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Immunohistochemical localization of intracellular plasma proteins in the human central nervous system

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Summary. The regional distribution of plasma protein immunoreactivity was studied in the postmortem central nervous system (CNS) of normal subjects 18 to 78 years old. Samples taken from various areas of brain and spinal cord were processed for peroxidase-antiperoxidase immunocytochemistry using polyclonal antibodies against plasma albumin, prealbumin, α_1-acid glycoprotein, α_2-macroglobulin, IgG, transferrin, haptoglobin, hemopexin, fibrinogen, as well against the glial fibrillary acidic and S-100 proteins. Many neurons of the spinal cord, cranial nerve nuclei, pontine nuclei, cerebellar dentate nucleus, red nucleus, thalamus and hypothalamus showed strong immunostaining for albumin and moderate to strong staining for α_1-acid glycoprotein, IgG, transferrin, haptoglobin, as well as relatively weak immunoreactivity against other plasma proteins. Less intense staining was seen in the nucleus basalis, putamen and Purkinje cells. In contrast, most cerebral cortical neurons were negative except for a few positively stained pyramidal neurons in the hippocampus and in layers III and V of the association neocortex, although more positive pyramidal neurons were observed in the motor and sensory neocortices. Reaction products were also seen in axons of motor and sensory long tracts. These findings suggest that plasma proteins may be transported to spinal cord and brain stem neurons by peripherally projecting nerves and that a series of anterograde and retrograde transneuronal transfers are responsible for the accumulation of plasma proteins in relay nuclei and in other CNS neurons.

Key words: Plasma protein – Brain – Blood-brain barrier – Neuron – Aging

The blood-brain barrier (BBB) normally restricts the passage of proteins and other large molecules from the blood into the central nervous system (CNS) [24, 25]. Conditions commonly associated with BBB breakdown are infarcts, trauma, cold injury, hypertension, lead poisoning, seizures and osmotic shock [2, 7, 13, 21, 23, 26, 32]. Immunohistochemical studies of brain infarcts and traumatic lesions have demonstrated that the bulk of the extravasated plasma proteins are endocytosed by reactive astrocytes and by a few neurons in the vicinity of the lesion [14, 16, 31]. Within astrocytes, plasma proteins co-distribute with glial fibrillary acidic protein (GFAP) and the S-100 protein [19].

Despite the traditional view that the occurrence of plasma proteins in brain parenchyma is pathological, several recent studies have demonstrated accumulation of plasma proteins in spinal cord neurons, brain stem nuclei and diencephalic nuclei in normal fetal and adult rat brain [8, 11, 20, 30]. However, it is not known if plasma proteins are found in the normal human CNS. An understanding of the occurrence and distribution of plasma proteins in the normal human CNS is important in interpreting the significance of plasma protein staining reported in brains of the elderly and in patients with Alzheimer's disease [1, 22, 33]. In the present study, we investigated the regional distribution of plasma protein immunoreactivity in formalin-fixed normal postmortem human CNS tissue. The mechanisms responsible for plasma protein accumulation and the possible physiological significance of these findings are discussed.

Materials and method

Postmortem samples of brain and spinal cord were obtained from 20 normal subjects ranging in age from 18 to 78 years. The causes of death were sudden cardiac arrest, stab wounds of the chest or rapid asphyxia. No subject had a history of prolonged illness, coma, neurological disease, head trauma, or alcohol or drug abuse. The interval between death and autopsy ranged

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from 5 to 12 h. The brains and cervical spinal cords were fixed in 20% buffered formalin for 10–24 days and samples were taken from the following regions: cerebral cortex (mid-frontal, superior temporal, motor, sensory, posterior parietal, and calcarine), hippocampus, amygdala, basal ganglia, nucleus basalis, thalamus, hypothalamus, mammillary bodies, midbrain, pons, medulla, cerebellum, corpus callosum and cervical spinal cord.

