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COMPARATIVE STUDIES ON GLIAL MARKERS

BY BETTY I. ROOTS
Department of Zoology and Erindale College, University of Toronto

SUMMARY

Macromolecular markers for glial cells have been sought for a variety of reasons. One of the earliest was the need for a means of assessing the purity of cell and subcellular fractions prepared from nervous tissue. While there is still a requirement for this kind of tool, emphasis has shifted towards seeking information on biochemical differentiation among cells and their functional interactions. A brief general review will be made of glial markers and two of these, 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNP) and glutamine synthetase (GS), will be considered in detail. Until recently studies of markers have been concentrated on the higher vertebrates and those on lower vertebrates and invertebrates have hardly begun. However, such comparative studies may lead to fresh insight into old problems. For example, CNP has long been regarded as a marker for myelin and oligodendrocytes but it has not been possible to attribute a functional role to it and its relation to myelination has remained obscure. The finding that it is present in the glia of a moth *Manduca sexta* which lacks myelin provides a stimulus for a fresh approach to the problem. Another example is provided by studies on GS. This enzyme is found in astrocyte feet and preliminary results indicate that it is localized also in the perineurial glia of *Aplysia* ganglia. These results lead to a reconsideration of the perennial question of the possible role of astrocyte feet in barrier mechanisms. Extension of comparative studies may not only raise new questions but also provide some answers.

INTRODUCTION

The early motivation for the search for macromolecular markers for specific glial cell types was the need to assess the purity of the fractions separated from the nervous system by various means. With the explosion of interest in the bulk preparation of cells, parts of cells, e.g. neuronal perikarya, and subcellular fractions derived from specific cell types, the search for markers was intensified. The development of cell lines in culture, especially those derived from tumours also demanded a means of assessing accurately the nature of the component cells. Often this proves to be a complex problem, as is the case with the C_6 rat glioma clone which manifests a bewildering combination of characteristics of both oligodendrocytes and astrocytes. The need for a tool to monitor fractions and tissue cultures still exists. For example, glial fibrillary acidic protein (GFAP) which is a marker for astrocytes has been used recently to check the purity of a preparation of Purkinje cells from developing rat cerebellum (Woodhams *et al.* 1980). Another somewhat different example is the use of GFAP and glutamine synthetase (GS), another astrocytic marker, to demonstrate

the presence of astrocyte feet on microvessels isolated from rat brain (White, Dutton & Norenberg, 1981). Cell type-specific markers for cultures of human neural cells have been reviewed recently by Kennedy and his co-workers (Kennedy, Lisak & Raff, 1980). However, emphasis has shifted towards seeking information on the differentiation and maturation of cells and on the functional interaction between cell types. Such studies have been hampered by the inability to identify cells at all times using morphological criteria alone. The development and use of biochemical markers as a complementary approach in studies of relationships between cells during development is beginning to be very productive. An example, which will be discussed in more detail below, is the study of Rhesus monkey brain development by Levitt & Rakic (1980) using localization of GFAP.

One of the limitations in the use of markers is that cells may express the molecule at only one stage in their development, e.g. GFAP in ependymal cells discussed below (Roessmann *et al.* 1980), or discontinuously according to their physiological milieu. An example of the latter is the failure of Schwann cells to synthesize galactocerebroside in culture although they do so *in vivo* (Raff *et al.* 1978). On the other hand where it is possible to identify cells morphologically, markers provide a tool for studying cell maturation and for probing physiological relationships. Several of the markers, e.g. GS, and carbonic anhydrase (CA), are enzymes and knowledge of their distribution and of their appearance during ontogeny should be particularly useful in giving insight into the differentiation of cell function and clues to the relationships between cells in the nervous system.

Morphological characterization of glial cell types in the invertebrates is in its infancy (Roots, 1978; Lane, 1981) and would profit greatly from the application of biochemical markers. The definition of cells and their function in phylogeny as well as ontogeny should lead to a better understanding of neurone-glia interactions. Preliminary results of studies on GS in invertebrate glia, discussed below, indicate that comparative studies may provide fresh insight into old problems.

The development of immunocytochemical techniques has led to a rapid burgeoning of studies using glial markers so that much progress has been made since the reviews of Varon (1978) and Varon & Somjen (1979). Immunological techniques have various problems associated with them, for example heterogeneity of the material used to raise the anti-sera, and cross-reactivity. Although detailed discussion of these problems is beyond the scope of the present review, some consideration will be given where appropriate.

A general review of advances made in the field of glial markers follows with particular attention given to the contributions made by comparative studies.

