Experimental Superficial Siderosis of the Central Nervous System.
I. Morphological Observations

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Abstract. Autologous washed red blood cells were injected weekly over a period of three to six months into the cisterna magna of adult New Zealand white rabbits. After three months, the surface of the brain stem, cerebellum, and pia-form cortex showed a distinct brown color, and staining of the gross specimens for iron produced an intense blue color which extended for a distance of 1–2 mm into the brain parenchyma. Enhanced iron stains of vibratome sections revealed the accumulation of reaction product in microglia and Bergmann glia of the cerebellar cortex, and in microglia and astrocytes of the pia-form cortex. Ferritin immunocytochemistry revealed reaction product in cerebellar microglia and Bergmann glia which strongly resembled that obtained by the enhanced iron stain. In the pia-form cortex, only microglia were reactive with anti-ferritin. Electron microscopy confirmed the accumulation of electron-dense ferritin granules only in the cytoplasm of microglia. Bergmann glia in the cerebellum and astrocytic processes in the pia-form cortex were replete with intermediate filaments and contained an excess of glycogen. After six months, small granules of hemosiderin began to appear in cerebellar and pia-form cortices. The observations support that the sequence of conversion of hemoglobin to ferritin and hemosiderin occurs in brain as in other organs.

Key Words: Electron microscopy; Ferritin; Hemoglobin iron; Histochemistry; Immunocytochemistry; Intracisternal injection; Superficial siderosis.

INTRODUCTION

Single episodes of subarachnoid hemorrhage generally leave no trace of surface pigmentation but recurrent extravasations over extended periods coat the exposed surfaces of cerebrum, brain stem, cerebellum, 8th cranial nerves, and spinal cord with a dark brown pigment which penetrates for 1–2 mm into the tissue. This rust-brown discoloration of a vast surface area of the central nervous system (CNS) is called superficial siderosis. It is an uncommon condition but may be representative of the cellular response of brain when it comes into contact with extravasated blood pigment. Clinical, neuropathological, and some neurochemical features of the disease were previously reported by Koeppen and Barron (1) and Koeppen and Dentinger (2). The clinical manifestations in an advanced case are quite characteristic and include profound neurosensory hearing loss, cerebellar ataxia, and spastic paraparesis. Laboratory confirmation is now possible by magnetic resonance imaging which reveals signal depression in T2-weighted images and directly visualizes the accumulation of iron (3).

Recent immunocytochemical and affinity cytochemical work on superficial sid-
erosion (2) suggested the following pathogenesis of human superficial siderosis: When hemorrhagic cerebrospinal fluid (CSF) continues to contact the surface of the brain, microglial activation (hypertrophy and hyperplasia) takes place in the subpial parenchyma. This cellular response is especially vigorous in the molecular layer of the cerebellum. Microglia can be demonstrated with antisera to heavy (H)-ferritin and light (L)-ferritin, and by their reactivity with the biotinylated lectin Ricinus communis agglutinin 1 (RCA-1). Bergmann glia may serve as transport conduits of hemoglobin iron from the CSF to the microglia of the molecular layer. Ferritin is locally converted to hemosiderin which accounts for the brown pigmentation of the brain, and for advanced cases the term "incrustation" is appropriate. In an effort to examine the pathogenesis of superficial siderosis, repeated intracisternal injections of autologous red blood cells (RBC) were performed over three- and six-month periods in adult New Zealand white rabbits.

MATERIAL AND METHODS

Adult male New Zealand white rabbits (3–4 kg) were anesthetized by an intramuscular injection of fentanyl (0.4 mg/ml) and droperidol (20 mg/ml). The usual injected dose was 0.15 ml/kg.

Preparation of Autologous Red Blood Cells

Blood from the ear artery was collected into heparinized tubes and centrifuged. The pelleted cells were suspended in lactated Ringer’s solution, centrifuged and washed three times. The packed red blood cells (RBC) were sealed in sterile serum bottles and stored at −20°C until use. Rubber septum and crimp permitted the removal of small volumes of RBC for intracisternal injection. Since the purpose of the injections was the delivery of measured amounts of blood pigment, it was not considered essential that RBC remained intact.

Intracisternal Injections

The cisterna magna was punctured as described by Pette (4) and Plaut (5), and weekly injections of 0.1 ml RBC were made for three months. One animal received RBC injections at weekly intervals for six months. The only untoward effect was transient opisthotonus. The intracisternal injection of lactated Ringer’s solution without RBC commonly produced minor bleeding and therefore was not an acceptable control. Accordingly, rabbits which received no intracisternal injections served as normal controls.

