METABOLIC BALANCES OF SULFUR IN PATIENTS WITH METACHROMATIC LEUCODYSTROPHY

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Metachromatic leucodystrophy (MLD) is a lipidosis characterized by the storage of sulfatides (Austin [2], Svennerholm [10]). Moser, Moser and McKhann [9], working with ^{35}S, showed that this accumulation is the result of a deficient degradation of the sulfatides. Austin [4] evidenced a low sulfatase (particularly type A) activity in the brain, liver and kidney. In urine, the mean arylsulfatase A specific activity of control children was found to be 47 times that of MLD children (Austin [3]).

In three cases of the late juvenile type of MLD studied by Canelas, Escalante, Iriya and De Jorge [5] the sulfur content of urine was determined. The total sulfur concentrations fell within the normal range, but the esterified sulfur was increased.

In this report the metabolic balances of sulfur in two of those patients are analyzed.

MATERIAL AND METHODS

Two cousins (O. A. and M. A. R.) with the late juvenile type of MLD were studied. The diagnosis was based on the clinical picture plus the finding of metachromatic bodies in urine (Austin [1] test) and metachromatic material in biopsies of peripheral nerve, liver and kidney. The clinical and laboratorial data on these cases are reported in full elsewhere (Canelas et al. [5]). The concentrations of esterified sulfur in urine were, respectively, 207 and 275 mg/24 hr. (normal range, 60-120 mg/24 hr.).

Patient O. A. was submitted to a mixed diet containing 850 mg of sulfur each day; patient M. A. R. was submitted both to mixed and vegetarian diets, the latter containing 500 mg of sulfur by day.

Patients O. A. and M. A. R. were kept in metabolic balance for 19 and 37 days (including 17 days on mixed diet and 20 days on vegetarian diet), respectively. Each balance was divided in periods of from three to six days.

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At the end of each period faeces and alimentary residues, wich were collected in glass containers kept in a refrigerator, were weighed, mixed and homogenized in a Wharing Bendor. Duplicate samples of each material were then submitted to the analytical method described below. The ingesta were the difference between the known sulfur content of each type of diet and the concentration in the alimentary residues. Urine was collected daily under the same conditions as faeces, and the 24-hour volume determined.

Total sulfur was determined by the following turbidimetric method (De Jorge, Silva and Cintra [7]):

Reagents — (1) Five percent barium chloride solution in 0.5% polyvinylic alcohol: exactly 500 mg of polyvinylic alcohol grade 52.22 are weighed, and dissolved in bidistilled water at 70ºC until a homogenous solution is obtained, all in a 100 ml volumetric flask, completing the volume with bidistilled water; the solution must be homogenized by shaking; exactly 5 gm. of dehydrated barium chloride are weighed and dissolved in the 0.5% polyvinylic alcohol solution, and then filtered through Whatman paper No. 42. (2) Ten per cent solution of trichloroacetic acid (10% TCA): 100 gm. of trichloroacetic acid R.A. are dissolved in bidistilled water, completing the volume to 1,000 ml, and homogenized by shaking. (3) Standard solution of ammonium sulfate 0.01 M: exactly 1.3214 gm. of anhydrous ammonium sulfate are weighed and dried during 4 hours at 100ºC and dissolved in bidistilled water, until a final volume of 1,000 ml is obtained; this solution contains 320 mg of sulfur per liter. (4) Standard solution for use: in a 100 ml volumetric flask 10 ml of the 0.01 M ammonium sulfate solution are diluted with bidistilled water, shaking the solution; this solution contains 32 mg of sulfur per milliliter. (5) Benedict solution for sulfur: 200 mg. of crystallized copper nitrate, 50 gm. of sodium or potassium chlorate and bidistilled water to 1,000 ml.

Determination of total sulfur in urine — In a porcelain capsule of 50 ml capacity, 2.0 ml of urine are pipetted, and in another capsule 2.0 ml of bidistilled water are pipetted. To each capsule 5.0 ml of the Benedict solution for sulfur are added. The material is well mixed, dried in a Bunsen burner or hot plate, put in a furnace regulated to 530ºC and left overnight. The furnace is turned off and the capsule allowed to reach room temperature. The material of the capsule is dissolved in 10.0 ml of 1:4 hydrochloric acid solution, and diluted to a final volume of 50.0 ml with bidistilled water. It is well homogenized and the solution filtered through a Whatman paper no. 42. One ml of the filtrate is pipetted, added 3.0 ml of 5% TCA, homogenized and then 1.0 ml of the barium chloride solution is added. The material is well shaked, let stand for 10 minutes and the turbidity compared to a blank prepared with water instead of urine, in a Klett-Summerson colorimeter with a No. 42 filter, or in a spectrophotometer, in 360 micron wavelength, adjusting the blank in maximal transmission. If the filtrate is not completely clear, the reading against the blank should be made before adding the barium reagent and any reading obtained should be subtracted of the reading after the addition of the reagent.

