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Laser-Spectrographic Analysis of the Cation Content in Fahr's Syndrome

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Summary. The cation content and composition of mineral deposits in cerebral vessels in a case of Fahr's syndrome were studied by laser-spectrographic analysis. It was shown that in addition to Ca and Fe, other minerals such as Mg, Al, and Zn accumulated in pathologic deposits. The occurrence of cations other than Ca and Fe in these deposits had not been previously reported. The observations made in this study may contribute to a better understanding of the etiopathogenesis of Fahr's syndrome.

Key words: Fahr's syndrome – Laser analysis – Cations in brain – Mineralization of blood vessels.

Introduction

Processes leading to mineralization of cerebral vascular walls are currently a focus of scientific interest [1, 3, 5, 11, 12], despite the fact that over one-hundred years have passed since the first case of calcification in the brain was reported by Virchow [13]. Subsequent studies revealed that calcium is not the only mineral deposited in the affected vessel walls, as the lesions frequently become incrusted with iron as well [8, 10].

Deviations in the cerebral calcium and iron metabolism are more intriguing, as the presence of these minerals is limited to certain brain regions. The pathomechanisms of these abnormalities are obscure despite many efforts that have been made to determine their nature. The question also arises whether calcium and iron are the only minerals accumulated in cases denoted as Fahr's syndrome, in which siderocalcification of cerebral vessel walls is the outstanding pathognomic feature, or whether deposition of other minerals occurs as well.

To answer this question, we investigated the cation content of the affected cerebral vessels in a case of Fahr's disease by means of laser-spectrographic

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analysis. This method is known for its great sensitivity and selectivity, which far surpass that of other histochemical staining procedures [6a and b].

Case History

The patient, a female, suffered her first epileptic fit at the age of 56. After this fit, a discrete right-sided hemiparesis was observed, which disappeared the following day. The clinical picture revealed psycho-organic symptoms, such as lack of allopsychic orientation, psychomotoric sluggishness, difficulties in perception and concentration, slowness of thinking, and defects in all memory functions. From the very beginning of the illness, the patient displayed periodic tremors of the left-hand digits and some motoric restlessness in all fingers, resulting in such involuntary motions as buttoning, fondling, shaking off, and wiping. At this time, the patient demonstrated a positive grasping reflex on the left-hand side and bilateral Rossolimo signs in the upper extremities. When ordered to perform a certain motion, she frequently displayed motoric perseveration. Her appearance showed extreme restlessness, and she would get up suddenly and aimlessly from her chair and then sit down at the next moment. Sometimes she persevered in standing for a longer time. When questioned about the reason for her action, she could not explain, but insisted she was somehow forced into such behavior.

In 1944 she underwent surgery owing to hyperthyreosis with exophthalmus. In 1960 she was operated upon for cataract of the right eye and, in 1975, of the left one. There were no signs of impaired function of the internal organs. The patient had a blood pressure of 120/80 mm Hg and a pulse rate of 64/min. Skull X-rays and left-carotid angiography were normal. Lumbar punction: cytosis 1 lymph, protein—21 mg%, pressure of the CSF—189/160 mm H_2O.

Electroencephalography: Against the background of a changed basic function shown as dispersed single theta waves. bilateral series of paroxysmal discharges were observed that consisted of rather irregular waves of 4—5 Hz and up to 50 mV. As a rule, discharges originating from the left side were of higher amplitude. Serum calcium level was 4 mEq/l.

During hospitalization the patient suffered from tonic epileptic seizures followed by slight paresis of the right extremities. Her condition deteriorated quickly and she died showing symptoms of paralysis of the respiratory center 10 days after admission to the hospital.

Material and Methods

Sections of all cortical regions and basal nuclei, the cerebellum, pons, and medulla oblongata were taken for microscopic examination. Paraffin sections, 7 μ thick, were stained with Nissl's, H+E, Kanzler's, and van Gieson's stains. Calcium deposits were demonstrated by the method of Kossa.

Laser-spectrographic analysis was performed on 15 μ thick sections mounted on plexiglass plates obtained from the same brain regions. Some of these sections were stained with hematoxylin for topographic delineation of the lesions, the remainder were analyzed unstained by a Zeiss microanalyzer, type LMA-1, and a Zeiss spectrograph, type PGS-2, using Kodak type 103-a-0 plates.

Identification of elements detected in the analyzed preparation was derived from the spectrogram with the aid of the Abbe comparator and respective spectrographic tables. Controls for eliminating eventual contamination introduced by the staining reagent, the plexiglass plate, or the electrodes were included in the experimental procedure.

Quantitative evaluation of cation content was essentially based on the method ΔS as described by Kozik et al. [6a and b] for the quantitative assay of acid phosphatase activity, a slight modification being introduced by application of an external standard in the form of an illuminated, developed, stripped film (Kodak, type AR-10).

