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Immunohistochemical Localization of Extravasated Serum Albumin in the Hippocampus of Human Subjects with Partial and Generalized Epilepsies and Epileptiform Convulsions*

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Summary. Human serum albumin-like immunoreactivity was detected by the peroxidase-antiperoxidase method in histological sections of the hippocampus from epileptic and control brains obtained on routine autopsies. In the hippocampi of epileptic patients immunoreactive astrocytes were found, the number of which was increasing with the severity of the manifest convulsions. The highest numbers of immunoreactive astrocytes were observed in those patients who died in status epilepticus. Hippocampi from control patients with no neurologic disorders in life were devoid of immunoreactive astrocytes. The results are discussed in terms of the breakdown of the blood-brain barrier during epileptic seizures.

Key words: Blood-brain barrier – Immunohistochemistry – Astrocyte – Epilepsy – Hippocampus

Introduction

The permeability of the blood-brain barrier (BBB) increases during chemically and electrically induced seizures (Hedley-Whyte et al. 1977; Petito et al. 1977; Mihály and Jójárt 1982; Nitsch and Klatzo 1983). However, no causal correlation has been found so far between the BBB breakdown and other (neurochemical and neuropathologic) alterations, although it has been shown that different convulsants cause different patterns of Evans blue (EB) extravasation (Nitsch and Klatzo 1983), with not only the gross topography, but also the histological localization of the EB differing. These observations strongly suggested that different mechanisms were operative during the opening of the BBB, depending perhaps on the biochemical action of the convulsant used.

According to Klatzo (1981), the fate of extravasated proteins is of equal importance for an interpretation of the effect of edema on brain tissue (Klatzo 1981). A number of morphological investigations have proved the active role of astrocytes in the clearing of serum proteins from the brain intercellular space (Blakemore 1969; Baker et al. 1971; Trachtenberg 1983). Moreover, Vise et al. (1975) suggested an appreciable contribution of astrocytes to the genesis of protein leakage by showing that damaged, protein-containing neurons were connected to blood vessels by fine astrocytic processes containing the same tracer protein. In this context, astrocytes containing the tracer or the endogenous serum protein might indicate the locus of the BBB damage.

Tracing BBB disturbances with macromolecules is not possible in postmortem human brains, although quantitative immunochemical methods have been used to detect serum proteins (particularly IgG) in post-mortem human brains with multiple sclerosis (Tourtellotte 1970). More recently, immunohistochemical studies proved that damage of the BBB in rats resulted in serum albumin extravasation and strong albumin-like staining of the astrocytes if the brains were removed several hours after the death of the animal (Oehmichen et al. 1979). This albumin-like immunostaining of the astrocytes seemed to be characteristic of the BBB damage (Oehmichen et al. 1979). In the present experiments we detected serum albumin immunohistochemically and tried to use the presence of immunoreactive astrocytes as the marker of in vivo BBB breakdown. We investigated the hippocampuses of epileptic and control patients (i.e., patients with no neurologic disorders in life). The susceptibility of Ammon's horn in various kinds of epilepsies (Corsellis and Meldrum 1976) and experimental seizures (Griffiths et al. 1983; Nitsch and Klatzo 1983) is well documented. Since there was no possibility for the exact localization of the epileptic lesion during the hospitalization, we investigated the hippo-

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Table 1. Short clinical history and gross brain pathology of the autopsy patients

Autopsy code (age)	Case history (clinical diagnosis)	Cause of death	Gross pathology of the brain (brain weight)
1745/81 (26 years)	Heart attack and ventricular fibrillation 2.5 h before death (myocardial infarct)	Cardiac arrest	Normal (1,420 g)
289/82 (56 years)	Heart attack 2 days before death (myocardial infarct)	Cardiac arrest	Moderate atherosclerosis in the carotid-basilar arteries (1,350 g)
421/81 (17 months)	Tonic-clonic movements of the arm 6 days before death (transposition of great vessels)	Sepsis	Normal (650 g)
428/80 (17 months)	Syncope, cyanosis and tonic-clonic convulsions twice in the last month; cardiac arrest 2 days before death (transposition of great vessels)	Cardiac arrest	Normal (940 g)
1048/83 (29 years)	Syncope and tonic-clonic convulsions 19 days before death; spastic hemiparesis on the left side (thrombus of the basilar artery)	Thrombosis of the basilar artery and necrosis of the pons	Thrombosis of the basilar and right posterior cerebral arteries; infarction in the right thalamus and hippocampus (1,350 g)
1367/81 (61 years)	Hospitalization because of temporal lobe epilepsy; Jackson-type motor convulsions beginning 9 days before death (partial epilepsy)	Cardiac and pulmonary failure	Cerebral atrophy; ventricular dilatation; microgyria in the right temporal lobe (1,000 g)
581/81	Chronic alcoholism; recurrent grand mal convulsions on the last day (cardiac failure)	Brain edema	Atherosclerosis in the carotid-basilar arterias (1,450 g)
352/82	8-year history of grandmal type partial seizures; left occipital lobectomy (tumor of the occipital lobe)	Hemorrhagic shock	Healing left occipital lobectomy; tumor histology: oligodendroglioma (1,440 g)
440/83 (79 years)	Several grand mal type seizures during hospitalization (10 days); mental decomposition; some grand mal seizures in previous years (it was not possible to clarify the early history because of impaired consciousness during hospitalization) (generalized epilepsy)	Pulmonary embolism	Moderate atherosclerosis in the carotid-basilar arteries; cerebral atrophy (1,160 g)
868/81 (70 years)	30-year history of grand mal type generalized seizures (generalized epilepsy)	Pneumonia	Cerebral atrophy; ventricular dilatation (1,100 g)