Determination of total sulfur in faeces and foods — An exact amount from 200 to 500 mg of material is weighed in a porcelain capsule and then the determination proceeds as described for total sulfur in urine.

RESULTS

The results are summarized in Tables 1 and 2.

Diet	Period (1964)	Days	Ingesta (mg/day)	Excreta (mg/day)		Balance (mg/day)	Blood serum (mg/100 ml)
				Urine	Feces		
Mixed	11 May-14 May	4	850	395	180	+285	1.889
	15 May-18 May	4	809	475	125	+209	—
	19 May-21 May	3	820	625	185	+ 10	1.860
	22 May-25 May	4	840	525	120	+195	—
	26 May-29 May	4	842	634	145	+ 63	1.915
	Total	19				+160	

Table 1 — Metabolic balance of sulfur in patient O.A.

Diet	Period (1964)	Days	Ingesta (mg/day)	Excreta (mg/day)		Balance (mg/day)	Blood serum (mg/100 ml)
				Urine	Feces		
Mixed	27 Jan-29 Jan	3	715	542	120	+ 53	1.915
	30 Jan- 1 Feb	3	786	601	130	+ 55	1.705
	2 Feb- 5 Feb	4	790	585	96	+109	—
	6 Feb- 8 Feb	3	500	305	215	− 20	—
	9 Feb-12 Feb	4	582	428	135	+ 19	—
	Total	17				+ 46	
Vegetarian	17 Mar-19 Mar	3	450	186	246	+ 18	1.840
	20 Mar-23 Mar	4	490	186	185	+119	—
	24 Mar-26 Mar	3	455	177	165	+113	2.218
	27 Mar-30 Mar	4	490	236	198	+ 56	—
	31 Mar- 5 Apr	6	480	248	226	+ 6	2.287
	Total	20				+ 56	

Table 2 — Metabolic balance of sulfur in patient M.A.R.

COMMENTS

As it is seen in Tables 1 and 2, a positive sulfur balance was evidenced in both patients, particularly in O. A., who showed an average daily sulfur balance of + 160 mg. In patient M.A.R., a significant difference was not found between the balances on vegetarian (+56) or mixed (+46) diets.

These figures are definitely higher than the average found by De Jorge and Cintra [6] in 8 normal adults submitted to mixed (mean metabolic balance of sulfur + 23.2 mg ± 17.3) or vegetarian (+ 23.5 mg ± 29.3) diets. If the results in the two cases of MLD under mixed diet (average 103.0 mg ± 80.6) are compared with the normal values, a statistically significant difference is found ($t = 3.069$; $P < 0.02$).

In the blood serum, the contents of inorganic sulfur were always above the upper normal limit.

The evidence of a positive sulfur balance in these two patients with MLD is in accordance with the facts already known on the pathogenesis of the disease, which is essentially characterized by a storage of sulfatides, probably due to a deficiency of the sulfatase activity (Austin [3, 4], Jatzkewitz [8]).

This finding, allied to the deficiency of an enzyme (sulfatase), leading to a storage of sulfatides in several body tissues, owing to deficient degradation, the increased urinary excretion of esterifield sulfur, and the high values of inorganic sulfur (sulfate) in the blood serum, build up a picture with a striking similarity with that of hepatolenticular degeneration. In Wilson's disease, likewise, copper absorption is raised, a copper oxidase (cerulplasmin) is lacking, copper accumulates in the body tissues, cupriuresis is high, and the blood serum contains higher levels of direct reacting (non-ceruloplasmin) copper than in control subjects.

SUMMARY

The metabolic balances of sulfur in two cases of the late juvenile form of metachromatic leucodystrophy were studied. A positive balance of sulfur was found in both patients, apparently not influenced by the type of diet (either mixed or vegetarian).

This finding is in accordance with the current views on the pathogenesis of the disease, namely a sulfatidosis with low sulfatase activity.

RESUMO

Balanços metabólicos do enxôfre em pacientes com leucodistrofia metacromática

Foram estudados os balanços metabólicos do enxôfre em dois pacientes com a forma juvenil da leucodistrofia metacromática. Foi verificado, em ambos os casos, um balanço positivo dêsse metalóide, aparentemente não influenciado pelo tipo de dieta (geral ou vegetal).

Êste resultado está de acôrdo com a atual concepção patogênica dessa moléstia, que consiste, essencialmente, em uma sulfatidose com diminuição da atividade das sulfatases.

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