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Homocystinuria:

A Study with Low-Methionine Diet in Three Patients

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HOMOCYSTINURIA is an inborn error of methionine metabolism which was first described in 1962.[1, 2] Since then, over 100 cases have been discovered[3] and a preliminary estimate suggests that its frequency is next to that of phenylketonuria. The disorder is inherited as an autosomal recessive. The clinical picture of malar flush, dislocated eye lenses, mental retardation, osteoporosis, various associated skeletal deformities and a tendency to vascular thromboses is found in the majority of the patients.[4, 5] The basic metabolic error is a deficiency of the enzyme cystathionine synthetase,[6] as a consequence of which plasma levels of methionine and homocystine are elevated and their urinary excretion is increased. In addition, plasma cystine levels are low and deficiency of cystathionine has been demonstrated in the liver and brain of these patients.[7, 8]

It is possible that either the accumulation of methionine, homocystine and their metabolites in the body fluids and tissues or deficiency of cystathionine, or both, is responsible for the clinical and the pathological manifestations of the disease. Therefore the aim of the treatment should be to correct the biochemical abnormality, i.e., to prevent the accumulation of methionine and homocystine and to restore the concentration of cystathionine and cystine in the tissues. To achieve this, it would be logical to provide the patients with a low-methionine diet supplemented with cystine and cystathionine.[9] As cystathionine for dietary supplementation is not available, treatment with a low-methionine diet supplemented only with cystine is the best alternative at present and has been used by several investigators with variable results.[3, 10-13]

This report describes a study with a low-methionine–high-cystine diet in three siblings with homocystinuria to demonstrate the long-term effect of this diet on the biochemical abnormalities and the nutritional states of these patients.

Case Reports

The three patients are members of the same sibship. The parents are healthy, of Irish origin and unrelated.

The diagnosis of homocystinuria was first made in the youngest sib, M.C., a female, who was first seen by us at the age of 13 months because the pneumonia from which she was suffering had failed to respond to antibiotics. Some delay in her motor development was noticed, so a random urine sample was screened for aminoaciduria. This revealed homocystinuria, which was confirmed by more detailed studies.

Examination of urine samples from her six sibs led to the diagnosis of homocystinuria in a 9½-year-old brother (W.C.) and a 5-year-old

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sister (T.C.). The rest of the sibs and the parents were normal in this respect.

The physical findings at the time of diagnosis and the previous history of the three patients are summarized below.

CASE 1.—M.C. was delivered normally at term. Her birth weight was 3 kg. Neonatal progress was uncomplicated. She was not breast-fed and solid foods were introduced at 4 months of age. There was no history of convulsions. She reached early milestones at the expected times, but did not sit up until the age of 13 months.

On examination she was well nourished and physically well developed. The hair was normal in colour, distribution and texture. There was no malar flush, and a consultant ophthalmologist found that the lenses were not displaced. There was no deformity of the skeleton. Clinical examination of the chest revealed no abnormality, but repeated roentgenograms showed a persistent shadow in the right upper lobe. The liver was uniformly enlarged, palpable 3 cm. below the subcostal margin, but it was of normal consistence. The spleen was not palpable. Neurological examination was negative. Her mental age was that of an infant of 9 months.

In view of the persistent shadow in the right upper zone on chest roentgenograms, bronchoscopic examination was performed which found doubtful bronchial compression. However, an aortogram did not demonstrate any vascular abnormality in that region. Roentgenograms of the skull and long bones were normal, but the spine showed osteoporosis. Full hemogram, routine urine analysis, liver function tests, serum iron and iron-binding capacity, plasma magnesium, serum calcium and inorganic phosphate, fasting blood sugars, blood urea nitrogen and bone marrow examination were all normal. Cyanide nitroprusside test of the urine was strongly positive and the urinary amino acid chromatogram showed a heavy spot of homocystine.

CASE 2.—T.C., a female, was born at term following a normal pregnancy and weighed 3.4 kg. Her neonatal course was normal. She was fed milk formula, and solid foods were introduced in the diet at 4 months of age. All her milestones were very much delayed. Her speech at 5 years of age consisted of only a few monosyllables, but her verbal comprehension was quite good. She had not had convulsions.

On clinical examination at 5 years of age she showed the classical features of homocystinuria: mental retardation, malar flush, bilateral dislocated lenses, slightly depressed sternum, mild kyphosis of the upper thoracic spine and genu valgum. In fact, the genu valgum was so marked that a surgical correction had been performed at a local hospital three months previously. The liver was palpable 2 cm. below the subcostal margin but its consistence was normal. Examination of the respiratory, cardiovascular and nervous systems did not demonstrate any abnormality.

Investigation revealed an abnormal electroencephalogram with non-specific diffuse changes and excess of slow-wave activity. A radiograph of the skeleton showed moderately severe osteoporotic changes in the long bones, but the spine was not undermineralized. Her I.Q. was 36. Other investigations, as described for her sister, gave normal results. The urinary amino acid chromatogram showed a heavy spot of homocystine.

CASE 3.—W.C., a male, was born normally at 36 weeks' gestation and weighed 2.9 kg. During the last six weeks of pregnancy the mother had edema of both legs but no other features of pre-eclampsia. The infant was breast-fed for nearly six months and solid foods were introduced at about that time. The milestones were reached at normal ages. There was no history of convulsions.

When examined at age 9½ years, the patient was well nourished and well developed, and appeared mentally normal with an I.Q. of 93. His performance at school was average. His eyes were examined by a consultant ophthalmologist and the ocular lenses were not found displaced. Malar flush was quite pronounced. Some of his hair had turned grey but the texture was normal. The sternum was slightly depressed and there was a mild degree of kyphosis of the upper thoracic spine. Examination of the major systems was normal except for an occasional ventricular extrasystole, confirmed on the electrocardiogram.

