THE DIAGNOSIS OF METACHROMATIC LEUCODYSTROPHY DURING LIFE

METACHROMATIC LIPIDS IN SALIVA AND CEREBROSPINAL FLUID SEDIMENTS, AND IN THE PAROTID GLANDS

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Metachromatic leucodystrophy is deserving an increasing interest from neurologists and pediatricians, mainly because it is the only disease in the whole group of diffuse cerebral sclerosis amenable to an *intra vitam* diagnosis by means of bloodless procedures.

The merit for this belongs to Austin [1], who introduced a simple test to demonstrate metachromatic bodies in the urine sediment. Although Hagberg and Svennerholm [8, 9] later showed that the test might be positive even in normal subjects, it is evident that it must be valued if associated with the proper clinical picture and eventually with the familial incidence.

The preence of similar metachromatic bodies was demonstrated in the saliva sediment of 4 patients with metachromatic leucodystrophy belonging to the same family; our investigations were extended to the study of the parotid gland of 2 patients, the presence of metachromatic bodies being evidenced in them. In these 2 patients metachromatic bodies were also found in the cerebrospinal fluid sediment. A simple chromatographic test (Denny-Brown et al. [6]) was used for the demonstration of metachromatic lipids in saliva and cerebrospinal fluid.

Thus, the examination of cerebrospinal fluid and mainly of saliva sediment may easily complement or strengthen a laboratorial diagnosis of metachromatic leucodystrophy made on the basis of the Austin test only.

MATERIAL AND METHODS

Material — Our study was performed in a group of 4 patients with the juvenile form of metachromatic leucodystrophy and belonging to the same family (Canelas et al. [5]). In all these cases the diagnosis was supported, besides the positive

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Austin test in urine, by the characteristic clinical symptomatology; in 2 of these cases the diagnosis was ratified by the demonstration of metachromatic bodies in peripheral nerve, kidney and liver biopsies. In a fifth case (sister of one of those 4 patients with metachromatic leucodystrophy) all the examinations resulted negative, in spite of a similar neurologic picture.

The results of the study of saliva sediment in these 5 cases (group 1, table 1) were compared with three control groups of subjects: (a) asymptomatic direct relatives of these patients; (b) patients with various and mostly chronic diseases of the nervous system, not related to any lipidosis; (c) normal subjects. In all these three groups the Austin test in urine proved negative.

Methods — Fasting saliva was collected either spontaneously or through catheterism of the Stenon and Wharton ducts. No drug stimulation was used. A sample of saliva was kept at room temperature and other in a refrigerator; both were examined after periods of 8, 12 and 24 hours, and the conclusion was drawn that temperature and examination time did not influence the results. The sediment was obtained by centrifugation at 2,000 rpm for 15 to 20 minutes. A drop of the sediment was placed in a microscopic plate and stained with a drop of blue toluidin "O" (Grüber) in a 2 per cent acetic acid solution. The preparation was examined in the microscope with magnifications of 100 and 400 times.

Cerebrospinal fluid in an amount of 200-300 ml. was sampled by lumbar puncture before pneumoencephalography. The sediment was obtained by centrifugation at 2,000 rpm for 15 to 20 minutes. The staining technique was the same as for saliva sediment.

Denny-Brown et al.[6] devised a simple test for the chromatographic demonstration of metachromatic lipids in the urine sediment. This method, easier than Austin's[2], was used also for the study of saliva and cerebrospinal fluid. The sediment was mixed with a 2:1 chloroform-methanol solution, in which a filter paper Whatman No. 1 was immersed and then stained with 0.01 mg of blue toluidin in a 2% acetic acid solution. The presence of metachromatic lipids was disclosed by the appearance of a pinkish red spot in the paper.

From each biopsy material of parotid gland two specimens were cut out, one for routine histopathological examination and other for histochemical study. For this purpose frozen sections with an average thickness of 15 μ were stained with Scarlach and cresyl violet, this last one for the study of metachromasia. At the time of use the dye was diluted (5 drops of the solution in 10 ml of destilled water); the slices were kept in the dye solution for 2, 24 and 48 hours, no significant variation on the dependence of the immersion time being noticed.

RESULTS

In group 1 (table 1) the examination of saliva sediment stained with acid blue toluidin showed, in all cases but one, the presence of large and free metachromatic granular bodies, similar to those found in urine (Fig. 1A). They were easily distinguishable from other elements commonly found in the saliva sediment, like the epithelial cells, the salivary bodies (Retterer and Lelièvre[11]), and polymorphonuclear leucocytes. Better results were achieved in saliva sampled by catheterism of the parotid or submaxillary glands. In the three control groups no metachromatic bodies were found.

The same metachromatic bodies were also found in the cerebrospinal fluid of 2 out of 3 cases from group 1.