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP)

Astrocytes, both fibrous and protoplasmic, are characterized by the presence of fine filaments in their cytoplasm which, as the name suggests, are particularly abundant in the fibrous form of the cell. The protein which composes these filaments was first isolated from multiple sclerosis plaques (Eng *et al.* 1971) which consist almost entirely of fibrous astrocytes and thus provide an excellent starting point for the preparation of GFAP. Antibodies to GFAP were prepared and immunological studies of its

distribution were launched (Uyeda, Eng & Bignami, 1972). Subsequent preparations from other material, normal human brain tissue, other mammalian brains and nervous tissue of lower vertebrates were contaminated to varying degrees with tubulin and neurofilament protein leading to the suggestion that the proteins may contain similar subunits (Yen *et al.* 1976, see also the review by Bignami, Dahl & Rueger, 1980), which has not however been substantiated (Davison & Jones, 1981).

However, as pointed out by Eng (1979) tubulin is much less antigenic than GFAP and antisera raised against a mixture of highly antigenic GFAP and weakly antigenic tubulin may still be very useful for immunocytochemical studies provided proper control absorptions are made. The problem of tubulin contamination has been overcome by the development of a method of preparation using immunoaffinity chromatography (Rueger, Dahl & Bignami, 1978).

Recently the question of the specificity of GFAP for astrocytes has been raised again by Jessen and Mirsky (1980) who have demonstrated that enteric glial cells in the rat myenteric plexus are rich in GFAP and by Yen & Fields (1981) who report the occurrence of either GFAP or a cross-reacting antigen in rat sciatic nerve. Bignami & Dahl (1977) investigated the occurrence of GFAP in human and rabbit sciatic nerves and found that non-specific fluorescence may occur *in vitro* with GFAP antisera.

Artefactual staining in sections has not been observed (Bignami & Dahl, 1977) and the immunoperoxidase staining used in electron microscopy permits the precise localization of GFAP with, so far as is known, no redistribution. It is with the use of these techniques with this glial marker that recent advances in the analysis of neurone–glia relations have been made.

The main thrust of earlier work, mainly immunofluorescence studies, was the establishment of the specificity of GFAP for mature astrocytes (Bignami & Dahl, 1974*a*, 1977) including those in tissue culture (Lim *et al.* 1978; Sensenbrenner, 1978), and for Bergmann glia, a specialized form of astrocyte found in the cerebellum (Bignami & Dahl, 1974*b*; Schachner *et al.* 1977). The chief application of these and similar studies being the identification of these cells in normal and pathological tissue and the elucidation of the nature of cell lines in culture. Some interest in development was also manifested, e.g. GFAP was shown to be present in radial glia (Antanitus, Choi & Lapham, 1976) lending credance to the view that these cells are related to astrocytes. It is this aspect of development which is receiving attention at the present time.

Roessman and his colleagues (Roessman *et al.* 1980) have followed the occurrence of GFAP in human ependymal cells during development. Adult ependymal cells lack GFAP but between the 15th week of gestation and full term the protein is present in the foetal cells. Thus the appearance of GFAP marks a stage in the differentiation of ependymal cells. A curious feature is that adult ependymal cells do possess filaments which are similar to those in astrocytes. Possible explanations are that the filaments lose their antigenicity in the adult cell, or that two distinct types of filament are produced at different stages in development. This question has not been resolved. In this study it was shown also that GFAP appears transiently in tanycytes* at a time in

* Tanycytes are glial cells in mammalian brain whose perikarya are in a ventricular position and which have long tapering processes which may reach the pial surface.

development which led the authors to conclude that tanycytes represent a form of ependymal differentiation and are not related to radial glia.

In tracing cell lineages during development a major problem has been the inability to distinguish cells at early stages of development. Radial glial cells which are responsible for guiding neurones in their migrations during development (Rakic, 1972) are very difficult to distinguish from the elongated migrating neurones. As Levitt & Rakic (1980) point out knowledge of the precise number and distribution of radial glia during development would lead to better understanding of the organization of the central nervous system and define the extent of glial participation in the guidance and direction of neurones. The sensitivity and precision of the immunoperoxidase localization of GFAP at the electron microscopic level makes such quantitative studies possible and it has been applied to the problem by Levitt & Rakic (1980) in a study of radial glial cells and astrocytes in developing Rhesus monkey brain. Several classes of glial cells were found to be present at considerably earlier stages of development than previously recognized in primate brain. Hitherto it had been thought that only one class of cell was present in early development. Radial glia are the first class of astrocytic cell to be detected, appearing in the first dimester of the 165-day gestational period. In all parts of the brain the ventricular and pial surfaces are constantly connected by elongated glia. Many radial cell processes also penetrate the axonal tracts at right angles, thus a scaffold of pathways is provided for the guidance of neurones through increasingly complex terrain. The pattern of the distribution of radial glia processes was found to be unique to each area of the brain and to change systematically during development. When their role in guidance is no longer required radial glia transform into astrocytes, both protoplasmic and fibrous, thus transitional cells are found first in areas where cell migration first ceases. Thus the use of the marker GFAP has provided new insight into neurone-glia interactions during development.

