cretory granule (*10*) during axonal transport, one might expect to find previously unreported labeled peptide products transported to the pituitary as well. Indeed, we have detected, in addition to labeled oxytocin and vasopressin, at least four major ^{35}S-labeled peptides (ranging between 700 and 2,500 daltons) that are transported to, stored in, and released by the posterior pituitary (*11*). The release of these peptides, which appear to be derived from the putative precursor, raises the question of whether they mediate some still unknown function of the posterior pituitary.

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6. The acid-urea polyacrylamide gels were prepared as described by Y. P. Loh and H. Gainer [*Brain Res.* **92**, 181 (1975)] except that the separating gel contained 15 percent acrylamide and 0.1 percent Triton X-100. Electrophoresis was toward the cathode, and the running *p*H of the gel was 2.7.
7. The appearance of labeled proteins in the posterior pituitary 1.5 hours after injection of [^{35}S]cysteine in the supraoptic nucleus of the rat is consistent with the time course found by A. Norström and J. Sjostrand [*J. Neurochem.* **18**, 29 (1971)] after supraoptic nucleus injection and by B. T. Pickering and C. W. Jones [in *Subcellular Organization and Function in Endocrine Tissues*, H. Heller and K. Lederis, Eds. (Cambridge Univ. Press, New York, 1971), pp. 337–351)] after intracisternal injection of the radioactive amino acid. In addition, the kinetics of appearance of labeled protein in the supraoptic nucleus and median eminence (*11*) are consistent with the axonal transport rates of protein found in the rat hypothalamo-neurohypophyseal system [tabulated by H. Valtin, J. Stewart, and H. W. Sokol, in *Handbook of Physiology*, E. Knobil and W. H. Sawyer, Eds. (American Physiological Society, Washington, D.C., 1974), sect. 7, vol. 4, pp. 131–171].
8. Twelve hours before bilateral injection of [^{35}S]cysteine into the rat supraoptic nucleus, approximately 100 μg of colchicine dissolved in 0.9 percent NaCl was injected intraventricularly. This treatment completely prevented the transport of labeled proteins from the supraoptic nucleus to the median eminence and posterior pituitary. Similar effects of colchicine were demonstrated by A. Norström [*Ann. N.Y. Acad. Sci.* **248**, 46 (1975)] and H. Sachs, D. Pearson, and A. Nureddin (*ibid.*, p. 36).
9. The apparent molecular weights of the putative precursor and neurophysin peaks were calculated to be 20,000 ± 2,000 and 12,000 ± 2,000 daltons, respectively. The three methods used for these determinations included sodium dodecyl sulfate (SDS) gel electrophoresis, Ferguson plot analysis of acid-urea gels, and Sephadex G-75 gel chromatography (*11*).
10. The fact that the vasopressin content of secretory granules is fivefold greater in the neurohypophysis than in the hypothalamus [H. Sachs, *J. Neurochem.* **10**, 289 (1963)] led to the suggestion that this peptide is generated from the precursor protein in the axonal region between the neuron perikarya in the hypothalamus and the nerve terminals in the neurohypophysis. Morphological observations indicating a "maturation" of the secretory granule during axonal transport have been reported by M. A. Cannata and J. F. Morris [*J. Endocrinol.* **57**, 531 (1973)] and J. F. Morris and M. A. Cannata (*ibid.*, p. 517). The possible relationship between this morphological "maturation" and an intragranular conversion of precursor protein to neurophysin and peptides is discussed by B. A. Cross, R. E. Dyball, R. G. Dyer, C. W. Jones, D. W. Lincoln, J. F. Morris, and B. T. Pickering (*Recent Prog. Horm. Res.* **31**, 243 (1975)].
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12. The Coomassie blue staining pattern of proteins extracted from the neurohypophysis and separated on acid-urea gels reveals a number of stained bands; the most rapidly migrating of these bands corresponds in mobility to the labeled peak (insets, Fig. 2). This rapidly migrating stained band is the one which dramatically decreases in intensity when rats are given 2 percent saline to drink for 5 days (*11*). This indicates that it is this protein (or proteins) which is released by the pituitary during osmotic stress. The labeled neurophysin, which consistently migrates as a single peak on acid-urea gels (and SDS gels), may in fact be heterogeneous, since separation of the labeled posterior pituitary proteins by isoelectric focusing revealed two major peaks of radioactivity with isoelectric points of 4.6 and 4.8 (*11*). Using another gel system, G. D. Burford and B. T. Pickering [*Biochem. J.* **128**, 941 (1972)] reported the transport of two major peaks of labeled neurophysin to the rat neurohypophysis after intracisternal injection of [^{35}S]cysteine.
13. The labeled putative precursors and products (corresponding to peaks in slices 31 and 43, respectively) in Figs. 1 and 2 were specifically precipitated by antibodies to rat neurophysin (antibodies were obtained from Dr. A. G. Robinson). The labeled proteins of higher molecular weight (such as those in slice 10 in Fig. 1) were not precipitated by antibody.

