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A STUDY OF PHENYLKETONURIA BY PAPER CHROMATOGRAPHY (*)

M. KOROURIAN, F. GHARIB AND M. PAKDEL

SUMMARV:

Two twin sisters of I8 months old were presented from the Children Medical Center (by Dr. Rafyi and Dr. Soleimaniha) to the Razi Institute for blood and urine analysis, on Sep. I975. The children were from cousins parents, having fair hair and blue eyes, suffering from mental and physical retardation.

Several paper chromatographic atudies of serum samples, showed a remarkable increase of phenylalanine. The quantitative determination of serum samples from the two patients showed 25.07 and 27.35 mg. per I00 ml. of the above aminoacid, which were much more than the normal levels.

The phenistix test on the urine was also highly positive.

INTRODUCTION:

Phenylketonuria is an inborn error of metabolism. Absence or deficiency of the liver enzyme, phenylalanine hydroxylase, prevents the conversion of phenylalanine to tyrosine (3). Normally 80 % of dietary phenylalanine is converted to tyrosine (4). Although the mechamism is not clearly defined (2), it is known that this disease results in mental retardation unless treatment is initiated . It has been shown that a diet low in phenylalanine effectively prevents mental retardation if started at the early stage of the disease. It must be realized that phenylalanine is an essential aminoacid for growth and its deficiency would result in growth failure, anemia, bone changes, or death (5).

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MATERIAL AND METHOD

PURIFICATION OF SERUM:

One ml. of the fresh serum was put into a 50 ml. beaker and was allowed to dry at 45° C. To the dried residue was added 5 ml. solution of aceton containing 5 per cent of 6 N hydrochloric acid, using a magnetic stirrer at room temperature, for at least 30 minutes. The mixture was filtered and the filter paper was washed 3 times with 5 ml. of the above solution and added to the first filtrate. The obtained solution was dried again at 45° C and the residue was dissolved in 3 ml. of chloroform solution. The amino acids were extracted by Io ml. of I% hydrochloric acid.The extraction was repeated one more time to obtain a solution free of lipid, and finally it was dried at 45° C and redissolved in one ml. of double-distilled-water (I).

METHOD OF CHROMATOGRAPHY:

The amino acid chromatography was accomplished with the purified blood serum, using an ascending and two - dimentional method in Shandon universal chromatank, on Whatman No. I paper (*) of 20 × 20 centimeters.

The first and second phases were normal butanol - acetic acid - water, and phenol - ammonia - water, respectively (6).

Samples of 50 to I00 microliters were spoted on the paper and the chromatograms were rum for 6 to 7 hours, for each phase at room temperature. Chromatograms were, finally, developed in a solution of 0 . 02 per cent ninhydrin in aceton (I).

RESULTS:

The spectras of Io microliter of a standard solution (0 . 0I molar) of phenylalanine in water containing Io percent isopropanol, and Ioo microliters of purified serum chromatograms were obtained by Shandon Chromatograph Recording Reflectance Densitometer apparatus, using a sensitive Elpher planimeter to determine the surface areas of the spectras, table I.

TABLE I, surface areas of the standard solution of phenylalanine and the purified sera of the patients,

Sample	Surface area
10 u lit. of standard sol.	1.45 mm2
100 u lit. of the purified serum patient 1	2.2 mm2
100 u lit, of the purified serum patient 2	2.4 mm2

(*) it is possible to use whatman No. 4 or 20 in this method.

With regard to the molarity of the standard solution of phenylalanine, however, the amount of phenylalanine was determined to be 25. 07 and 27.35 mg. per I00 ml. of sera of the patients No. I and No. 2 respectively (6).

In figure I and 2 the spectrograms of I00 microliters (in both cases) of the above sera are shown.