S-100 PROTEIN

This highly soluble, highly acidic protein has had a chequered career as a putative glial marker. Early studies appeared to indicate that it was located specifically in glia although present in both astrocytes and oligodendrocytes. Then followed reports of its presence in neurones. It was then speculated that the production of S-100 rather than its localization constituted the true glial marker (Varon & Somjen, 1979). Now, this year, Ghandour and his colleagues have re-examined the status of S-100 (Ghandour *et al.* 1981). The results of their experiments using light and electron microscope immunocytological techniques show that the protein is present only in astrocytes. They argue that previous reports of its occurrence in oligodendrocytes and neurones are due to a number of different technical artifacts which they analyse and describe. These new findings await confirmation, meanwhile the functional significance of S-100 remains obscure.

GLYCEROL-3-PHOSPHATE DEHYDROGENASE (GPDH)

The soluble cytoplasmic enzyme L-α-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) in rat brain is exclusively located in oligodendrocytes which appear to be

target cells for glucocorticoids (De Vellis *et al.* 1978; Leveille *et al.* 1980). While in rat brain GPDH and its inducibility by hydrocortisone should be viewed as oligodendroglial markers it is by no means clear that this is the case in mouse brain or for primary rat brain glial cultures (see Varon & Somjen, 1979). Its low activity in multiple sclerosis plaques is consistent with a predominantly oligodendrocyte location in human brain (Hirsch, Blanco & Parks, 1980).

GALACTOCEREBROSIDE (GC)

Galactocerebroside, the major glycolipid component of myelin, is found also in oligodendrocytes and Schwann cells and has been investigated as a possible marker for these cells, particularly in culture (Raff *et al.* 1978; Steck & Perruisseau, 1980). It is of interest in the present context as an example of a marker whose expression depends upon the physiological environment of the cells. Thus Raff and his colleagues found that cultured Schwann cells derived from neonatal rat sciatic nerve do not react with anti-galactocerebroside antisera. On the basis of unpublished observations, they considered it unlikely that the Schwann cells do not make detectable amounts of GC before myelination or that GC although present is inaccessible to the antibody and favoured the explanation that Schwann cells normally synthesize GC *in vivo* but stop doing so in culture.

CARBONIC ANHYDRASE (CA)

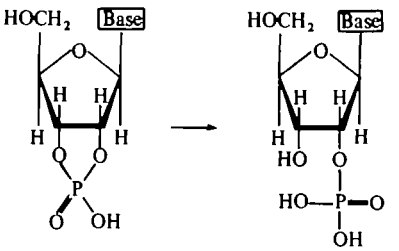
Carbonic anhydrase (EC 4.2.1.1) exists in several isozymic forms all of which are monomeric 30000 dalton proteins. The two major isoenzymes CAI and II have different amino acid sequences and exhibit different enzyme kinetics (Sciaky *et al.* 1976) and are immunologically distinct (Wistrand & Rao, 1968). Not all studies have distinguished between the isoenzymes but recent reports (Ghandour *et al.* 1980*a*, *b*) indicate that only CAII is intrinsic to nervous tissue where it is present in both soluble and insoluble membrane-bound fractions.

Carbonic anhydrase was first proposed as a general glial marker by Giacobini (1961, 1962). Subsequently several groups of investigators have reported its presence in primary cultures of rat brain astrocytes (Sensenbrenner, 1978; Kimelberg *et al.* 1978; Church, Kimelberg & Sapirstein, 1980). However, it should be noted that such cultures contain other cells which may be oligodendroglial in nature (see review by Van Calker & Hamprecht, 1980). The insoluble form of CA was found to be associated with myelin (Sapirstein, Lees & Trachtenberg, 1978) which would indicate an oligodendroglial location. Staining of both astrocytes and oligodendrocytes by an immunohistochemical reaction was reported by Roussel and his co-workers (Roussel *et al.* 1979). Recently, Ghandour and his colleagues (Ghandour *et al.* 1980*a*, *b*, *c*) found CA to be located exclusively in oligodendrocytes in rat cerebellum and claim (1980*a*) that the astrocyte location found by Roussel *et al.* is erroneous due to the incorrect identification of cells in their electron micrographs. It is difficult to resolve this question from an examination of published micrographs, so clearly more work needs to be done. The answer may well lie in the physiological conditions in which glial cells of different types express their potential for CA production. (Cf. discussion of GC above.)

