Latent Cystathioninuria

L. J. SCHNEIDERMAN*

From the Department of Biochemistry, University of London King's College, London

Harris, Penrose, and Thomas (1959) described a mentally retarded individual whose urine contained large amounts of an unusual amino acid clearly visible on two-dimensional chromatography and identified as cystathionine. A survey of over a thousand mentally retarded patients at the time failed to uncover another example of cystathioninuria, and its rarity can be surmised by the paucity of case reports that have followed (Frimpter, Haymovitz, and Horwith, 1963; Berlow, 1966; Mongeau, Hilgartner, Worthen, and Frimpter, 1966; Perry, Robinson, Teasdale, and Hansen, 1967).

To provide for the more sensitive resolution of cystathionine, Harris and his co-workers employed a single-dimensional solvent system of methanol-water-10N HCl-pyridine (32:7:1:4). With this system they discovered no additional cases of cystathioninuria; however, two brothers from their survey sample showed a slight trace of a substance that ran with cystathionine when overloads of their urine were applied. Subsequently it was discovered that methionine feeding caused this spot to increase considerably so that it became easily visible on two-dimensional chromatography. When the two brothers were returned to their ordinary diet, this spot disappeared from their two-dimensional urinary amino acid chromatograms and became only barely detectable once again on the single-dimensional system.

The studies reported here indicate that the unusual substance appearing in the urine of these brothers after methionine ingestion is cystathionine, and because of the latency of this phenomenon it is referred to as 'latent cystathioninuria'. Further studies of these brothers as well as similar investigations of 50 mentally retarded patients at the same institution and 50 apparently healthy, normal volunteers, along with family studies, are herein described.

Materials and Methods

Subjects. The propositi were studied on two occasions separated by three years (Fig. 1). The second study confirmed and enlarged upon the initial investigation to include observations on other subjects. The mother and brother of the propositi were studied: both appeared of normal intelligence and were in good health, sharing the same house and similar diets; the father was not available for study.

Two control population samples were also studied: 50 subjects, all males of ages comparable with the propositi, were chosen from the same mental institution; of these, 25 were from the same ward and shared the same facilities and diet. The second sample comprised 50 volunteer medical students. All were in good health and had independent facilities and diets.

Available relatives of subjects demonstrating a positive response to methionine loading were also studied.

![Response to Methionine](image)

Fig. 1. The P. family. I. 1: born 1882, suffers from bilateral cataracts, otherwise in reasonably good health for age. I. 2: information unavailable, other than date of birth, 1879. II. 1: born 1905, mentally retarded from birth, admitted mental hospital at age 30, single IQ determination at age 21 was 36. Present stature not remarkable, height 160 cm. (5 ft. 3 in.), no other illnesses. II. 2: born 1907, in good health, works actively as salesman. II. 3: born 1911, killed in military service. II. 4 and 5: 'abnormal pregnancy', ?ectopic. II. 6: born 1918, retarded from birth, admitted mental hospital at age 34, no IQ determination, but considered 'severely subnormal', stature not remarkable, height 173 cm. (5 ft. 8 in.), no other illnesses. II. 7: miscarriage, first trimester. No known consanguinity or familial diseases.

Procedures. Two-dimensional urinary chromatography was carried out on Whatman No. 4 papers (18° × 22°) with phenol-water (80:20) in an atmosphere of ammonia followed by lutidine-water (65:35). Urine aliquots were calculated from 24-hour collections to represent 10-second samples. They were applied and run in descending fashion for 17 hours in each direction. The single-dimensional solvent system described
latent cystathioninuria

261

above (methanol-water-10N HCl-pyridine) for the more sensitive resolution of cystathionine was employed both before and after methionine feeding experiments. In order to assure the detection of trace amounts of cystathionine overloaded urine samples were applied. Descending runs were made over 17 hours on Whatman No. 1 papers (10’’ x 20’’).

A synthetic cystathionine marker was run simultaneously with every study. Chromatogram development was with 0.2% ninhydrin in 95% acetone, heated at 100° C. for a few minutes.

