CASE REPORT

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Creutzfeldt-Jakob Disease (CJD) in a Case of Suspected Chronic Heavy Metal Poisoning

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ABSTRACT: We describe a patient who died of suspected heavy metal poisoning after a nine-month history of rapidly worsening dementia. Autopsy at a forensic-pathological institute established the postmortem diagnosis of sporadic Creutzfeldt-Jakob disease (CJD) based on demonstration of the proteinase-resistant prion protein (PrP^{Sc}) in Western-Blot on native brain tissue. Microscopic examination of the macroscopically largely inconspicuous brain revealed marked spongiform changes in the gray matter—mainly affecting the cerebral cortex, nucleus caudatus, and putamen—with confluent vacuoles. Patchy or perivacuolar deposits of PrP^{Sc} were found as well as granular PrP^{Sc} deposits. The cerebellum contained focal PrP^{Sc} deposits. There was an astrogliosis in the white matter and a proliferation of microglia in the gray matter with a simultaneous clear reduction in neuronal elements. The differential diagnosis is discussed, as is the potential risk to those performing autopsy on forensic cases with a clinical picture of rapidly progressing dementia, especially in cases where a prion disease is not initially suspected.

KEYWORDS: forensic science, Creutzfeldt-Jakob Disease, prion protein, dementia

Human prion diseases include the familial Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia (FFI), and familial forms of Creutzfeldt-Jakob disease (CJD). CJD is a rare, fatal, degenerative disease that occurs once in a one million population per year and which, though it usually occurs spontaneously, can also be genetically determined or acquired (1). Ten to fifteen percent of CJD cases are dominantly inherited. These disorders are transmissible diseases although the histological features do not express an inflammatory process. With the appearance of the new variant of CJD (nvCJD), which is apparently caused by transmission of the bovine spongiform encephalopathy (BSE) pathogen to humans (2–5), prion diseases have caught the public's interest.

At present, all findings indicate that the aetiology and pathogenesis of sporadic, genetic, and infectious (iatrogenic) forms of these diseases are due to a conformational change of the prion protein (PrP), a normal cell membrane protein expressed at particularly high levels in nerve cells (6). The pathological type of this protein, the protease-resistant, scrapie-like isoform of the prion protein (PrP^{Sc}) accumulates within the gray matter of the brain and is responsible for the conversion of normal cellular PrP molecules into the pathologic isoform (7–9).

To date no case of CJD has been described from a specifically forensic point of view. A recent survey, however, did hint at the potential forensic aspects, drawing attention to, among other things, the differential diagnosis with acute dementias of other genesis as well as to the safety measures that need to be observed during autopsy on suspected victims of CJD (10). The present case was autopsied because of a forensic question and represents an example of the differential diagnosis of an acute dementia process as well as the potential risk to perform an autopsy, especially in cases where a prion disease is not initially suspected.

Case Report

Anamnesis

The 57-year-old man had worked for 21 years in the metal industry. Several cases of heavy metal poisoning had occurred among his coworkers. In September 1996 the man visited his family physician complaining of fatigue, weight loss, memory loss, restlessness, pain in the extremities, and headache. Infection with a flu virus was diagnosed, although the differential diagnosis included suspicion of chronic heavy metal poisoning.

The patient's condition did not improve; examinations for trace elements and heavy metals were negative. In January 1997 the symptoms took a dramatic turn for the worse, with a psychosis-like disease picture, disorientation, speech impairment, ataxia, pareses, and convulsions. Alzheimer's disease was diagnosed and the patient was admitted to a nursing home. He died in the home nine months after onset of the disease. With suspicion of chronic heavy metal poisoning, an autopsy was performed. The differential diagnostic possibilities included Alzheimer's disease and Creutzfeldt-Jakob disease.