Immunohistochemistry

Sections were incubated in 3% H_2O_2 for 15 min to eliminate endogenous peroxidase, then in 3% goat serum for 30 min to block nonspecific binding. Sections were then incubated, with a 15-min rinse with phosphate-buffered saline (PBS) between incubations, in the following solutions: (a) primary polyclonal antibodies (1:500 to 1:2,000 dilutions: Dako Co.) to plasma proteins (albumin, prealbumin α_1-acid glycoprotein, α_2-macroglobulin, IgG, transferrin, haptoglobin, hemopexin and fibrinogen) or glial marker proteins (GFAP, S-100), for 16 h at 4°C; (b) goat anti-rabbit IgG (1:100 dilution), 40 min at 25°C; and (c) rabbit peroxidase-antiperoxidase (PAP) (1:100 dilution), 40 min at 25°C. The sections were washed for 10 min in PBS and were reacted with 3-amino-3-ethylcarbozol in 0.03% H_2O_2 for 15 min, counterstained with hematoxylin and mounted.

Results

The immunoreactivity of neurons was consistently strongest using albumin antisera, whereas staining using α_1-acid glycoprotein, IgG, transferrin and haptoglobin antisera was moderate. Staining for prealbumin, α_2-macroglobulin, hemopexin and fibrinogen was relatively weak. Astrocytes, identified by the positive staining for GFAP and S-100, were positively stained for plasma proteins. The intensity of intraglial plasma protein immunoreactivity was comparable to that observed intraneuronally (e.g., in both glia and neurons immunostaining of albumin was strong whereas that for prealbumin was weak).

Intraneuronal plasma proteins

The regional distribution of plasma protein immunoreactivity is illustrated in Fig. 1, and the estimated percentage of positively-staining neurons in each region is presented in Table 1.

Cerebral cortex. Reaction products to antisera against plasma albumin, α_1-acid glycoprotein, IgG, transferrin and haptoglobin were seen in a few pyramidal neurons in layers III and V of the cerebral cortex of normal adult brains (Fig. 2). Betz cells in the motor cortex and large pyramidal cells in the sensory cortex were more frequently and heavily stained than were neurons in other areas of the cerebral cortex. In the hippocampus, groups of large pyramidal neurons were stained. No substantial age-related differences in the number of immunoreactive neurons or the relative intensity of staining were observed.

Diencephalic nuclear groups. The largest amount of intraneuronal plasma protein was observed in the thalamus and red nucleus (Table 1). Thus, nearly all the neurons in the ventral lateral thalamic nuclei and

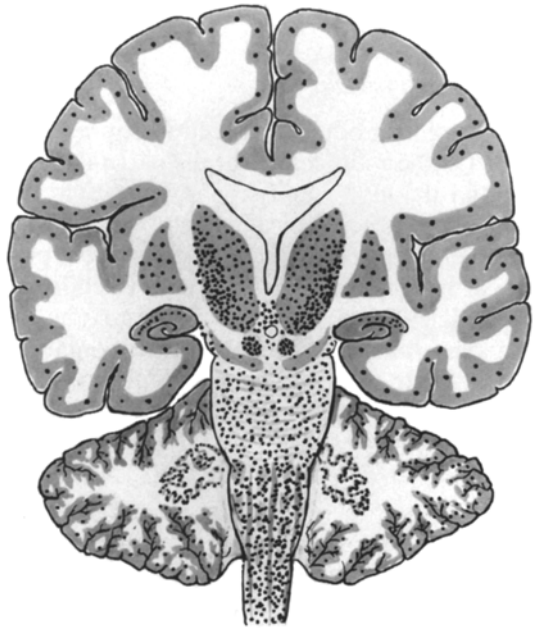


Fig. 1. Schematic representation of the distribution of intraneuronal plasma protein immunoreactivity in various regions of normal human brain and spinal cord

Table 1. Percentage of neurons immunostained for plasma proteins

Region		Immunostained neurons (%)
Cerebral cortex		1
Hippocampus		2– 5
Diencephalic nuclei	N. Basalis	10– 40
	Caudate N	5– 10
	Putamen	20– 25
	Globus pallidus	50– 70
	Thalamus and hypothalamus	75– 90
	Red nucleus	100
	Substantia nigra	5– 10
	Mammillary body	10– 20
	Lateral geniculate N	80– 90
Cerebellum	Dentate N	80– 90
	Purkinje cells	25– 75
	Granular cells	0
Pons	Cranial N	75– 80
	Pontine N	80– 90
Medulla	Cranial N	85– 90
	Olivary N	5– 10
Spinal cord	Motor neurons	90–100