Identification of cystathionine in the urine was based on a variety of electrophoretic and chromatographic methods. Any spot migrating with marker cystathionine was eluted and further compared by high-voltage electrophoresis at pH 1.9 and 4.0, and low-voltage electrophoresis at pH 8.6, in addition to the chromatographic systems described above. Elution of the substance from urine was achieved by painting electrolytically desalted urine in a narrow strip across Whatman No. 3MM paper in 5 ml. amounts, then employing single-dimensional descending chromatography first in the phenol NH₃ system (4’’ x 22’’, 17 hours), then after drying, continuing the run in the same direction in the methanol-water-HCl-pyridine system (17 hours). Because of its slow migration the spot (and marker cystathionine) could easily be eluted free from other ninhydrin-reacting substances. The appearance of cystathionine in the urine of one of the P. brothers following methionine loading was also confirmed by Moore-Stein ion exchange chromatography (Dr. D. C. Cusworth).

Methionine Feeding Experiments. Preliminary studies were done on members of the P. family and five other subjects in order to ascertain the dose response curve to methionine ingestion. After a control 24-hour urine collection, 5 g. D-L-methionine powder suspended in juice was fed in a single dose. Six-hour urines were collected over the next four days. Those subjects who showed a positive response to methionine by the production of cystathionine excreted the greatest concentration at about eight hours; those who showed a negative response excreted no detectable cystathionine over the entire four days. Thus, from these observations, we were able to devise a simple screening test. A sample of urine was obtained about 3 p.m. of the first day, following which 5 g. D-L-methionine powder was fed. A second urine sample was obtained on the morning of the second day. The period of maximum excretion was timed to coincide with the overnight concentrated urine. Overloads of these urine specimens were then run on the methanol-water-10N HCl-pyridine solvent system and examined for the appearance of cystathionine. No ill effects were experienced by any subjects during the feeding experiments.

Results

Under the conditions described, three different kinds of response were observed.

Strongly Positive. In the two propositi, a prominent degree of cystathionuria, amounting to about 500 mg./l. urine at the height of the response to methionine feeding, was evident. On the two-dimensional chromatographic system described, the spot was as prominent as the other amino acids normally visible, such as alanine, glycine, histidine, glutamine, and serine (Fig. 2). Except for the
added presence of methionine, their urinary chromatograms resembled that of a patient with cystathioninuria. No other subjects in this study demonstrated a response of similar magnitude.

**Positive.** In the mother and brother of the propositi, both apparently normal, a response of approximately one-fifth the magnitude occurred following methionine ingestion. This same response was evident in 3 out of 50 male patients of the same mental institution, plus the apparently normal brother of one of these patients, and in 1 out of 50 healthy volunteers, plus his brother. In these subjects, the cystathionine spot became detectable on two-dimensional chromatography, but was fainter than the other amino acids. It was easily seen, however, on the single-dimensional system.

**Negative.** The vast majority of subjects studied showed no detectable cystathioninuria following methionine ingestion either by the two-dimensional or single-dimensional systems. The response was negative, therefore, in 47 out of 50 mental patients and 49 out of 50 healthy volunteers.

**Discussion**

The unique feature of the P. brothers is the latent nature of their aminoaciduria. On an ordinary diet their two-dimensional urinary amino acid chromatograms are normal, and in this respect they differ from the usual species of aminoacidurias reported. On the other hand, they differ from the general population in the marked cystathionuria they produce following methionine ingestion.

So far as is known cystathionine derives exclusively from the pathway involving transulfuration from methionine to cysteine (Fig. 3). Evidence from these studies suggests that the P. brothers handle the amino acid, cystathionine, somewhat differently from the majority of the population, though within the control populations studied here variations of a lesser degree were noted in cystathionine excretion following methionine loading. These observations, plus the similar responses observed in close relatives, suggest that a genetic polymorphism may exist with respect to this phenomenon. The exact definition of the mechanism of this response, however, is not known. Whether the alterations exist primarily in the renal clearance of the substance or its enzymatic cleavage (Frimpter, 1965), or indeed whether the mechanisms are the same in all the individuals cannot be determined from this study. Nevertheless, the P. brothers seem to form a unique group of people as defined by the magnitude of their methionine-induced cystathioninuria. Their close relationship, the persistence of the phenomenon over several years, its apparent independence of other environmental factors, its appearance, albeit to a lesser extent, in other family members, all suggest an underlying genetic basis for the condition.