Autopsy

At autopsy, the safety measures recommended for postmortem examination of victims of prion diseases were taken (10). The au-

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topsy findings were as follows:

> Diminished nutritional state, pressure sore above the right greater trochanter, terminal aspiration of chyme, chronic, gastric ulcer, one 1.5 cm large cortical cyst in the left kidney, incipient fibrosis of the pancreas, slight coronary arteriosclerosis, slight general arteriosclerosis.

Neuropathology

The initial diagnosis was made on a specimen of brain tissue and the macroscopic findings were documented at the Institute for Neuropathology of the University of Göttingen (Prof. Dr. H. A. Kretzschmar).

The brain weighed 1350 g prior to fixation. The interior meninges exhibited a slight cloudy discoloration above the convexity and the brain was mildly atrophied with enlargement of the internal and external CSF spaces. Grossly, no alterations of the cerebral cortex were seen.

Microscopic Investigation and Final Diagnosis

Blocks of tissue were taken from 14 regions of the cerebrum, midbrain, medulla oblongata, and cerebellum in accordance with the standard for postmortem examination for dementia (modified according to 11). The blocks were decontaminated with formic acid, embedded in paraffin, and prepared according to routine methods. The following stains were applied: Hematoxylin and eosin (H and E), periodic acid Schiff, silver staining for demonstration of Alzheimer's fibrils, and senile plaques (12). The following proteins were demonstrated immunohistochemically:

> Glial fibrillary acidic protein (monoclonal antibody - GFAP, Dako Diagnostika GmbH, D-22047 Hamburg/Germany), PGM_1 (monoclonal antibody CD68 - Dako Diagnostika GmbH, D-22047 Hamburg/Germany); β-amyloid precursor protein (β-APP, monoclonal antibody, Dako Diagnostika GmbH, D-22047 Hamburg/Germany); protease resistant prion protein (monoclonal antibody GÖ 138 - Kretzschmar et al. 1996).

Microscopic examination—The cerebral cortex exhibited severe spongiform changes with confluent vacuoles (Fig. 1). Marked astrocytic gliosis and moderate to severe nerve cell loss were seen. Spongiform changes with confluent vacuoles, gliosis and nerve cell loss (Fig. 2*c*) were also found in the basal ganglia, predominantly in the anterior part and in thalamic nuclei. Spongiform changes and mild gliosis were noted in the molecular layer of the cerebellum. The cerebellar granular layer showed nerve cell loss. In contrast, the Purkinje cell layer was relatively well preserved. There was some proliferation of the Bergmann glia. Additionally, some amyloid plaques, but no tangles, were detected in the frontobasal cortex.

Immunohistochemistry—Microtome sections were placed on silanized slides and pretreated with hydrolytic autoclaving in 2 mMol HCI for 30 min (13). The monoclonal antibody Gö138 (14) directed against a synthetic peptide corresponding to amino acids 138 to 152 of the human prion protein was used (Fig. 2). Marked perivacuolar staining was seen in the cortical areas (Fig. 2*a,b*) and a more granular distribution in the basal ganglia. Granular prion protein deposits (Fig. 2*a*) were observed in thalamic nuclei and substantia nigra. Focal granular and plaque-like PrP^{Sc} deposits were found in the cerebellum, mainly in the molecular layer and sparsely in the granular layer (Fig. 2*d*). There were no PrP^{Sc} deposits in the white matter (Fig. 2*a,d*). An antibody against beta A4 revealed sparse amyloid plaques of Alzheimer type in the cortex.