Roentgenograms of the spine showed well-marked osteoporosis, but those of long bones were normal. An electroencephalogram was reported as showing non-specific diffuse abnormality. Other investigations, as described for his sisters, were normal. The urinary amino acid chromatogram showed a heavy spot of homocystine.

METHODS OF INVESTIGATION

Chromatography of Amino Acids

Qualitative. — Two-dimensional paper chromatography was used. Random urine specimens, usually collected in the morning, were desalted on a short Dowex 50 (50-100 mesh) column. A sample corresponding to a constant amount of creatinine was loaded on to the paper. Pyridine, 3N ammonium hydroxide, acetone (5:2:3) and isopropanol, formic acid and water (8:1:1) were the first and second solvents respectively. The chromatograms were stained with the ninhydrin reagent.

Quantitative.—Measurements of amino acids in plasma and urine were performed by the Piez-Morris procedure of ion exchange column chromatography, using a 140 cm. micro column in a Technicon automatic amino acid analyzer. Venous blood, after an overnight fast, was drawn in a syringe containing a minute quantity of

crystalline EDTA* to prevent clotting. The plasma was separated by immediate centrifugation and the proteins were precipitated within 30 minutes by adding 1 volume of plasma to 5 volumes of saturated picric acid solution. After centrifugation the supernatant was stored in the refrigerator. Preliminary to amino acid chromatography, the picric acid was removed by a "Rexyn" AG 1 column. The clear picric-acid-free eluate containing amino acids was then evaporated to dryness in a vacuum evaporator. The amino acids were redissolved in an acid-sucrose solution (0.02N HCl and 12.5% sucrose) and an aliquot corresponding to between 0.5 and 1 ml. of plasma was loaded on the column. In the case of urine, a volume corresponding to 1-minute excretion from a 24-hour collection was acidified with the same volume of 0.02N HCl, evaporated to dryness and redissolved in acid sucrose solution as described above, before being loaded on the column. In view of the low reliability of homocystine, its recovery was tested and was found to be 90% of the expected at a concentration which was three times the highest concentration in the plasmas of these patients.

The method for quantitative measurements of amino acids was standardized by the use of analytical grade L-forms of amino acids. In the case of homocysteine-cysteine disulphide (HCD) the following procedure was used: L-homocysteine thiolactone and L-cysteine HCl were each dissolved in 0.02N sodium hydroxide solution under nitrogen to yield solutions of equimolar concentration. Equal volumes of these two solutions were mixed and then air was bubbled through for two hours to allow the formation of the disulphides. The solution was then neutralized and an aliquot put on the column. The peaks corresponding to cystine, HCD and homocystine were identified. Assuming that all the cysteine originally added to the solution was present either as cystine or as HCD, the amount of HCD was calculated by subtracting the amount of cystine which we were able to measure. This figure was used as the basis for calibration.

The renal clearances of amino acids were calculated from quantitative measurements of amino acids in a timed urine specimen and the mean plasma levels of amino acids in specimens obtained at the beginning and at the end of the timed urine collection. The patients had not taken protein by mouth for 8 to 10 hours before the test was begun. Endogenous creatinine clearances were also measured during the same periods. The clearances were expressed as ml./minute/1.73 square metre of body surface.

*Ethylenediamine tetracetic acid disodium salt.

Methionine loading test.—L-methionine loads of 100 mg. per kg. body weight dissolved in fruit juice or water were given orally and by nasogastric tube to W.C. and T.C. respectively. The tests were performed when both patients were on low-methionine diets and the plasma methionine levels had returned to normal and homocystine had virtually disappeared. After the patient had fasted for about 10 hours, plasma amino acid levels were measured, immediately before the load and at 2, 4, 6, 9 and 12 hours after the load, on the first day. Subsequent measurements of fasting plasma amino acids were made every day until the levels of methionine had returned to normal and homocystine had cleared. The patients were kept on the low-methionine diets throughout the test period.

Low-methionine diet supplemented with cystine.—Foods rich in methionine, such as meat, fish, eggs, milk and milk products, were excluded from the diet. Instead a soybean preparation* and another low-methionine preparation (L.M.P.)† based on lupine protein were used as the main source of protein.[14] The protein intake was curtailed to the minimum necessary. Of the total protein in the diet, 50-60% was derived from the low-methionine preparations while the remainder came from certain vegetable foods such as carrots, beans, peas and tomatoes.‡ The low-methionine preparations were mixed with strained fruits to mask their somewhat unpleasant taste and smell. The caloric requirement was made up by allowing a liberal intake of fat (mainly butter) and carbohydrate. L-cystine was added to the diet or mixed with drinks. The diet was also supplemented with a polyvitamin preparation.§ During each period of the study, the methionine intake was kept constant by keeping the diet constant.

TABLE I.—COMPOSITION OF LOW-METHIONINE DIETS

Patient	*W.C.*	*T.C.*	*M.C.*
Age	9½ yrs.	5 yrs.	15 months
Weight, kg.	38	19	9
Protein, g./kg./day	1-1.2	1-1.7	1.8-2.4
Methionine, mg./kg./day	6-13	10-18	16-23
Added cystine, g./day	2.0	1.5	0.9
Main source of protein	Sobee* and L.M.P.**	Sobee*	Sobee*

*Soybean preparation (Mead Johnson).
**Low-methionine preparation based on lupine protein.[14]

The main source of protein and the dietary intake of protein, methionine and cystine supplement in each of the patients are given in Table I.

*Sobee (Mead Johnson).
†The composition of "L.M.P." was as follows: Protein 48-50 g. and methionine 90-135 mg. in 100 g. powder.
‡The figures of protein and methionine were obtained from "Amino Acid Content of Foods" by Orr and Watt.[18]
§"Paramette" (Ayerst Laoratories) 1 teaspoonful three times a day.

