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The Association of Amaurotic Idiocy and Metachromatic Leucodystrophy: a Histochemical and Biochemical Study

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With 7 Figures

The problem of the pathogenic relationship between amaurotic family idiocy and metachromatic leukodystrophy has frequently been discussed in the neuropathological and neurochemical literature of the last few years. The aim of the present report is to illustrate this problem on the basis of histochemical and biochemical studies carried out on three cases, which morphologically combined the full picture of both metachromatic leukodystrophy and amaurotic idiocy.

The cases now reported are three sibs (two sisters and one brother) coming from a sibship of five. Their parents are healthy and not consanguinous. No neurological diseases have been observed in their families.

All three children were delivered normally following a full term uncomplicated pregnancy. All of them developed normally until the age of 15 to 24 months. At that time in each of them, a progressive neurological disorder developed; this was characterized by arrest and retreat of their somatic and mental development, blindness, deafness, muscular rigidity and a terminal marantic state. Death occurred after 11, 20 and 45 months of illness at ages 3, 5 and 5 years.

In one case, we had at our disposal only small pieces of brain tissue obtained at surgical biopsy; in two others, full postmortem examination was carried out.

Widespread accumulation of lipid within the cytoplasm of neurones was found throughout the neuraxis on histological examination (Figs. 1 and 2). The neuronal changes were accompanied by glial proliferation of corresponding degree. There were large antler-like distortions of the Purkinje cells of the cerebellum and the torpedo-shaped deformities of their axons (Figs. 3 and 4).

Table I

Histochemical properties of substances accumulated in nervous system

Method used		Gray matter		White matter		
		Neurones	Metachr. neurones	Large macrophag.	Small macrophag.	Free deposits
Sudan IV	Frozen	—	—	—	—	—
Sudan Black B	Frozen	+ + +	+ + +	+	+ +	+ +
PAS	Frozen	+ + +	+ + +	+ + +	+ + +	+ + +
PAS	Paraffin	+ +	+ +	+ +	+ +	+ +
PfAS	Paraffin	—	—	—	—	—
Acetic-acid cresyl violet	Frozen	—	+	+ + +	+ + +	+ + +
Toluidine blue-standard	Frozen	—	+	+ + +	+ + +	+ + +
Feyrter's "mounting" method	Frozen	+	+ +	+ + +	+ + +	+ + +
Orcin-hydrochloric acid	Frozen	+	+	—	—	—
Orcin-hydrochloric acid	Paraffin	+	+	—	—	—
Copper-phthalocyanin	Paraffin	+ +	+ +	+	+	+
Alcian blue	Paraffin	+	+	—	+	+
Okamoto meth.	Frozen	+ +	+ +	+ + +	+ + +	+ + +
Okamoto meth. after pyridine	Frozen	+ +	+ +	—	+	—
Okamoto meth. after ether	Frozen	+	+	—	+	—
Coupled tetrazonium	Paraffin	+ +	+ +	+ +	+ +	—
Coupled tetrazonium, benzoyl	Paraffin	+ +	+ +	+ +	+ +	—
Millon's meth.	Paraffin	+	+	+	+	—

+ Faintly positive + + Positive + + + Strongly positive — Negative 0 Test not done

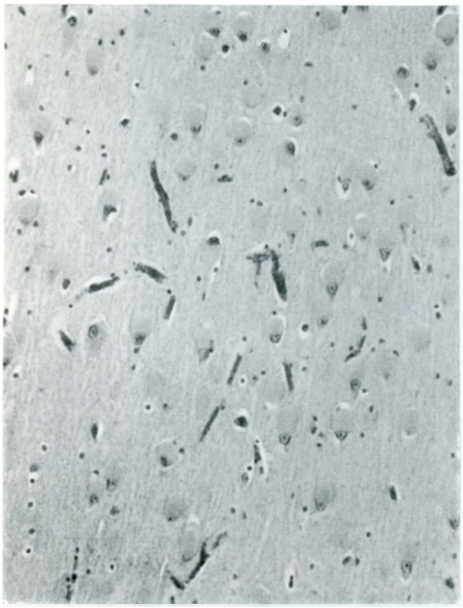

Fig. 1

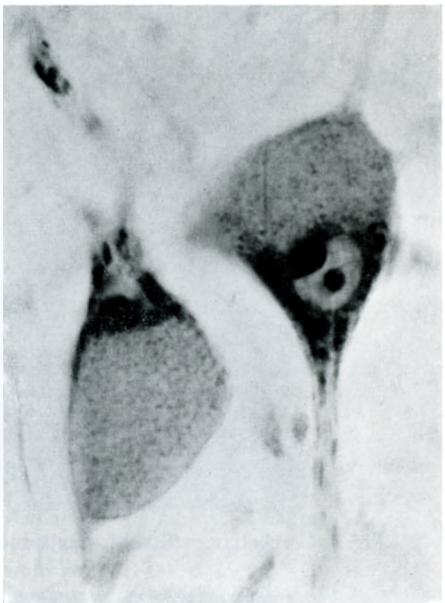

Fig. 2

Fig. 1. Hippocampal cortex showing voluminous cytoplasm and nuclear eccentricity of neurones. Haematoxylin and eosin.