The phenomenon of latent cystathioninuria uncovers an interesting example of the interaction of genetics and environment. Had the two P. brothers normally consumed a diet rich in methionine their aminoaciduria presumably would have been detected as have all the other aminoacidurias by routine screening urinary chromatography. An interesting comparison might be made with phenylketonuria. If the amino acid, phenylalanine, were not present in the ordinary diet to the extent that it is, subjects defective for phenylalanine hydroxylase might never have been recognized. Indeed, the aminoaciduria of phenylketonuria can be 'cured' simply by the reduction of the dietary intake of phenylalanine. Hence the expression of genetic differences depends once again on the environmental conditions operating. How far this analogy can be extended to the mental consequences associated with the aminoaciduria is uncertain. It is of interest, however, that most of the reported cases of cystathioninuria, including the latent form reported here, are associated with mental or affective disorders. Also of interest is the observation that cystathionine is highly concentrated in brain tissue (Moore and Stein, 1951). From our own studies, slight variations in cystathionine excretion following methionine ingestion had no correlation with intellectual function. The positive response was
nearly as frequent in the normal, healthy population (1/50) as in the mentally retarded population (3/50). In addition, there were no distinguishing features associated with the three patients of the control group who showed cystathioninuria following methionine loading. One of these was an adult patient with Down’s syndrome; however, five other patients with Down’s syndrome were negative. The positive excretors ranged between 20 and 80 years of age, hence simple age effects seem unlikely. Interesting observations have been made of striking reductions in plasma and urinary cystathionine following pyridoxine administration by Frimpter et al. (1963) and Mongeau et al. (1966) on their patients. Although pyridoxal phosphate is a co-factor in the enzymatic cleavage of cystathionine, neither the P. brothers nor any reported patients with cystathioninuria have exhibited signs of vitamin B6 deficiency. More recently, results from liver homogenate studies on two patients with gross cystathionuria have led Frimpter (1965) to postulate a structural alteration in the apoenzyme, cystathionase, causing a failure to combine normally with pyridoxal phosphate. Similar studies on Berlow’s patient, however, led Finkelstein, Mudd, Irrevere, and Laster (1966) to suggest the possibility of non-enzymatic degradation of cystathionine in the presence of pyridoxal phosphate.

The genetics of cystathionuria, both gross and latent, are still uncertain. Hereditary factors are suggested by the clustering of increased cystathionine excretors in relatives of all the patients described. In this study, two brothers of positive responders as well as the mother and brother of the propositi showed cystathioninuria following methionine ingestion. This phenomenon was evident despite differences in environment that had extended over many years; hence the familial clustering is more likely due to genetic causes than to simple environmental ones. As yet, however, no specific pattern of inheritance has been adduced from any of the family studies reported. Nor can it be assumed that the genetic mechanism is the same in all reported cases. In fact it should be emphasized that the mode of responses observed in this study is in no way interpreted to represent discrete genetic phenomena. Such a conclusion would necessarily require a larger understanding of the normal variation of urinary excretion of cystathionine as well as a more precise knowledge of the mechanisms accounting for such variations. In the case of the P. family we were unable to test the father of the two propositi. Had he proved to be a positive responder it would have raised the tempting hypothesis that he and his wife carried genes (not necessarily at the same locus) whose interaction led to the observed latent cystathioninuria of the propositi.

**Summary**

Two mentally retarded brothers are described who excrete large quantities of cystathionine in their urine following methionine ingestion, yet produce insufficient amounts of the amino acid to be detectable by the usual methods of two-dimensional chromatography when they are on normal diets. This entity, called ‘latent cystathioninuria’, is distinguished from previously reported cases of cystathionuria in which similar amounts of the amino acid were excreted without methionine loading. A study of 50 mentally retarded patients and 50 normal, healthy adults fails to disclose any other examples of this latent form of cystathioninuria. The majority of subjects responded to methionine loading with no detectable production of cystathionine. However, one normal, healthy volunteer showed a small degree of cystathioninuria in response to methionine, as did his brother. Similarly, three mentally retarded subjects showed the same response, as did the normal, healthy brother of one of these. A response of the same magnitude was also observed in relatives of the two propositi. The relation of this response between individuals and families is not known, nor is the genetics of the phenomenon clear. However, there appears to be a variation in cystathionine excretion following methionine loading that shows familial clustering. Pooling the two control samples reveals an incidence of the positive trait to be about 4%.

The work was carried out in the laboratories of Professor Harry Harris, whose advice the author gratefully acknowledges. The author also wishes to express his appreciation to Drs. D. H. H. Thomas and G. E. B. Scott for permission to investigate their patients, and to the Cell Barnes Hospital nursing staff for their helpful co-operation, and to Mrs. Nona Parry-Jones for her technical assistance.

**References**