Histology and Immunocytochemistry

After three or six months, the experimental rabbits were reanesthetized and killed by intracardiac perfusion with various fixatives and buffers. For iron, aldehyde-fixed cerebellum and piriform cortex (see electron microscopy below) were cut on a vibratome at 40 μm and incubated in Perl’s solution, followed by enhancement of the reaction product with diaminobenzidine tetrahydrochloride and hydrogen peroxide (6, 7). Gomori’s solution (8) was used for iron stains of the gross specimens. Optimal immunocytochemical results were obtained with neutral buffered formalin followed by acetic acid–ethanol (1:3) and paraffin embedding. Freeze-substitution and paraffin embedding (9) were used only for ferritin immunocytochemistry.

Rabbit liver ferritin was isolated from an iron dextran-treated rabbit by standard procedures and a final purification by preparative polyacrylamide gel electrophoresis. Two rats were inoculated with 50 μg of rabbit liver holoferitin in complete Freund’s adjuvant. Two additional injections of 50 μg each in incomplete adjuvant followed. One week after the last injection, the animals were anesthetized and bled from the heart for the collection of plasma. The presence of antibody to holoferitin was checked by double immunodiffusion. The rat anti-rabbit liver ferritin and a commercially available rabbit anti-human liver ferritin (Boeh-
ringer-Mannheim, Indianapolis, IN) worked well on freeze-substituted, paraffin-embedded sections but not on paraffin sections from acetic acid-ethanol fixed tissues. An anti-human liver ferritin donated by Dr. James Drysdale (Boston) proved most successful for paraffin-embedded sections after perfusion with acetic acid-ethanol mixtures. All antisera were diluted 1:1,000, and the binding sites were visualized by the avidin biotin peroxidase complex (ABC) method (Vector Laboratories, Burlingame, CA) (10). Diaminobenzidine tetrahydrochloride was the chromogen.

For glial fibrillary acidic protein (GFAP), a commercial polyclonal antiserum (DAKO, Santa Barbara, CA) was used.

Electron Microscopy

For electron microscopic observations, the anesthetized rabbits were perfused with aldehydes (11). The brain was removed promptly and hemisected. Iron histochemistry was performed as described above on one half of the brain. The other half was immersed in fixative for an additional two hours and washed in sodium cacodylate-sucrose buffer. Minced tissues and vibratome-sectioned (100 μm) blocks of cerebellum and piriform cortex were postfixed in osmium tetroxide and either mordanted en bloc in tannic acid (12) or processed without the tannic acid treatment. All specimens were dehydrated in graded concentrations of ethanol and propylene oxide and embedded in an epoxy resin. Plastic-embedded blocks were cut at 1 μm thickness and stained with toluidine blue-ryonycin Y. Blocks were trimmed to the area of interest, and ultrathin sections were cut and collected on grids. Ultrathin sections that had received en bloc tannic acid treatment were stained with lead citrate. Those ultrathin sections obtained from blocks in which the tannic acid step was omitted were either left unstained or were stained with uranyl acetate-lead citrate. Some sections were treated with bismuth subnitrate for ferritin enhancement (13).

RESULTS

Gross Appearance and Macroscopic Iron Stain

After intracisternal injections over three months, a faint brown color appeared on the surface of the brain stem, cerebellum, and base of the cerebral hemispheres. Immersion of the specimen in Gomori’s solution (8) produced a bright blue coloration of brain stem and cerebellum. The quadrigeminal plate was most intensely stained whereas cerebellum and cerebral hemispheres revealed less intense staining. In the cerebrum, the medially located piriform cortex showed the most intense iron stain. The paraflocculus which has its own bony chamber remained nearly unstained (Fig. 1). The extension of the blue color for a short distance into brain parenchyma was readily seen on sagittal sections of the brain stem (Fig. 2). After six months, the brown color of the perfusion-fixed brain was unmistakable. The general distribution of the pigmentation was not different from the appearance in the three-month specimen.

Light Microscopy

Hematoxylin and eosin stains of paraffin sections revealed no pathological changes at three months and only some thinning of the molecular layer near the crest of the cerebellar folia at six months. No remarkable change occurred in the piriform cortex but there was some thickening of the overlying arachnoid membrane. At three months, routine iron stains of paraffin sections revealed no reaction product. Perl’s stain with diaminobenzidine/hydrogen peroxide enhancement was also negative. At six months, fine blue granules were found in the perivascular spaces of the cerebellar molecular layer and the superficial piriform cortex. Scattered, much coarser hemosiderin granules appeared in the arachnoid membrane.

