METACHROMATIC LEUCODYSTROPHY: STUDY OF THE FREE AMINO ACIDS IN BLOOD, URINE, SALIVA AND CEREBROSPINAL FLUID

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In the study of 2 cases of metachromatic leucodystrophy, late juvenile form, an increase of the total amino acid content in urine was found. The results of the study of the free amino acids in blood, urine, saliva, and cerebrospinal fluid (CSF) by means of circular and two-dimensional chromatography, and high voltage electrophoresis, are the subject of this communication.

MATERIAL AND METHODS

Material — Two familial cases (cousins) of the late juvenile type of metachromatic leucodystrophy were studied. The diagnosis was based on the clinical picture plus the finding of metachromatic bodies in urine (Austin test [1]), saliva, and CSF (Canelas et al.[12]), as well as in biopsies of peripheral nerve, liver, and kidney. The clinical and laboratorial data on these cases are extensively reported elsewhere [11].

The following samples were used: (a) recently emitted or 24-hour urine preserved with thymol; (b) 10 ml of recent blood serum; (c) whole saliva obtained by mechanic stimulus (rubber ball chewing) for periods from 8 to 12 hours; (d) 10 ml of recent CSF collected by lumbar puncture. The fluids were kept in a refrigerator at 5°C.

Methods — (a) Deproteination, according to Awapara [3], Troll and Cannan [57], Stein and Moore [54], and Campos et al.[10]. Two milliliters of each sample are added to 6 ml of ethanol and then transferred to a glass-stoppered tube and left at room temperature for one hour. The volume now is centrifuged for 10 minutes at 2,000 rpm; 2.5 ml of the supernatant are transferred to a graduated cylinder and completed up to 10 ml with chloroform. The cylinder is shaken and then left in rest until the layers are separated. The acqueous upper layer contains amino acids (A).

(b) Hydrolysis of the biologic fluids: 0.5 ml of the upper layer (A) are transferred to a 10 ml ampoule; 0.5 ml of 6N hydrochloric acid are added; the ampoule is closed and put in a stove at 100-110°C for 24 hours. The ampoule is then opened

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and 0.9 ml are transferred to a porcellain capsule immersed in water bath. The material is concentrated with ethanol and the hydrochloric acid is evaporated. The residuum is redissolved in 0.3 ml of 10% isopropanol. In this way a sample of hydrolyzed amino acids is concentrated three times (B).

(c) Standards for comparison with the biologic samples were artificially obtained according to the most frequent amino acids in urine [7,8,20,36,38,52,59], blood serum [23, 25,39,51,54,61], saliva [7,8,19,21,28,42,47], and cerebrospinal fluid [24,27,30,31,33,35,45,50,56,60].

(d) The chromatographic technique followed the general rules of Block et al.[9] and Lederer [29], using as solvents a mixture 4:1:1 of butanol, acetic acid and water, and a mixture 80:20 of phenol and water. For circular chromatography Whatman paper No. 1 was used. A 0.4 ninhydrin solution in 95% acetone in water was used for color development. Circular chromatographies in wooden cases, as well as two-dimensional chromatography, always developed, in each chromatogram: the direct sample (A), the hydrolyzed sample (B), and the standard of the corresponding biologic fluid (C).

(e) High voltage electrophoresis was used in accordance to H. Michl (apud Lederer [29]), Smith [49], Block et al.[9], Visakorpi and Puranen [59], Efron [17], and Mabry and Todd [36]. The controle were made with 4 kv, 60 mA, 5 Lb, 20 minutes each, in an apparatus of high voltage *. The buffer solution was prepared with 2.5 ml of pyridine, 10 ml of acetic acid, and water up to 2,500 ml (pH 5.3). Whatman paper 3 MM, 12×50 cm, was used. The samples were placed in the center of the paper moistened with the buffer solution, and equally distant from the two poles. The lirect sample (A), the hydrolyzed sample (B), and the corresponding biologic fluid (C) were always used.