campus and attempted to find some correlation between the hippocampal damage and the clinical status of the patients. The role of postmortem autolysis in the genesis of the immunohistochemical staining pattern was studied in animal experiments.

Material and Methods

The investigations were performed on human brains obtained from routine autopsies 8–12 h after death. The clinical history and the neurologic diagnosis of each case are briefly summarized in Table 1. Two cases (1745/81 and 289/82) who had no signs of any neurologic disease served as controls (Table 1). Classification of seizures was based on that proposed by the International League against Epilepsy (1969, cit. Sutherland and Eadie 1980).

About 1 cm thick coronal slices of whole brains were fixed in 10% buffered (pH 7.4) formalin for 4–20 days at room temperature. The hippocampus, together with the surrounding brain tissue (including the parahippocampal gyrus), was excised and fixed in fresh fixative for an additional 1–2 days. Tissues were embedded in paraffin, and 10 μm sections were cut, mounted on slides, deparaffinized, and stained with human serum albumin (HSA) antibodies raised in rabbit. Consecutive sections were stained with hematoxylin and eosin (HE). We adapted the peroxidase-antiperoxidase (PAP) method of Sternberger (1974) in the following manner:

The deparaffinized sections were

1. washed in Tris-buffered saline (TBS; Tris-HCl buffer containing 0.8% NaCl, pH 7.6) for 15 min;
2. incubated with 3% normal sheep serum for 30 min;
3. incubated with HSA antibodies (1:800, containing 1% normal sheep serum) for 2 h;
4. washed in TBS for 2 × 15 min;
5. incubated with anti-rabbit IgG (1:15) for 30 min;
6. washed in TBS for 2 × 15 min;
7. incubated with the rabbit PAP complex (1:80, containing 1% normal sheep serum) for 30 min;
8. washed in TBS for 2 × 15 min;
9. incubated with diaminobenzidine tetrahydrochloride (DAB) and H_2O_2 for 3–4 min (0.25 mg/ml DAB and 0.01% H_2O_2);
10. washed in distilled water, dehydrated, and mounted in Entellan (Merck).

As in the procedure of Sternberger (1974), the specificity of

the immunostaining was controlled by substituting the specific antisera (steps 3 and 5) and the PAP by 3% normal sheep serum. Furthermore, the diluted HSA antibodies were absorbed with undiluted HSA (100 µl of undiluted HSA was added to 1 ml of diluted antibody) and the sections were incubated with the absorbed anti-HSA solution. The sera and the DAB were dissolved or diluted in TBS (pH 7.6).

The effects of postmortem autolysis were investigated in four rats killed with an overdose of Nembutal (Abbott), and left at room temperature for 12 h. The brains of two of these rats were removed, and 5 mm thick coronal slices were fixed in 10% buffered (pH 7.4) formalin for 2 days. The other two brains were left in the skull, fixed for 4 days in 10% buffered formalin, and then removed; 5 mm coronal slices were cut and fixed for an additional 2 days. The tissues were embedded in paraffin, and 10-µm sections were stained with rat serum albumin (RSA) antibodies, according to the PAP method. RSA antibodies were diluted to 1:500. In the immunohistochemical procedure, several dilutions of the primary antibody were tried (1:50, 1:200, 1:500, 1:800). The best results were obtained with the 1:200, 1:500, and 1:800 dilutions. Therefore, in later experiments 1:800 for anti-HSA and 1:500 for anti-RSA were used routinely.

The position and density of the immunoreactive astrocytes were plotted against a free-hand drawing of the hippocampus obtained with the aid of an overhead projector. Due to the simplicity of the hippocampal cytoarchitectonics, accurate transfer of the distribution of HSA-reactive astrocytes to the drawings was relatively easy. No counting procedures were used. In the drawings (Figs. 2, 3) each dot represents approximately 5–10 HSA-reactive astrocytes. Every section was evaluated four times in a blind manner, i.e., without knowledge of the clinical history of the patient.

In cases 1745/81, 428/80, 1048/83, 352/82, 440/83, and 1367/81, sections from both hippocampi were stained with the astrocyte impregnation technique of Gallyas (1981). This silver method is based on the catalytic activity of the tissue strucure in the reaction of silver ions and reducing molecules dissolved in a special photographic solution (physical developer) (Gallyas 1980, 1981). The method is very reliable and stains proliferating astrocytes only (Gallyas 1981). These sections were evaluated similarly to the immunohistochemical ones.