Fig. 2. High power view of distended neurones. Cresyl violet.

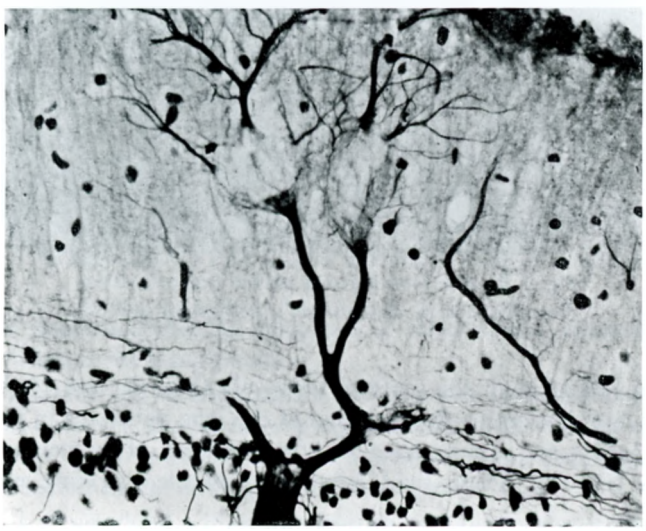

Fig. 3. Cerebellar cortex showing distortion of *Purkinje* cell dendrites. *Bielschowski*'s method.

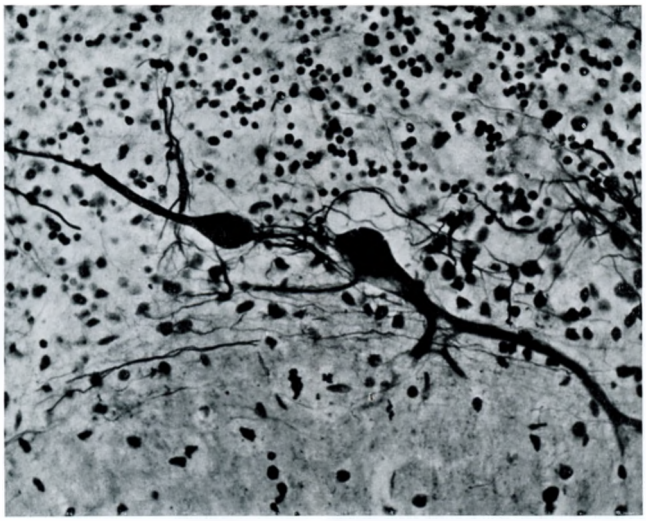

Fig. 4. Cerebellar cortex showing spindle shaped swelling of *Purkinje* cell axon. *Bielschowski*'s method.

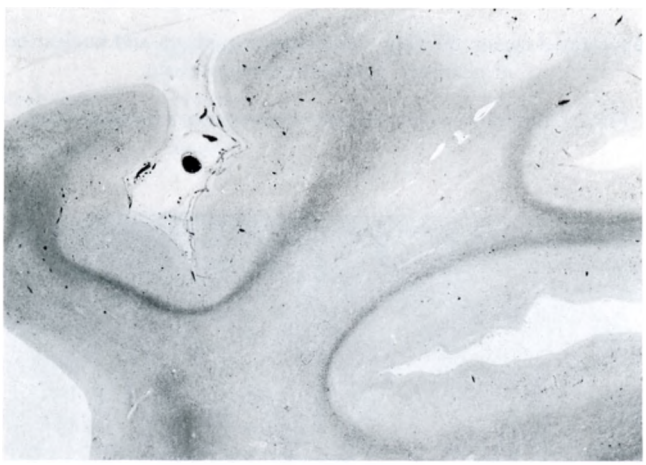

Fig. 5. Frontal lobe showing partial demyelination. Paraffin section. *Heidenhain's* method for myelin.

Partial demyelination of the centrum semiovale (Fig. 5) and of the cerebellar white matter and descending degeneration of the long pathways in the brain stem and spinal cord were accompanied by the presence of numerous lipid-laden macrophages (Fig. 6). These lipid substances revealed striking metachromasia (Color slide 1).