The primary function of CA is thought to be the catalysis of the reversible hydration of carbon dioxide. From this stems the potentiality of its involvement in the regulation of pH, secretory activities, photosynthesis and the movement of ions (Giacobini, 1961; Bourke *et al.* 1975; Bundy, 1977; Kimelberg, Biddlecome & Bourke, 1979). In cultures of astrocytes both norepinephrine and cAMP increase the activity of soluble CA and ($Na^+ + K^+$) ATPase. The effects of inhibitors indicate that the response to norepinephrine is mediated by changes in intracellular cAMP. Since the release of norepinephrine is stimulated by increased extracellular concentrations of K^+ ions, it has been suggested that in the nervous system soluble CA plays a role in K^+ ion homeostasis and that its activity is modulated by the putative transmitter norepinephrine via cAMP (Kimelberg *et al.* 1978; Church *et al.* 1980). It appears that membrane-associated CA is not affected and its function is enigmatic.

2′,3′-CYCLIC NUCLEOTIDE 3′-PHOSPHOHYDROLASE (CNP)

2′,3′-cyclic nucleotide 3′-phosphohydrolase (EC 3.1.4.37) is also referred to as 2′,3′-cyclic nucleotide 3′-phosphodiesterase and has erroneously been given the Enzyme Commission number 3.1.4.16. It is perhaps the most curious of the markers. The reaction it catalyses is the hydrolysis of 2′,3′-cyclic nucleotides of each of the four bases, adenine, guanine, cytidine and uridine to the 2′-nucleotides. The rate is greatest for adenine and decreases through the bases in the order given. The reaction is



Although CNP does occur in other tissues, in fact it was first discovered in bovine spleen (Whitfield, Heppel & Markham, 1955), it is found predominantly in the nervous system in myelin and has long been regarded as a myelin marker. Drummond and his co-workers (Drummond, Iyer & Keith, 1962) first showed that the nervous system contained at least ten times the level of CNP as other tissues. Subsequently its distribution was shown to coincide with that of myelinated fibres (Kurihara & Tsukada, 1967) and to be associated with myelin fractions from brain homogenates (Kurihara & Tsukada, 1967, 1968). It was later shown to be enriched in purified myelin preparations (Kurihara, Nussbaum & Mandel, 1971).

Additional evidence was derived from developmental studies in which it was found that CNP increases in parallel with myelinogenesis (Kurihara & Tsukada, 1968; Sarlieve *et al.* 1976; Toews & Horrocks, 1976) and from studies of myelin-deficient mutant mice, such as Jimpy and Quaking, in which the level of CNP is low (Kurihara *et al.* 1971; Sarlieve *et al.* 1976; see also reviews by Sims & Carnegie, 1978; Varon & Somjen, 1979).

The myelin association of CNP was reinforced by the fact that it could not be

detected in the nervous systems of the Dungeness crab (*Cancer magister*), the squid (*Rossia pacifica*), the octopus (*Octopus dolfeini*), the pink starfish (*Pisaster brevis spinosus*) and the Pacific prawn (*Pondalus platyceros*)* all of which are unmyelinated (Drummond, Eng & McIntosh, 1971).

Like other myelin components, e.g. GC (see above) and myelin basic protein, CNP is found also in oligodendrocytes isolated from the nervous system (Raine, Poduslo & Norton, 1971; Poduslo, 1975; Szuchet & Stefansson, 1980). However, long term cultures of these cells do not show CNP activity. This could be due to their having dedifferentiated although they still exhibit most of the morphological characteristics of oligodendrocytes, or they could simply stop expressing this function in culture (Szuchet & Stefansson, 1980). If the latter is the case it is parallel to that of Schwann cells and the synthesis of GC (see above).

The relationship between CNP and myelin, however, is not a simple one. In a number of elasmobranch fishes, the spiny dogfish, *Squalus acanthias* (Drummond *et al.* 1971), the lemon shark, *Negaprion brevirostris*, the nurse shark, *Ginglymostoma cirratum*, the brown shark, *Carcharhinus milberti*, and the sting rays, *Dasyatis sayi* and *D. sabina* (Trams & Brown, 1974) the forebrain exhibits a higher CNP activity than more myelinated medulla or spinal cord. In the dogfish the ratio of CNP activity to cerebroside, a myelin lipid, was higher in the forebrain than in medulla or spinal cord (Drummond *et al.* 1971). A closer examination of the association of CNP with myelin in mammalian brain reveals a similar apparent anomaly. Although CNP activity is indeed higher in heavily myelinated areas than in regions containing fewer myelinated tracts, for example it is high in the spinal cord, the medulla and cerebral white matter but low in grey matter, it is not related to the amount of myelin as measured by the amount of cerebroside. The CNP/cerebroside ratio in the spinal cord of the dog is about one-fifth of that for both white and grey matter (Drummond *et al.* 1971). From these observations it may be concluded that there are regional differences in myelin composition and that CNP cannot be used as a quantitative marker for myelin.

