Spontaneous Vascular Mineralization in the Brain of Horses

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ABSTRACT. Cerebral vascular mineralization was found in 12 (60%) of 20 3- to 10-year-old healthy horses collected at an abattoir. It was variable in degree and occurred mostly in the pallidal arteries showing two types of lesions; small globoid bodies along capillaries, and amorphous deposits in the wall of arterioles, small- or medium-sized arteries and veins. Both types were strongly positive for periodic acid-Schiff reaction, and weakly positive for von Kossa's and Berlin blue stains. Elemental analysis of the deposit revealed the presence of large amounts of aluminum, moderate amounts of phosphorus, zinc, calcium and iron, and a small amount of sodium. — KEY WORDS: blood vessel, equine, globus pallidus, mineralization.

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Frequent occurrence of periodic acid-Schiff (PAS) positive cerebral mineralization has been reported in humans [1, 5, 12], monkeys [19], cows [18], and mice [3, 16]. The cerebral mineralization occurs in close association with vascular walls, and aging influenced the prevalence and severity in humans and mice [1, 16]. In horses, there have been a few reports on the occurrence of cerebrovascular mineralization [6, 13]. However, the pathogenesis of the lesions remains unclear and elemental analytical studies of the mineralized materials have not been applied.

In the present paper, cerebral mineralization in horses is described with special emphasis on incidental aspects in the animals under 10 years of age, morphological features of the lesions, and elemental analysis of the mineralized deposit.

MATERIALS AND METHODS

The brains of 20 horses, aged 3 to 10 years, were examined in this study (Table 1). These consisted of 17 heavy horses of Percheron-cross breeds used for farming and carrying, and of 3 light horses of Arabian-cross breeds used for horse racing.

Grossly normal brains were sampled immediately after killed at the municipal abattoir in Kumamoto city, Japan and fixed with 10% buffered formalin. The brains were routinely embedded in paraffin, and sections were stained with hematoxylin and eosin (HE). Selected sections were also subjected to PAS, alcian blue, von Kossa, Berlin blue, Turnbull blue, Congo red, phosphotungstic acid hematoxylin (PTAH), elastica Van Gieson, and Watanabe's silver impregnation. Tissue sections were prepared from various levels of the cerebral cortex, Ammon's horn, caudate nucleus, globus pallidus, thalamus, pons and medulla oblongata.

Severity of mineralization on microscopic examination was graded as follows: none = (-); a few globoid mineralization in capillaries, venules and arterioles = (+), mild; many globoid mineralization / mineralization of larger vessels, particularly in the media = (++), moderate; more extensive / large mineralization, = (+++), severe.

For X-ray microanalysis, formalin-fixed and paraffin-embedded tissue samples from an affected pallidum (No. 18) were de-waxed and fixed in 2% cacodylate buffered glutaraldehyde. Tissue blocks were processed through graded ethanol series and embedded in epoxy resin in a routine manner. Sections at 1 μm thick were cut and stained with toluidine blue to find mineralized lesions. Thin sections at 100 nm thick were made and mounted on uncoated beryllium grids, and examined with a Hitachi H-800 electron microscope at an accelerating voltage of 20 kV combined with an energy-dispersive X-ray microanalyzer (EDAX system, model 9100/60), using a sample holder made of spectrographically pure carbon. The specimens were screened in the scanning transmission (STEM) mode and appropriate portions were defined for elemental analysis. The spectra were smoothed and the background was subtracted using a dedicated microprocessor (including in EDAX system). Net peak intensities were then used for quantitative evaluation.

RESULTS

Under light microscopy, 12 (60%) of 20 horses examined revealed mineralization of varying degrees in the brain (Table 1). The incidence increased with aging; 3 of 8 horses at 3 years old and 9 of 12 cases over 4 years old had the lesions. Animals with moderate lesions were first found in cases over 4 years of age, and a 7-year-old female showed severe changes. There was no apparent sex difference in the incidence.