(f) The fluids were carefully led to the same concentration, each one being applied in the amount of 25 μl for high voltage electrophoresis, 50 μl for circular chromatography, and 100 μl for two-dimensional chromatography.

RESULTS

The results are summarized in Table 1. Emphasis will be given to the amino acid concentrations specifically altered in the CSF of the two patients in study. Plasma, urine, and saliva amino acid levels will be used as qualitative data.

Plasma — Seventeen of the 21 normal amino acids were found. The most increased amino acids were cystine, aspartic acid, glycine, glutamic acid, alanin, proline, tyrosine, and phenylalanin. Besides, 3 still unidentified substances (peptides? amino acid derivatives?) were found: (1) between taurine and histidine; (2) between arginine and aspartic acid; (3) between serine and glutamic acid.

Urine — Seventeen of the 21 amino acids normally present in this fluid were found. The most increased amino acids were cystine, aspartic acid, alanin, proline, tyrosine, and phenylalanin. Besides, 3 still unidentified substances were found: (1) between taurine and histidine; (2) between arginine and aspartic acid; (4) between glutamine and threonine.

* Locarte Company, 10 kv, 100 mA.

Table 1 — Study of the free amino acids in two cases of metachromatic leucodystrophy, juvenile type (with quantitative data on the most concentrated amino acids in the cerebrospinal fluid — μg/ml).

Amino acids	Blood serum	Saliva	Urine	Cerebrospinal fluid		
				O.A.	M.A.R.	Normals*
1. Cystine	++	+	++			
2. α-γ-diaminobutyric	+	++				
3. Lysine	+	+		+	+	+
4. Taurine	+		+			
5. Unidentified No. 1	+	+	+	+	+	
6. Histidine	+	+	+			
7. Arginine	+	+	+	+	+	+
8. Unidentified No. 2	...		...			
9. Aspartic acid	++	+	++	50.3	13.2	0.4-1.2
10. Glycine	++	+	+	+	+	+
11. Serine	+	+	+	++	++	+
12. Unidentified No. 3	...					
13. Glutamic acid	++	++	+	+	+	+
14. Glutamine	+		+	+	+	+
15. Unidentified No. 4		...	...	...	...	
16. Threonine			+	+	+	+
17. Alanin	++	++	++	56.1	17.1	2.9-3.4
18. Proline	++	++	++	8.8	3.0	0.1
19. Tyrosine	++	+	++	+	+	+
20. Unidentified No. 5		...				
21. Tryptophan	+		+			
22. Valine			+	+	+	+
23. Phenylalanin	++		++	40.4	12.3	1.5-1.8
24. Unidentified No. 6				...	...	
25. Leucines	+	+	+	+	+	+

..., traces. * Logothetis [30,31], Logothetis and Bovis [33], Perry and Jones [45].

Saliva — Thirteen of the 21 normal amino acids were found. The most concentrated amino acids were α-γ-diaminobutyric acid, glutamic acid, alanin, and proline. Besides, 3 still unidentified substances were found: (1) between lysine and histidine; (4) between glutamic acid and alanin; (5) between tyrosine and leucines.

Cerebrospinal fluid — Fourteen of the 21 amino acids most constant in the normal controls were found. The most concentrated amino acids were aspartic acid, alanin, proline, and phenylalanin, mainly in patient O.A. (Table 1, Fig. 1). The remaining amino acids were present in levels paralleling the normal averages reported by most authors. Besides, 3 still unidentified substances were found: (1) between lysine and arginine; (4) between glutamine and threonine; (6) between phenylalanin and leucines.