Myelin is not homogeneous and several fractions can be isolated on sucrose gradients. Compact, multilamellate myelin separates out in the lightest fractions, whereas the heaviest fraction contains single membrane fragments which are derived from inner and outer lamellae, oligodendroglial membrane, and paranodal segments of myelin, and vesicles of unknown origin. The latter fraction has the highest CNP activity and the lowest myelin basic protein content, while compact myelin has a very low CNP activity and a high basic protein content (Shapira *et al.* 1978).

Waehneldt & Lane (1980) studied the distribution of CNP in membrane fractions during development and found that it shifted gradually to heavier fractions. They suggest that CNP is localized in oligodendroglial membranes, where it precedes myelin basic protein in development, and is gradually extruded from maturing forms of myelin. Thus in fully mature compact myelin the concentration of CNP is very low.

Very recently Wells & Sprinkle (1981) have purified rat CNP and shown that it is a basic hydrophobic protein with a MW of 48000–50000. Since it shows similar

* Note that only the cerebral and visceral ganglia of the prawn were examined. If this species is similar to others, e.g. *Leander serratus*, myelin-like wrappings may occur round axons in the optic peduncles, circumoesophageal connectives and abdominal cords. However, it is not known whether this myelin-like material shows CNP activity.

electrophoretic migration and protein band pattern as Wolfgram proteins and has similar amino acid composition they consider CNP to be a, if not the, primary component of the Wolfgram proteins W_1 and W_2 of myelin. Moreover, not only do antisera to CNP recognize both W_1 and W_2 but some CNP activity is preserved in Wolfgram proteins isolated using protective conditions. The Wolfgram proteins W_1 and W_2 have been localized in the dense line of the innermost and outermost myelin lamellae and in the plasma membrane and peripheral cytoplasm of oligodendroglial cells in rat brain by the use of immunocytochemical methods at both light and electron microscopical levels (Roussel *et al.* 1977, 1978; Mandel *et al.* 1978). Although Roussel and his colleagues (Roussel *et al.* 1978) considered the limitation of staining to the inner and outermost lamellae to be due to the inaccessibility of Wolfgram proteins in the compact central lamellae to the antibodies, in view of the finding that there is little CNP in compact myelin the demonstrated localization probably represents accurately the disposition of the proteins.

Supporting evidence for the Wolfgram identify of CNP comes from studies on acclimation to environmental temperature changes in gold fish. As may be seen from Table 1 the CNP activity of brain myelin isolated from goldfish acclimated to 5 °C is significantly higher than of that from fish acclimated to 25 °C (D. F. Matheson,

Table 1. *Effect of temperature acclimation on the CNP activity of brain myelin of goldfish*

Acclimation temperature	CNP activity* Mean ± S.D.
5 °C	$2{\cdot}337 \pm 0{\cdot}434^{6}$
25 °C	$1{\cdot}832 \pm 0{\cdot}153^{5}$

* μmoles 2′-AMP formed/min/mg myelin protein. Numbers in superscript are number of determinations. Value for 5° is significantly higher than 25 °C: $P < 0{\cdot}01$ (Student's t test, double-tailed).

R. Oei & B. I. Roots, unpublished observations). A 50000 MW protein in goldfish brain myelin which shows similar electrophoretic migration in SDS gels to a rat Wolfgram protein is also present in a larger amount in myelin from fish acclimated to 5 °C (D. P. Selivonchick, K. Fujimoto, H. C. Agrawal & B. I. Roots, unpublished observations). Since the amounts of cerebroside and total myelin protein do not change with acclimation temperature (Selivonchick & Roots, 1976), both the CNP/cerebroside and Wolfgram(?)/cerebroside ratios are greater in 5° than in 25° goldfish brain myelin. This confirms the observations made above that myelin composition may vary and that CNP activity cannot be used as a quantitative marker.