During these studies the terminology of Lorente de Nó (cit. Stephan 1975) was used to define the cellular fields.

Normal sheep serum, anti-rabbit IgG, and concentrated HSA solution were obtained from HUMAN (Hungary). The PAP complex was acquired from DAKO (Denmark). Anti-HSA serum and anti-RSA serum were from Nordic Immunology (FRG).

Results

Two cases (1745/81 and 289/82) which presented no signs of neurologic diseases in their history and no gross neuropathologic alterations (except for some degree of atherosclerosis of the carotid-basilar arteries in case 289/82) were taken as the controls in these investigations. Except for the staining of the astrocytes, the immunohistochemical staining pattern in these cases was more or less characteristic for the other cases as well.

Control Brains

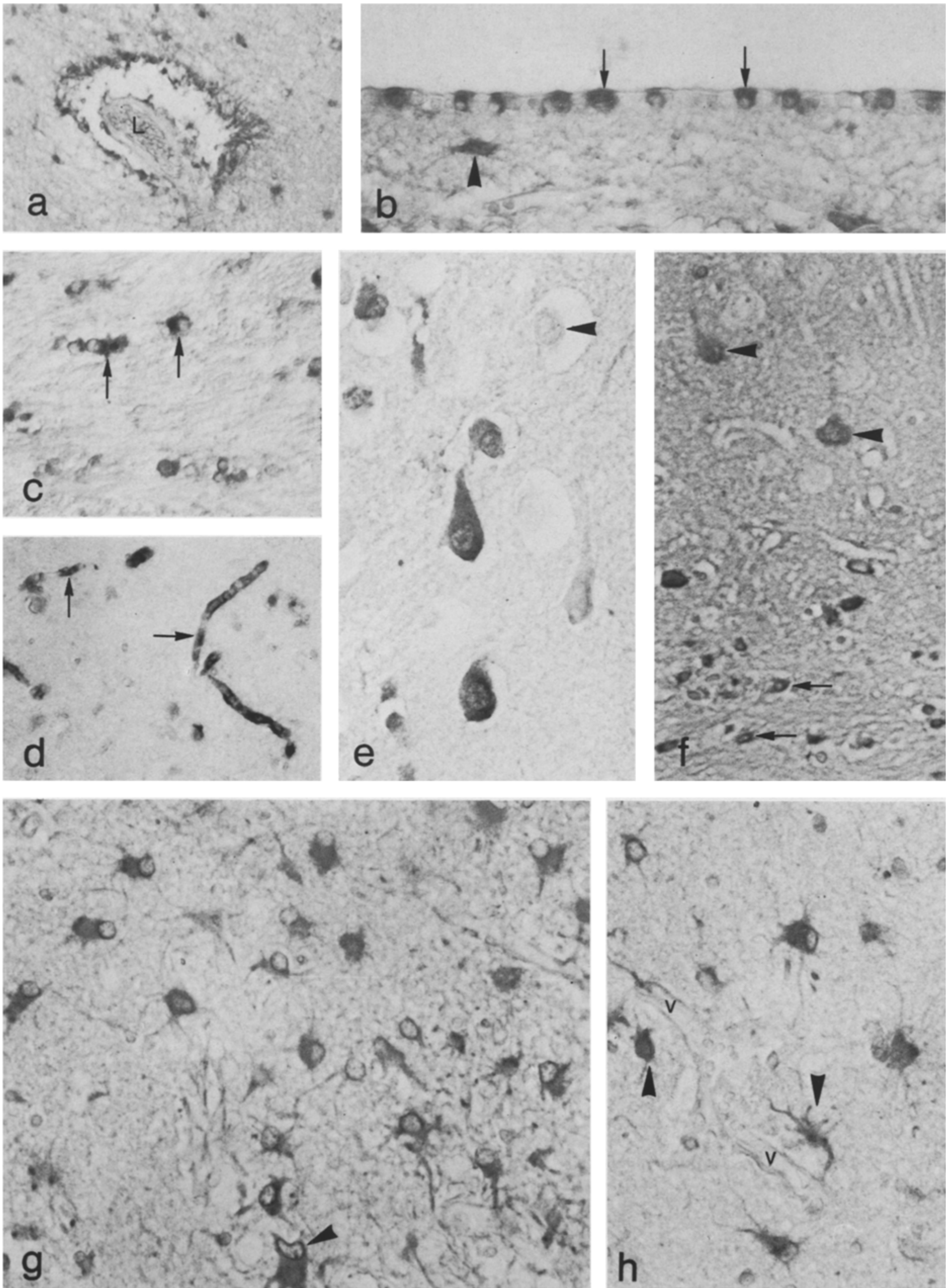
HSA-like immunoreactivity could be encountered around medium-sized vessels (Fig. 1a). The wall of the vessels was sometimes stained. Such vessels were seen mainly in the subpial and subependymal regions. However, this perivascular staining was not common – some sections were completely devoid of it. Immunoreactivity was detected in the ependymal cells (Fig. 1b) and in the cells and vessels of the piaarachnoid membrane. A 40–50 µm thick subpial and subependymal zone of the neuropil was sometimes stained homogeneously. In the white matter, and mainly in the fimbria hippocampi, scattered immunoreactive oligodendrocytes were found (Fig. 1c). In some sections neuronal staining was observed (Fig. 1e). The controls were almost completely devoid of immunoreactive astrocytes (Figs. 2a, b and 3a, b). If any, astrocytes were present in the dentate fascia, in CA 4 and in the subependymal (Fig. 1b) and subpial regions (Figs. 2a, b and 3a, b). HE-stained sections of the controls showed a normal cytoarchitectonic picture, without loss or proliferation of cells. Astrocytic impregnation (Gallyas 1981) revealed slight gliosis in the dentate gyrus and CA 4. In the subpial and subependymal regions and in the fimbria hippocampi impregnated glial fibers were seen (Fig. 4b).