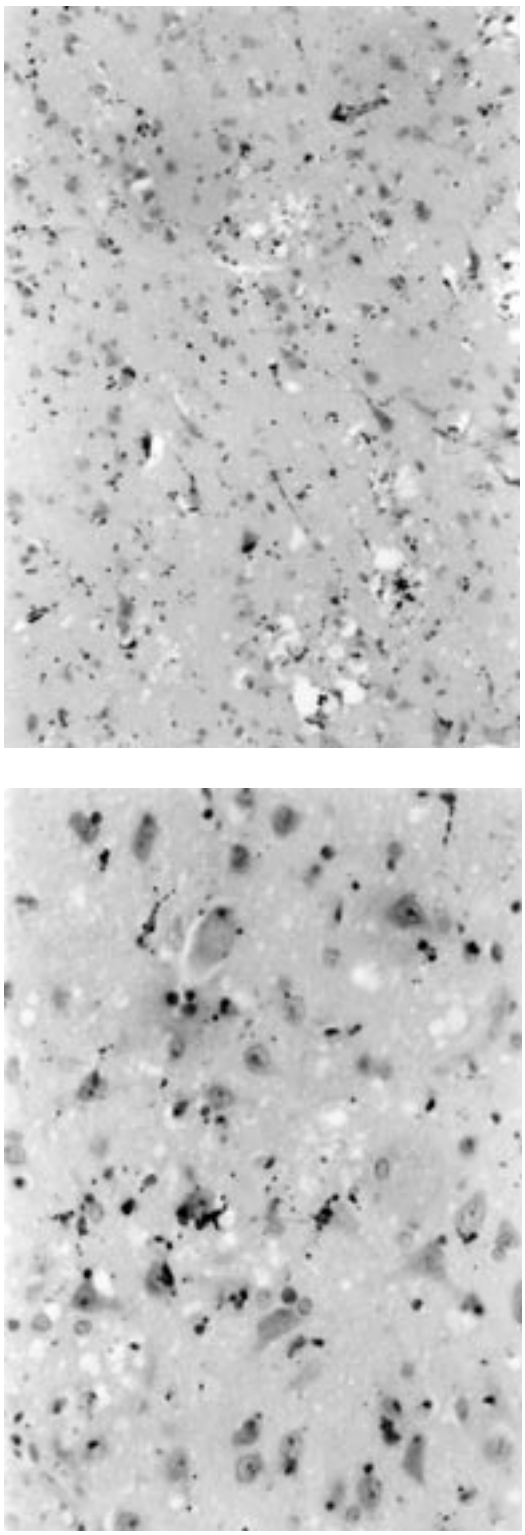

FIG. 1—*Spongiform changes with confluent vacuoles within the cortex* (a) *H and E, magnification × 25;* (b) *H and E, magnification × 200.*