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Glutamine Synthetase: Glial Localization in Brain

Abstract. *Light microscopy immunohistochemical techniques were used to examine the distribution of glutamine synthetase in rat brain. Glutamine synthetase was found to be localized in the glial cells. Neuronal cell bodies, endothelial cells, and choroid epithelium contained no enzyme. The findings indicate that glia have a crucial role in glutamic acid, γ-aminobutyric acid, and ammonia metabolism in brain.*

Glutamic acid participates in a number of important metabolic processes in brain (*1*) and may also serve as an excitatory neurotransmitter (*2, 3*). It is generally accepted that glutamate metabolism in brain is compartmentalized (*4*). A large compartment has been linked with energy metabolism, whereas a small compartment with a fast turnover has been associated with glutamine synthesis, ammonia detoxification, and metabolism of the putative inhibitory neurotransmitter, γ-aminobutyric acid (GABA). The site of these metabolic compartments has not been established, although indirect studies suggest that glia may contain the small glutamate compartment (*5*).

Glutamine synthetase (E.C. 6.3.1.2) is a key enzyme of the small glutamate compartment which catalyzes the amidation of glutamate to glutamine (*1*). If the location of this enzyme could be determined, then this would provide strong evidence for the site of the small compartment. Glutamine synthetase is present chiefly in the microsomal fraction of brain (*6*), although a small amount has also been found in the synaptosomal fraction (*7*). Attempts to determine the location of glutamine synthetase more precisely by bulk cell isolation techniques have been unsuccessful (*8*). By using immunohistochemical techniques specific for glutamine synthetase, we have found that the enzyme is localized in the glial cells of rat brain.

Three adult albino rabbits were immunized by weekly subcutaneous injections of 0.1 mg of glutamine synthetase (purified from sheep brain, Sigma G-6632, lot 35C-8650, 120 unit/mg) dissolved in 0.5*M* phosphate-buffered saline, *p*H 7.4, and mixed with Freund's adjuvant (1 : 1 by volume). Serum was collected weekly, and at the end of 8 weeks the rabbits were killed. A rat brain extract containing glutamine synthetase antigen was obtained from the supernatant fraction by homogenization in sodium barbital buffer, *p*H 8.6, containing 1 percent Triton X-100, and subsequent centrifugation at 40,000*g* for 1 hour. The rabbit antiserum was characterized against both sheep glutamine synthetase and the rat brain extract by double immunodiffusion and immunoelectrophoretic studies (*9*). By immunodiffusion, both antigens (from sheep and rat) gave a single fused precipitin line indicating antigenic identity (Fig. 1A). Immunoelectrophoresis demonstrated the monospecificity of the

antibody (Fig. 1B). We took advantage of this antigenic identity between rat and sheep glutamine synthetase and used rat as the experimental animal.

Rats were killed by decapitation, and tissue blocks of cerebral cortex, basal ganglia, and cerebellum were immediately frozen. Sections (10 μm thick) were cut and then stained with the peroxidase-labeled antibody by means of the indirect method. The procedures used for conjugation and incubation were described previously (*10*). To avoid the background normally found when antiserum is reacted with nervous tissue (*11*), the antibody was purified by affinity chromatography (*12*). Normal rabbit serum and acrylamide-purified rabbit antibody to mouse basement membrane (*13*) were used as controls.

The enzyme-labeled antibody method disclosed that all positive cells were glial cells (Fig. 2), but it could not be determined whether the positive cells were astrocytes or oligodendroglia, or both. In glia of gray matter, the antigen had a perinuclear distribution and was usually more intense toward one pole of the cell (Fig. 2C). In white matter, a perivascular distribution of the positive glial cells was more evident. In addition, there was a more diffuse and dense cytoplasmic reaction in the perivascular cells that obscured nuclear detail (Fig. 2D). Occasionally, a small amount of stain was observed adjacent to vessel walls. In all areas studied, neurons, endothelial cells, and choroid plexus epithelium were consistently negative. In control sections reacted with the acrylamide-purified antibody to basement membrane, reaction product was exclusively restricted to the vascular basement membrane. Sections reacted with normal rabbit serum showed a slight nonspecific reaction in white but not in gray matter glia.