The mineralized lesions were observed mainly in the globus pallidus and rarely in the dentate nucleus. The other parts of the brain, such as the putamen or hippocampus,

Table 1. Incidence and severity of mineralization in the brains of horses

No.	Breed	Age (years)	Sex	Body weight (kg)	Mineralization	
					Pallidum	Dentate
1	Heavy	3	Gelding	850	–	–
2	Heavy	3	Gelding	750	–	–
3	Heavy	3	Gelding	860	+	–
4	Heavy	3	Gelding	850	–	–
5	Heavy	3	Gelding	780	–	–
6	Heavy	3	Gelding	850	+	–
7	Heavy	3	Female	800	+	–
8	Heavy	3	Female	780	–	–
9	Heavy	4	Male	940	+	–
10	Heavy	4	Gelding	890	++	–
11	Heavy	5	Female	700	+	–
12	Heavy	6	Gelding	970	++	–
13	Heavy	6	Gelding	800	–	–
14	Heavy	6	Female	800	–	–
15	Light	7	Male	450	+	–
16	Heavy	7	Gelding	700	+	–
17	Heavy	7	Female	780	+	–
18	Heavy	7	Female	900	+++	–
19	Light	8	Female	450	–	–
20	Light	10	Male	500	++	+

–, No lesion; +. mild, ++, moderate; +++, severe.

were spared. Mineralization occurred in or around the vessel walls, showing two types of lesions: small globoid bodies along capillaries (Fig. 1) and amorphous depositions in and around the walls of arterioles, small- or medium-sized arteries and veins (Figs. 2–5). The former type was deeply basophilic, usually round, varying in size, approximately 3 to 10 μm in diameter, and frequently coalesced forming chains or conglomerates of globules surrounding the capillaries. The small globoid deposits were occasionally found to be free in the parenchyma, having no relation to capillaries. In the other type, faintly basophilic or acidophilic amorphous deposits were observed in and around the vascular walls, usually round to oval in shape, and had a uniform appearance, often showing concentric lamellar structures (Fig. 3). In a severe case, the deposits in the vascular walls grew and fused each other, and eventually the media of vessels appeared as a ring or sheath of faintly basophilic substance. The lumina of the involved vessels were frequently narrowed or occluded by the protruding deposits (Fig. 7). Dilatation of perivascular space was usually observed (Figs. 6 and 8). As mildly mineralized lesions corresponding to initial stages, fine or amorphous basophilic materials were present in the media of small- and medium-sized arteries (Figs. 4 and 6), and acidophilic