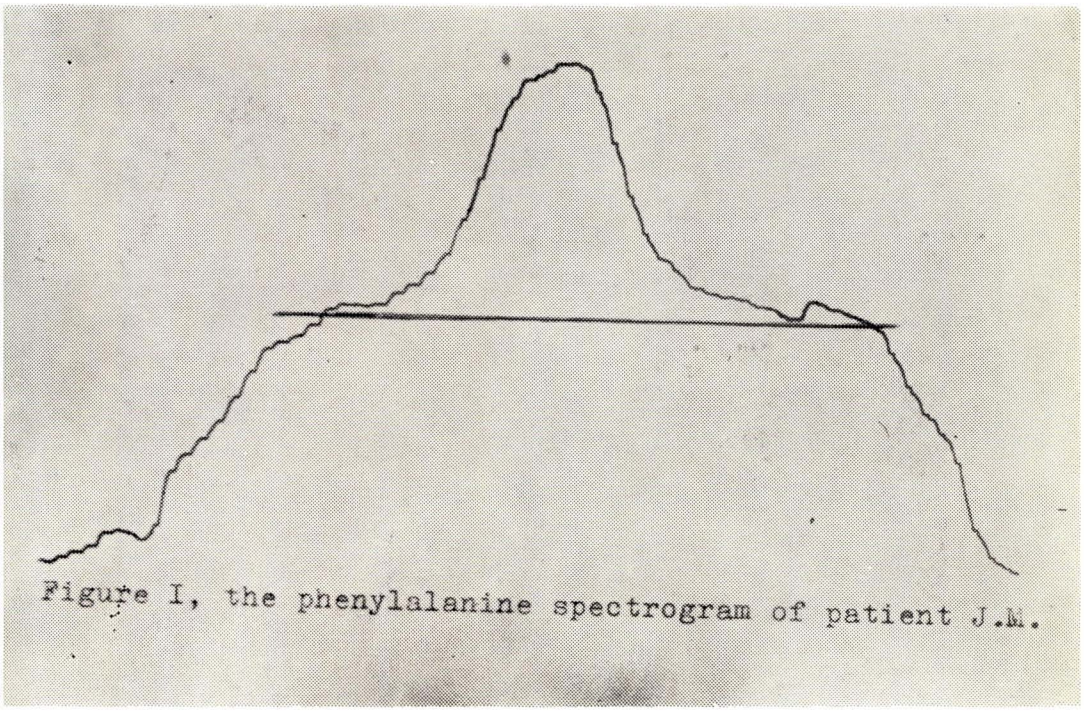

Figure I, the phenylalanine spectrogram of patient J.M.

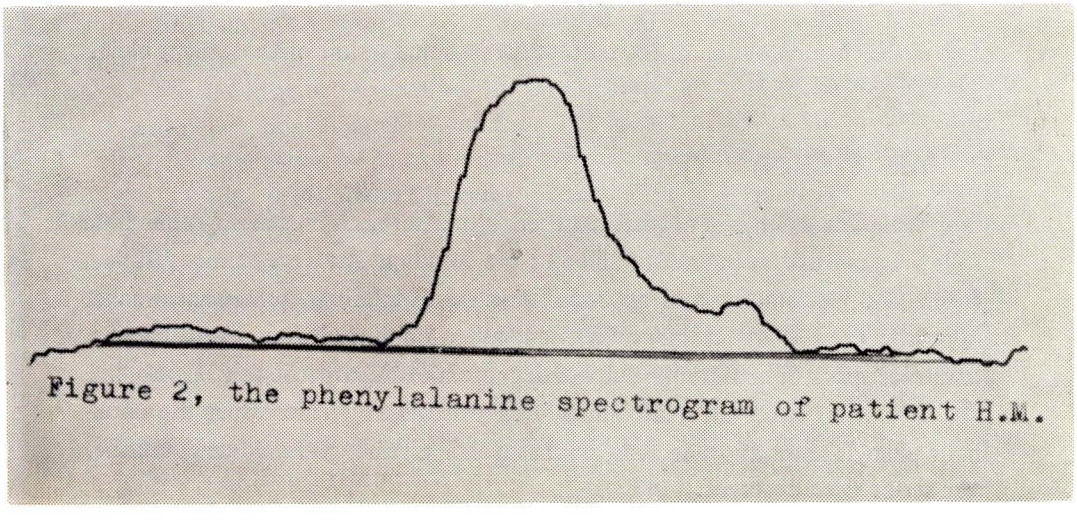

Figure 2, the phenylalanine spectrogram of patient H.M.

I00 u liters of purified blood serum was chromatographed and the obtained amino acids related to the patients (in both cases) were as follows: the very intense and dominant spot is phenlalanine, somewhat weaker appearance spots were alanine, glycine, serine, taurine, glutamic acid, lysine, valine , leucine , and trace apots of glutamine, proline , threonine, and methionine sulphoxide, as shown in figure 3.