Our finding that glutamine synthetase is localized in glia indicates that the small glutamate pool associated with glutamine synthesis resides in glia. This finding substantiates the view that glia participates actively in glutamic acid and GABA metabolism (*8, 14, 15*). Glutamic acid and GABA are presumed to be neurotransmitters (*2, 3*). Therefore, glial regulation of these amino acids may provide a way for glia to modulate brain excitability, a view proposed by several investigators (*2, 14, 16*).

In the nervous system, glutamine formation is the principal pathway for ammonia elimination (*17*). Consequently, our studies also signify that glia are responsible for ammonia detoxification. This function is critical, because ammonia, although a normal by-product of brain metabolism (*18*), is toxic in high concentrations, causing seizures or coma (*19*). Moreover, increased ammonia in the blood has been implicated in the pathogenesis of human hepatic coma (*20*). This and other disorders in humans, as well as in experimental animals, in which hyperammonemia is present are characterized by a glial alteration known as the Alzheimer type II change (*21*). While the precise significance of the Alzheimer type II change is unknown, the findings of this study serve to link further this glial alteration to abnormalities in ammonia metabolism.

The findings of this study are also germane to epileptogenesis. Abnormal glia (*22*) and irregularities in glutamic acid, GABA, and ammonia metabolism (*3*, p. 141; *23*) have been associated with seizures. Impaired glial regulation of these metabolites may represent one mechanism by which glial abnormalities result in seizures. This view correlates well with the preictal glial morphological change observed in rats following the administration of the convulsant methio-

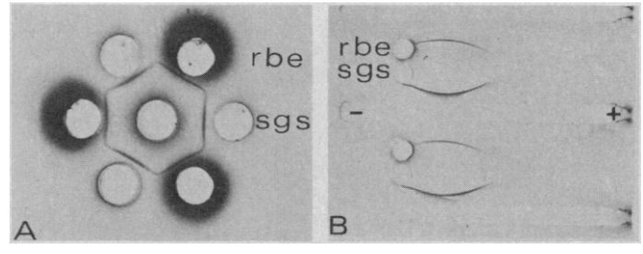

Fig. 1. Immunologic comparison of purified sheep brain glutamine synthetase (*sgs*) and a rat brain extract (*rbe*). (A) Immunodiffusion: 1 percent agarose in barbital buffer, *p*H 8.6, containing 1 percent Triton X-100. Antiserum in center well. A single precipitin line is obtained against both antigens, and the fusion of this line indicates antigenic identity. (B) Immunoelectrophoresis of *sgs* and *rbe* in barbital buffer, *p*H 8.6; electrophoresis at 25 volts for 2 hours. A single precipitin line indicates a monospecific antiserum. Immunoelectrophoresis and immunodiffusion plates stained with 0.5 percent Coomassie blue.