Pigmentary degeneration of the retina with lipid storage in retinal ganglion cells was found in one case. Microscopical examination of the body organs revealed

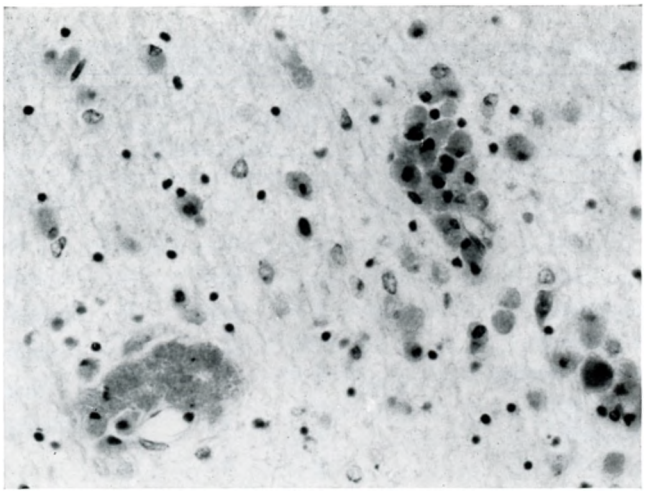

Fig. 6. White matter of centrum ovale showing groups of macrophages. Paraffin section, haematoxylin and eosin.

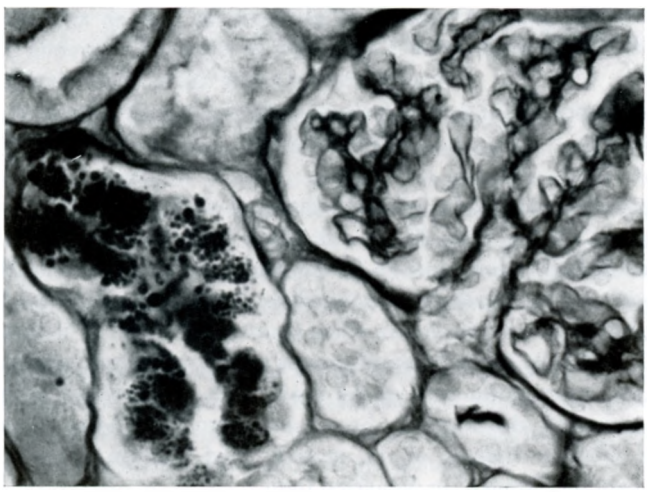

Fig. 7. Kidney showing PAS positive granules in Aubular cytoplasm.

abnormal lipid deposits within the cytoplasm of renal tubular epithelium (Fig. 7) and in macrophages in the biliary tract.

In order to establish the chemical nature and probable relationship of substances accumulated in neurones and in macrophages, histochemical studies were carried out on brain tissue of two cases and on the retina and relevant body organs. Table 2 summarizes the histochemical findings on the brain tissue.

The material accumulated within the neuronal cytoplasm was strongly positive in Sudan Black B (Color slide 2) and negative in Sudan IV, positive in periodic acid-Schiff (Color slide 3), negative in performic acid-Schiff and strongly positive

Table 2
Histochemical properties of substances accumulated in viscera and retina

Method used	Kidneys / cases 2 + 3 /	Gall bladder / case 3 /	Retina / case 2 /
Sudan IV	—	0	—
Sudan Black B	+	+ +	+ + +
PAS	+ + +	+ + +	+ + +
PfAS	—	—	—
Acetic acid cresyl violet	+ + +	0	+ +
Toluidine-blue standard	+ +	0	0
Feyrter's "mounting" method	+ + +	0	+
Orcin-hydrochloric acid	—	—	±
Copper-phthalocyanin	+	+	+ +
Alcian blue	±	+	±
Okamoto meth.	+ + +	0	0
Okamoto meth. after pyridine	+	0	0
Okamoto meth. after ether	+	0	0
Coupled tetrazonium	+ +	+ +	+ +
Coupled tetraz. benzoyl.	+ +	+ +	+ +
Millon's meth.	+	+	+

+ Faintly positive
+ + Positive
+ + + Strongly positive
± Doubtful result
— Negative
0 Test not done

in copper phthalocyanin (Color slide 4). Staining with alcian blue gave a weakly positive reaction (Color slide 5) as did Bial's test. Reactions for amino acids (*Danielli* and *Millon)* were positive (Color slide 6). The positive *Okamoto*'s test remained positive after incubation in pyridine, but it was weakened by ether. All neurones showed beta metachromasia when stained in *Feyrter*'s "mounting" method (Color slide 7), but only some neuronal groups such, for instance, as dentate nucleus, globus pallidus, large motor cells of the brain stem and anterior horn cells of the spinal cord, revealed also a strong brown metachromasia when stained in acetic cresyl violet (*Peiffer-Hirsch*) or in tartaric acid thionin (*Feyrter*). Neuronal deposits were not birefringent in polarized light.