The relative amount of the respective element was calculated from the following equation:

$$\Delta S = S_{Ag} - S_{\bar{x}},$$

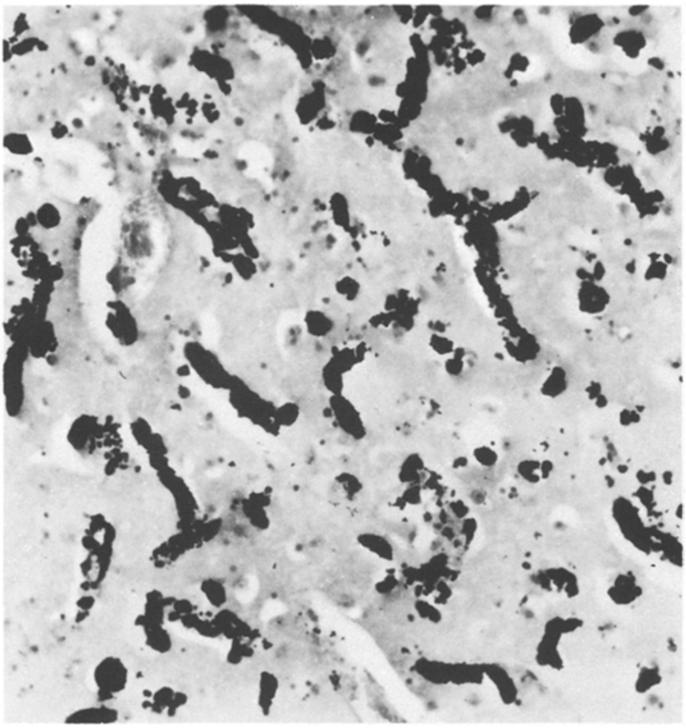

Fig. 1. Nucleus caudatus. Widespread calcification of capillaries and precapillaries. Stained according to Kossa. 160×

where: S_{Ag} = degree of blackening of the Ag line,
S_x = degree of blackening of the spectral line corresponding to the element, under-study, and
ΔS = the difference between S_{Ag} and S_x.

The densities of the spectrographic lines were measured on the logarithmic scale of the Zeiss G-2 spectrophotometer.

Results

Macroscopic Examination

The cerebral hemispheres were symmetric, and the gyri and sulci clearly defined. The meninges were unchanged. In the blood vessels of the cerebral base, single atherosclerotic plaques were seen. When sectioned frontally, the hemispheres cut with increased resistance at the level of the basal nuclei and the thalamus. On palpation of the section surface, some distinct nodular induration was detected in the regions of the nucleus caudatus and the lateral thalamic nuclei. Similar changes were observed in the nucleus dentatus of the cerebellum and in some regions of the cerebellar cortex. Otherwise, neither the cerebral hemispheres nor

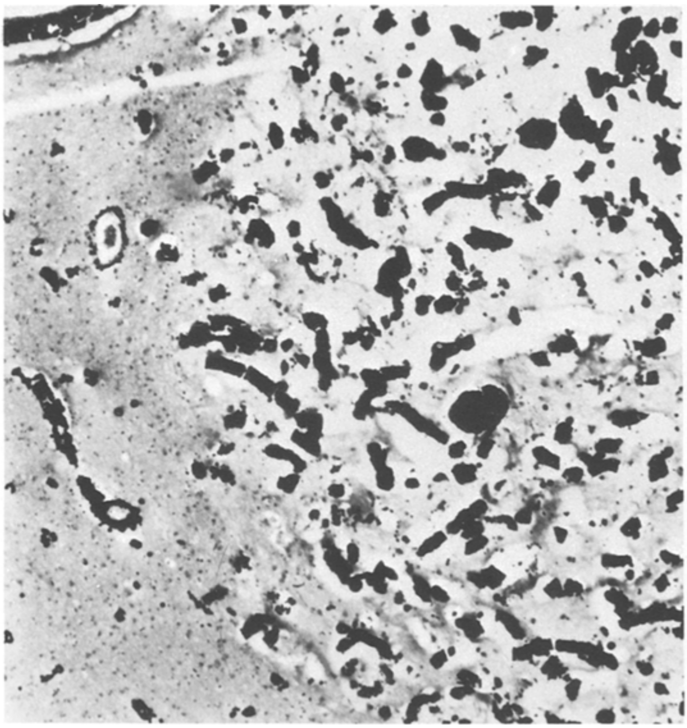

Fig. 2. Lateral nucleus of the thalamus. Besides calcified blood vessels, calcium deposits are observed outside the vascular network. Stained according to Kossa. 95×

the back brain revealed any macroscopic abnormalities. The lateral ventricles were slightly distended.