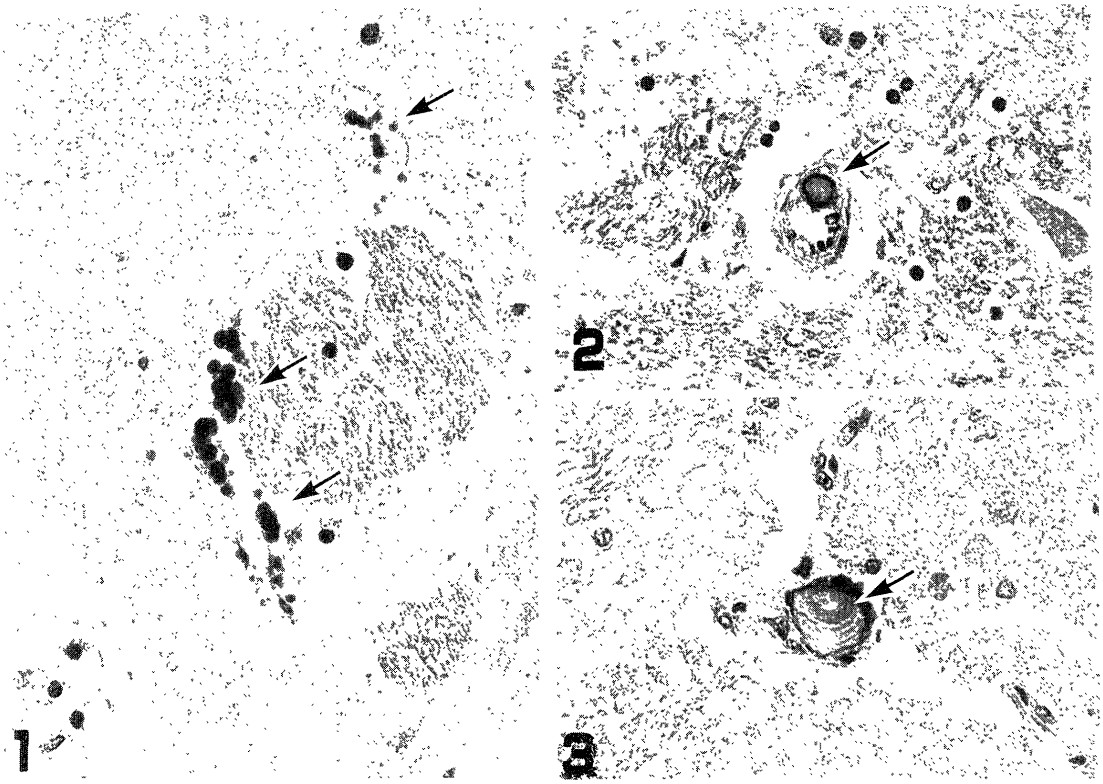

Fig. 1. Globus pallidus (No. 18). Multiple deeply basophilic small globoid bodies (arrows) along capillaries, some of which coalesce and form conglomerate of globules. HE stain. × 450.
Fig. 2. Globus pallidus (No. 6). A small amorphous deposit (arrow) in the wall of an arteriole. HE stain. × 450.
Fig. 3. Globus pallidus (No. 6). A deposit with concentric lamellar structure (arrow) in the wall of an arteriole. HE stain. × 450.

amorphous substances were deposited in the perivascular space of venules (Fig. 5). No inflammatory or glial reaction was seen around both types of the mineralized lesions.

The mineralized lesions were either faintly to intensely basophilic or acidophilic with HE stain, and were intensely positive for PAS reaction after diastase digestion. They were also positive for acid mucopolysaccharides by alcian blue stain. Weakly positive reaction was observed in the

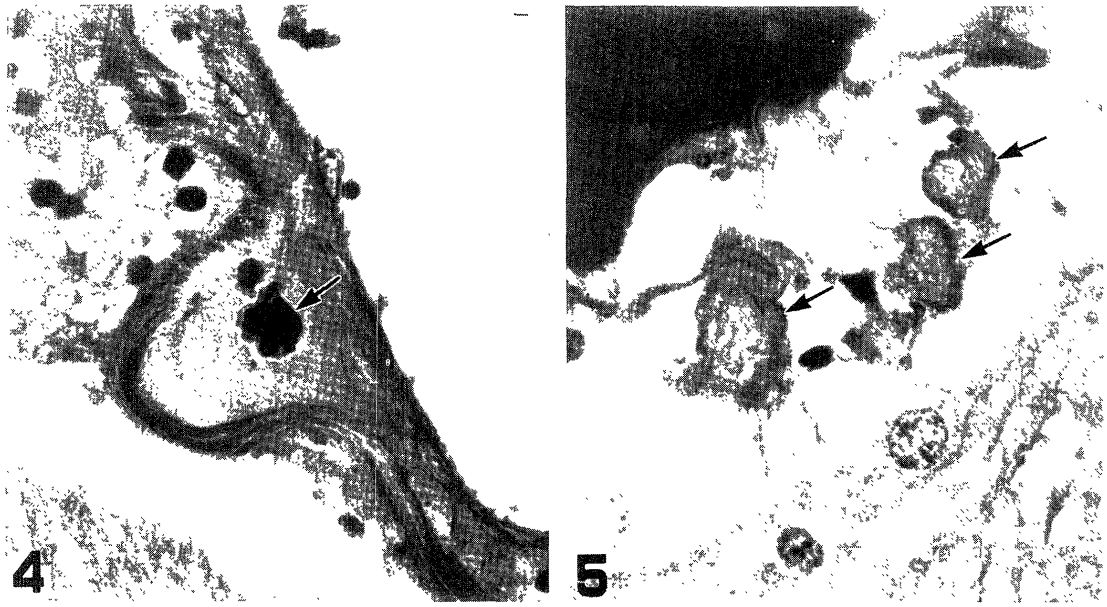

Fig. 4. Globus pallidus (No. 17). A small deposit (arrow) occurs in the focally thickened wall of a blood vessel. Perivascular edema is found. HE stain. × 900.