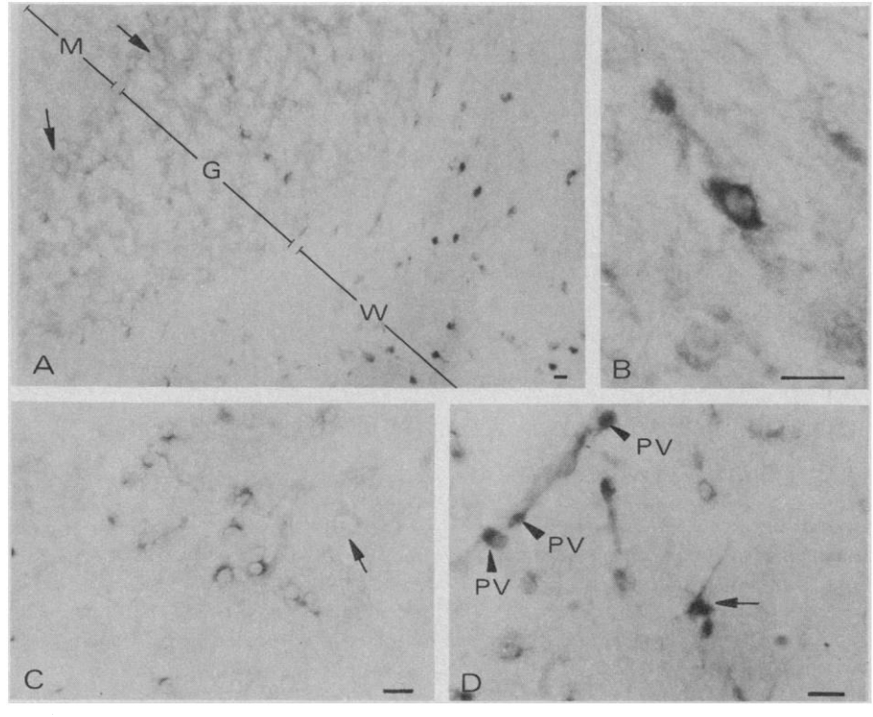

Fig. 2. Photomicrographs of frozen sections of rat brain stained for glutamine synthetase by means of peroxidase-labeled antibody. Scale bar, 20 μm. (A) Cerebellum (*M*, molecular layer; *G*, granule cell layer; *W*, white matter). Numerous positive-staining cells are present in the white matter, while only isolated positive-staining cells are found in the granule cell layer. Purkinje cells (arrows) are negative. No positive-staining cells are present in the molecular layer. (B) Basal ganglia illustrating a glial cell with strongly positive cytoplasmic reaction. (C) Basal ganglia showing numerous positive-staining glial cells. Note the polarity of the cytoplasmic stain. A neuron (arrow) contains no reaction product. (D) White matter. Perivascular cells (*PV*) with a strong reaction product obscuring nuclear detail. Abundant reaction product is evident in a cell with radiating cytoplasmic processes characteristic of astrocytes (arrow).

nine sulfoximine (*24*), whose primary biochemical effect appears to be the inhibition of glutamine synthetase (*25*).

Few definitive data are available regarding glial physiology. The finding that glutamine synthetase is restricted to glia defines a metabolic function for these cells and may clarify their role in certain neurologic disorders of man.

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Visual Pigment Changes in Rainbow Trout in Response to Temperature

Abstract. *Lower water temperature (6°C in comparison to 16°C) favors a higher proportion of porphyropsin in the retina of rainbow trout (*Salmo gairdneri*), regardless of the light conditions (constant darkness or 12 hours of light and 12 of darkness). This response to temperature does not follow a* Q_{10} *relation, namely an increase in the rate of a reaction produced by raising the temperature 10°C.*

Many species of fishes have paired visual pigments in their retinas (*1, 2*). The visual pigment composition in several of the paired-pigment species changes seasonally or during the spawning migration (*2*).

Both light (quality, intensity, and photoperiod) and temperature have been implicated as factors involved in changes in visual pigment composition (*2–8*). Light (as compared with darkness) is associated with decreases in the percentage of porphyropsin in some of these species [rudd, *Scardinius erythrophthalmus*; golden shiner, *Notemigonus crysoleucas*; and common shiner, *Notropis cornutus* (*3, 4, 6, 8*)] and increases in others [redside shiner, *Richardsonius balteatus*; rainbow trout, *Salmo gairdneri*; and brook char, *Salvelinus fontinalis* (*5, 7*)]. On the other hand, higher temperatures favor decreases in the proportion of porphyropsin in golden shiner (*6*), rainbow trout, and common shiner (*8*). From this, it has been suggested that temperature is an important factor in the similar seasonal alteration of visual pigment composition (higher porphyropsin in winter) in species that do not respond in the same way to light and darkness (*7*).

A question with respect to the effect of temperature is whether temperature directly influences a change in visual pigment composition or only modifies the increasing rate of a chemical reaction with each 10°C increase in temperature (a Q_{10} effect) irrespective of the direction of the change (relative increase or decrease in porphyropsin). We now report evidence that temperature is directly associated with a change in the molar percentage of porphyropsin in rainbow trout.

Juvenile rainbow trout (35 to 140 g) were obtained from the Alberta Provincial Government Rearing Station at Crammond, Alberta, in January 1975. The water temperature at the rearing station was 5°C. The fish were divided into two groups; one group was used immediately in the first set of temperature experiments, and the other group was exposed to constant darkness at 16°C for 30 days. During the experiments, the fish were held in 120-liter aquamarine, fiber glass tanks covered with recessed, lightproof lids painted white on the inside. For fish exposed to light, two 20-watt fluorescent tubes (General Electric Cool White) were placed in the top of the lid, and the photoperiod, 12 hours of light and 12 hours of darkness (LD 12 : 12), was controlled by an automatic timer (Tork). A 7.5-watt red bulb was used in the continuously dark tanks for daily (1- to 2-minute) inspection of the fish. The total radiant energy at the water surface in the illuminated tanks, recorded from the remote probe of a spectroradiometer (Isco model SR), was from 50 to 87 μw/cm^2 over the range of 380 to 750 nm. For the red bulb it was very low (0.6 to 0.9 μw/cm^2 from 380 to 750 nm). Water temperature was controlled by a thermoregulator (CanLab C-Series, T3345) in combination with a relay connected to a solenoid valve on a warm (18°C) water line. Cold (5°C) water constantly flowed into the tank, and the desired temperature (6° or 16°C) was attained by adding warm water from the controlled solenoid valve. Total flow was adjusted to provide about 150 liter/hour. Fish were not fed during any experiment.

At the beginning of each set of experiments, the trout were dark-adapted for 2 hours and anesthetized with tricaine