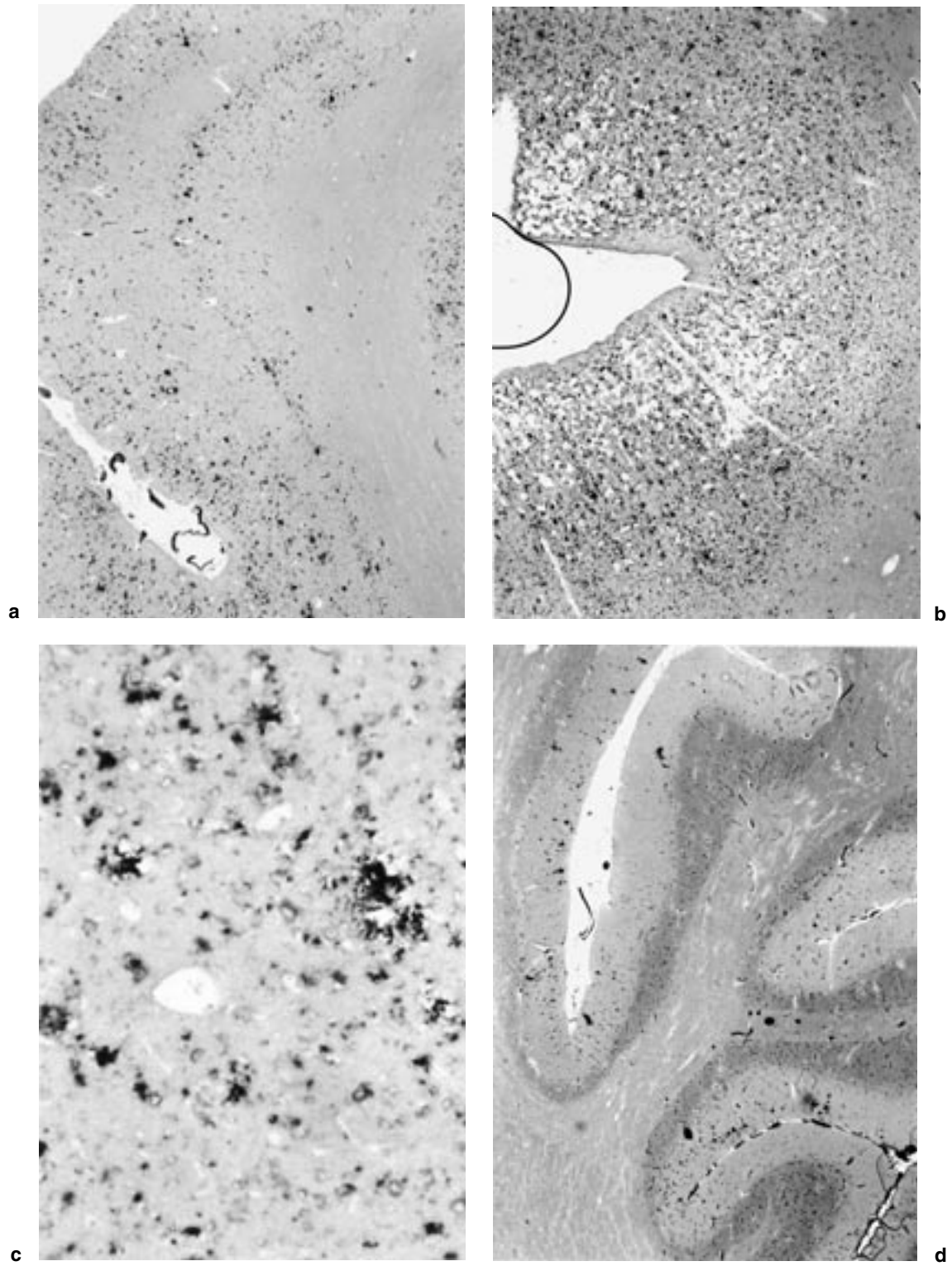

FIG. 2—*Deposits of human prion protein—partly laminar distribution* (a), *partly diffuse distribution* (b); *the deposits are sometimes granular* (a), *sometimes perivacuolar* (c). *In the cerebellar cortex the deposits are mostly plaque-like, occasionally granular* (d) *(monoclonal antibody Gö138; magnification: [a, b, d] × 25, [c] × 400).*

Also conspicuous was the pronounced axonal injury, which was seen almost exclusively distributed symmetrically in the tegmental part of the pons (Fig. 3). Activation of the astrocytes was found throughout the cerebral medulla (Fig. 4). In addition to vacuolization and the presence of PrP^{Sc}, the gray matter showed a proliferation of microglia and an increase in their activation (Fig. 5).

Molecular genetic analysis—Genomic DNA was prepared from brain tissue using the QIAamp Kit (Qiagen). The open reading frame of the prion protein gene (PRNP) was amplified by PCR using the primers 895W and 896W as described by Nicholl et al. (15). The resulting 874 bp PCR product was purified with the QIAquick PCR Purification Kit (Qiagen) and digested with restriction en-