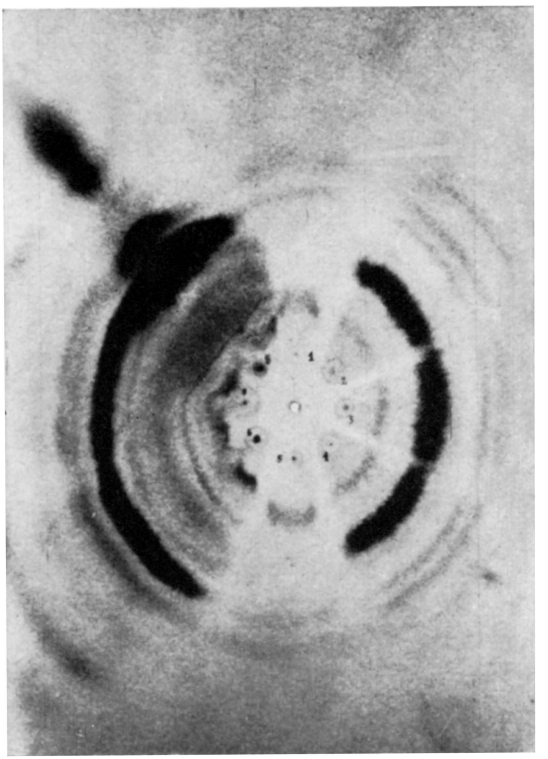

Figure 1 — Circular chromatogram of the free amino acids in the cerebrospinal fluid in a case (O.A.) of metachromatic leucodystrophy. The standard and the samples were applied in amounts of 500 µl. No. 1 and 5, standards of amino acids; No. 2, 3 and 4, free amino acids of the sample (direct extraction); No. 6, 7 and 8, hydrolysis of the amino acids of the sample.

Of particular interest is the marked increase of aspartic acid and proline, a finding not yet reported. From the unidentified components, the most frequent were (1) and (4). Through high voltage electrophoresis the component (1) was disintegrated into three parts (Fig. 2), one of them be-

having like a polypeptide [9, 29, 49], and was seemingly identifiable with a compound substance containing glutamic acid, glycine, and aspartic acid, which prevails in the chromatographic and electrophoretic splittings.

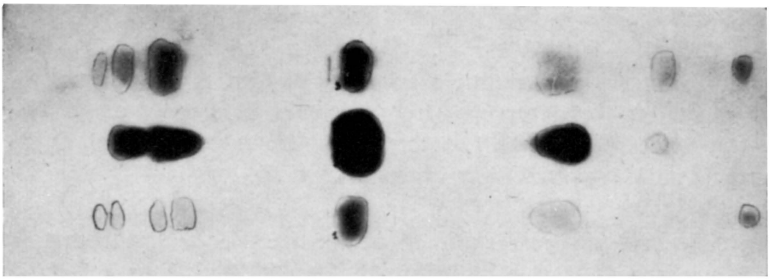

Figure 2 — High voltage electrophoresis of the free amino acids in the cerebrospinal fluid in a case (O.A.) of metachromatic leucodystrophy. No. 1, direct extraction of the amino acids (25 μl); No. 2, standard of glutamic acid (25 μl); No. 3, hydrolisis of the amino acids (25 μl), showing a splitting in spots (peptides) migrating to the negative pole and corresponding to the unidentified spots No. 1 and 2 of the circular and two-dimensional chromatographies.

COMMENTS

The two cases with juvenile metachromatic leucodystrophy showed a marked aminoaciduria: patient O.A. 1,346.6 mg/day, volume 1,380 ml; patient M.A.R. 411.5 mg/day, volume 1,350 ml (normal values, according to McEvoy-Bowe and Lugg [38], and others: 64 to 110 mg/day). In the cerebrospinal fluid, the total concentrations of amino acids were, respectively, 266 and 128 μg/ml as compared to the normal range from 65 to 95 μg/ml [30, 31, 33, 45].

Liver and kidney function tests disclosed no definite impairment in these two cases; this is in accordance with the literature, the finding of kidney damage in metachromatic leucodystrophy being exceptional [43, 62]. So, the increased aminoaciduria in these cases could be ascribed to a genetic error in the metabolism of amino acids, probably a specific enzymatic disorder. The physical and chemical characteristics make it similar to the renal aminoacidurias, either inborn or acquired [5, 6, 18, 20, 22, 23, 38, 49, 51].