Epileptic Brains

Patients with Epileptiform Convulsions: 421/81. Very few HSA-positive astrocytes were seen in the left hippocampus (Fig. 2c). In the right hippocampus, the white matter of the hippocampal gyrus and subiculum contained a moderate number of HSA-positive astrocytes (Fig. 2d). The cytoarchitectonic picture was normal except for some pyknotic neurons in the subiculum on both sides.

428/80. HSA-stained astrocytes were found in high numbers in the left hippocampus, mainly in CA 2 and CA 3 (Fig. 2e). The alveus, subiculum, CA 1 and CA 4 also contained numerous HSA-positive astrocytes (Fig. 2e). At the same time, sectors CA 2 and CA 3 of Ammon's horn were destroyed, and very few neurons were seen in CA 1 and CA 4. The right side showed more moderate alterations. Astrocytes were found in CA 3, in the white matter of the subiculum, and in the alveus (Fig. 2f). HE-stained sections exhibited no alterations except for some neuronal pyknosis in CA 3.

1048/83. HSA-positive astrocytes could be found in every sector, except for the hilus of the dentate fascia and the subiculum. Both sides showed roughly the same staining pattern. HE-staining revealed that on the left side about 70% of the dentate granules had disappeared. Otherwise, the neurons of Ammon's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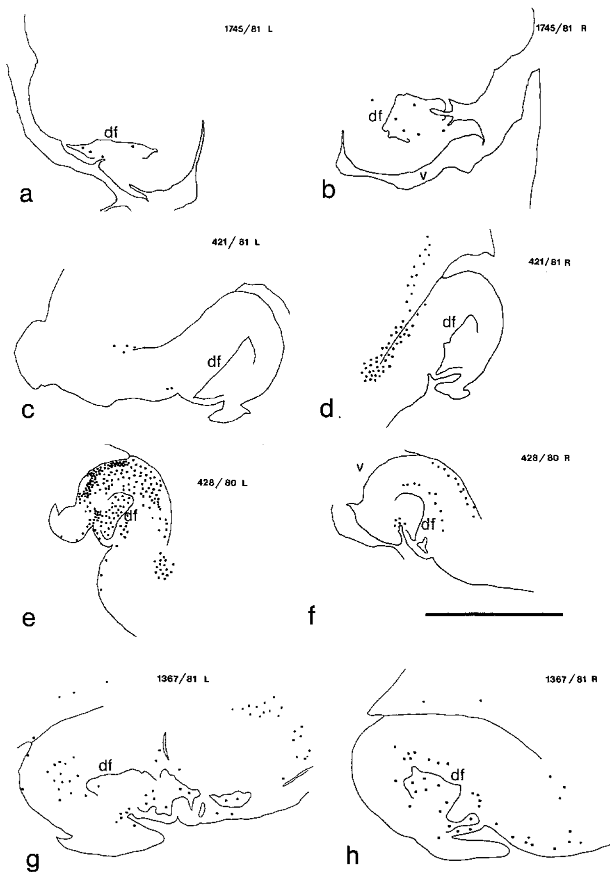

Fig. 2a–h. Camera lucida drawings of human hippocampuses stained with the PAP method. The numbers on each drawing are the autopsy codes, while *L* and *R* denote left and right hippocampi, respectively. *Black dots* represent immunoreactive astrocytes (*one dot*=5–10 astrocytes). Bar in **f**: 1 cm (*df* dentate fascia; *v* lateral ventricle)

horn were destroyed completely, except for some pyknotic ones in CA 3 and CA 4. The parahippocampal gyrus displayed normal cytoarchitectonics. On the right side, only a few pyknotic dentate granules were seen; otherwise, the hippocampal neuropil was replaced by a loose tissue containing small, round cell nuclei, possibly microglial cells. The border of this tissue destruction was the parahippocampal gyrus, where a gradual build-up of the normal cytoarchitectonics was seen.