Table 1 — Metachromatic bodies (blue toluidin dye) in saliva and urine

	Name	Sex	Age (years)	Diagnosis	Urine	Saliva	CSF
Group 1	M. A. R.	F	17	Metachromatic leucodystrophy	+	+	+
	O. A.	M	34	Metachromatic leucodystrophy	+	+	+
	N. A. C.	F	32	Non determined leucodystrophy	O	O	O
	D. A. M.	M	32	Metachromatic leucodystrophy	+	+	−
	J. A. M.	F	35	Metachromatic leucodystrophy	+	+	−
Group 2	A. M. R.	F	37	Normal relative of ML cases	O	O	−
	Ra. A.	M	46	Normal relative of ML cases	O	O	−
	Ru. A.	M	21	Normal relative of ML cases	O	O	−
	L. C. R.	M	15	Normal relative of ML cases	O	O	−
	A. G. C.	M	8	Normal relative of ML cases	O	O	−
	V. R.	M	5	Normal relative of ML cases	O	O	−
Group 3	A. M.	M	50	Progressive spinal muscular atrophy	O	O	−
	A. P. C.	F	47	Degenerative chronic myelopathy	O	O	−
	G. A. M.	M	45	Cerebrospinal cysticercosis	O	O	−
	A. F.	M	36	Epilepsy plus Peripheral neuropathy	O	O	−
	Z. S.	F	35	Amyloid neuropathy	O	O	−
	H. P.	M	28	Hepatolenticular degeneration	O	O	−
	J. A. N.	M	18	Friedreich's ataxia	O	O	−
	A. M. N. S.	F	12	Sydenham's chorea	O	O	−
	F. R. A.	F	2	Little's disease plus ichthyosis	O	O	−
Group 4	C. F. C.	M	32	Normal control	O	O	−
	R. T. F.	M	30	Normal control	O	O	−
	O. D. E.	M	27	Normal control	O	O	−
	G. D. E.	F	24	Normal control	O	O	−
	R. F.	M	10	Normal control	O	O	−
	I. F.	F	7	Normal control	O	O	−
	O. A. E.	M	4	Normal control	O	O	−
	J. F. E.	M	3	Normal control	O	O	−
	M. G. E.	F	1	Normal control	O	O	−
	R. F. F.	M	1	Normal control	O	O	−

M, male; F, female; ML, metachromatic leucodystrophy; +, positive; O, negative; −, not performed.

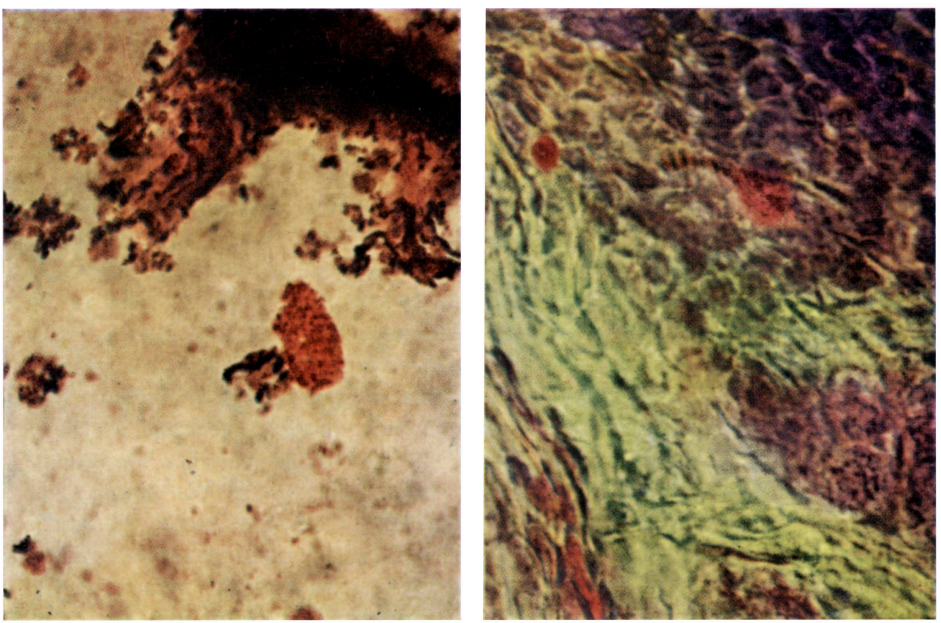
Furthermore, in these 2 patients the chromatographic test of Denny-Brown et al. [6] was positive in urine, saliva, and cerebrospinal fluid sediments.