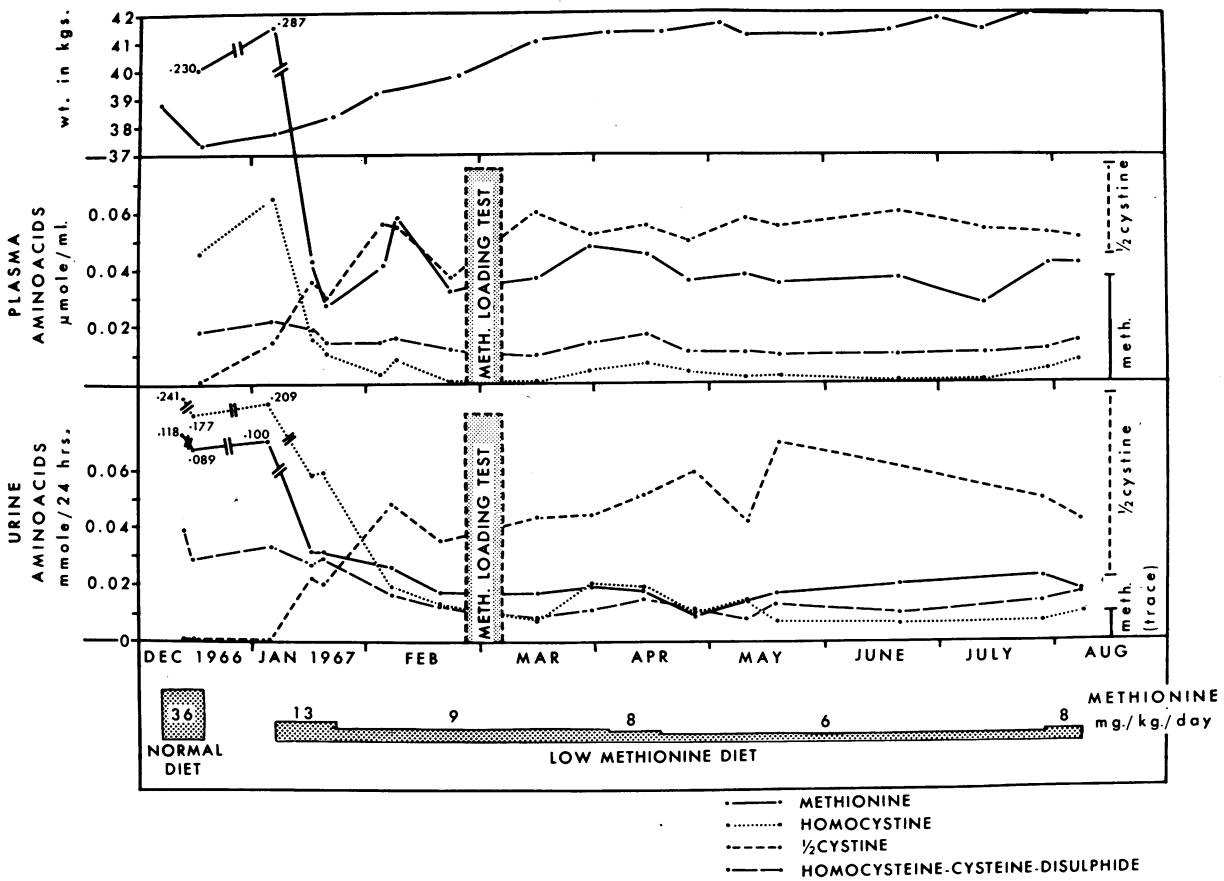

Fig. 1.—Plasma levels and 24-hour urinary excretions of methionine, homocystine, homocysteine-cysteine-disulphide and cystine (expressed as half cystine) in patient W.C., aged 9½ years, on a normal diet and on low-methionine diets supplemented with cystine. The majority of the points are the mean values of the two determinations performed on two consecutive days. The vertical lines on the right represent the normal ranges of methionine and half cystine. The uppermost section shows the changes in body weight.

Parameters of nutrition.—At frequent intervals, measurements of height and weight were recorded and detailed clinical examinations were carried out. In addition, the levels of hemoglobin, total serum proteins and serum albumin were measured. At least two determinations of plasma amino acid levels and 24-hour urinary excretion of amino acids were made during any one period that the patient was taking a particular low methionine diet. Nitrogen balance studies were not done.

These children were admitted to the Clinical Investigation Unit where the major part of the study was conducted and the diets and the various collections were carefully controlled.

RESULTS

Initial Studies on Normal Diet

The results of the serial measurement of the plasma levels and the 24-hour urinary excretions of the relevant amino acids in W.C. and T.C., together with the changes in body weight, are illustrated in Figs. 1 and 2 respectively.

While the two older patients, W.C. and T.C., were on a normal diet containing 36 mg. and 48 mg. per kg. per day of methionine respectively (December 1966 to January 6, 1967), the plasma levels of methionine were markedly elevated (Figs. 1 and 2). The levels of homocystine and homocysteine-cysteine-disulphide (HCD) were increased in both, as were also 24-hour urinary excretions of the same amino acids. The plasma levels and urinary excretions of cystine were very low.

In the youngest patient, M.C., on a normal diet containing 67 mg. per kg. per day of methionine, the results were similar to those

TABLE II.—PLASMA LEVELS AND 24-HOUR URINARY EXCRETION OF METHIONINE, HOMOCYSTINE AND HOMOCYSTEINE-CYSTEINE-DISULPHIDE IN M.C. ON A NORMAL DIET CONTAINING 67 MG. METHIONINE/KG./DAY

	Methionine	*Homocystine*	*HCD**
Plasma µmole/ml.			
Patient	0.021-0.026	0.040-0.049	0.014-0.027
Normal	Trace - 0.037	Not detectable	Not detectable
Urine mmole/24 hr.			
Patient	0.010-0.021	0.28 - 0.40	0.027 - 0.034
Normal	Trace	Not detectable	Not detectable

*Homocysteine-cysteine-disulphide.