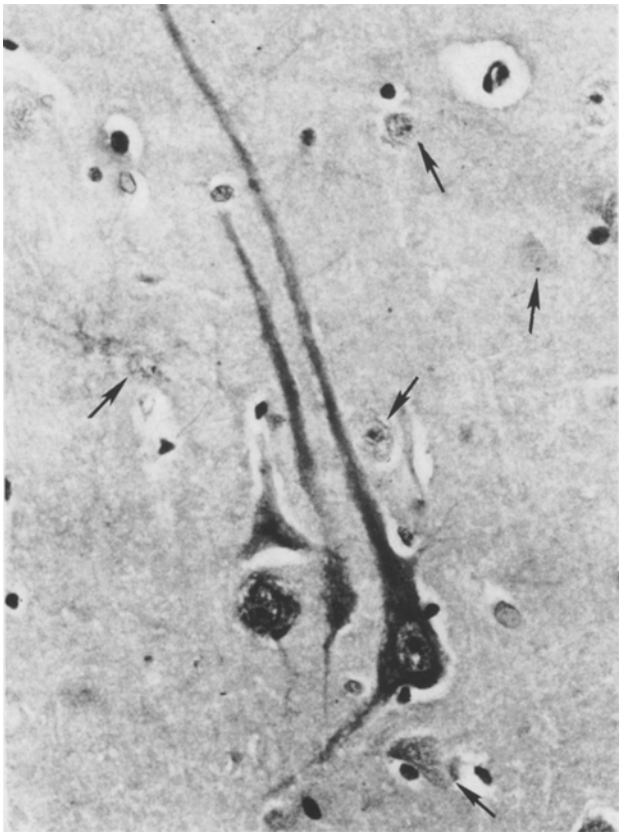


Fig. 2. Parietal lobe cortex stained with anti-albumin serum to show the presence of plasma albumin in a few large pyramidal neurons. Unstained neurons are indicated by *arrows*. The pattern of neuronal staining was consistent throughout the CNS, i.e., immunopositive neurons always stained strongest with antisera against albumin (+ + +), there was moderate (+ +) staining using α_1-acid glycoprotein, IgG, transferrin, and haptoglobin antisera whereas the staining of prealbumin, α_2-macroglobulin, hemopexin and fibrinogen antisera was weak (+)

approximately two-thirds of those in the medial thalamic nuclei were positively stained (Fig. 3). There was also consistent positive staining of neurons in lateral geniculate bodies, globus pallidus, hypothalamus and the preoptic and paraventricular nuclei. Less intense staining was seen in neurons of nucleus basalis and putamen. Weak intraneuronal staining was observed in the caudate nucleus, mammillary bodies and substantia nigra. As with the cortical neurons, no clear age-related changes were seen.

Cerebellum. Nearly every neuron in the dentate nucleus of all brains showed positive staining for albumin, α_1-acid glycoprotein, IgG, transferrin and haptoglobin. The amount of reaction product in Purkinje cells was less consistent, with positively stained cells in some, but not all, cases (Fig. 4). The granular cells were always unstained.

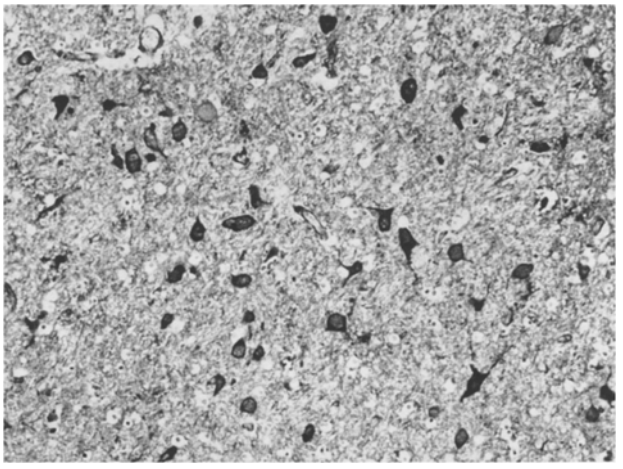


Fig. 3. A group of thalamic neurons the majority of which are stained with anti-albumin serum