In these 2 cases (table 2) parotid gland biopsies stained with cresyl violet dye showed the presence of fine brownish granular bodies inside the cells revesting the intralobular ducts of the gland. In the connective tissue surrounding the larger ducts histioid cells containing in the cytoplasm dark brown granular bodies were also found (Fig. 1 B). Although the metachro-

Table 2 — Metachromatic bodies found through the histochemical study (cresyl violet dye) in three cases of leucodystrophy

Biopsy	*M.A.R.*	*O.A.*	*N.A.C.*
Kidney	+	+	O
Liver	+	+	O
Peripheral nerve	+	+	−
Brain	—	−	O
Muscle	O	O	−
Parotid gland	+	+	−

+, present; O, absent; −, not perfomed



A **B**

Figure 1 — *A:* Saliva sediment, patient M.A.R.; a 15.5×28.8 μ metachromatic granular body stained with acid blue toluidine dye (400×). *B:* Parotid gland, same patient; the metachromatic material is seen in the cytoplasm of macrophages and in the connective tissue (cresyl-violet dye, 400×).

masia in the parotid glands was not so evident as in kidney and liver, the staining attributes of the granular bodies were closely similar. The degree of metachromasia showed an increase when the plates were examined 24 hours after they had been prepared; however, four weeks later colour decay started.

COMMENTS

In spite of extensive studies on the saliva sediment [3, 4, 4, 10, 11, 12], never the presence of foreign metachromatic granular bodies was reported. The same is true regarding the cerebrospinal fluid.

In all our cases of metachromatic leucodystrophy large and free granular bodies were found in saliva, staining with a pinkish red colour with acid blue toluidin dye. In 2 of these cases the study of the cerebrospinal fluid sediment disclosed similar findings.

The evidence of metachromatic bodies in the parotid glands of 2 cases of metachromatic leucodystrophy, staining in brown with cresyl violet dye, did corroborate the results in the study of the saliva sediment.

Moreover, in 2 cases the presence of metachromatic lipids in urine, saliva, and cerebrospinal fluid was demonstrated by a chromatographic technique.

The conclusion was drawn that the search for metachromatic bodies in cerebrospinal fluid and mainly in saliva sediment may be helpful for the elucidation of the diagnosis of metachromatic leucodystrophy.

SUMMARY

The authors report the study of saliva sediment in 4 cases of juvenile metachromatic leucodystrophy belonging to the same family (and else in a sister of one of these cases presenting the characteristic neurological picture but with no metachromasia demonstrable by the Austin test in urine or by biopsies), in 6 normal relatives of the patients with Scholz disease, in 9 cases of various diseases of the nervous system, and in 10 normal subjects. The presence of metachromatic bodies staining in a pinkish red colour with acid blue toluidine dye was demonstrated in the saliva sediment of the 4 cases of metachromatic leucodystrophy. In 2 of these patients biopsies of the parotid gland, stained with cresyl violet dye, showed the presence of intracellular brownish metachromatic bodies. In these 2 cases the study of cerebrospinal fluid sediment also disclosed the presence of metachromatic bodies. Furthermore, a chromatographic qualitative test for metachromatic lipids yielded positive results in saliva, cerebrospinal fluid, and urine sediments.

The conclusion was drawn that the search for metachromatic bodies in cerebrospinal fluid and mainly in saliva sediment may be of help in disclosing or ratifying the diagnosis of metachromatic leucodystrophy during life.

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RESUMO

Diagnóstico intra vitam da leucodistrofia metacromática: Lípides metacromáticos nos sedimentos da saliva e do líquido cefalorraqueano, e nas parótidas

Os autores relatam o estudo do sedimento salivar em 4 casos de leucodistrofia metacromática juvenil pertencentes à mesma família (assim como em uma irmã de um dêstes casos, que apresentava o quadro neurológico característico, porém com ausência de metacromasia demonstrável pelo teste de Austin ou mediante biopsias), em 6 parentes normais dos pacientes com a moléstia de Scholz, em 9 casos de várias afecções do sistema nervoso, e em 10 indivíduos normais. A presença de corpúsculos metacromáticos corando-se em róseo com o azul de toluidina, foi demonstrada no sedimento salivar dos 4 casos de leucodistrofia metacromática. Em 2 dêstes pacientes, biopsias da parótida, coradas com cresil violeta, demonstraram a presença de corpúsculos intracelulares com metacromasia em marrom. Nestes 2 casos também foram encontrados corpúsculos metacromáticos no sedimento do líquido cefalorraqueano. Além disso, um teste cromatográfico qualitativo para lípides metacromáticos forneceu resultados positivos na saliva, líquido cefalorraqueano e urina.

Os autores concluem que a pesquisa de corpúsculos metacromáticos no líquido cefalorraqueano e principalmente no sedimento salivar pode contribuir para estabelecer ou ratificar o diagnóstico de leucodistrofia metacromática durante a vida.

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