Patients with Epilepsy: 1367/81. On the left side HSA-positive astrocytes were found in the parahippocampal gyrus, the subiculum, and the stratum moleculare of CA 1, CA 2 and CA 3 (Fig. 2g). On the right side HSA-stained astrocytes were seen in the stratum moleculare of CA 1 and CA 2, in the dentate fascia, and in the parahippocampal gyrus (Fig. 2h). On the right side, HE-staining revealed pyknotic neurons in sectors CA 1, CA 2, and CA 3, and neuronal loss in the parahippocampal gyrus. On the left side, neuronal cell loss was seen in the parahippocampal gyrus.

581/81. Only the left hippocampus was investigated. Many HSA-positive astrocytes were observed in the dentate gyrus, in CA 4, and in the white matter of the subiculum. A moderate number of astrocytes were present in all cellular fields. As a peculiar feature, capillary staining (Fig. 1d) was seen in every region. HE-staining revealed patchy cell losses in the dentate fascia. Ischemic cell change (Brierley 1976) was observed in the subiculum, CA 1, and CA 2. In these regions, a mixed population of degenerating and normal neurons was observed.

352/82. HSA-positive astrocytes were found in the whole territory of the right dentate fascia, in CA 1, the subiculum, and the parahippocampal gyrus. On the left side, CA 1, the dentate fascia, the subiculum, and the parahippocampal gyrus contained immunoreactive astrocytes. On the right side, most of the neurons of CA 1 and the subiculum had disappeared, and cell loss was observed in the parahippocampal gyrus as well. On the left side, no clear-cut cell losses were observed, but abundant neurons in CA 1 and CA 2 showed neuronal pyknosis.

440/83. In the left hippocampus, HSA-positive astrocytes were encountered in the dentate fascia and in the white matter and molecular layer of the parahippocampal gyrus (Fig. 3c). Neuronal depopulation was found in CA 1, the subiculum, and the parahippocampal gyrus. The right hippocampus contained numerous HSA-stained astrocytes located in the dentate fascia, in CA 2 and CA 3. A smaller number was found in the white matter and in the molecular layer of the subiculum and the parahippocampal gyrus (Fig. 3d). By HE-staining, neuronal depopulation was observed in the subiculum and in CA 1.

Fig. 1a–h. Light-microscopic localizaton of HSA-like immunoreactivity in human and rat hippocampuses. **a** Perivascular staining of the neuropil in case 1745/81 (*L* lumen of a vessel, ×500). **b** Ependymal cells containing HSA-like immunoreactivity in case 1745/81 (*arrows*). The *arrowhead* points to an immunoreactive astrocyte in the subependymal region. ×970. **c** Immunoreactive oligodendrocytes (*arrows*) in the fimbria of case 289/92. ×970. **d** Immunostaining of the capillaries (*arrows*) in case 581/81. ×500. **e** Neuronal staining in case 581/81 (*arrowhead* – unstained neuron, ×970). **f** Albumin-like staining in the hippocampus of control rat brain (*arrowheads* RSA-containing pyramidal cells; *arrows* RSA-containing oligodendrocytes in the alveus, ×970). **g, h** HSA-like immunoreactivity of the astrocytes in case 1048/83. *Arrowhead* in **g** points to hypertrophied astrocyte. *Arrowheads* in **h** point to immunoreactive astrocytes in close proximity of blood vessels (*v*). ×1,000

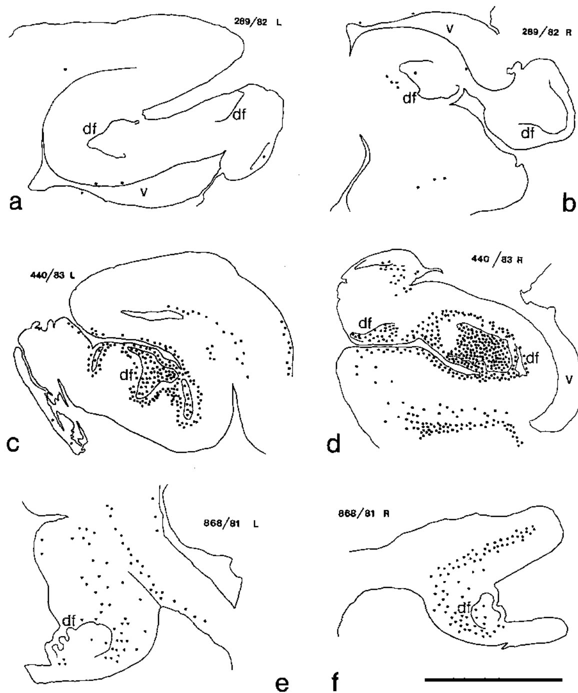

Fig. 3a–f. Camera lucida drawings of human hippocampuses stained with the PAP method. Explanation as in **Fig. 2a–h**

868/81. HSA-positive astrocytes were seen in the parahippocampal gyrus and subiculum, in CA 1 and CA 3 on the left side (Fig. 3e). The right hippocampus contained immunoreactive astrocytes scattered evenly in moderate numbers in every region (Fig. 3f). On the left side, patchy cell loss was seen in the dentate fascia. Most of the neurons of CA 2 had disappeared. There were only a few neurons in the parahippocampal gyrus. Neuronal pyknosis was observed in CA 1 and CA 3. On the right side, patchy cell losses were seen in the dentate fascia. The neurons of CA 1 and CA 2 had disappeared completely, while the neurons of CA 3 exhibited pyknosis. Neuronal cell loss was observed in the parahippocampal gyrus.