Pons. Nearly all of the neurons in the III, IV and V cranial nerve nuclei and in the pontine nuclei consistently showed strong staining. Many axons in the corticospinal tract, the medial and lateral lemniscus and the medial longitudinal fasciculus were also strongly stained.

Medulla. There was strong staining of most neurons in the cranial nerve nuclei. Some of the neurons in the olivary nucleus also showed moderate staining.

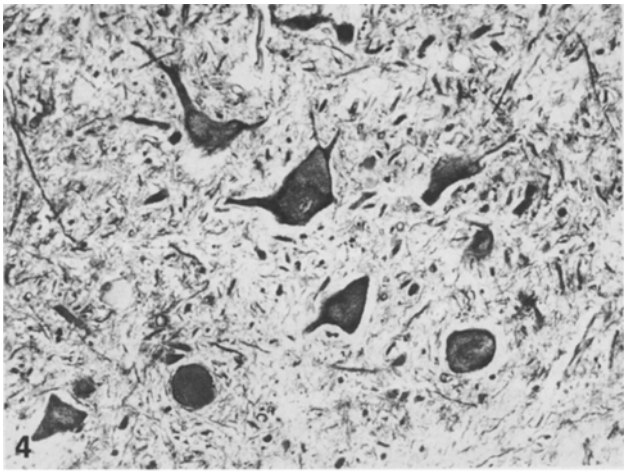
Spinal cord. Almost all of the motor neurons and neurons in Clark's column were strongly stained (Fig. 5). There was also strong staining of neurons in the dorsal horns.

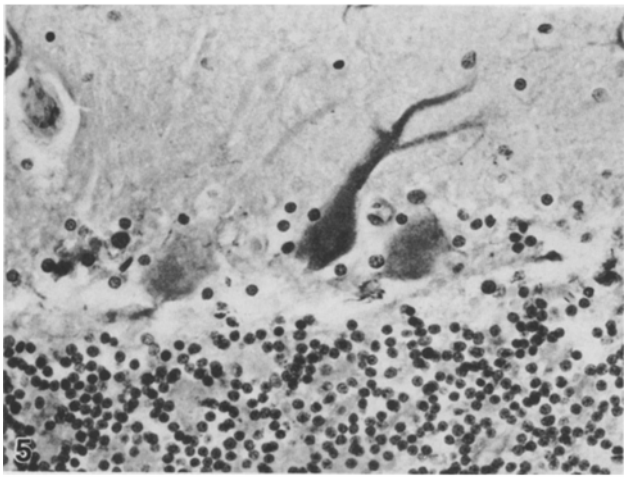
Intraaxonal plasma proteins

Abundant reaction product was seen in some large-caliber axons in the white matter of the parietal lobes, in and around the motor and sensory cortex. Many thick axons in the corpus callosum were likewise stained. Numerous positively stained axons were present in the corticospinal and corticobulbar tracts in the cerebral peduncle (Fig. 6). In the pons, approximately half of the axons in the corticospinal tract contained reaction product. In the medulla, most of the axons in the pyramids and in the lateral lemniscus were stained.

Intraglial plasma protein immunoreactivity

In all of the brains, the subpial and subependymal astrocytes showed strong staining for albumin, α_1-acid glycoprotein, IgG and transferrin (Fig. 7). The distribution of intraglial plasma protein was similar to that of GFAP and the S-100 protein at the light microscopic level. In brains from young and middle-aged individuals, the astrocytes in gray and white mat-