Microscopic Examination

The vessel walls of capillaries and small-to-medium sized arteries were incrusted with calcium deposits, which sometimes occupied only the adventitia or media. Usually, however, they were found in all layers of the vessel wall, particularly in those of the affected capillaries (Fig. 1). The capillaries were often completely occluded. In addition, calcium deposits were seen outside the vascular network (Fig. 2). In regions in which calcification was particularly intense, nerve cells were totally absent, while areas with less intense calcification showed partial loss of neurons. These changes were accompanied by a moderate proliferation of fibrillar glia.

Calcification was found in the following topographic brain regions: caudate nucleus, lateral nuclei of the thalamus, cerebral cortex, and to a lesser degree in the gyri of the frontal lobe. In addition, considerable calcification was observed in the nucleus dentatus and in the granular layer and white matter of many cerebellar gyri (Fig. 3). Small calcium deposits were also found in the choroid plexus of the IV ventricle and locally in the meninges covering the frontal lobe.

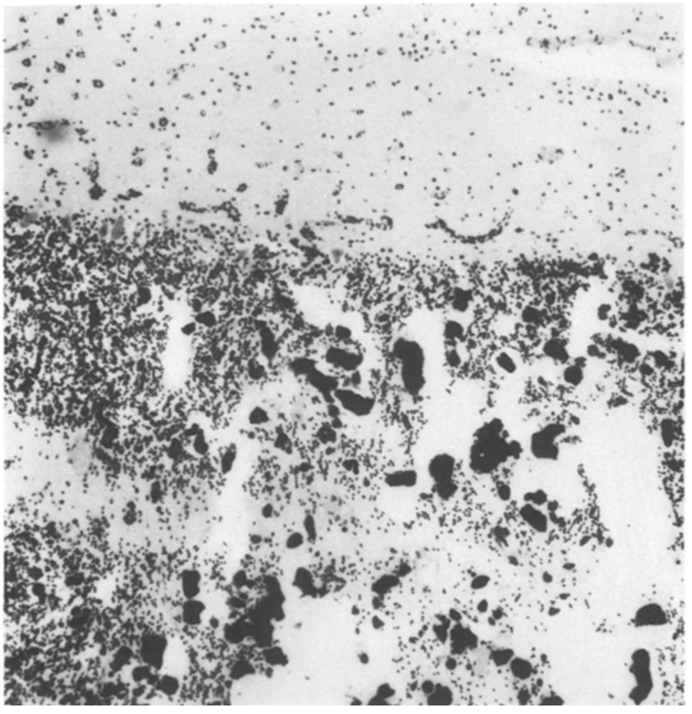

Fig. 3. Cerebellar cortex. Calcium deposits in the granular layer and white matter. Stained according to Kossa. 70×

Table 1. Qualitative results of laser-spectrographic analysis

Brain region	Cations and corresponding wavelengths				
Nucleus caudatus	Ca = 3933.66 3968.46	Fe = 3581.19 3719.93	Mg = 2802.69 2852.12	Zn = 3345.57	Al = 3082.15 3944.03 3961.52
Thalamus	Ca = 3933.66	Fe = 3020.64 3581.19 3719.93	Mg = 2802.69	Zn = 2138.56 3345.57	
Cerebral cortex	Ca = 3933.66	Fe = 3020.64 3581.19	Mg = 2802.69	Zn = 2138.56 3345.02 3345.57	
Cerebellar cortex	Ca = 3933.66 3968.46 4226.72	Fe = 3020.64	Mg = 2802.69	Zn = 3345.57	
Nucleus dentatus of the cerebellum	Ca = 3933.66 3968.46 4226.72	Fe = 3020.64	Mg = 2802.69	Zn = 3345.57	
Pons	Ca = 3933.66	Fe = 3020.64	Mg = 2802.69	Zn = 3345.57	

Table 2. Quantitative results of laser-spectrographic analysis, calculated on the basis of the 'ΔS' method (Kozik et al., 1970b)