It is difficult to conceive of a function for CNP activity in myelin or indeed in any other tissue. The possibility that the substrate for CNP *in vivo* is something other than the 2′,3′-cyclic nucleotides was raised by Olafson and his associates (Olafson, Drummond & Lee, 1969). Since there are no reports of 2′-3′-cyclic nucleotides in mammalian tissues (Sims & Carnegie, 1978) this would seem to be highly probable, at least for mammals. As yet, no viable alternative substrate has been suggested. Several lines of evidence point towards a function in membrane reorganization. Firstly there is the close association of CNP activity with myelinogenesis. Secondly, it has been found that CNP activity changes in the optic nerve of goldfish concommitant with the remodelling of the nerve which occurs during acclimation to environmental temper

ure changes. With acclimation the axon diameter distribution spectrum changes, thus in the optic nerve of 5 °C acclimated fish there is a greater proportion of axons larger than 0·9 μm in diameter than in 25 °C fish nerves (cf. 71 % versus 22 %) The thickness and periodicity of the myelin also change (Matheson *et al.* 1978; D. F. Matheson, M. S. Diocee, S. T. Hussain & B. I. Roots, unpublished observations). The CNP activity is significantly higher in the optic nerves of 5° than 25 °C acclimated fish (Matheson, Oei & Roots, 1979). Thus there is both a considerable reorganization of myelin and a change in CNP activity. Finally, Sims & Carnegie (1978) report several instances of changes in CNP activity in cases where membrane structure is known to be altered, e.g. in viral transformation of normal tissue. However, there is nothing in any of these lines of evidence to preclude the possibility that CNP is purely a structural protein whose enzyme activity is coincidental. That this may not be so is indicated by some other information. CNP activity is increased two-fold in C_6TK^- and C_6DE clones of the C_6 rat glioma by the neurotransmitter norepinephrine. An effect which is mediated by a β-adrenergic receptor and an increase in intracellular cyclic AMP (McMorris, 1977). Whether norepinephrine regulates CNP activity *in vivo* is unknown. In the tobacco horn moth, *Manduca sexta*, norepinephrine causes a 133-fold increase in cyclic AMP levels in the glia. These cells also show CNP activity although at a much lower level than is found in myelin (Taylor, Dyer & Newburgh, 1976). It would be interesting to know whether or not CNP activity is also stimulated by norepinephrine in *Manduca*. There is increasing interest in the role of cyclic AMP as a second messenger and in its relationships with neurotransmitters (Van Calker & Hamprecht, 1980; Levitan & Benson, 1981). One might speculate that somehow CNP is involved in these interactions and their postulated roles in membrane phenomena, and suggest that a fresh approach to the problem of the function of CNP be made along these lines.

GLUTAMINE SYNTHETASE (GS)

Glutamine synthetase (L-glutamate: ammonia ligase; ADP-forming; EC 6.3.1.2) catalyses the biosynthetic reaction

$$\text{glutamate} + \text{ammonia} + \text{ATP} \xleftrightarrow{Mg^{2+}} \text{glutamine} + \text{ADP} + P_i.$$

This reaction serves to detoxify ammonia and is involved in the metabolism of the neurotransmitters γ-aminobutyric acid (GABA) and glutamic acid. Both these functions are associated with the smaller, fast turnover glutamate compartment of mammalian brain (Berl & Clarke, 1969), which, it has been suggested, may be represented by the glia (Balázs, Patel & Richter, 1970). Localization of GS in glia by light microscopical immunohistochemical techniques (Martinez-Hernandez, Bell & Norenberg, 1977) provided evidence that glia do indeed constitute the smaller glutamate compartment in mammalian brain. The subsequent demonstration by ultrastructural immunocytochemistry that GS is not only restricted to glia but confined to astrocytes established the enzyme as an astrocyte marker (Norenberg & Martinez-Hernandez, 1979). It is present in both fibrous and protoplasmic astrocytes, in Bergmann glia, and in Müller cells in the retina which are also astrocytic in nature (Riepe & Norenberg, 1977). High GS activity is shown also by astrocytes in culture (Schousboe, Svenneby

and Hertz, 1977). In rat brain trace amounts were also seen in some ependymal cells but not in tanycytes, thus confirming the findings from studies of GFAP that ependymal cells and astrocytes are related, but that tanycytes represent another form of ependymal differentiation. Comparative studies in a number of vertebrates, man, dog, chick, toad and goldfish on the distribution of GS have shown that in all these animals the enzyme has been confined to astrocytes or ependymo-glial cells (M. D. Norenberg, personal communication). In the lower vertebrates ependymal cells predominate and the main evolutionary development has been a reduction in their number with a progressive increase in other cell types (see Roots, 1978). Thus it would appear that astrocytes have assumed the ependymal cell functions associated with GS.