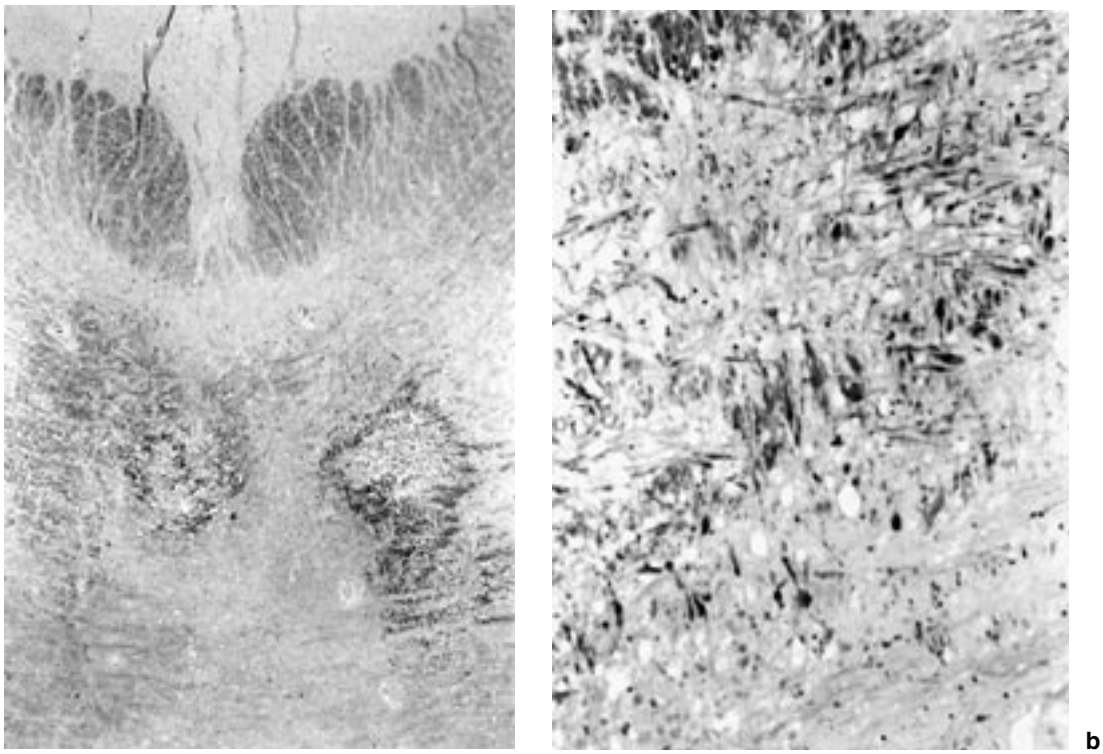

FIG. 3—*Axonal injury as demonstrated by antibodies against β-APP; magnification:* (a) × *25,* (b) × *400.*

zymes *Nsp* I and *Mae* II. Depending on the occurrence of methionine or valine at codon 129, the PCR product was digested at this site by restriction enzymes *Nsp* I or *Mae* II (16). The digested PCR product was analyzed by 1.5% agarose gel electrophoresis. Mutation analysis of the PRNP was performed using single strand conformational polymorphism analysis (17). The present case showed methionine homozygosity at codon 129 of the PRNP. No mutation was detectable.

Biochemical analysis—For the prion protein typing, brain tissue was homogenized in nine volumes of lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P40, 0.5% sodium deoxycholate, 10 mM Tris pH 7.4). Aliquots were digested with proteinase K at a concentration of 50 μm/mL at 37°C for 1 h. Digestion was terminated by addition of PMSF (2 mM final concentration). Samples were boiled in electrophoresis buffer (3% sodium dodecyl sulfate in 60 mM Tris pH 6.8) and run on a 12% SDS-PAGE. Western blotting was performed by semi-dry transfer to a nitrocellulose membrane (0.45 μm, BioRad) and immunodetection was done using the monoclonal antibody 3F4 followed by goat antirabbit IgG (Dako) coupled to alkaline phosphatase. Enzymatic activity was visualized using the CSPD chemiluminescent system (Tropix Inc.) as described by the manufacturer. The present case showed PrP^{Sc} Type 2 according to Parchi et al. (18).