A homogeneous HSA-like immunoreactivity filled the cytoplasm and the processes, but not the nuclei of the astrocytes (Fig. 1g, h). Some astrocytes seemed to be hypertrophied (Blackwood 1976; Fig. 1g), but no binucleated forms were observed. The astrocyte impregnation technique of Gallyas (1981) revealed that areas containing immunoreactive astrocytes were heavily occupied by the proliferating glia (Fig. 4a). The overlap between proliferated and HSA-containing glial cells was quite tight in cases 428/80 (Fig. 4c), 1048/83, and 440/83 (Fig. 4e), and in the right hippocampus of case 352/82. In case 1367/81 (Fig. 4d), the region of astrocytic proliferation was far greater than that of the astrocytic immunoreactivity. Areas of neuronal cell loss were occupied by proliferated astrocytes.

Rat Brains

A characteristic staining pattern was observed in the rat brain. Besides the spotty staining of the neuropil, the walls of the small- and medium-sized vessels stained conspicuously. The pial membranes and the ependymal lining of the ventricles were stained, too. Sometimes, a 40–50 μm thick subpial zone of the neuropil was stained homogeneously. As a peculiar feature, some neurons and oligodendrocytes contained RSA-like immunoreactivity (Fig. 1f). Staining of the oligodendrocytes was most pronounced in the corpus callosum and in the fimbria hippocampi. Brains removed from the skull before fixation were more damaged during removal. In these brains more neurons and oligodendrocytes containing RSA-like immunoreactivity were observed (Fig. 1f) than in those fixed in the skull before removal. No astrocytic staining was found in the rat brain.

Sections treated as the specificity controls of the immunohistochemical procedure (see Material and Methods) displayed no immunostaining.

Discussion

Validity of the Method

To use postmortem human brains in immunohistochemical (Gaspar et al. 1983) and metabolic studies is a standard procedure (Hardy and Dodd 1983), although some caution has to be exercised when dealing with enzymatic activities or transmitter levels (Hardy and Dodd 1983). Since the aim of the present study was the assessment of the BBB breakdown, we have to discuss the validity of our method and, particularly, the effect of autolysis. While the presence of extravascular serum proteins in the central nervous system (CNS) indicated the breakdown of the BBB in brains fixed by transcardiac perfusion (Nitsch and Klatzo 1983), this was not the case in postmortem tissues fixed by immersion (Rinder 1968). The macromolecular tracer (Evans blue-albumin: EBA) which was in the cerebral vessels at the moment of death slowly penetrated into the surrounding neuropil after death, although no dysfunction of the BBB was induced (Rinder 1968). The postmortem penetration of EBA resulted in the staining of the vessel wall, the surrounding neuropil, neurons, and unspecified glial cells (Rinder 1968). Oehmichen and Gencic (1980) found the same pattern of postmortem serum albumin leakage, but they noted that postmortem albumin leakage in otherwise normal brains never resulted