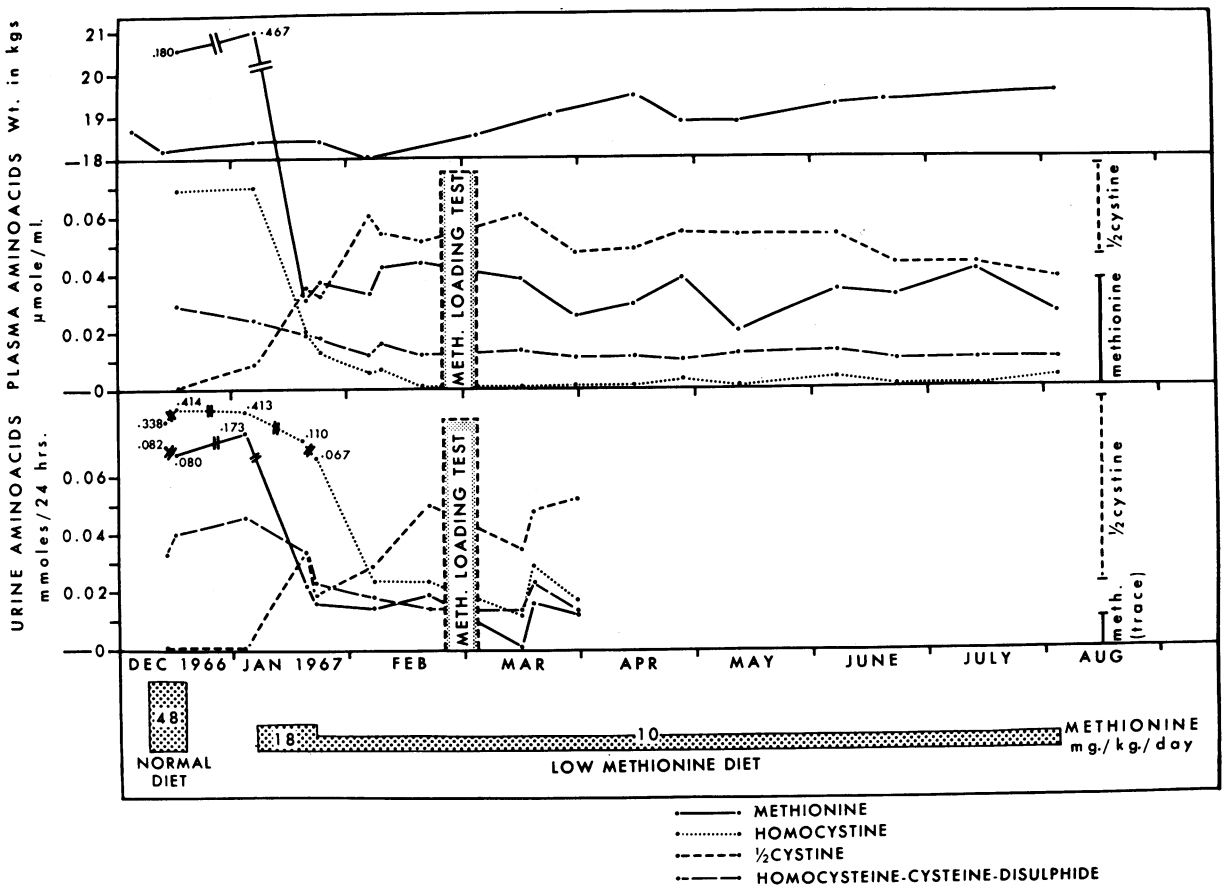

Fig. 2.—Plasma levels and 24-hour urinary excretions of methionine, homocystine, homocysteine-cysteine-disulphide and cystine (expressed as half cystine) in patient T.C., aged 5 years, on a normal diet and on low methionine diets supplemented with cystine. Majority of the points, before April 1967, are the mean values of two determinations performed on two consecutive days. The vertical lines on the right represent the normal ranges of methionine and half cystine. The uppermost section shows the changes in body weight.

found in the older siblings except that the plasma methionine level and its urinary excretion were within the normal range. The relevant data are shown in Table II. The blood levels and urinary excretions of the other amino acids were within the normal range in all three patients.

Renal Clearance Studies

The renal clearances of endogenous methionine, homocystine and creatinine measured in the three patients while they were on a normal diet are shown in Table III. The clearances of methionine and creatinine were within the normal range in all three. The renal clearances of homocystine were quite low in the two older patients (W.C. and T.C.). Calculation of the percentage of filtered load reabsorbed, based on the creatinine clearances, yielded 97% for W.C. and 92% for T.C. The clearance of homocystine was higher in the youngest patient M.C. (15 months of age). It amounted to a reabsorption of 85% of the filtered load. The renal clearances of all other amino acids were normal in all three patients.

TABLE III.—RENAL CLEARANCE (ml./min./1.73 m²)

Patient	*W.C.*	*T.C.*	*M.C.*	*Normal*
Homocystine	3.3	3.4	14.1	—
Methionine	0.88	0.29	2.01	1 - 3.4
Creatinine	105	85.2	99.5	70 - 157*

*Figures obtained from Henry.[19]

Methionine Loading Tests

Brenton, Cusworth and Gaull[15] have carried out oral methionine loading tests in normal persons using a dose of 100 mg. L-methionine per kg. body weight. They found that the plasma levels of methionine rose to a peak between 0.4-0.8 μmoles per ml. at six hours and subsequently fell to normal levels within 24 hours. Perry[16] observed a fall to normal levels by about 8 hours after the oral methionine loading, using the same dose in normal persons. Two of our patients (W.C. and T.C.) had oral methionine loading tests performed with a dose