In astrocytes the enzyme is distributed throughout the cytoplasm (see Fig. 1) and is often associated with the endoplasmic reticulum. Astroglial processes surrounding synaptic endings and pericapillary end-feet (Fig. 2) are heavily stained. There are, however, significant regional variations in the intensity of astrocyte staining by immunohistochemical methods, the hippocampus and the molecular layer of the cerebellum showing the heaviest staining (Norenberg, 1979). As Norenberg points out these are areas of high glutamergic activity. There is thus a close correlation between the distribution of GS and the proposed role of astrocytes in transmitter metabolism (see Hertz, 1979 for review).

Some preliminary observations indicate that GS is localized in the perineurial glial cells of the abdominal ganglion of *Aplysia californica* (M. D. Norenberg & B. I. Roots, unpublished observations). The perineurium lies beneath the connective tissue capsule surrounding the ganglion and is composed of two kinds of glial cell. One of these resembles astrocytes in having relatively clear cytoplasm, glycogen and bundles of filaments (Colonnier, Tremblay & McLennan, 1979). It will be interesting to see whether GS is restricted to this glial cell type. There is no intraganglionic blood supply, thus the position of the perineurial cells interposed between blood and neurone is analogous to that of astrocyte feet in the mammalian nervous system.

Astrocyte feet have long intrigued scientists, beginning with Cajal who envisaged astrocytes pulling on the walls of capillaries with their end feet thus enlarging the lumen and increasing blood flow. Suggestions that the endfeet constitute a barrier between brain and blood have been discounted on many grounds, including the fact that they do not surround the capillaries completely, there being a few oligodendroglial and neuronal processes as well as extracellular space. Nevertheless it is tempting to think that the astrocyte feet have a special functional, as well as morphological, relationship with blood vessels. The level of ammonia and glutamate is closely regulated in the brain and the finding that GS, which is involved in the metabolism of both of these substances, is prominent in astrocytic feet lends credence to the idea of a special functional relationship. The location of GS in perineurial cells which in *Aplysia* occupy a position analogous to astrocyte feet is yet another tantalizing piece to fit in the puzzle.

CONCLUSIONS AND PERSPECTIVES

The development of immunocytochemical methods in conjunction with glial markers has led to significant advances in knowledge of cell differentiation and of the