Figs. 1, 2. Macroscopic iron stain of brain stem and cerebellum after intracisternal injections of autologous RBC for three months. Intense blue reaction product is present over the brain stem. The cerebellum is also stained except for the paraflocculus (Fig. 1). The sagittal section of the specimen in Figure 1 shows that reaction product penetrates for a short distance into the parenchyma (Fig. 2).
Figs. 3–8. Iron histochemistry of siderotic and normal cerebellum and piriform cortex (vibratome sections). Reaction product was enhanced with diaminobenzidine and hydrogen peroxide. The experimental rabbit received intracisternal injections for three months. The injections produced focal accumulation of iron in the cerebellar molecular layer (Fig. 3) which was absent in the normal rabbit (Fig. 4). The dense reaction product was due to iron reactivity in microglia (arrows) and Bergmann glia (arrow heads) (Fig. 5). The cytoplasm of Golgi epithelial cells was also reactive (not illustrated). The normal cerebellar cortex revealed only occasional iron-reactive microglia (arrow) (Fig. 6). Figure 7 is from the siderotic piriform cortex and shows reaction product in microglia (arrows) and astrocytes (arrow heads). Iron-reactive microglia were very rare in normal piriform cortex (arrow in Fig. 8). Magnification markers: 1 mm in Figures 3 and 4; 50 μm in Figures 5–8.

Siderotic and normal tissues are compared in Figures 3–20. In each of the composites, normal cerebellum and piriform cortex are shown on the right side.

In contrast to paraffin sections of the three-month survivors, vibratome sections of the same animal revealed dense focal reaction product at or near the crest of cerebellar folia. At higher magnification, reaction product for iron occurred in hypertrophic microglia and in Bergmann glia (Fig. 5). Very sparse iron reactivity was

Figs. 9, 10. Iron histochemistry of siderotic cerebellar cortex (vibratome sections). Reaction product was enhanced with diaminobenzidine and hydrogen peroxide. The experimental animal received intracisternal injections for six months. Microglial cells in the cerebellar molecular layer reveal refractile cytoplasmic inclusions interpreted as incipient hemosiderin granules (arrows) (Fig. 9). In the normal cerebellar cortex (Fig. 10), these inclusions are absent, and iron reactivity is very light and diffuse. Magnification markers: 10 μm.

present in the normal cerebellar molecular layer. An occasional microglial cell was iron-reactive (Fig. 6). The superficial piriform cortex of the injected animals revealed reaction product in microglia and possibly astrocytes, though staining of the latter was pale (Fig. 7). The reaction produced a peculiar reticulated pattern in the subpial cortex (Fig. 7). In the normal piriform cortex only a few scattered microglia stained for iron (Fig. 8). Prior immersion of the vibratome sections in a chelating solution of 2,2'-dipyridyl and sodium hydrosulfite in acetate buffer (pH 5.6) for 15 minutes (2) eliminated all iron reaction.

Vibratome sections obtained from cerebellar and piriform cortices after weekly injections for six months gave similar results. However, there were also small refractile non-birefringent concretions within the processes of microglia (Fig. 9). They were similar to the iron deposits seen in paraffin sections and therefore thought to represent incipient hemosiderin. The normal cerebellar and piriform cortices never revealed similar inclusions though faint iron reactivity could be detected in microglial cytoplasm (Fig. 10).

The result of ferritin immunocytochemistry was quite similar to iron histochemistry. In the molecular layer of the cerebellar cortex, reaction product was visualized in hypertrophic and hyperplastic microglia, Bergmann glia and occasional Purkinje cell dendrites (Fig. 11). The normal molecular layer contained occasional microglia with ferritin immunoreactivity (Fig. 12). In the piriform cortex, hypertrophic and hyperplastic cells thought to be microglia reacted with ferritin antiserum (Fig. 13). Normal piriform cortex also contained scattered ferritin-positive microglia (Fig. 14). The arachnoid over siderotic cerebellum and piriform cortex was always densely ferritin-positive.