The polypeptide (glutaminyl-glycyl?) found in the CSF could be bound to an abnormal fraction also produced by an enzymatic inborn error, giving way to a specific polypeptide-lipid.

It must be reminded that deposits of mucopolysaccharides have been demonstrated in the white matter of cases with metachromatic leucodystrophy [16, 58]. Furthermore, in the granular cells of the brain in adult cases of this disease, Diezel [16] found a glycolipid-proteid complex. In another neurolipidosis, gargoylism, the cerebral cells store gangliosides, while the liver and kidney cells store mucopolysaccharides, which are heavily excreted in urine [40, 55].

Regarding the disorders of amino acid concentrations in CSF, it deserves mentioning that in Niemann-Pick disease Logothetis and Bovis [34] found an increase of aspartic acid, tyrosine, glycine, valine, arginine, alanin, leucine, serine, and lysine. In one case of cerebromacular degeneration the same authors found an increase of glutamic and aspartic acids, tyrosine, arginine, alanin, serine, and glutamine.

While in 12 cases of multiple sclerosis Walker et al.[60] found no quantitative or qualitative differences from normals, in 4 cases of "diffuse sclerosis" (type not mentioned) Logothetis and Bovis [34] evidenced an increase of glutamic and aspartic acids, tyrosine, valine, arginine, alanin, leucine, serine, and lysine.

The increased concentrations of γ-aminobutyric acid, tyrosine, glycine, and serine in the CSF of a patient with familial spastic paraplegia, studied by Logothetis and Bovis [34], must be emphasized. Actually, Scholz [48], Curtius [14], Poser and van Bogaert [46], and Kastan [26] found several cases of Strümpell's paraplegia in families of patients with metachromatic leucodystrophy, suggesting a genetic relationship between the two diseases. In 2 cases of hereditary ataxia, a nosologic group closely related to familial spastic paraplegia, Logothetis [32] found a significant increase of aspartic acid, while alanin, serine, glycine, lysine and threonine appeared moderately elevated. In 3 further cases of "heredoataxias", Logothetis and Bovis [34] evidenced an increase of γ-aminobutyric acid, glutamic acid, aspartic acid, arginine, and serine.

One can easily admit that the metabolism of amino acids in the central nervous system is deeply impaired in those disease processes, since the blood-brain barrier would prevent the passage to the CSF of the most concentrated amino acids in blood plasma.

Concerning the disorders of amino acid concentrations in urine, we remind that, in Wilson's disease, an increased excretion of threonine, cystine, lysine, serine and tyrosine, and less marked of glutamine and alanin, is found [6, 13, 49, 53]; in the CSF the amino acid concentrations are only slightly changed, perhaps because the disorder depends more on the kidney than on the brain damage.

In "maple syrup" disease, where the presence of mental impairment, spasticity and myoclonus reflects the nervous involvement, an increase of valine, leucine, and isoleucine is found; the evidence of a great amount of indole components points to an interference in the metabolism of tryptophan [15, 18, 22, 37].

In Hartnup disease — a hereditary condition characterized by progressive mental deterioration and transient cerebellar ataxia, besides of other manifestations — an increase of alanin, serine, glutamine, valine, leucines, tyrosine, phenylalanin, tryptophan and histidine is found [5, 18, 22, 41, 49].

In phenylketonuria, the urinary excretion of phenylalanin and its metabolites is increased [2, 18, 44, 49]; this metabolic disorder and the mental impair-

ment are two features in common with our cases of metachromatic leucodystrophy.

It must be emphasized that in phenylketonuria, "maple syrup" disease and in Lowe's syndrome (another disease involving a disorder of the metabolism of amino acids), retarded myelination and gliosis have been found [4].