Brain region	Cations detected in the blood vessel deposits	*S* values obtained in the individual analyses									
		1	2	3	4	5	6	7	8	9	10
Nucleus caudatus	Ca	2.7	27.9	18.1	22.5	25.5	30.4	27.3	13.6	18.9	21.1
	Fe	4.0	30.0	19.8	24.2	27.0	32.2	29.5	15.5	20.4	22.8
	Al	11.2	37.5	26.6	31.1	34.2	39.4	36.5	22.7	27.3	39.0
	Mg	14.3	41.6	30.5	35.1	38.3	34.6	41.0	26.3	30.8	49.3
	Zn	34.4	68.8	60.0	64.9	69.2	65.0	70.2	56.6	60.3	80.1
Thalamus	Ca	14.5	27.4	5.5	11.6	30.2	6.4	24.3	9.5	28.7	17.8
	Fe	16.0	29.9	7.7	19.0	31.8	8.4	26.1	11.0	29.8	19.6
	Mg	19.8	33.7	10.9	14.0	34.2	11.8	29.4	14.2	34.0	23.5
	Zn	49.9	63.5	40.7	44.2	74.3	42.2	59.7	34.5	64.0	54.3
Cerebral cortex	Ca	22.4	29.5	14.4	19.0	28.0	25.7	30.8	26.6	4.8	5.6
	Fe	24.1	31.2	16.0	20.5	30.1	27.4	32.2	28.2	6.5	7.3
	Mg	28.2	34.2	19.8	20.7	33.2	30.7	35.5	31.2	9.7	10.4
	Zn	58.5	64.4	40.4	57.7	64.1	61.0	65.7	62.2	40.1	40.7
Cerebellar cortex	Ca	10.5	22.0	7.8	29.0	12.7	20.0	25.9	3.1	30.0	16.8
	Fe	12.3	23.7	9.9	30.5	14.5	21.6	27.7	4.7	31.5	18.2
	Mg	15.3	26.8	12.8	33.4	17.7	24.8	31.8	7.6	34.2	21.7
	Zn	36.2	57.1	33.8	63.7	47.9	55.0	62.1	33.2	64.7	52.8

Observe: The relative content of a given element is inversely proportional to the ΔS value

Laser-Spectrographic Analysis

By means of laser spectrography, calcium and iron was detected in all brain regions with microscopically evident changes in the blood vesssel walls. In addition to the considerable optical density of spectrographic lines characteristic of Ca and Fe, there was also sufficient evidence in the spectrographic spectrum for the presence of other metals, such as Mg, Zn, and Al (Table 1).

The optical densities of the respective spectrographic lines of Mg, Al, and Zn were always much lower than those of Ca and Fe. When expressed in relative quantitative terms, the individual values representing the content of the respective cation in 10 probes from a given brain region appeared widely scattered (Table 2).

Discussion

The widespread symmetric calcification of basal nuclei, together with focal calcification of the cerebellar and cerebral cortex described by Fahr [4] has since become known as Fahr's syndrome or Fahr's disease. Despite the characteristic anatomopathologic picture of these cases, the clinical symptoms may be quite diverse [2, 10]. That is why many authors believe that the term 'Fahr's syndrome' is unreasonable and should be avoided, both in neurology and in neuropathology [9, 10]. However, these authors did not suggest a more pertinent term to define these cases. Notwithstanding the nomenclatural difficulties, we do believe that the present case corresponds closely to those described by Fahr [4], and for reasons of convenience we use the term Fahr's disease. The relevant question regarding Fahr's syndrome is its etiopathogenesis. In Fahr's own opinion, the evaluation of the chemical composition of deposits accumulated in the brain may be of considerable aid. In many cases of Fahr's syndrome, not only siderocalcification of blood vessels and deposits located outside the vessel network was observed, but also deposits giving a positive reaction for iron only and having a similar localization and density [3, 8, 10]. In the available literature on Fahr's syndrome, no mention is made of the occurrence of other minerals in the deposits, excepting calcium and iron.

The present study, conducted with the aid of laser-spectrographic analysis, gave evidence of the occurrence of cations other than calcium and iron in the pathologic deposits, such as Mg, Zn, and sometimes Al.

The relative quantitative data on the content of the individual cations in the deposits showed that concentrations of Mg and Zn were always decisively lower than those of Ca and Fe. These large quantitative differences between the single minerals in the deposits were most likely the reason for not detecting the minor components (Mg, Zn, Al) in the pathologically changed structures. They probably also could not be detected because of the low sensitivity of histochemical reactions for Mg and Zn, particularly in the presence of abundant amounts of Ca and Fe. The application of a highly sensitive analytic method, i.e., laser-spectrographic analysis through which picogram (1×10^{-12}) amounts of an element [7] can be detected in histologic preparations, extends our views on metabolic aberrations in Fahr's syndrome. The results of laser-microanalytic determination of the cation content and composition of pathologic deposits in the brain in Fahr's syndrome do not allow extensive conclusions to be made. However, based on our results, we feel justified in assuming that the pathomechanism of accumulation of minerals in the brain in Fahr's syndrome differs from mere absorption of cations from the surrounding areas. The occurrence of Mg, Zn, and Al in the pathologic deposits, and the presence of Ca and Fe as the prevailing components, seem to favor the assumption that disturbances in the metabolism of many cations are involved in the etiopathogenesis of changes observed in Fahr's syndrome.

Conclusions

1. The mineralized cerebral vessel walls in a case of Fahr's syndrome were shown to contain, besides Calcium and Iron, Aluminium, Magnesium, and Zinc.

2. The proportions of the elements in all studied areas of the diseased brain were characteristic. Calcium was found to show the highest concentration, followed by Iron. Content of Aluminium and Magnesium was much lower, whereas Zinc showed the lowest concentration.

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