In the siderotic cerebellum, focal hypertrophy of Bergmann glia occurred in patches at or near the crest of the affected folia (stain for GFAP, Fig. 15). At higher magnification, the dense reaction product was seen in expanded straight processes and in their lateral branches (Fig. 17). The normal cerebellum never showed the same focal hypertrophy of Bergmann glia (Figs. 16, 18). In the siderotic piriform cortex (Fig. 19), GFAP-reactive astrocytes extended over a wider cortical band than in the normal state (Fig. 20).
Figs. 11–14. Ferritin immunocytochemistry (paraffin sections; hematoxylin counterstain). The experimental animal received intracisternal injections for three months. In siderotic cerebellum (Fig. 11), reaction product visualizes microglia (arrows), Bergmann glia (arrow heads), and occasional Purkinje cell dendrites. The normal cerebellar molecular layer (Fig. 12) shows only occasional ferritin-reactive microglia (arrow). Hypertrophic microglial cells are present in the siderotic piriform cortex (arrows) (Fig. 13). Occasional microglia are visualized in the normal piriform cortex (arrow) (Fig. 14). Magnification markers: 50 μm.

**Electron Microscopy**

Electron microscopic findings on the molecular layer of the cerebellar cortex were highly characteristic. Microglia were distinguished from other glia, neurons, and endothelial cells by their rich content of electron dense particles measuring 5–6 nm in diameter. These were interpreted as ferritin granules. In normal rabbits, ferritin granules were scattered throughout the cytoplasmic matrix and served as a marker for the sparse population of microglia in the neuropil of the molecular layer (Fig. 21). Microglia were more frequent in the cerebellar cortex of the three-month siderotic rabbits. These microglia were replete with ferritin granules within their cytoplasm, and the electron dense particles extended into the cellular processes (Fig. 22). In addition, closely packed granules of ferritin formed a border of varying width around heterogeneous cytoplasmic inclusions (Fig. 22). In the specimen from the six-month siderotic rabbit, microglia in the molecular layer were hypertrophied. Their cytoplasm contained free particles of ferritin, ferritin micelles, and groups of heterogeneous inclusions associated with a network of ferritin aggregates (Fig. 23). In most cases, these inclusions extended irregularly throughout much of the length of the microglial processes (Fig. 24). The composition of these dense bodies was similar to ferritin and ferritin micelles. Clusters of ferritin of variable width bordered the inclusions. The dense bodies were interpreted as hemosiderin and corresponded
Figs. 15–20. GFAP immunocytochemistry (paraffin sections; hematoxylin counterstain). The siderotic cerebellar cortex shows focal increase of GFAP-reaction product in Bergmann glia (Fig. 15). Accumulation of GFAP-reaction product is present in the vertical shafts of Bergmann glia and in their lateral branches (Fig. 17). The normal cerebellar cortex has relatively slender Bergmann glia (Figs. 16, 18). The siderotic piriform cortex (Fig. 19) shows more numerous astrocytes in the deeper layers when compared to the normal piriform cortex (Fig. 20). Magnification markers: 1 mm in Figures 15 and 16; 50 μm in Figures 17 and 18; 100 μm in Figures 19 and 20.

Figs. 21–23. Electron microscopy of the normal and siderotic molecular layer. The occasional microglial cell found in the molecular layer of the normal rabbit cerebellar cortex has a typical nucleus (N) and contains ferritin particles in its cytoplasm (Fig. 21). Inset (Fig. 21): Cytoplasmic ferritin particles of the boxed area are shown at higher magnification. In the molecular layer of the cerebellar cortex of the siderotic rabbit (intracisternal injections of RBC for three months; Fig. 22), ferritin granules adjacent to the nucleus (N) outline the cytoplasm of a microglial cell and extend into its processes (arrow). In addition, these particles form aggregates of variable width around the periphery of cytoplasmic dense bodies and lipid-containing structures. Inset: Higher magnification of boxed area showing ferritin aggregates.
In the molecular layer of the cerebellar cortex of the siderotic rabbit after intracisternal injections over six months, hypertrophy of a microglial cell is apparent (Fig. 23). A small region of the cytoplasm adjacent to the nucleus (N) is shown and contains heterogeneous dense bodies (asterisk), ferritin micelles (arrows), and free ferritin particles. Insert: Higher magnification of the boxed area shows a cytoplasmic dense body with clusters of ferritin encircling its periphery and a portion of an adjacent ferritin micelle. Unstained sections. Magnification markers 500 nm; Insets: 50 nm.
Fig. 24. Electron microscopy of the cerebellar molecular layer after intracisternal injection of RBC for six months. A microglial process can be easily distinguished from other contents of the neuropil (NP) by the presence of free ferritin, ferritin aggregates, and electron dense inclusions. The denser inclusions are characteristic of hemosiderin granules. Unstained section. Magnification marker: 500 nm.

to the refractile inclusions seen by light microscopy (Fig. 9). The cytoplasm around these inclusions contained free ferritin and ferritin micelles. The ends of microglial processes were closely related to small blood vessels.