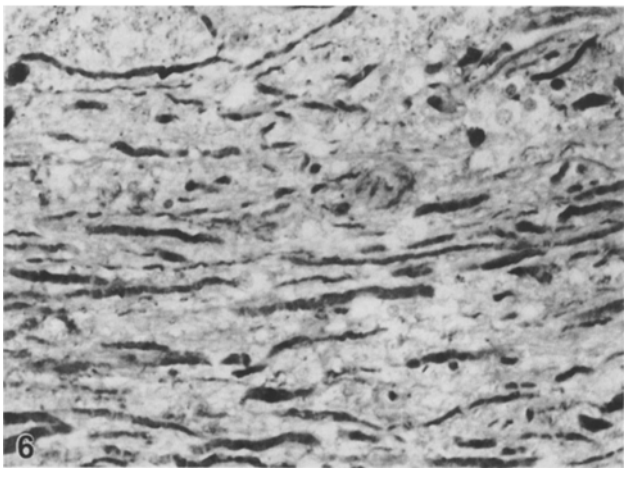


Fig. 4. Cerebellar cortex stained with anti α_1-acid glycoprotein serum. Different neurons contain various amounts of the protein

Fig. 5. Spinal cord motor neurons showing plasma albumin immunoreactivity in all neurons as well as in axons of the anterior horn

Fig. 6. Corticospinal tract at the midbrain level to show plasma albumin immunostained thick axons

Fig. 7. Subpial region of the medulla to show a zone of albumin-immunostained astrocytes and glial process in the subpial region.

ter were relatively small in size and showed relatively weak immunostaining for all the plasma proteins as well as GFAP and the S-100 protein (Fig. 8A). With aging, there was an increase in the number and size of the fibrous astrocytes (Fig. 8B). These cells stained strongly positive for GFAP and the S-100 protein.

Discussion

Intracellular plasma protein immunoreactivity has been demonstrated in the brain, spinal cord and sensory ganglia of developing animals, including humans, monkey, sheep, rat, mouse and chicken [8, 11, 20, 30]. It has been suggested that an immature BBB is responsible for the penetration of plasma proteins into the CNS [8, 11]. However, during late fetal stages (60 days in sheep embryos), when the BBB has reached maturity, aggregates of positively stained neurons are still visible in thalamic, hypothalamic and brain stem nuclei [8]. These findings suggest that the intracellular plasma proteins may be important in brain development [8, 11].

In adult animals, plasma proteins are regularly seen in perikarya of some neurons whose axons either project outside of the CNS or terminate in the

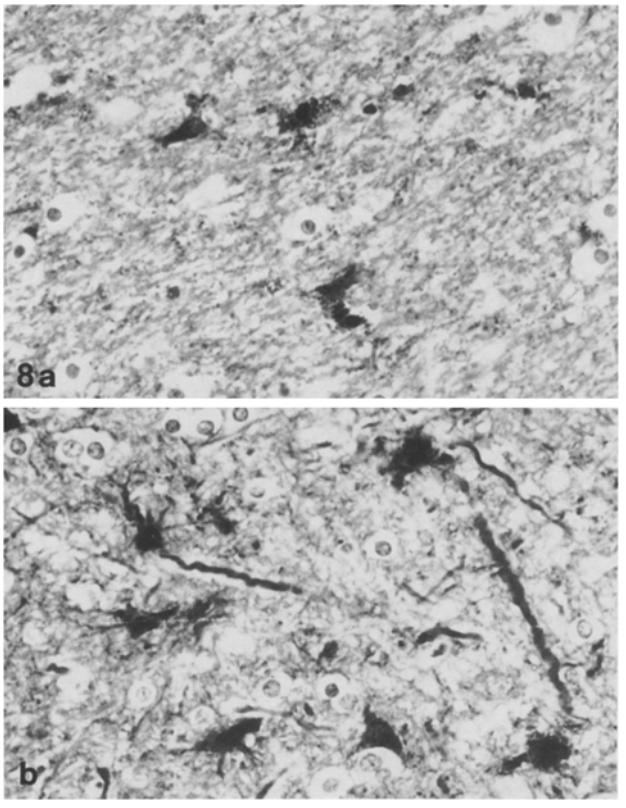


Fig. 8 **A** Parietal lobe white matter from a 27-year-old person. The glial cells have a relatively small cytoplasm which showed moderate immunostaining for albumin. **B** Parietal lobe white matter from a 78-year-old person showing an increase in number and size of the fibrous astrocytes. These cells contain strong plasma albumin immunoreactivity as compared with younger subjects

