

## THE CHROMATOGRAPHIC STUDIES OF A CASE OF CYSTINURIA IN IRAN (\*)

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### SUMMARY :

A patient suffering from kidney stone, and operated surgically two times, was presented by Dr. Khonsary, faculty of medicine, Daryoosh Kabir to the Razi Institute on 8/10/2535 for biochemical studies of urine and blood.

Different qualitative studies of urine samples by paper chromatography showed a notable increase of cystine in urine. The quantitative determination of urine samples showed 300 mg. of cystine per liter, i.e. about 10 times more than normal case (Medes, 1937).

### MATERIAL AND METHOD :

The tests were performed on the fresh twenty four hours collected urine and blood serum.

The chromatography of urine and blood were done by ascending and two-dimensional technique in Shandon universal chromatank, using Whatman No. 1 paper of 20×20 centimeters. The first and second phases were, normal butanol, acetic acid, water, and phenol, ammonia, water, respectively.

The amino acids chromatograms of urine were performed with 5 to 20 microliters of 5 times concentrated and purified urine. The purification of urine was done with a solution of acetone containing 5 per cent 6 N hydrochloric acid, and solving the residue in one ml. D.D. water. The amino acids chromatograms of blood were performed with 50 to 100 microliters of purified serum. The

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purification of serum was done with the same solution used for urine. The lipids of the product were extracted by chloroform solution. The dried residue, then, was dissolved in one ml. D.D. water, and the chromatograms were developed with a solution of 0.02 per cent ninhydrin in acetone.

## RESULTS :

The urine was turbid, albumin and sugar tests were negative. Microscopic observation showed a lot of ammonium urate, 1-2 uric acid, and 4-5 calcium oxalate crystals in each microscopic field.

The qualitative identified cystine was followed by studying its spectra with Shandon Chromatograph Recording Reflectance apparatus.

The spectras of 5, 10, and 20 microliters of a standard solution (100 mg. per liter) of cystine and 5, 10, and 20 microliters of a concentrated and purified urine chromatograms were obtained. The surface areas of these spectras were measured with a sensitive Elpher planimeter, with a very little error in comparison (table 1).

**Table I. surface area of different volume of cystine and urine;**

	sample	surface area
5	microliters of cystine	0.66 mm <sup>2</sup>
10	»	1.31 »
20	»	2.63 »
5	microliters of urine	0.23 »
10	»	0.44 »
20	»	0.87 »

Concentration and volume of the standard solution of cystine is known, then with considering the used volume of urine, the amount of cystine was determined 300 mg. per liter, i.e. about 10 times more than normal.

In figure I a spectrogram of 20 microliters of the above urine is shown. The first large peak belongs to cystine.

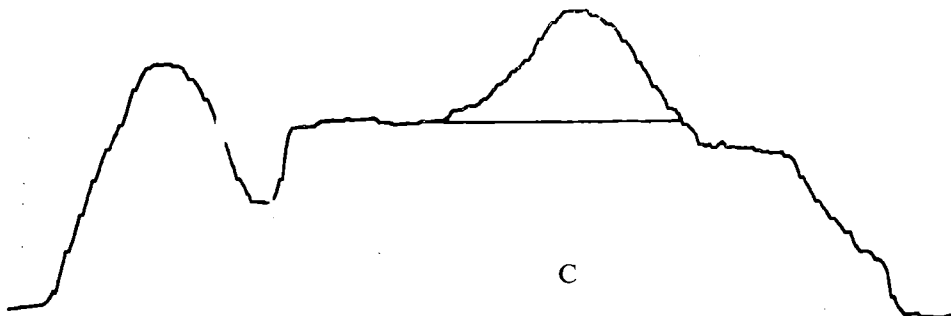


Figure I, the peak of cystine is shown by, C, in this spectrogram.

## DISCUSSION :

The amino acids chromatogram of 5 to 10 microliters of normal urine usually shows about 5 to 8 pale amino acids, with intensity of excretion which are related to, glycine, alanine, taurine, serine, methionine sulphoxide, glutamic acid, and beta amino isobutyric acid respectively. But in the studied case, using the same technique, 11 amino acids were obtained, with notable increase in cystine, lysine, histidine, arginine, and a decrease in taurine, serine, alanine, and glutamic acid. Some increase was also observed in proline and methionine sulphoxide (fig. 2).

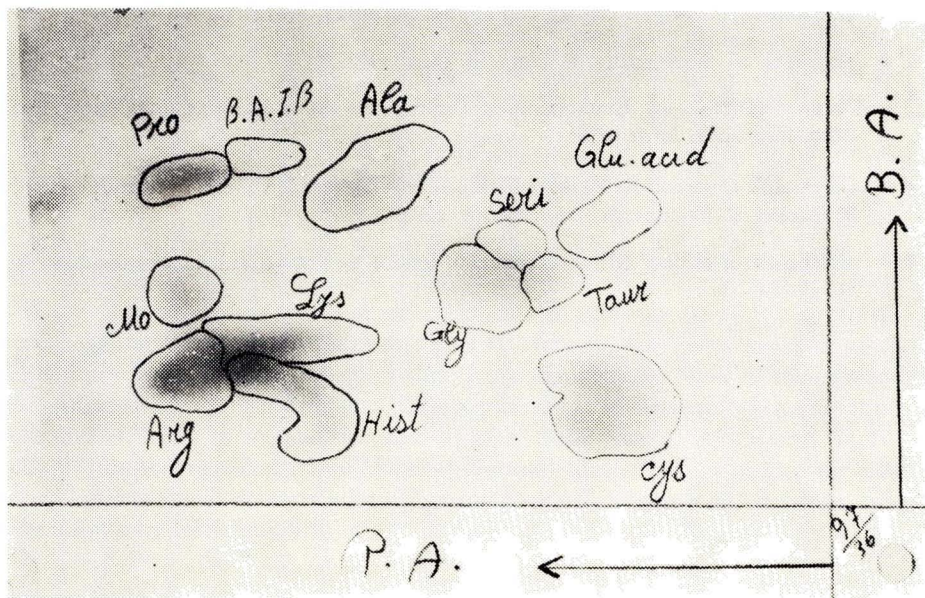


Figure 2, the urine chromatogram of the patient.

The amino acids chromatogram of normal serum showed the following amino acids: alanine, glutamic acid, taurine, serine, valine, leucine, glycine, glutamine, lysine, histidine, threonine, arginine beta amino-iso-butyric acid, proline, phenylalanine, and tyrosine. But in the serum of the patient the following amino acids were obtained: alanine, glutamic acid, taurine, serine, valine, leucine, threonine, glutamine, glycine, lysine, histidine, arginine, beta amino-iso-butyric acid, proline, and phenylalanine.

As have been determined, however, the blood chromatogram of the patient showed no cystine.

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