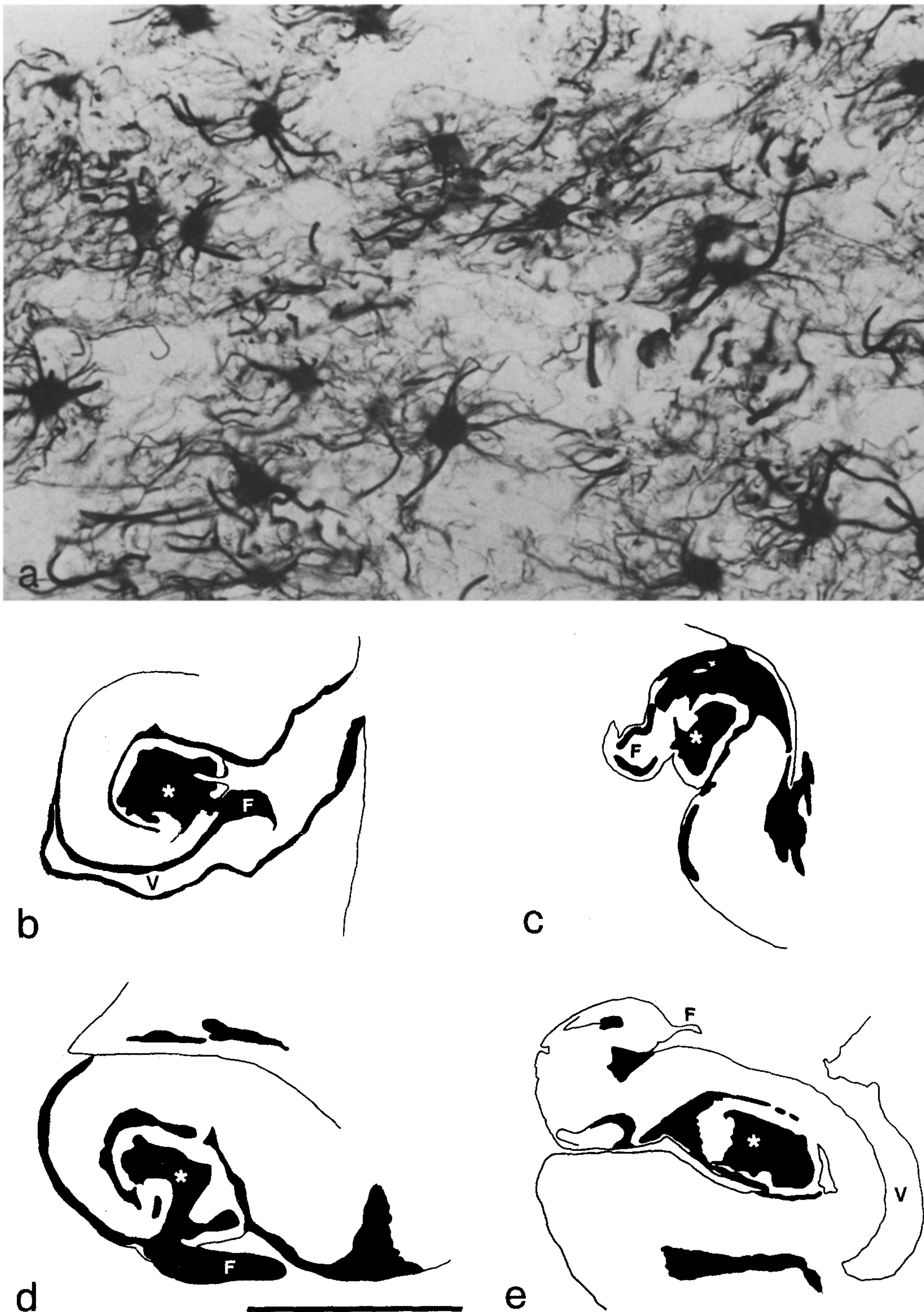

Fig. 4. a Proliferated astrocytes in the hippocampus of case 1048/83 as shown by the astrocyte impregnation technique of Gallyas (× 1,100). **b–e** Camera lucida drawings of human hippocampuses stained by astrocyte impregnation. *Black areas* indicate the occurrence of proliferated astrocytes and the presence of impregnated glial fibers (*asterisk* hilus of the dentate fascia; *F* fimbria hippocampi; *V* lateral ventricle; bar in **d** represents 1 cm). **b** Case 1745/81, right side. **c** Case 428/80, left side (cp. this diagram with **Fig. 2e**). **d** Case 1367/81, right side (cp. with **Fig. 2h**). **e** Case 440/83, right side (cp. with **Fig. 3d**)

in astroglial staining (Oehmichen et al. 1979). On the other hand, they observed a clear-cut albumin-like immunoreactivity in the astrocytes of postmortem brains when the breakdown of the BBB was induced in vivo (Oehmichen et al. 1979).

The staining pattern in control human brains was very similar to that in rat brains, and both corresponded to the literature descriptions (Rinder 1968; Oehmichen et al. 1979; Oehmichen and Gencic 1980). Furthermore, this staining pattern was found to some degree in every case, supporting its artifact origin. Since the specificity of the immunohistochemical reaction was strictly controlled (see Material and Methods), we must say that this artifact resulted from the postmortem diffusion of albumin into the cells and neuropil due to postmortem autolysis (Rinder 1968). In this respect, the capillary staining in case 581/81 must be referred to as an autolysis artifact.

In contrast, the astrocyte was the only immunoreactive cell which was (nearly) absent from the control samples. Furthermore, the number of immunoreactive astrocytes increased with the severity of the seizures (cp. the diagrams of cases 1367/81 and 440/83). These observations seem to support the idea that HSA-positive astrocytes in autopsy brains are a consequence of the in vivo breakdown of the BBB. However, we cannot say that the postulated in vivo protein extravasation was restricted to the areas occupied by HSA-stained astrocytes. As to the real extent of the BBB damage, quantitative data obtained by radioimmunoassay of HSA in hippocampal tissue might be more informative.

Ethiologic Considerations

Electrically and chemically induced convulsions are accompanied by an increase in the protein permeability of the BBB (Petito et al. 1977; Mihály and Jójárt 1982; Nitsch and Klatzo 1983). By electron microscopy, tracer proteins could be visualized in the perivascular astrocytic processes (Mihály and Joó, in preparation), supporting other experimental data on the role of astrocytes in the clearing of the extravasated proteins (Klatzo 1981).