The deposits in white matter, occurring both free and within macrophages, were negative in Sudan IV but positive in Sudan Black B (Color slide 8). Their strong PAS positivity (Color slide 9) was accompanied by entirely negative performic acid-Schiff reaction. The most striking feature was their marked brown and purple mentachromasia (*Peiffer-Hirsch* method) (Color slide 10). *Bial*'s test was negative. The positive *Okamoto*'s reaction was weakened after both pyridine and ether. Protein reactions (*Danielli* and *Millon*) were positive (Color slide 11) only in substances within macrophages. Lipid deposits of the white matter were strongly birefringent in polarized light (Color slides 12 & 13).

Table 2 illustrates the histochemical results in retina, kidney an gall bladder. In general the cytoplasmic content of the retinal ganglion cells had the same histo-

Table 3
Results of biochemical studies. Cerebral lipids in two of the cases.

Substance	Case 2. Age 5 years		Case 3. Age 3 years	
	White matter	Cortex	White matter	Cortex
Total phospholipids	5.45	6.31	14.18	17.81
Sphingomyelin	1.53	1.47	3.85	3.21
Total cholesterol	6.08	5.47	6.84	6.14
Esterified cholesterol	0.22	0.15	0.20	0.23
Total hexosamine	0.44		0.57	
Neuraminic acid		0.18		0.46
Neutral cerebrosides	2.3		3.43	
Sulphatides	1.8		6.30	
Water %	77.8	81.0	75.6	82.6

Results in g./100 g. dry tissue

chemical properties as that of neurones in dentate nucleus, globus pallidus and others, while abnormal products in the renal tubules, liver and biliary tract gave reactions similar to those of the white matter deposits.

As a result of our histochemical studies, we consider that both neurones and macrophages of the white matter had stored a mixture of lipid compounds. The main substance accumulated within neurones was characterized as being an acid glyco-lipid, partially free, partially bound with cellular protein. It seems that despite the weak Bial reaction, it could be considered as ganglioside (*Diezel, Klenk* and *Seitelberger*) or as an intermediate substance between ganglioside and cerebroside (*Diezel*). In addition, neurones stored other complex lipids such as sphingomyelins.

The histochemical properties of the lipids in the white matter enabled us to classify them as sulphuric acid esters of cerebroside, i.e., sulphatides (*Jatzkewitz, Wislocki* and *Singer, Austin, Cumings*). Additional lipid substances were also present here, among them sphingomyelin and other phospholipids.

Neurones exhibiting brown metachromasia also contained sulphatides as an additional product admixed with those already discussed. We feel that histochemical peculiarity of these neuronal groups in this disease is a reflection of some inherent biochemical difference present even in the healthy state.

Biochemical studies on brain tissue from two of our cases were carried out partially on formalin-fixed material, partially on deep frozen tissue. Results of these studies are presented in Table 3.

The two cases showed different degrees of biochemical abnormality. The case with the longer clinical course revealed a great loss of total phospholipids and less severe loss of sphingomyelins and of cholesterol. No significant amount of esterified cholesterol was present. The neuraminic acid level in cortex was normal. There was increase in hexosamine in white matter and a relative increase of sulphatides there.

In the second case, the fourfold increase in sulphatides of the white matter was accompanied by an increase of hexosamine in the white matter and a slight loss of total phospholipid content (less significant in cortex than in white matter) and of sphingomyelin and cholesterol. Neuraminic acid was slightly increased in the cortex.

The biochemical studies suggest that both cases fall into the group of metachromatic leukodystrophy and that the derangement is one in which there is some abnormality in one of the myelin components. This abnormality would appear less likely to be one resulting from an unusual demyelination than a consequence of a faulty formation of myelin in that a great amount of a lipid material normally present is found in the cerebral white matter.

These cases are considered as showing the histopathological and histochemical features of both metachromatic leukodystrophy and amaurotic idiocy. We would suppose that the inborn enzymatic disorder underlying the two conditions may produce changes in the whole group of sphingolipids, leading to disease states in which the biochemical and histochemical features varied according to the position of the defect in the enzymatic system. It might, for example, result in alteration mainly of cellular lipids as in amaurotic idiocy or of myelin lipids as in metachromatic leukodystrophy. Such a hypothesis suggests the possible existence of intermediate forms of the disease showing simultaneously alteration in both cellular and myelin lipids. We consider our cases exemplify just such a possibility.