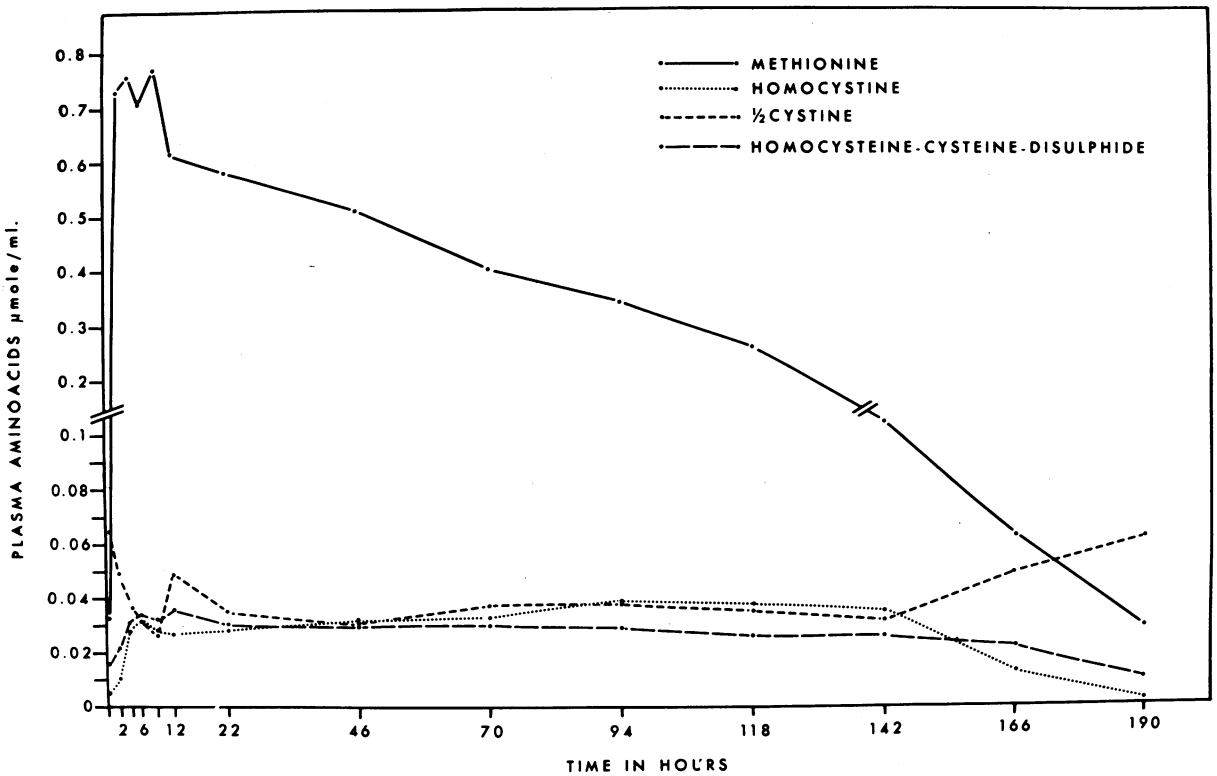

Fig. 3.—Changes in the plasma levels of methionine, homocystine, homocysteine-cysteine-disulphide and cystine (expressed as half cystine) in patient W.C. after oral methionine load of 100 mg. per kg. The patient was on a low methionine-added cystine diet containing 9 mg. per kg. per day of methionine.

of 100 mg. per kg. body weight. The tests were performed at the time when the plasma methionine levels were reduced to near normal and only trace amounts of homocystine and HCD were present in the plasma. This was achieved by dietary manipulation (to be described later). The results in terms of changes in the blood levels of the relevant amino acids are shown in Figs. 3 and 4.

In both patients between two and nine hours later there was a sharp rise in the plasma methionine to a peak level of about 0.8 µmole per ml., a level which is not significantly higher than that observed in the normals by both Brenton and his colleagues[15] and Perry.[16] However, in both our patients the fall in the levels of plasma methionine was very slow; by 46 to 72 hours they were still 10 to 15 times above the initial values. In the case of W.C. (Fig. 3) the fall to preloading levels occurred after eight days (190 hours) and in T.C. (Fig. 4) after six days (142 hours).

The homocystine levels, which were barely detectable before the administration of the load, rose in both cases. In W.C. there was a sharp but moderate rise which approached maximum at six hours and the level then remained around this point in spite of the falling levels of methionine. It did not start to fall until the methionine level fell below 0.12 µmole per ml. after 142 hours. Subsequently the methionine level returned to normal and homocystine disappeared from the plasma. In T.C., plasma homocystine levels continued to rise for a longer period of time, reaching a higher peak (0.072 µmole per ml.) at 70 hours in spite of falling levels of plasma methionine. Both patients achieved almost identical peak levels of methionine, but the decline was faster in T.C. than in W.C. and the homocystine levels rose higher in the former.

The plasma HCD levels rose in both patients; their eventual fall was slower than that of homocystine.

The plasma cystine levels changed in an interesting manner in both. With the initial rise in homocystine and HCD the cystine levels fell significantly. They remained low throughout but rose at the end when the methionine approached normal levels and homocystine fell to trace levels.

When plasma samples of our patients taken during the first 22 hours of methionine loading tests were tested by column chromatography for the presence of s-adenosylhomocysteine, none

