they fail to acquire dendritic spines (spines on the cell soma and on proximal portions of stout dendrites do appear; these are involved in synapse formation with climbing fibres). The staggerer thus is characterized by normal initial granule cell differentiation, strikingly aberrant Purkinje cell morphogenesis, and selective failure to form parallel fibre-to-Purkinje spine synapses. The granule cell degeneration in the granular layer begins in the second postnatal week and continues until virtually all have disappeared.

There is little reason on morphological grounds to suspect that an abnormality of astrocytes is involved in the abnormal cerebellar development in the staggerer, but

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Fig. 9. Vast gap junction on the lateral surface of an astrocytic process comprising the cerebellar glial limitans of a weaver mutant mouse. The structure is folded (as in the staggerer); a portion of the astrocytic membrane facing the cerebrospinal fluid, bearing assemblies, is identified by an asterisk. Glial filaments are evident in the cross-fractured astrocytic cytoplasm (arrow). (41 000 × .)

Fig. 10. Astrocytic processes comprising the cerebellar glial limitans in a staggerer mouse. At least four gap junctions are present (below asterisks) and several assemblies are also evident, associated with gap junctions or isolated (arrows). The number of assemblies here is roughly equivalent to the number found in astrocytic processes in normal neuropil. (58 000 × .)

Fig. 11. Scanning electron microscopic image of a primary astrocytic culture at 21 days. The flattened cells are found to contain filaments when thin-sectioned (Fig. 12) and their membranes contain assemblies (Fig. 13). The rounded cells are much less frequent (2–10% overall), and most are probably oligodendrocytes. (3400 × .)

Fig. 12. Glial filaments in the perikaryonal cytoplasm of a cultured astrocyte at 21 days. (26 000 × .)

Fig. 13. Assemblies in the membrane of cultured primary astrocytes at 21 days; about 10 assemblies are present μm^{-2}. (188 000 × .)

the widespread death of granule cell neurons in concert with the vestigial Purkinje cell dendritic arbors results in a collapsed molecular layer with numerous sheaves of astrocytic processes (Figs. 8, 10). During the third and fourth postnatal weeks these astrocytic processes appear to be involved in the phagocytosis of cellular debris resulting from granule cell death. It is difficult to be certain whether proliferation of astrocytic cells occurs, but the stacked lamellae of attenuated astrocytic processes in the superficial molecular layer resembles closely the astrocytic scars described in adult animals subjected to a variety of insults.

When freeze-fractured, staggerer astrocytes are seen to have normal numbers of assemblies in perivascular regions and in the glial limitans. The stacks of astrocytic processes are linked by numerous punctate gap junctions, but the concentration of assemblies is approximately that of normal astrocytic processes facing neuronal elements. It does not appear that 'reactive' astrocytic processes contain unusually large numbers of assemblies. These processes do contain many glial filaments and elaborate desmosome-like junctions. The very large gap junctions recognized in the weaver mouse were not encountered in the staggerer cerebellar cortex.

VI. MEMBRANE STRUCTURE IN CULTURED ASTROCYTES

As an initial step in biochemical characterization of assemblies, we have attempted to devise an *in vitro* system in which assemblies might be subjected to experimental manipulation and isolation procedures. However, C-6 rat glioma cells did not acquire assemblies under our conditions of culture. Two cell lines derived from human astrocytomas (generously supplied by Dr P. Kornblith) similarly were devoid of assemblies when freeze-fractured. These results suggested that assemblies might be a characteristic of differentiated cells, an interpretation which fits with observations on their selective deployment in normal tissue.

We employed mechanical dissociation techniques to isolate astrocytes from newborn pups and grew those cells in varying concentrations of rat or foetal calf serum. In such cultures, some flattened cells contained a few ($1-3/\mu m^2$) assemblies after 3 weeks of primary culture. Subsequently we employed the techniques of mechanical and enzymatic dissociation, primary culture, agitation to separate astrocytes and oligodendrocytes, and then re-plating as described by McCarthy & de Vellis (1980). More than 90% of the cells in such cultures appear flattened in light- and scanning-electron-microscopic preparations (Figs. 11, 12); when these flattened cells are freeze-fractured, virtually all are found to contain assemblies (Landis *et al.* 1980) (Fig. 13). The number of assemblies increases with duration of culture at least over 4 weeks, and the assemblies appear uniformly distributed over the cell surface. This is distinctly different from the *in vivo* situation, especially in that assemblies do not occur on astrocytic perikarya in tissue. Some of the cells in the cultures have a more stellate shape. These presumably represent contaminating oligodendrocytes and possibly a population of more differentiated astrocytes (a similar configuration can be induced by culturing astrocytes in the presence of dibutryl c-AMP; Kimelberg, Narumi & Bourke, 1978). Thus far it has proved difficult to obtain sufficient freeze fracture images of these stellate cells to analyse their membrane composition.