Ferritin granules in microglia were readily visualized in unstained ultrathin sections (Fig. 25) whereas their presence was masked by staining with uranyl acetate-lead citrate. However, use of bismuth subnitrate (13) enhanced the size and appearance of ferritin granules in microglia (Fig. 26). The presence of ferritin in microglia was not unique to the cerebellar cortex. Similar form, distribution, and association of ferritin were identified in microglia of the piriform cortex (Fig. 27). At the ultrastructural level, ferritin was not detected in astrocytes in either location. In the cerebellar cortex of three-month siderotic rabbits, processes of Bergmann glia were hypertrophied and contained abundant glycogen (Fig. 28) in comparison to Bergmann glia in normal specimens (Fig. 29).

DISCUSSION

Superficial Siderosis after Spontaneous and Experimental Subarachnoid Hemorrhages

The literature on experimental subarachnoid hemorrhage has been skillfully summarized by Butler et al (14) who pointed out that subarachnoid blood is extravasation of RBC and other blood constituents, including copious amounts of protein. These investigations emphasized the clearance of RBC from the CSF and ultimately concluded that most erythrocytes are lysed rather than transported intact back to the circulating blood via the arachnoidal villi. Little attention was paid to the tissue reactions in the CNS proper, and the rarity of superficial siderosis stands out in contrast to the common spontaneous hemorrhages.

Ferritin in the CSF rises in response to brain infarctions or hemorrhages (15, 16) but non-hemorrhagic lesions may also be associated with ferritin increase. The higher than normal levels of ferritin in CSF suggested that the protein was biosynthesized in larger than normal amounts by brain tissue which came into contact with hemoglobin or more likely hemoglobin iron. The intracisternal injection of washed autologous RBC did not duplicate spontaneous or one-time experimental subarachnoid hemorrhages but more likely equalled the minor recurrent subarachnoid extravasations which are thought to cause superficial siderosis. A very similar condition could be induced very readily by the intracisternal injection of soluble iron salts or compounds (17–20). In contrast to iron salts, hemoglobin delivers relatively little iron to the cerebral surfaces (0.34% by weight of hemoglobin), and this fact is the likely explanation why prolonged subarachnoid bleeding or repeated intracisternal injections of RBC are required for the induction of siderosis. In the experiments reported here, hemosiderin was demonstrable in paraffin sections only after injections for six months but not after three months.

Hemosiderin and Ferritin in the Brain

While the emphasis in superficial siderosis was on “incrustation” of cerebral surfaces by hemosiderin, the role of soluble ferritin and other “hemosiderin precursors” in the pathogenesis of superficial siderosis was not often considered. Köppen and Dentinger (2) reviewed the relationship of hemosiderin and ferritin in brain and other organs. Despite occasional dissenting publications, a precursor role of ferritin is now widely accepted. Blinzinger (20) detected the accumulation of ferritin in rabbit brain after the intracisternal injection of iron salts and concluded that the
Figs. 25, 26. Electron microscopy of ferritin-containing microglial cells after enhancement by bismuth subnitrate. In an unstained section of the cerebellar cortex of a three-month siderotic rabbit (Fig. 25), the ferritin particles, confined to the microglial process, are of uniform size and distributed in all portions of the process. In Figure 26, the ferritin particles in a similar microglial process from the molecular layer of a three-month siderotic cerebellum.
Figs. 28, 29. Electron microscopy of the siderotic cerebellar cortex after intracisternal injection of RBC for three months. Bergmann glia in the siderotic cerebellar cortex (Fig. 28) typically contain filaments (F) and numerous glycogen particles (arrows). In contrast, the Bergmann glia process from a normal rabbit (Fig. 29) contains filaments (F) but few glycogen granules (arrows). Uranyl acetate–lead citrate stain. Magnification markers: 500 nm.

Iron-containing protein was closely related to superficial siderosis. It proved somewhat difficult to document the relationship of ferritin to hemosiderin because ferritin iron is highly water soluble and not readily detectable in processed sections. In contrast, apoferritin may remain demonstrable by immunocytochemistry with spe-

have been enhanced by staining with bismuth subnitrate. Compare the size of the ferritin particles in Figures 25 and 26. Magnification markers: 100 nm.