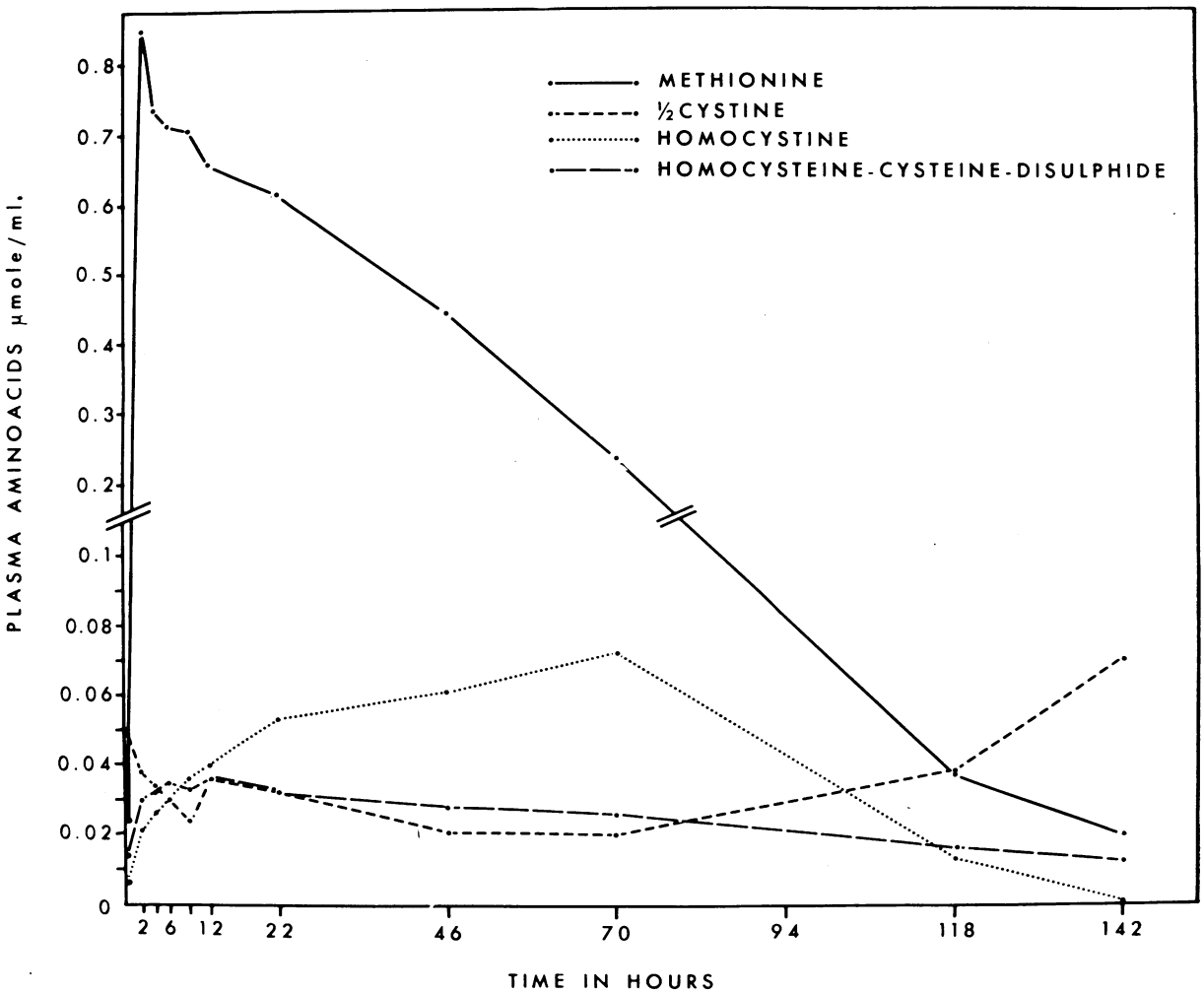

Fig. 4.—Changes in the plasma levels of methionine, homocystine, homocysteine-cysteine-disulphide and cystine (expressed as half cystine) in patient T.C. after oral methionine load of 100 mg. per kg. The patient was on a low methionine-added cystine diet containing 10 mg. per kg. per day of methionine.

was detected.* Paper chromatographic examination of corresponding urine samples failed to reveal the presence of s-adenosylhomocysteine or other unusual homocysteine containing disulphide compounds.[17]*

Effect of Low-Methionine Diet

At the end of the initial control period, all three patients were placed on the low-methionine diets. Starting on January 6, the intake of methionine was reduced to 13 mg. per kg. per day in W.C. and to 18 mg. per kg. per day in T.C., for a period of two weeks (Figs. 1 and 2). This resulted in a marked fall in the plasma levels of methionine and homocystine, but a less marked fall was noted in levels of HCD. Similar observations were made on the urine.

A further reduction in the dietary intake of methionine to 9 mg. per kg. per day in W.C. (Fig. 1) led to a more marked decrease in the plasma level of homocystine and eventually to barely detectable amounts. The plasma concentration of methionine showed some fluctuations but later settled close to the normal range. However, the plasma levels of HCD did not decrease further. When the methionine intake was decreased to 6 mg. per kg. per day there was no significant effect on the blood levels of methionine or homocystine or on the HCD levels. It is interesting to note that on a methionine-restricted diet the molar concentrations of HCD were persistently higher than those of homocystine. In this same patient the successive reduction of methionine intake from 13 to 9, 8 and 6 mg. per kg. per day led to a further but slight reduction in the 24-hour urinary output of methionine, homocystine and HCD. On the

*Performed by Dr. T. L. Perry, Vancouver, British Columbia.

lowest level of methionine intake the urinary excretion of methionine remained still slightly above the normal range, while homocystine and HCD were both present but in only barely detectable amounts.

For T.C. (Fig. 2) a methionine intake of 10 mg. per kg. per day restored the biochemical state to near normal but, as in her brother W.C., HCD persisted in the plasma. On April 14, she was discharged from the hospital to continue at home on a low-methionine–high-cystine diet containing 10 mg. per kg. per day of methionine. As judged by the frequently measured plasma amino acid levels determined at visits to the outpatient clinic, satisfactory biochemical control was maintained.

Our youngest patient, M.C., on a low-methionine diet providing her with 16 to 23 mg. of methionine per kg. per day, showed a response which was just as satisfactory as that of her sibs. Plasma methionine levels remained normal throughout the entire nine-month period of observation. Homocystine virtually disappeared from the plasma but, as in her sibs, measurable but low concentrations of HCD persisted. The urinary findings paralleled those in the plasma.

The addition of supplements of cystine to the low-methionine diet produced a desired rise in the plasma levels of cystine and this was subsequently maintained within the range of normal values. The urinary excretion of cystine also increased (Figs. 1 and 2).