VII. DISCUSSION

The technique of freeze-fracture has proved a powerful tool in the study of astrocyte structure and function. Because the plane of fracture follows membrane contours, the technique often can reveal patterns of organization that would have required tedious reconstruction of serial thin-sections to recognize. More importantly, utilization of freeze-fracture methodology in the study of several brain regions has led to the recognition of two distinct specializations of membrane structure which had simply been invisible in thin-sectioned preparations. One of these specializations, the assemblies, is positioned where astrocytic processes are juxtaposed to blood vessels and cerebrospinal fluid. These assemblies support a hitherto unappreciated function of astrocytes, and their discovery emphasizes how much remains to be learned of astrocytes.

Assemblies have not been recognized in oligodendrocytes, neurones, vascular structures, or macrophages within the brain, but they do occur in the ependyma lining the ventricular system (Brightman, Prescott & Reese, 1975). Essentially identical particle aggregates have been found outside the central nervous system. Hepatocytes (Kreutziger, 1968), small intestinal epithelial cells (Staehelin, 1972), tracheal epithelial cells (Inoue & Hogg, 1977), and some supporting cells of sympathetic ganglia (Elfvin & Forsman, 1978) manifest a few assemblies, but in the basolateral membranes of gastric parietal cells (Bordi & Perrelet, 1978) and light cells of the renal distal collecting duct (Humbert *et al.* 1975) their concentration approaches that of perivascular astrocytic processes. Assemblies have been recognized in a zone around mammalian neuromuscular junctions (Rash & Ellisman, 1974; Smith, Baerwald & Hart, 1975) and in cardiac myocytes (McNutt, 1975), but are not a feature of Schwann cells or peripheral axons. Interestingly, assemblies have been found immediately adjacent the frog neuromuscular junction (Heuser, Reese & Landis, 1974), but are not present in astrocytes of Muller cells in frog retina (Landis, unpublished). Thus far, we have not been able to identify a cell activity which parallels the distribution of assemblies in the body.

The study of astrocytic specializations during development has contributed comparatively little to our understanding of the functions subserved by these specializations. Assemblies in general increase in number in perivascular and glial limitans membranes over the first 2 postnatal weeks in mouse cerebellar cortex. During development the number of assemblies may be quite different in adjacent perivascular or glial limitans membranes, apparently reflecting some asynchrony in maturation. In both developing and mature systems, the number of constituent particles per assembly is on the average greater in membranes which contain more assemblies. Nonetheless, even in perivascular membranes packed with assemblies, individual assemblies range in size from 4 to 60 constituent particles. Thus far, no one has been able to recognize a situation in which the constituent particles have scattered from the orthogonal packing and remained single.

Astrocytic membrane structure has been examined in three mouse mutations affecting cerebellar structure (the third mutation, the reeler mouse, has not been described here in detail; see Landis & Landis, 1978, 1980), and it would seem that in these animals, assemblies are not markedly increased in 'reactive' astrocytic

processes. Anders & Brightman (1979) examined gliosis on the surface of developmentally mature rat medulla induced by transplantation of neural tissue, and clearly demonstrated an increase in the number of assemblies in the glial lamellae. It seems likely that astrocytic responses to cell death or other insult may be different in mature and developing systems.

Prescott & Brightman (1978) used cerebellar explant cultures to show that astrocytes could express assemblies *in vitro*. Subsequently, Anders & Pagnanelli (1979) have exploited primary astrocyte cultures to show that brief exposure to an inhibitor of protein synthesis, cycloheximide, is followed by a gradual, transient decline in the number of assemblies in the cell membrane. These experiments reaffirm that the intramembrane particles very likely have a protein component. This slow change in assembly number should not be confused with the very rapid, dramatic alteration of assembly morphology caused by circulatory arrest (Landis & Reese, 1979, 1981*a*). If more than 35 seconds elapse between decapitation of a mouse and the rapid freezing of an excised tissue slice, the number of assemblies in the frozen tissue declines precipitously. Virtually all assemblies are gone 15 minutes after decapitation. Cerebellar slices incubated *in vitro* in the presence of nitrogen or dinitrophenol also lose their assemblies, while slices incubated in the presence of oxygen maintain essentially normal numbers of assemblies over a 30 min interval. The lability of assemblies after anoxic insult is specific; we have recognized no changes in background particles or particles constituting other membrane specializations in the same tissues.