Fig. 5. Globus pallidus (No. 16). Acidophilic amorphous deposits (arrows) in the perivascular space of a blood vessel. HE stain. × 900.

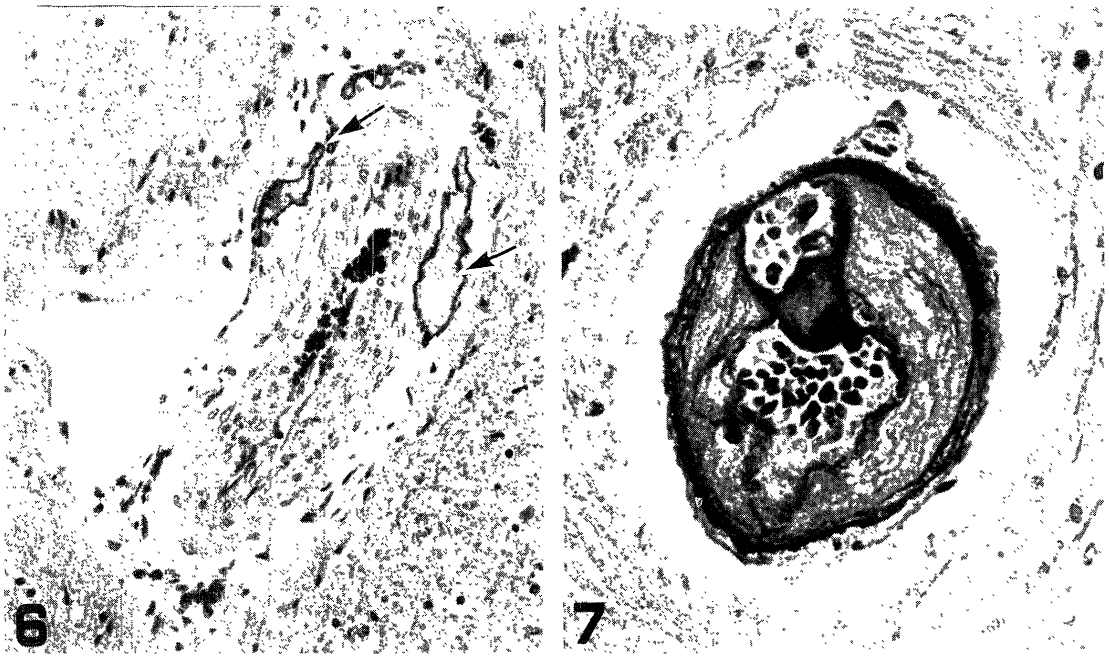

Fig. 6. Globus pallidus (No. 10). Mineralization (arrows) in the thickened wall of a small artery. Note dilatation of a perivascular space. HE stain. × 225.

Fig. 7. Globus pallidus (No. 18). Marked deposition of amorphous substance in the wall of a small artery. There is considerable narrowing of the arterial lumen due to protrusion of the deposits. HE stain. × 450.