In the CSF of our cases we found a marked increase of aspartic acid, alanin, proline, and phenylalanin, and a moderate rise of serine, the remaining amino acids being in normal levels; this pattern resembles those found by Logothetis and Bovis [34] in Niemann-Pick disease, cerebromacular degeneration, and in "diffuse sclerosis". In urine the prevailing amino acids were cystine, aspartic acid, alanin, proline, tyrosine, and phenylalanin, a pattern which resembles that of Hartnup disease.

It can not be taken for granted that the increased concentrations and abnormal presence of amino acids in urine and CSF of our cases of Scholz disease are caused by an inborn error, since we could not perform the same investigation in the youngest relatives of them. However, it can be presumed that the amino acid dysmetabolism would chronologically follow the lipid disorder.

SUMMARY

Increased aminoaciduria in metachromatic leucodystrophy is demonstrated, presumably for the first time. By means of circular and two-dimensional chromatography, the free amino acids of urine, blood, saliva, and cerebrospinal fluid of two familial cases of juvenile metachromatic leucodystrophy were studied.

In blood an increased concentration of cystine, aspartic acid, glycine, glutamic acid, alanin, proline, tyrosine, and phenylalanin was found. In urine, cystine, aspartic acid, alanin, proline, tyrosine, and phenylalanin showed increased levels. In saliva α-γ-diaminobutyric acid, glutamic acid, alanin, and proline were mostly concentrated. In cerebrospinal fluid aspartic acid, alanin, proline, and phenylalanin were found in greater concentrations.

Besides, 6 non-identified amino acids were present. One of them disintegrated into 3 components and one of these fractions behaved like a polypeptide, seemingly identifiable to a compound substance of glutamic acid, glycine, and aspartic acid. This substance could integrate a polypeptid-lipid complex resulting from a lipid dysmetabolism, already known in the leucodystrophies, allied to a proteid dysmetabolism, now reported.

The results are compared with the data reported in the literature regarding the concentrations of amino acids in the cerebrospinal fluid and urine of some nervous diseases showing demyelinating processes. In CSF the pattern of metachromatic leucodystrophy resembles those previously found in Niemann-Pick disease, cerebromacular degeneration, and "diffuse sclerosis". In urine the pattern resembles that of Hartnup disease.

RESUMO

Leucodistrofia metacromática: estudo dos aminácidos livres no sangue, urina, saliva e líquido cefalorraqueano.

É registrado, presumìvelmente pela primeira vez na literatura, o aumento da aminacidúria em casos de leucodistrofia metacromática. Mediante cromatografia circular e bidimensional foram estudados os aminácidos livres na urina, sangue, saliva e líquido cefalorraqueano de dois casos familiais de leucodistrofia metacromática.

No sangue foi verificado aumento da concentração de cistina, ácido aspártico, glicina, ácido glutâmico, alanina, prolina, tirosina e fenilalanina. Na urina encontravam-se elevados o ácido aspártico, cistina, alanina, prolina, tirosina e fenilalanina. Na saliva foi notado aumento do ácido α-γ-diaminobutírico, ácido glutâmico, alanina e prolina. No líquido cefalorraqueano o ácido aspártico, alanina, prolina e fenilalanina encontravam-se em maiores concentrações.

Além disso, estavam presentes 6 aminácidos não identificados. Um dêles desintegrava-se em três componentes e uma destas frações comportava-se como um polipeptídeo, provàvelmente identificável a uma substância composta de ácido glutâmico, glicina e ácido aspártico. Esta substância poderia integrar um complexo polipéptido-lipídico resultante de um dismetabolismo lipídico, já conhecido nas leucodistrofias, associado a um distúrbio do metabolismo protêico, agora relatado.

Os resultados são comparados com os dados referidos na literatura relativa às concentrações de aminácidos no líquido cefalorraqueano e urina de algumas afecções nervosas de caráter desmielinizante. No líquido cefalorraqueano o perfil da leucodistrofia metacromática se assemelha aos anteriormente encontrados na moléstia de Niemann-Pick, na degeneração cerebromacular e na "esclerose difusa". Na urina as alterações se aproximam das que ocorrem na moléstia de Hartnup.

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