Despite the mass of descriptive information, the function of assemblies remains unknown. It is reasonable to presume that assemblies in freeze-fractured preparations represent proteins wholly or partially intercalated in the hydrophobic interior of membranes (Branton, 1969; Pinto da Silva, 1972, 1979), though we have assembled no direct evidence about their chemical nature yet. Studies of deep-etched, rapidly frozen specimens indicate that some representation of assemblies extends to the true outer surface of the membrane (Landis *et al.* 1980). One would speculate, then, that assemblies are well positioned to participate in the transport of some material into or out of the astrocytic compartment at the interfaces with blood and cerebrospinal fluid. Unfortunately, even after 70 years of neurobiological investigation, we are able to do little more than repeat Lugaro's suggestion that glial processes may remove unwanted substances delivered to the brain by the blood and may dump wastes into the blood (Lugaro, 1907, as cited by Palay, 1979).

As one considers the list of properties ascribed to mammalian astrocytes, it is difficult to correlate one with assemblies. The intimate association of astrocytic processes with some synapses has led to the suggestion that astrocytes have a role in neurotransmitter metabolism (Palay, 1966). Subsequently, astrocytes *in vivo* and *in vitro* have been found to take up a variety of neurotransmitters or related metabolites (e.g. Henn & Hamburger, 1971; Haber *et al.*, 1978; Hertz, 1979). One might suppose that assemblies do not represent such systems because one would predict that the uptake systems would be disposed around neuronal elements (where assemblies are least concentrated). In fact, the available evidence from autoradiographic analysis of [^{3}H]GABA uptake (Kelly & Dick, 1978) and from histochemical analysis of glutamine synthetase activity (Norenberg & Martinez-Hernandez, 1979) indicates

that these functions are diffusely distributed. Astrocytes are also thought to participate in brain potassium homeostasis (e.g. Henn, Haljamae & Hamberger, 1972; Orkand, 1977; Prince, Pedley & Ransom, 1978; Franck *et al.* 1978) and one should consider the possibility that assemblies represent ouabain-sensitive astrocyte Na^+–K^+–ATPase. It is clear, however, that assemblies are not the sole intramembrane representation of the protein subserving Na^+–K^+–ATPase activity, since this transport function is ubiquitous in the body, while assemblies are limited to only a few cell types. Even within the brain, electron microscopic study of the localization of K^+-p-nitrophenyl-phosphatase activity (a marker for Na^+–K^+–ATPase) has shown a distinctly different pattern than that exhibited by assemblies (Broderson, Pattan & Stahl, 1979).

The second astrocytic specialization, the polygonal particle junctions, also has an unknown role in astrocyte metabolism. This junction is faintly visible in thin-sectioned preparations after the use of tannic acid as a mordant. The particle specialization is present in two apposed astrocytic membranes, and so is interpreted as a junction. The position of these junctions suggests that they may have a mechanical role. They are quite distinct from gap junctions in both thin-sectioned and freeze-fractured preparations. In freeze-fracture preparations, it seems that such junctions might 'zip up' the margins of adjacent astrocytic processes just below the cerebellar or cerebral surface. It is important to recall that there is no barrier to the movement of large molecules (like horseradish peroxidase) in the regions occupied by polygonal particle junctions (Brightman & Reese, 1969). These junctions are thus morphologically and physiologically distinct from tight junctions.

Analysis of astrocyte morphology in freeze-fractured preparations has clearly shown that astrocytic membranes contain distinctive specializations of intramembrane particles which are concentrated in portions of astrocytic processes apposed to certain extracellular elements. There must be a membrane-associated astrocyte function at the interface with blood and cerebrospinal fluid compartments, which is represented morphologically by assemblies. Anders & Brightman (1979) correctly point out that primary cultures of astrocytes are a system in which the biochemical properties of the membrane specialization may be rigorously examined. Flattened astrocytes in cultures, however, have assemblies distributed over their entire surface. We would suggest that electrophysiological studies seeking for a function of assemblies might be more profitably pursued in systems which manifest focal concentrations of the membrane specialization.

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