TABLE IV.—LEVELS OF HEMOGLOBIN, TOTAL SERUM PROTEIN AND SERUM ALBUMIN (g. per 100 ml.) ON NORMAL AND LOW-METHIONINE DIETS

		On normal diet	*On low-methionine diet*			
		Dec. 1966	*Feb. 1967*	*April 1967*	*June 1967*	*Sept. 1967*
W.C.	Hemoglobin.....	12.1	11.2	11.8	11.6	13.2
	Serum proteins...	7.9	7.5	7.5	7.2	8.1
	Serum albumin...	4.3	4.5	4.5	4.6	4.4
T.C.	Hemoglobin.....	12.1	10.8	11.9	10.6	12.0
	Serum proteins...	7.9	7.7	7.3	7.5	6.8
	Serum albumin...	5.2	4.4	4.4	4.3	4.2
M.C.	Hemoglobin.....	13.0	11.6	11.8	11.8	12.0
	Serum proteins...	7.6	7.7	7.0	7.1	7.5
	Serum albumin...	4.9	4.4	4.3	4.3	4.7

The nutritional state of all three patients on the low-methionine diet remained excellent. Periodic clinical examinations showed no malnutrition or evidence of vitamin deficiency. The children continued to gain weight (Figs. 1 and 2) and their hemoglobin, serum protein and serum albumin levels remained normal throughout the entire period (Table IV). The plasma levels of other amino acids, which were frequently measured, remained normal.

DISCUSSION

In the three patients studied by us a satisfactory control of the biochemical abnormalities was possible by rigidly controlled administration of a diet low in methionine with added cystine. The most effective diet contained 8 to 10 mg. methionine per kg. per day in the two older children and 16 to 23 mg. per kg. per day in the youngest patient who was between 15 and 22 months old. Satisfactory biochemical control was maintained for an eight- to nine-month period in all three children while they continued to grow adequately and did not show any signs of nutritional deficiency.

The results presented here also indicate that a substantial fall in the plasma levels of methionine can be produced by a moderate reduction of methionine intake in the diet but the fall in the levels of homocystine and HCD is not in the same proportion as that of methionine. It is therefore clear that to eliminate the homocystine from the plasma, the methionine levels must be reduced and maintained at near normal. Even at the lowest intake of methionine, when homocystine was virtually absent in the plasma, HCD still persisted and its molar concentrations were constantly higher than those of homocystine. A similar observation was made before and at the end of methionine loading tests. The implication of this finding is that for careful evaluation of the low methionine-added cystine diets it is necessary to measure not only methionine but also homocystine and HCD in either the plasma or in the urine or both.

There are several reports in the literature concerning the use of low-methionine diets with added cystine.[3, 10-13] The results were variable. In some instances it was not possible to restrict the intake of methionine to the extent necessary in our patients.[3, 10, 13] In others, with restriction of methionine in the diet, deficiencies in other amino acids developed and the patients' nutrition was adversely affected.[16] We are tempted to suggest that the successful dietary management in our cases can be attributed to (1) the rigid dietary control which was achieved under ideal circumstances in a clinical investigation unit and continued at home by most co-operative and intelligent parents and to (2) meticulous control of the effect of the diet by frequent monitoring of the biochemical and nutritional states of the patients. However, this may not be the only explanation, and one must consider the possibility of differences between patients, which may partly explain the excellent results in our patients and the failure of a similar type of management in patients studied by others.

Some of the unusual findings in our patients merit close consideration.

On a normal diet M.C., the youngest of the three sibs, had normal blood levels of methionine, yet significantly raised urinary excretion. At the same time her homocystine and HCD plasma levels, as well as the urinary excretion of these compounds, were extremely high. The two older sibs, on the other hand, did have the typical high methionine plasma levels while on a normal diet. It may be of significance that during the control period when the homocystine plasma levels in M.C. were just as high (0.046 μmole per ml.) as in W.C. (0.046 μmole per ml.), the methionine plasma level was normal in M.C. but markedly elevated in W.C. Therefore the difference in methionine plasma levels cannot be explained only on the basis of the observed increased renal clearance of homocystine in M.C., since it is the actual concentrations of the reactants which are important and these were the same. The renal clearance of methionine in M.C. was not increased, so that in her case we must assume more efficient metabolism of methionine by an alternate pathway or an alteration in the equilibrium constants in the metabolic pathway of methionine to homocysteine resulting in a lesser degree of reversibility of this reaction sequence. In either case, the unusual finding of normal plasma levels of methionine in M.C. was not present in the other two sibs and it has been observed in other patients with otherwise typical homocystinuria.[9]

The fact that Perry *et al.*[17] have not been able to find s-adenosylhomocysteine in the urine and plasma of our two older patients during a methionine loading test, whereas this compound was detected in the urine of their own patients, may also suggest that our patients are not in every way comparable to others.

Another interesting feature in our family of three homocystinuric patients is the marked variation of the phenotypic manifestations. T.C. had the classical features of severe mental retardation, dislocation of lenses and genu valgum. W.C., her 10-year-old brother, escaped all of these and had only a number of minor clinical features of the disease. Our third patient, M.C., is too young to be assessed in this respect. The reasons for the marked phenotype variation in the older sibs is difficult to understand. One possibility is that W.C., who is clinically less affected, was kept on breast milk for nearly six months whereas T.C. was fed on a cow's milk formula for the same period and longer. Human milk contains much less methionine (6-36 mg. per 100 ml.) than cow's milk (50-140 mg. per 100 ml.). Could it be that this was the reason that W.C. who was fed on breast milk escaped early damage?

A second possibility is that the biochemical defect in W.C., who has so far escaped many of the crippling features of this disease, was less pronounced than in T.C. The fact that T.C. on a normal diet had higher plasma levels of homocystine than W.C. and that in response to a methionine load the plasma homocystine level rose higher in T.C. than in W.C., may be regarded as evidence in favour of this contention. However, we do not feel that this important question can be answered on the basis of the results presented here.