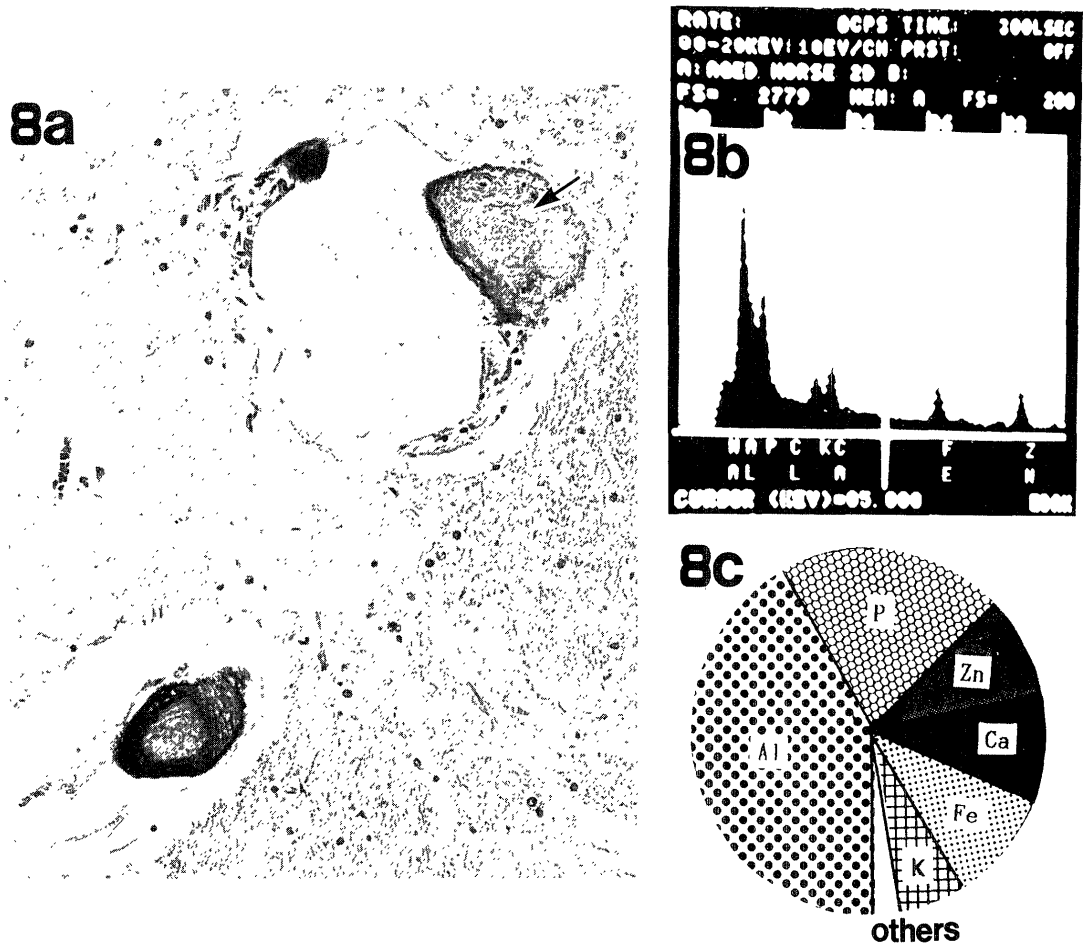

Fig. 8. Globus pallidus (No. 18). a. Amorphously mineralized deposits in the wall of small arteries. A deposit (arrow) in serial sections cut at 50 μm was subjected to X-ray microanalysis. HE stain. × 225. b. Elemental spectra over identical field shown in Fig. 8a. Elemental spectra indicated calcium (CA), phosphorus (P), aluminum (AL), iron (FE), zinc (ZN) and sodium (NA). c. The circular "pie chart" represents a quantitative analysis (weight percent) of the peaks shown in Fig. 8b.

deposits with von Kossa's stain. The deposits were positive for iron by both Berlin blue and Turnbull blue stains, and negative for the Congo red, PTAH, elastica Van Gieson and silver impergnation stains.

Elemental spectra on the deposition in a severe case (No. 18) revealed the presence of large amounts of aluminum and phosphorus, moderate amounts of calcium, iron, zinc and potassium (Fig. 8-b and 8-c). In quantitative analysis, the deposition in the case contained aluminum of 41.5%, phosphorus of 20.5%, calcium of 8.9%, zinc of 10.0%, iron of 9.4%, potassium of 8.0% and sodium of 1.7% by percentage weight.

