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Histochemical, Electron Microscopic and X-ray Microanalytic Studies of Pseudomelanosis Cerebelli

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ABSTRACT. Abnormal pigments in the cerebellum of a patient with Creutzfeldt-Jakob disease were examined histochemically, electron microscopically and X-ray microanalytically. The pigments, which were 2 to 10 microns in diameter and bright brown in color, were observed in astrocytes and Bergmann glia which had proliferated in the dentate nucleus and cerebellar cortex. Morphologically and elementally they were different from calcification, ferrugination, neuromelanin, lipofuscin and melanosis coli pigments. They were presumed to be an intermediate between neuromelanin and lipofuscin occurring in senility and composed mainly of proteinaceous compounds containing sulfur and chlorine. The term "pseudomelanosis cerebelli" was proposed for this abnormality.

Key words : pseudomelanosis cerebelli — abnormal pigments — histochemistry — electron microscopy — X-ray microanalysis

Neuromelanin and lipofuscin are two pigments commonly seen in the central nervous system.[1] We have preliminarily reported on abnormal pigments, other than these two pigments, in the dentate nucleus of an elderly woman with Creutzfeldt-Jakob disease.[2] The purpose of this paper is to describe extensively the findings of histochemical, electron microscopic and X-ray microanalytic studies of these cerebellar pigments in the same patient.

MATERIALS AND METHODS

The patient was a 74-year-old woman (B 23015, A 81-156) who showed progressive visual disturbance, mental confusion, rigidity of the extremities and myoclonic jerks during the three-month course of the illness. Prominent nerve cell loss, protoplasmic astrocytosis and stromal spongiosis were observed in the cerebral cortex, especially, in the occipital and temporal lobes. There were similar, but mild to moderate changes in the basal ganglia and thalamus also. In the cerebellum, Purkinje cells and granule cells were moderately decreased in number. The Bergmann glia had proliferated in the Purkinje cell layer. Nerve cells in the dentate nucleus, however, were well preserved with a mild proliferation of protoplasmic astrocytes corresponding to the patient's age.

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Other than these findings consistent with the subacute spongiform encephalopathy type of Creutzfeldt-Jakob disease, numerous small brown pigments were found, mainly in the dentate nucleus. Other detailed clinicopathological observations have been described elsewhere.[2)]

After routine histological examinations of the brain, tissue sections of the cerebellum, substantia nigra and inferior olives were stained with periodic acid-Schiff (PAS), Berlin blue, Kóssa, luxol fast blue, Masson-Fontana, Mallory's basic fuchsin, Lillie's ferric ferricyanide and Schmorl. Similar staining procedures were performed in a different case of melanosis coli.[3)] Bleaching of the pigments was tested with permanganate, peroxide and peracetic acid, and dissolving of the pigments with 5% hydrochloric acid, 5% ammonium hydroxide and ethyl ether. The pigments were examined further for autofluorescence under dark field illumination. Small specimens from the dentate nucleus fixed in formalin for several weeks were embedded in epoxy resin. Thin sections were observed electron microscopically. Unstained semi-thin sections were analyzed as previously described[4-6)] using the EDAX computerized energy dispersive X-ray microanalysis system attached to a Hitachi HSE-2 scanning electron microscope.

RESULTS

The pigments in question were found mainly in the dentate nucleus of both cerebellar hemispheres, predominantly in the dorsolateral portion opposite the hilus (Fig. 1). Small quantities were found also in the Purkinje cell and granular

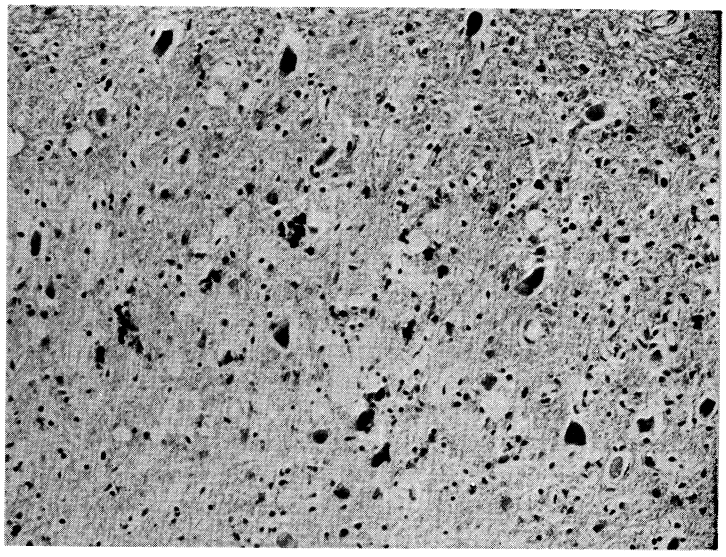

Fig. 1. Photomicrograph of the dentate nucleus scattered with numerous bright brown pigments of 2 to 10 microns in diameter. Nerve cells are well preserved. HE, ×158.

layers of the cerebellar cortex. These pigments, 2 to 10 microns in diameter, were homogeneous or coarsely granular, and bright brown in color with hematoxylin and eosin staining. Those in the cerebellar cortex were generally smaller. The pigments seemed to be situated frequently in the cytoplasm of the astrocytes and Bergmann glia, and sometimes were free in the parenchyma. They were not seen in the cytoplasm of nerve cells. These pigments were not found in other parts of the brain including the occipital cortex.