It is still not known which one of the biochemical alterations that are produced by the basic genetic defect in this disease is responsible for the manifestations of the disease (e.g. mental deficiency, dislocated lenses, thromboses, etc.). It may be important that in M.C., our youngest patient, who had normal plasma methionine levels, there was nevertheless delay in motor development. It seems that the accumulation of homocystine and its various known and unknown metabolites, or the lack of cystathionine, is the biochemical alteration that is in more direct causal relationship with the features of this disease. The fact that the homocystine plasma levels were higher in T.C. than in W.C. and that the former was clinically more severely affected than W.C. points to this conclusion.

The results presented here leave little doubt that in some patients with homocystinuria, many of the biochemical alterations, such as accumulation of methionine, homocystine, HCD and their metabolites and the borderline deficiency of cystine, can be corrected. This can be achieved by a carefully controlled administration of a diet low in methionine and supplemented with cystine. Whether this form of treatment will ultimately prove to be beneficial to the patients is an open question. The evidence we have in this respect is very limited. None of our patients have suffered vascular thromboses since the institution of the therapeutic diet. W.C.'s abnormal electroencephalogram became normal. M.C. appears to have made good progress in terms of motor and mental development. However, a much longer period of observation will be necessary before a satisfactory assessment of the therapeutic effect of the diet can be made.

Summary The clinical and laboratory findings in three patients with homocystinuria who are members of one sibship are presented. After the achievement of satisfactory biochemical control with a low-methionine diet, oral loading

with a single dose of 100 mg. per kg. L-methionine resulted in a rise of plasma levels of methionine, homocystine and homocysteine–cysteine disulphide (HCD) which returned to the baseline values after five to seven days in the two patients so tested. By carefully controlled restriction of methionine in the diet and supplementation with cystine it was possible: (a) to achieve a sustained normal plasma level of methionine and cystine, (b) to bring about the virtual disappearance of homocystine from the plasma and (c) to reduce greatly HCD levels in both plasma and urine. These effects of the therapeutic diet were maintained for an eight- to nine-month period.

Résumé Nous présentons ici les observations cliniques et les résultats des épreuves de laboratoire obtenues chez trois malades consanguins souffrant d'homocystinurie. Après qu'un control biochemique raisonable fut obtenu par un régime pauvre en méthionine, la surcharge oral effectuée avec une dose unique de 100 mg/kg de L-méthionine fut traduite par une augmentation des concentrations plasmatiques de méthionine, d'homocystéine (DCH), ces concentrations étant revenues à leur niveau préalable après cinq à sept jours, chez les deux malades soumis à cette épreuve. Grâce à une restriction judicieusement contrôlée de méthionine dans le régime alimentaire et l'apport exogène de cystine, il nous a été possible: a) de réaliser une concentration normale et soutenue de méthionine et de cystine dans le plasma, b) d'entraîner la disparition virtuelle d'homocystine dans le plasma et c) de réduire considérablement les concentrations de DCH dans le plasma et dans l'urine. Les effets de ce traitement diététique ont pu être maintenus pendant une période de huit à neuf mois.

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ADDENDUM

Since the completion of this manuscript, Perry *et al.* (*Lancet*, 2: 474, 1968) published their most recent experiences in the treatment of homocystinuria with a low-methionine diet, supplemental cystine and a methyl donor.

REFERENCES

1. FIELD, C. M. B. *et al.*: Homocystinuria. A new disorder of metabolism. *In*: Xth International Congress of Pediatrics, Lisbon, September 9-15, 1962, abstract of papers, Lisbon, 1962, p. 274 (abstract).
2. GERRITSEN, T., VAUGHN, J. G. AND WAISMAN, H. A.: *Biochem. Biophys. Res. Commun.*, **9**: 493, 1962.
3. SCHIMKE, R. N., MCKUSICK, V. A. AND WEILBAECHER, R. G.: Homocystinuria. *In*: Amino acid metabolism and genetic variation, edited by W. L. Nyhan, McGraw-Hill Book Company, New York, 1967, p. 297.
4. CARSON, N. A. J. *et al.*: *J. Pediat.*, **66**: 565, 1965.
5. SCHIMKE, R. N. *et al.*: *J. A. M. A.*, **193**: 711, 1965.
6. FINKELSTEIN, J. D. *et al.*: *Science*, **146**: 785, 1964.
7. GERRITSEN, T. AND WAISMAN, H. A.: *Ibid.*, **145**: 588, 1964.
8. BRENTON, D. P., CUSWORTH, D. C. AND GAULL, G. E.: *Pediatrics*, **35**: 50, 1965.
9. WAISMAN, H. A.: *Amer. J. Dis. Child.*, **113**: 101, 1967.
10. CARSON, N. A. J.: *Ibid.*, **113**: 95, 1967.
11. KOMROWER, G. M. *et al.*: *Arch. Dis. Child.*, **41**: 666, 1966.
12. PERRY, T. L. *et al.*: *Pediatrics*, **37**: 502, 1966.
13. PERRY, T. L.: Unsolved problems in homocystinuria. *In*: Amino acid metabolism and genetic variation, edited by W. L. Nyhan, McGraw-Hill Company, New York, 1967, p. 279.
14. SABRY, Z. I. *et al.*: Manuscript in preparation.
15. BRENTON, D. P., CUSWORTH, D. C. AND GAULL, G. E.: *J. Pediat.*, **67**: 58, 1965.
16. PERRY, T. L.: Personal communication.
17. PERRY, T. L. *et al.*: *Clin. Chim. Acta*, **15**: 409, 1967.
18. ORR, M. L. AND WATT, B. K.: Amino acid content of foods, United States Department of Agriculture, Home Economics Research Report No. 4, Superintendent of Documents, U.S. Government Printing Office, Washington, 1957.
19. HENRY, J.: Clinical chemistry: principles and techniques, Harper & Row, Publishers, New York, 1964, p. 892.