DISCUSSION

Diagnostic and pathologic significance on cerebral mineralization in humans differed in each report [1, 5, 12, 14, 15]. Hurst [6] examined the morphologic features of cerebral mineralization in horses which died of various disease conditions and concluded that it was an age-related change. Saunders [13] reinvestigated the lesions of healthy horses over 6 years of age obtained from abattoirs, and thought them to be no correlation with aging. Although he described the morphological features in detail, the incidence of the lesions or their accurate location in blood vessels was not shown. Hence, it was felt that a re-evaluation of lesions conducted on a random series of horses from an abattoir would be of value.

The incidence rate of the brain mineralization was 60% in the present cases, which was almost identical to that in the previous reports; 60% (15/25) in healthy horses [13] and 77.8% (14/18) in aged horses [6]. The frequency of the lesions in the present cases was higher in horses over 4 years of age than that in animals at 3 years of age. Therefore, aging may be an important factor in the manifestation of pallidal vascular mineralization in the

horse. The cerebral mineralization has been found in 80% of brains in man at and over 70 years old [1, 12]. In B6C3F1 mice, the incidence and severity were age dependent, and 60% of mice at 109 weeks of age had lesions [16]. As for the site of the involvement, Hurst [6] found severe mineralization in both the globus pallidus and dentate nucleus in a horse over 20 years old. In humans, cerebral mineralization always occurs in the globus pallidus and less frequently in the dentate nucleus, and the severe degree of the lesions is often called "striopallidodentate calcification" [7].

The morphology and histochemistry of cerebral mineralization in the present horses closely resemble those of humans and monkeys in which two types of lesions are commonly found in the globus pallidus: basophilic globoid bodies in or around the capillaries, arterioles or venules (type A), and sheath-like basophilic depositions mainly in the media and adventitia of small- or medium-sized arteries (type B) [5, 12, 14, 19]. In mice, type A lesions were observed in the vascular wall of the thalamus [16, 20]. In cows, type B alterations with prominent intimal thickening were found in the globus pallidus [18].

The present histochemical and elemental analytical studies have shown that the depositing material consisted mainly of aluminum, phosphorus and calcium, with smaller amounts of other minerals embedded in PAS positive mucopolysaccharide matrix. These components resemble those observed in monkeys [19], rats [17] and mice [20]. A number of studies have demonstrated aluminum in high concentration in the neurofibrillary degeneration of the Alzheimer type which in the amyloid cores of senile plaques, which may play an active role in the promotion of diseases and contribute to the molecular disorder which eventually results in altered function and cell death [8].

The pallidal mineralization seems to be common in humans, cows and horses, but the actual evaluation of this phenomenon is still unclear. Hurst [5] regarded cerebral mineralization as an aging process, not directly related to the cause of death, but correlated with a high level of iron in the basal ganglia in which it occurred. Slager and Wagner [14] considered the perivascular location of the deposits to be significant and postulated them to be a vascular rather than a nervous tissue origin. The developmental process of the deposits was regarded as being possibly due to fatigue and anoxia resulting in an accumulation of mucopolysaccharide ground substance around capillaries, and the lesions were thought to appear first in susceptible areas such as the globus pallidus, which is highly sensitive to anoxia [11]. Gometz *et al.* [4] stressed an abnormality of the vascular endothelial basement membrane preceding mineral accumulation in human cerebral mineralization.

PAS-positive intimal asteroid bodies were frequently observed in equine small arteries of the gastrointestinal tracts [2, 10]. Although small globoid mineralization around pallidal capillaries resembled the intimal asteroid bodies in size and shape, the locations in the blood vessel differed: The former was located in the periphery of capillaries, while the latter occurred in the subendothelial space of small arteries, usually bulged into the lumen. Elemental analysis revealed that the asteroid bodies contained a fairly much amount of aluminum as well as phosphorus and calcium [2]. The pathogenesis of these asteroid bodies is also unknown, but they may have a relationship with equine calcium metabolism. Blood calcium values in horses are approximately 10% higher than those in other animal species [9]. Although the intimal asteroid bodies differ from the pallidal mineralization in the morphology and involved site in blood vessels, they seem to be a common factor causing the two different types of vascular mineralized lesions containing aluminum as well as phosphorus and calcium.

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