Fig. 27. Electron microscopy of the piriform cortex after intracisternal injection of RBC for three months. A microglial cell with nucleus (N) contains ferritin particles singly or in clusters of variable forms within the cytoplasm. Inset: Higher magnification of the boxed region to detail ferritin appearance. Unstained section. Magnification marker: 100 nm; Inset: 50 nm.
cific antisera applied to ordinary paraffin sections of human brain (2, 21). However, ferritin is a ubiquitous protein and should in theory be detectable in many neuronal and glial cells. It is not fully understood why microglia stain readily with anti-ferritin whereas other cell types rarely show reaction product in paraffin sections.

The Role of Glial Cells in Experimental Superficial Siderosis

The utility of ferritin immunocytochemistry for the visualization of microglia (2, 21) was confirmed for the rabbit although immunoreactive cells were very rare in normal rabbit brain. Successful visualization of ferritin at the ultrastructural level was made possible only by omission of the customary stains for electron microscopy such as uranyl acetate, lead citrate, and tannic acid. However, bismuth subnitrate enhanced the appearance of ferritin granules (13). It was of interest that bismuth improved ferritin resolution in naturally occurring ferritin accumulation though it was originally intended for ferritin-labeled antibodies and ferritin deposits after intravenous injection (13).

The injection of RBC quite obviously induced ferritin biosynthesis in these cells. Their numerical increase does not necessarily imply hyperplasia since they may have existed in equal numbers before the injection but may not have been detectable with ferritin antisera. However, hyperplasia was strongly suggested by the ease with which they could be found by scanning the sections with the electron microscope. Iron and ferritin stains correlated well, and it appeared acceptable to equate the iron excess on vibratome sections with ferritin accumulation.

The role of astrocytes in superficial siderosis is much less certain. Iron stains showed reaction product in Bergmann glia and the astrocytes of the piriform cortex. However, ferritin was detectable only in Bergmann glia, raising doubt about the nature of the iron in other astrocytes. Also, electron microscopy could not confirm ferritin particles in either type of astrocyte, possibly because this method does not recognize apoferritin, i.e. iron-free ferritin. Koeppen and Dentinger (2) previously proposed that the long apical processes of Bergmann glia took up hemoglobin iron from the CSF due to their proximity to the subarachnoid space. Iron was then thought to be transported as though in conduits to the cell bodies of Golgi epithelial cells where tiny hemosiderin granules developed. In the process of iron transport, the cytoplasm of Bergmann glia became ferritin-reactive. Microglia reacted to the local iron excess by similar increase of ferritin biosynthesis and ultimate formation of larger hemosiderin granules. In the course of this transformation, microglia became macrophages. This mechanism implied that hemosiderin was made inside the cell. The concept of a required cellular response in the formation of hemosiderin was first pronounced by Neumann (22) who coined the term “Hämosiderin”. The dense granules in the microglial processes of the siderotic cerebellar cortex after intracisternal injection of RBC over a six-month period also support this mechanism (Figs. 9, 24).

Enhanced biosynthesis of GFAP in Bergmann glia in experimental siderosis is thought to be a non-specific response.

Biochemical Considerations

Considerable progress has been made in our understanding of ferritin biosynthesis during iron excess (23–31). It has been known for many years that much of the soluble normal brain iron is ferritin (32). This observation implies that brain has the biochemical apparatus to biosynthesize ferritin since uptake of such a large molecule from the blood is unlikely. In brain, as in other organs, accelerated ferritin
biosynthesis occurs at the site of local and immediate contact with iron. In superficial siderosis, this iron derives from hemoglobin, and it is likely that some transport is required to deliver it to cells endowed with ferritin-messenger ribonucleic acid (mRNA). Nothing in this work addresses the possible role of brain transferrin and transferrin receptors though both are known to be quite abundant in brain (33, 34). The predominance of transferrin (protein and mRNA) in oligodendroglia raises doubt that transferrin is essential in the pathogenesis of superficial siderosis.

Ferritin is thought to be an iron detoxicant, and the metal may not be noxious to tissues until ferritin biosynthesis can no longer keep pace with the iron load. Though hemosiderin has been considered to be “tissue-toxic” it may actually be innocuous. However, an excess of ionic iron may stimulate lipid peroxidation which is responsible for delayed tissue necrosis (35, 36).

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