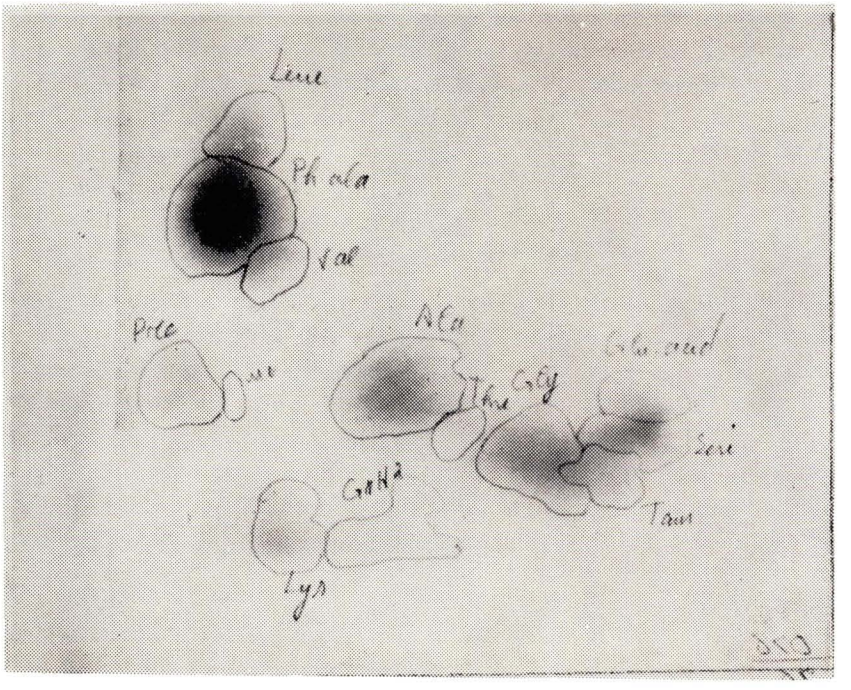

Fig. 3, the chromatogram of 100 u lit. of the puridied serum of the patient No.1.

DISCUSSION:

When I00 u lit. of a normal purified blood serum is chromatographed the following picture is obtained (I and 6) : the dominant spots are alanine, glycine, serine, and glutamic acid of approximately equal intensity, somewhat weaker appearance spots are taurine, valine, leucine, glutamine, lysine, phenylalanine, and trace spots of methionine sulpholxide, proline, and aspartic acid, figure 4.

Regarding to the chromatograms of the patients a high increase in the amount of phenylalanine and a decrease in the level of glycine, glutamic acid, taurine, and serine were observed.

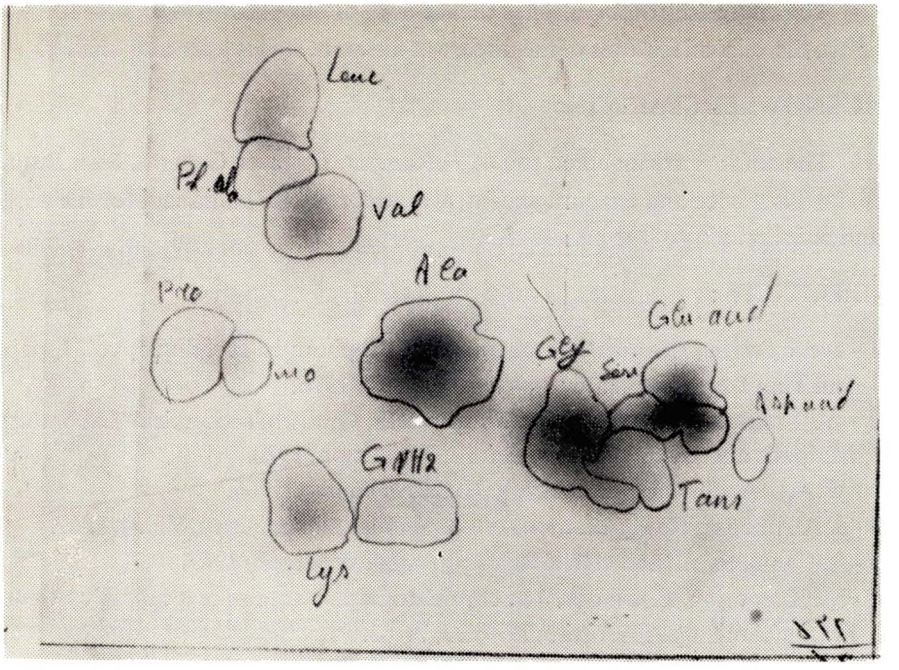

Fig. 4, the chromatogram of 100 u lit. of a normal purified blood serum.

The metabolism and the relationship between phenylalanine and tyrosine is complicated and of considerable interest. Phenylalanine is an essential amino acid, whereas tyrosine is not. The conversion of phenylalanine to tyrosine is irriversible, but tyrosine cannot wholly replace phenlalanine in the diet.

The amont of phenylalanine in normal serum is about one mg. per I00 ml. (4).

In hereditary tyrosinaemia (tyrosinosis), the amount of phenylalanine and tyrosine is approximately 4 and 5 mg. per I00 ml. of blood serum, respectively (I). In this disease p - hydroxyphenylpyruvate hydroxylase enzyme is abnormal.

In hyperphenylalaninaemia the amount of phenylalanine is 4 to 20 mo. per I00 ml. of serum. At the critical stage of high level of phenylalanine., low protein diet is proposed as treatment (I).

In phenylketonuria, the increased amino acid of plasma is phenylalanine with a quantity more than 20 mg. per I00 ml. In this disease phenylacetic and phenylpyruvic acide are also highly present in the urine (phenistix test positive) (2). In our studies the clinical features of the patients were mental retardation, fits, fair hair and skin, which were similar to those features reported by others in patients with phenylketonuria (I,8).

The interesting observation is that in both of our patients who were identical twins, the clinical features of the disease at different stages were also similar.

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