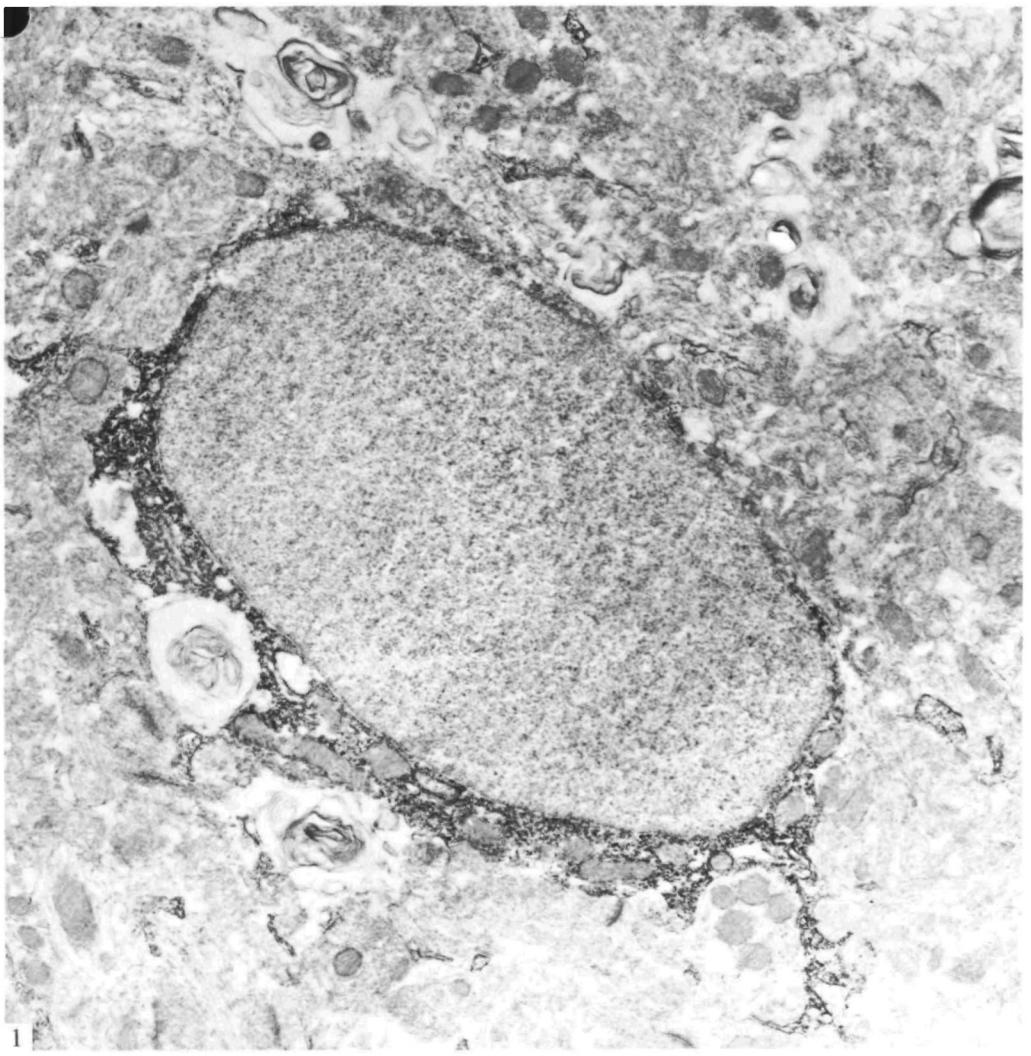


Fig. 1. Astrocyte in rat corpus striatum. The dense granules seen throughout the cytoplasm are reaction product indicating the presence of glutamine synthetase. × 14450. (M. D. Norenberg original.)

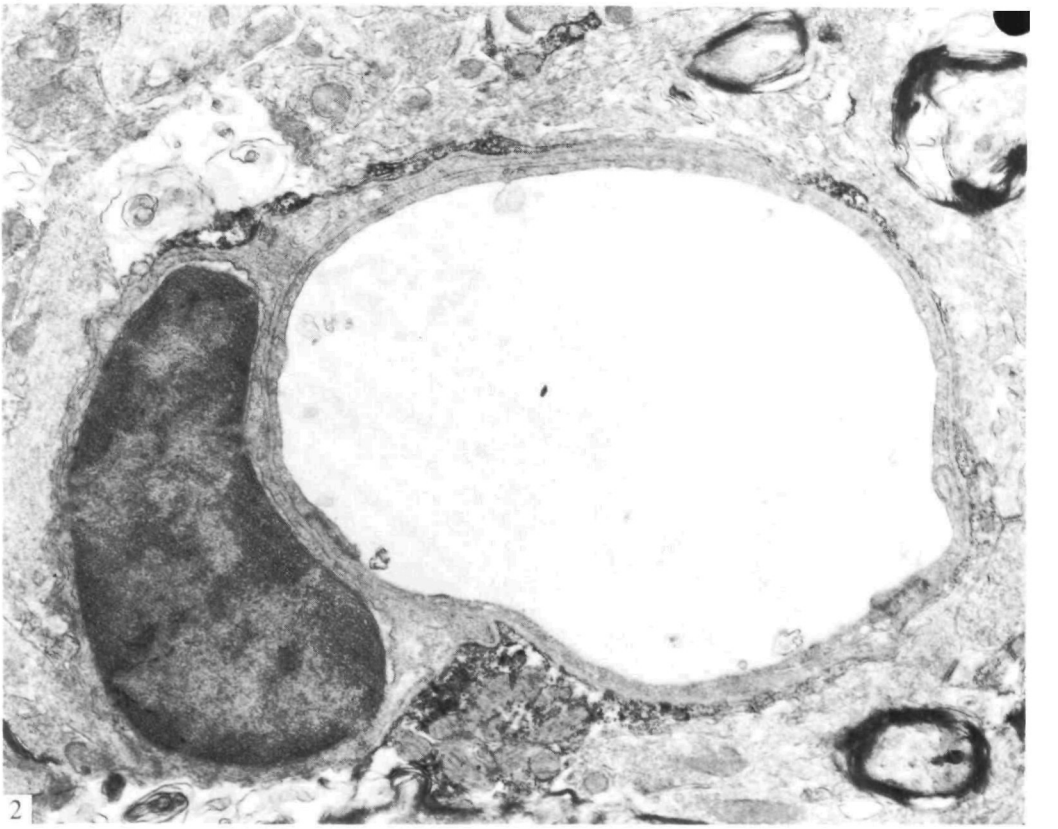


Fig. 2. Capillary surrounded by astrocyte-feet containing reaction product indicating glutamine synthetase. × 15300. (M. D. Norenberg original.)

ble of glia in the development of the nervous system. An outstanding example is the study of the role of radial glia during the development of Rhesus monkey brain. It is to be expected that many more studies will be made using similar techniques.

Another fruitful line of research using markers, which has barely begun, is the study of the effects of hormones and transmitters on glial cells. Such studies, as the example of the induction of CA by norepinephrine shows, may give clues as to how neuronal activity may be linked metabolically to that of glia. The pursuit of this line of enquiry has exciting prospects.

Finally, there is the vast potential of comparative studies yet to be realized.

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