circumventricular organs, where the BBB does not exist [30]. Thus, motor neurons of the spinal cord, and neurons of the cranial nerve nuclei in the medulla and the hypothalamic supraoptic, paraventricular, and arcuate nuclei regularly contain plasma protein immunoreactivity. These findings suggest that plasma proteins may reach neuronal perikarya by retrograde transport subsequent to endocytosis by nerve terminals at sites where the passage of plasma proteins from the blood is not as restricted as at the BBB [30]. Furthermore, transneuronal transfer may be responsible for the presence of plasma proteins in neurons not in direct communication with the peripheral tissues.

The concept of retrograde transport followed by transneuronal transfer has been supported by findings from experimental tracer studies [5, 9, 15, 28, 29]. Thus, it has been shown that following intravascular injections of horseradish peroxidase in mice, the tracer passes rapidly across permeable vessels in muscle, peripheral nerve ganglia, and in the circumventricular organs in the brain [5]. Within 16 h, the tracer appears in neurons of the hypothalamus, cranial nerve nuclei III, V, VI, VII, X, XII, nucleus ambiguus and in the ventral horns of the spinal cord at all levels [5]. The same findings were obtained by intraperitoneal injection of IgG in rats [9]. With longer survival time (3–5 days), the tracer appears in Purkinje cells, pons and diencephalic neurons, presumably by transneuronal transfer [9].

In the present study, the distribution of intraneuronal plasma proteins in human CNS was found to be similar to that reported for experimental animals following intravascular or intraperitoneal injections of protein tracers. In addition, we observed strong plasma protein immunoreactivity in neurons of the thalamus, red nucleus and cerebellar dentate nucleus, not previously reported in experimental animals. Further, we found plasma proteins in large axons of the motor and sensory long tracts at the brain stem level, in the parietal lobe white matter and in the corpus callosum. More pyramidal neurons in motor and sensory cortices and in hippocampus contained plasma proteins than did neurons in other areas of the cerebral cortex. These findings are consistent with a transneuronal transfer of plasma proteins from brain stem and spinal cord neurons via the relay nuclei to the cortical and other CNS neurons [6, 10].

The observation that the subependymal and subpial astrocytes in all age groups were strongly immunostained suggests that these astrocytes endocytose plasma proteins normally present in the cerebrospinal fluid (CSF). This interpretation is based on the findings that proteins and dyes injected into the lateral ventricles can be taken up by neurons and glia located along the perivascular spaces [4] and that cerebellar Purkinje neurons readily accumulate small and large molecules that enters CSF from blood [3]. With respect to CSF plasma protein, the relatively selective regional distribution of plasma protein immunoreactivity in the human CNS suggests that the staining observed in the present study does not merely reflect uptake of proteins normally present in the CSF (and therefore also present in the extracellular fluid).

The possibility that the observed intracellular uptake of plasma protein is the result of a postmortem artifact must be considered; plasma proteins could have escaped into the neuropil and been passively absorbed by the neurons during the postmortem interval. However, their selective localization in certain neuronal groups, which is similar to that reported in experimental animals is strong evidence that these findings are not artifactual.

Our findings of extensive plasma protein immunoreactivity in the normal CNS at all ages suggest that there is a continuous influx of plasma proteins into the CNS under physiological conditions. The present

observations suggest that the plasma protein immunoreactivity observed in brains of Alzheimer's disease and in non-demented aged subjects may not be the result of BBB breakdown [1, 33]. Indeed, CSF-protein and positron-emission tomography studies indicate that the BBB is not compromised in Alzheimer's disease [12, 27].

Although the function of intracellular plasma proteins in the CNS is unknown, we have recently shown that several plasma proteins, including α_1-acid glycoprotein and prealbumin, have in vitro neurotrophic activities [17, 18]. Consequently, it is possible that plasma proteins normally enter the CNS where they may have a role in maintaining neuronal function.

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