The results of histochemical examinations of the pigments as compared with those of neuromelanin, lipofuscin and melanosis coli pigments are summarized in Table 1. The pigments of the cerebellum were positive for Masson-Fontana and Mallory's basic fuchsin, but negative for PAS, Berlin blue, Kóssa, luxol fast blue, Lillie's ferric ferricyanide and Schmorl. They were negligibly bleached by permanganate, peroxide and peracetic acid, and they were not dissolved by hydrochloric acid, ammonium hydroxide and ethyl ether. They did not autofluoresce.

TABLE 1. Histochemical examinations of the pigments.

Stain	"Pigment" (Cerebellum)	Neuromelanin (Substantia nigra)	Lipofuscin (Olive)	Pigment of melanosis coli (Colon)
HE (granule)	bright brown (coarse)	dark brown (fine)	straw yellow (fine)	dark yellow-brown (fine)
PAS	−	−	+	+
Berlin blue	−	−	−	−
Kóssa	−	−	−	−
Luxol fast blue	−	+	+	−
Masson-Fontana	+	+	−	−
Mallory	+	−	−	+
Lillie	−	+	−	−
Schmorl	−	+	+	+
Bleaching :				
permanganate	+~−	+	+~−	+
peroxide	−	+	−	+~−
peracetic acid	−	+	−	+~−
Dissolving :				
hydrochloric acid	−	−	−	−
ammonium	−	−	−	−
ethyl ether	−	−	−	−
Autofluorescence	−	−	+	−

Electron microscopically, the pigments were composed of mulberry-shaped accumulations of homogeneously electron-dense substances occasionally containing curvilinear bodies, although their fine structures were not well preserved because of the rather long formalin fixation (Fig. 2).

X-ray microanalysis of these pigments demonstrated the presence of sulfur

and occasionally revealed chlorine; silicon and copper artifacts also appeared (Fig. 3). Metals such as aluminum, manganese and zinc were not detected.

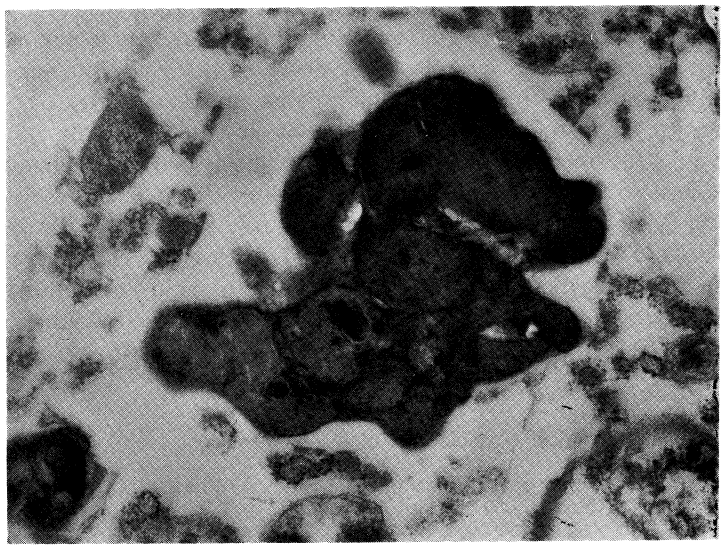

Fig. 2. Electron micrograph of the pigment in the dentate nucleus. It is composed of mulberry-shaped accumulations of homogeneously electron-dense substances with occasional curvilinear bodies. × 24,000.

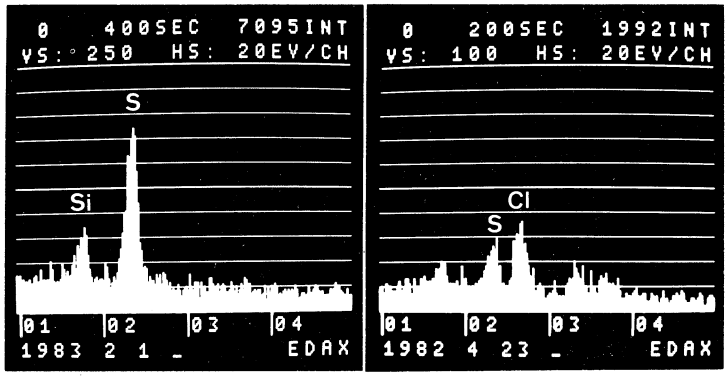

Fig. 3. Spectra of the pigments showing peaks of sulfur and chlorine, in addition to a concomitant peak of silicon.