Discussion

Based solely on the clinical picture of a rapidly progressing dementia, the differential diagnosis in the present case included a prion disease, namely CJD. Given this suspicion, all recommended precautions against infection were taken at autopsy.

Before further work-up, native and formalin fixated brain tissue was sent for molecular genetic analysis to the German Reference Center for Prion Disease in Göttingen, where the brain was additionally subjected to histological examination and anti-prion-protein immunohistochemistry and Western blot.

The histological lesion pattern was dominated by confluent vacuoles in the cerebral cortex. Combined with the localization of PrP^{Sc} and the predominant perivacuoloar deposition pattern, we could demonstrate the typical neuropathological features of the cortical form of a case of sporadic CJD, which is homozygous for methionine at codon 129 of the PRNP and accumulates PrP^{Sc} Type 2 (18). The polymorphism at codon 129 in combination with the biochemical PrP^{Sc} type determines the clinical appearance and the neuropathological lesion pattern of sporadic CJD (19). Based on these criteria and the neuropathological lesion pattern, the different forms of sporadic CJD (20–23) can be distinguished from the new variant of CJD, which is thought to be caused by the BSE agent.

The present case constitutes a cautionary tale for forensic pathologists and neuropathologists confronted with cases of rapidly progressing dementia. Regardless of the clinical diagnosis, because CJD is among the differential diagnostic possibilities in such cases, precautions must always be taken at autopsy to shield the participants against possible infection (protection of eyes, respiratory passages, and open sores or wounds). Since CJD is not highly contagious, however, a special isolation room is not necessary for autopsy. It should also be pointed out that effective decontamination cannot be achieved with formalin, but only with formic acid in formalin-fixated tissues. For instruments, decontamination with NaOH or autoclaving with the prion-program (134°C, 3 bar) is sufficient. The best policy in such cases is certainly to let a suitable reference center make the diagnosis; such centers are also equipped to deal with epidemiological issues.

