THE IDENTIFICATION OF HOMOCYSTINE IN THE URINE*

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Methionine is an essential amino acid present in human blood plasma and normally excreted in small amounts in the urine. The normal pathway for the metabolism of methionine involves demethylation to homocysteine which in turn combines with serine to form cystathionine, an intermediate along the pathway to cysteine and cystine.

During biochemical investigations of children with congenital anomalies, mental retardation and failure to thrive, several significant abnormalities in the pattern of the sulfur amino acid excretion were observed in a one year old male infant.

To our knowledge, the presence of homocystine in urine has not been previously reported. This communication will serve to describe the isolation and identification of this amino acid. A complete clinical description of the patient and evaluation of the metabolic data will be published later.

Experimental

Twenty four hour urine collections were obtained at frequent intervals, preserved with thymol and kept at -15° C. Amino acid analysis was performed on an automatic amino acid analyzer following essentially the standard procedure of Spackman et al. (1958), except for specific modifications necessary for the separation of homocystine.

Several abnormalities were found in the amino acid pattern. The urine contained an exceptionally high amount of methionine, some samples contained methionine sulfoxides, while analysis on the 150 cm column showed an unidentified

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peak on the chromatogram in the area where normally β-alanine is eluted. This peak disappeared on hydrolysis or oxidation of the urine with performic acid, indicating that it was not β-alanine.

The following analyses were performed in order to demonstrate that this substance was homocystine:

1. The standard procedure on the 150 cm column was modified so that the temperature change from 30 to 50°C took place after 7 1/2 hours instead of after 10 1/2 hours; the pH of the second buffer was adjusted to 4.00 instead of 4.25, and the buffer change performed after 10 1/2 hours. The pH of the first buffer was kept at 3.25. The result was that β-alanine was eluted after 680 ml, β-amino-iso-butyric acid after 730 ml and the unknown compound between 780 and 790 ml. The position of this substance was greatly influenced by the pH of the buffer. At both pH levels 4.00 and 4.25 the position of the unknown compound was identical with the position of homocystine on the 150 cm column chromatogram. The addition of l-homocystine to the urine before analysis produced a symmetrical and additive peak.

2. A concentrate of the unknown compound was prepared from 30 ml of urine using the technique described in detail for the isolation of homocitrulline by Gerritsen et al. (1961). After performic acid oxidation (Schram et al. 1954) of the isolate the unknown compound disappeared, and a new sharp peak was obtained at 55 ml, the position of the inseparable peaks of cysteic acid and homocysteic acid. Since the isolation technique precluded the presence of cystine, the oxidation product was assumed to be homocysteic acid. Thin layer chromatography of this oxidized concentrate on neutral silica gel in n-butanol, acetic acid/water, 60:20:20 showed the presence of a ninhydrin positive compound with the same Rf value as homocysteic acid.

3. Paper chromatography of the unknown compound and of standard solutions of homocystine was performed on Whatman 3MM paper. In n-butanol/acetic acid/water, 60:20:20, the Rf value of both was found to be 0.28, while in isopropanol/formic acid/water, 30:2:15, the Rf value of the unknown and of homocystine was 0.70.

4. Reduction of the compound with zinc powder in 4N HCl, or treatment with HCN (Greenstein and Winitz, 1961) resulted in formation of a new compound which was identified as homocysteine-thiolactone. This identification was made by compariso with a standard homocysteine-thiolactone HCl solution by thin layer chromatography

and paper chromatography. By spraying the thin layer plate with 0.2% ninhydrin solution in acetone an immediate yellow color developed, which became purple after being exposed to the air overnight.

After treatment of the urine with performic acid not only did the homocystine peak disappear from the chromatogram, but the methionine peak also diminished considerably; larger peaks appeared at the positions of methionine sulfoxide and methionine sulfone. This was considered additional proof for the presence of more than normal amounts of methionine in the urine.

Table 1

Urinary excretion of homocystine and creatinine in an infant with congenital anomalies, mental retardation and failure to thrive.

Urine Sample No.	Homocystine mgm/24 hrs.	Creatinine mgm/24 hrs.
1.	14.1	44
2.	7.2	32
3.	8.2	33
4.	16.0	35

Discussion

The combination of increased amounts of methionine in plasma and urine, and the presence of homocystine in the urine only, points to a "normal overflow" of methionine, as well as a rapid clearance of homocystine by the kidneys. If the defect is in the decreased ability of homocysteine to combine fast enough with serine, the surplus homocysteine moves rapidly from the plasma into the urine and is lost to the body. Oxidation to homocystine must take place somewhere along this route, possibly in the kidneys. This loss of intermediate in the metabolism of the sulfur amino acids may represent an error of metabolism. At this time no explanation is available for these findings nor how homocystine excretion relates to the physical and mental abnormalities in this infant.

References

Gerritsen, T., Lipton, S. H., Strong, F. M. and Waisman, H. A., Biochem. Biophys. Res. Comm., 4, 379, 1961.

Greenstein, J. P. and Winitz, M., "Chemistry of the Amino Acids", vol. 3. p. 2667; John Wiley & Sons, New York 1961.

Schram, E., Moore, S. and Bigwood, E. J., Biochem. J., 57, 33, 1954.

Spackman, D. H., Stein, W. H. and Moore, S., Anal. Chem., 30, 1190, 1958.