However, three of our cases presenting epileptiform convulsions had another illness, the effects of which must be discussed. While cardiac malformations are known to cause convulsions (Stephens 1962), the pathogenetic factors (anoxia, brain infarcts, etc.; Stephens 1962) per se are serious enough to cause various neuropathologic alterations. Cardiac arrest (which happened in case 428/80) is known to result in severe hypoxic nerve cell damage (Brierley 1976). Hippocampal cell loss and glial proliferation might be attributed to this hypoxic episode (Brierley 1976). However, the high number of immunoreactive astrocytes in the left hippocampus of case 428/80 cannot be explained exclusively on the basis of hypoxic insults, as the BBB has proved to be resistant to hypoxia in several animal experiments (Bradbury 1979). The effect of the grand mal convulsions therefore has to be considered.

In case 1048/83, the occlusion of basilar and right posterior cerebral arteries (PCA) occurred. Occlusion of the middle cerebral artery in cats seriously damaged the BBB (Olsson et al. 1971). Since the PCA supplies the major part of the human hippocampus (Stephan 1975), we may say that the BBB was damaged by the severe ischemia due to the PCA occlusion. Indeed, the tissue was much more damaged on the right side than on the left, as seen in HE-stained material. The immunohistochemical staining pattern was roughly the same on both sides; therefore, it must be assumed that occlusion of the basilar artery was sufficient to induce severe ischemia of the left hippocampus (since PCA originate from the basilar artery). The severe protein extravasation in both hippocampi might be a consequence of ischemia and tissue destruction.

In cases 581/81 and 440/83, more than two grand mal convulsions occurred without recovery of consciousness, which might be regarded as status epilepticus (Oxbury and Whitty 1971). Status epilepticus is known to induce serious damage to the brain by causing neuronal necrosis, mainly in the neocortex, hippocampus, thalamus, and cerebellum, both in man (Corsellis and Meldrum 1976) and in experimetal animals (Meldrum et al. 1973). Prolonged convulsions in cats resulted in a more than threefold increase in the uptake of radioiodinated serum albumin in the hippocampus (Lorenzo et al. 1972). Hippocampal BBB damage was observed by Nitsch and Klatzo (1983) in bicuculline and methoxypyridoxine convulsions, EB being used as a tracer. Although these seizures cannot be considered as status epilepticus due to the short observation period, the authors point out the possibility of BBB damage in the hippocampus during generalized convulsions. On the basis of these literature data, and since no cerebrovascular damage was detected, we assume that in cases 581/81 and 440/83 the high number of albumin-containing astrocytes indicated a large increase in the protein permeability of the hippocampal vessels. If this is true, it would be the first direct evidence of the increase of protein permeability in the hippocampus of human subjects with status epilepticus. Efforts are in progress to verify this suggestion in animal experiments.

In case 1367/81, we found microgyria in the right temporal lobe, which could be the pathologic substrate of a partial epilepsy (Corsellis and Meldrum

1976). The minor neuronal alterations in the hippocampus and the moderate number of albumin-containing astrocytes suggested that at least this segment of the hippocampus was not involved in the seizure process. On the other hand, Jacksonian motor seizures of the upper extremities are mainly prerolandic in origin (Sutherland and Eadie 1980). Parts of the prerolandic frontal cortex were investigated with the Gallyas stain and with HSA immunohistochemistry, and diffuse gliosis and many HSA-positive astrocytes were found (Mihály and Bozóky, unpublished data). This focal change, which could be detected on both sides (more pronounced on the left side), would explain the Jacksonian fits observed during hospitalization.

In cases 440/83 and 868/81, the topography of HSA-stained astrocytes did not follow the areas of neuronal destruction, i.e., some gliosed areas did not contain HSA-reactive glial cells. Both patients had several grand mal seizures in the previous years (Table 1), which could result in hippocampal neuronal death and glial proliferation. We think that these possibly several years old lesions are not necessarily reflected by the presence of HSA-containing astrocytes.

The ischemic neuronal change observed in several cases possibly reflects an in vivo neuronal damage (Brierley et al. 1973) and can be the consequence of status epilepticus (Corsellis and Meldrum 1976) in case 581/81. The neuronal damages in other cases are not necessarily related to epilepsy. These neurons might have been affected by terminal ischemia.

The results obtained from the Gallyas-stained sections are in accordance with literature descriptions, i.e., areas of neuronal destruction are occupied by proliferated astrocytes (Corsellis and Meldrum 1976).

Conclusions

In the present investigations, the severity of the seizures and the amount of HSA-positive astrocytes observed showed parallelism. Supposing that these astrocytes represent a true breakdown of the BBB, the question arises as to whether the BBB damage was a *sui generis* alteration or a consequence of the death of the neurons. Since the breakdown of the BBB was observed at a rather early stage in animal experiments (Nitsch and Klatzo 1983), and in some cases there was only a poor correlation between the areas of neuronal loss and those of containing immunoreactive astrocytes, we think that the ongoing seizure activity was able to open the BBB to serum proteins.

As to the mechanism of the BBB damage, we cannot draw definite conclusions. At present we can only state, that the immunohistochemical detection of extravasated serum proteins might be a useful tool of neuropathologic diagnosis and might act as a supplementary source of interesting details as to the origin of epileptic brain damage.

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