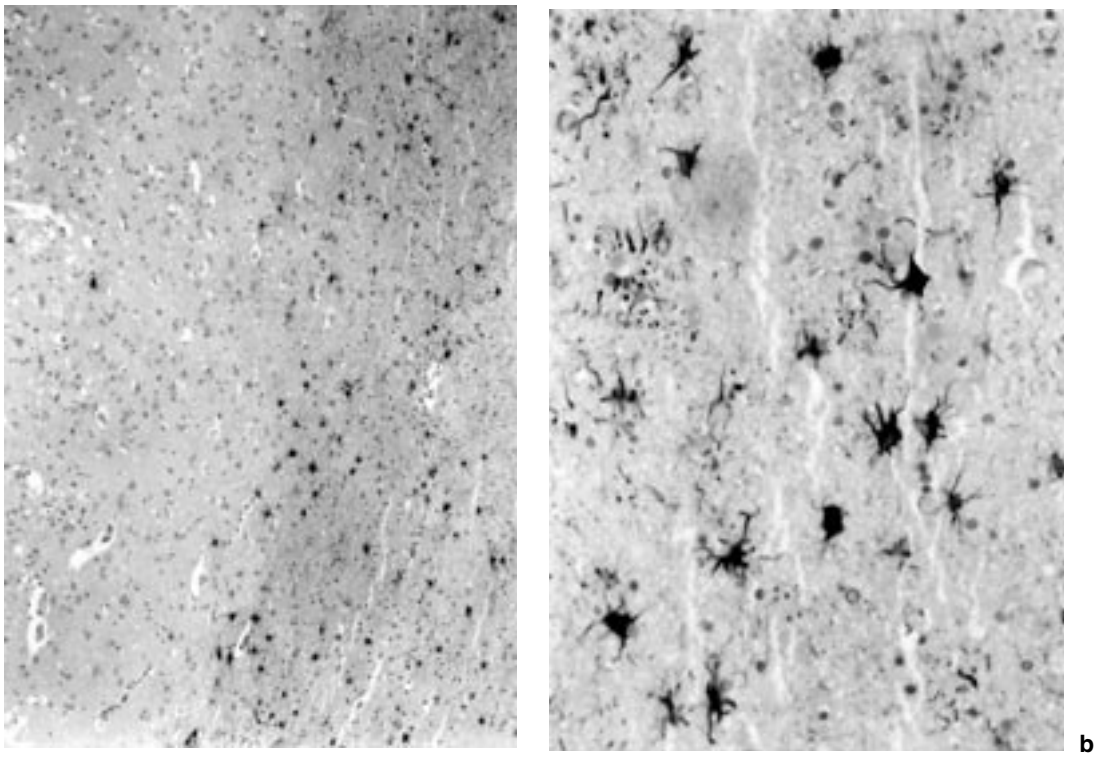

FIG. 4—*Demonstration of an astrocyte gliosis in the white matter; GFAP, magnification:* (a) *× 100,* (b) *× 400.*

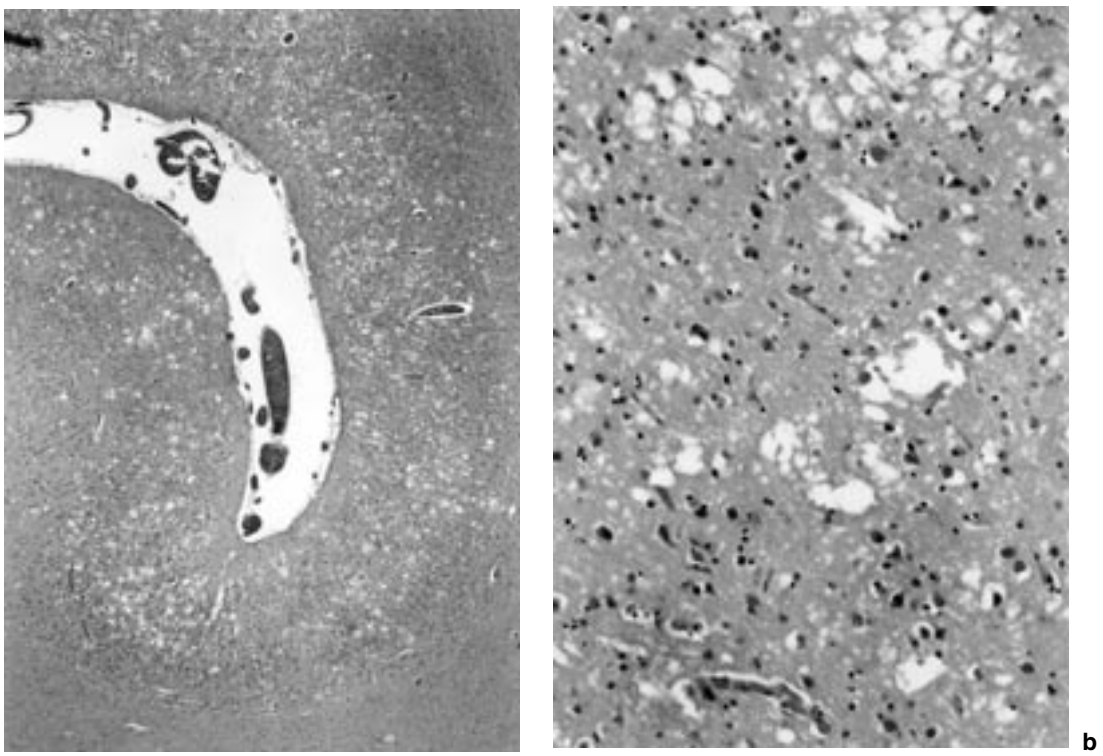

FIG. 5—*Microglial reaction within the gray matter as well as a reduction of neurons and demonstration of vacuoles; CD68 antibody; magnification:* (a) *× 200,* (b) *× 400.*

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