DISCUSSION

The abnormal pigments in this case were scattered mainly in the dentate nucleus and to a small extent in the Purkinje cell and granular layers of the cerebellar cortex. These cerebellar pigments were homogeneous or coarsely

granular and bright brown in color, in contrast to the pigments of the substantia nigra, inferior olives, and the case of melanosis coli, which are all finely granular, and dark brown, straw yellow and dark yellow-brown, respectively. Histochemically the abnormal pigments did not contain calcium and iron as components. They were different from neuromelanin in the substantia nigra in that they were positive to Mallory's basic fuchsin, and negative to luxol fast blue, Lillie's ferric ferricyanide and Schmorl. They were not bleached by peroxide and peracetic acid. In contrast, neuromelanin is negative to Mallory's basic fuchsin, positive to luxol fast blue, Lillie's ferric ferricyanide and Schmorl, and bleached by peroxide and peracetic acid. The abnormal pigments were different from lipofuscin in the inferior olives in that they were positive to Masson-Fontana and Mallory's basic fuchsin, and negative to PAS, luxol fast blue and Schmorl. They did not autofluoresce as does lipofuscin. They were different from the melanosis coli pigments in that they were positive to Masson-Fontana and negative to PAS and Schmorl.

Electron microscopic findings were also different from those of neuromelanin and lipofuscin in that the abnormal pigments did not contain electron lucent homogeneous lipid-like droplets surrounded by a limiting membrane. X-ray microanalysis of the pigments demonstrated the presence of sulfur and occasionally revealed chlorine.

These results suggest that the pigments in our case include more proteinaceous than lipid or carbohydrate components.

Such abnormal cerebellar pigments as in our case are of very rare occurrence, with only nineteen cases having been reported in the literature.[7-15] Most of them have been described under the name of melanosis cerebelli, and almost all the patients were over fifty years of age. Associated diseases are varied, including cerebral infarction, senile dementia, carcinoma and ischemic heart disease, all of which are common to the aged person. The pigments do not seem to be related to specific diseases or specific clinical symptoms, except that they seem to appear in connection with senility. These pigments probably are not related to Creuzfeldt-Jakob disease, although there is another case of this disease having these pigments.

Some authors describing the occurrence of the pigments as melanosis cerebelli believe these pigments to be neuromelanin, but others, despite their use of the name of melanosis, think that they are not identical with neuromelanin, at least histochemically. Best et al.[14] have examined seven patients with melanosis cerebelli and concluded from their size and intensity that these pigments are derived from lipofuscin in the protoplasmic astrocytes, and appear first in the dentate nucleus, then in the cerebellar granular layer, and finally in the cerebral cortex from the occipital cortex forward. The pigments in our case were most prominent in the dentate nucleus, sparser in the cerebellar cortex, and were not observed in the cerebral cortex at all. If our case is assumed to be in a state before the cerebral cortex becomes involved, it supports their theory.

Fan et al.[11] observed the coexistence of extraneuronal neuromelanin and

glial lipofuscin in the dentate nucleus and suggested the interconvertability of these two pigments. In addition, Shuangshoti et al.[16,17] described a unique case of melanosis of the choroid plexus in a 74-year-old man, and another case of intracranial melanosis in which the dentate nucleus and choroid plexuses were melanotic. The proposed pathogenesis was the conversion of choroidal epithelial lipofuscin into melanin. Since the results of our pathological examinations indicate that the pigments in question are different from neuromelanin and lipofuscin, the pigments were presumed to be an intermediate between them probably due to the melanization of lipofuscin.[18]

Recently, abnormal pigmentation of the astrocytes of the striatum, pallidum and substantia nigra has been reported.[19,20] These pigments were judged to be melanin by histochemical and electron microscopic findings, and hypothesized to be derived from anomalous catecholamine metabolism. As the cerebellar pigments do not seem to have any pathological significance in relation to catecholamine, they probably do not result from such abnormal catecholamine metabolism. The pigmentation of the extrapyramidal system may be due to a different entity from the cerebellar pigments.

In conclusion, these cerebellar pigments may be an intermediate between neuromelanin and lipofuscin involved in senility, and are composed mainly of proteinaceous compounds containing sulfur and chlorine. The name "pseudomelanosis cerebelli" would appear to be more suitable than melanosis cerebelli, because the pigments do not seem to be identical with neuromelanin.

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