

Treatment of Homocystinuria with Pyridoxine

A Preliminary Study

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Homocystinuria is the result of an inborn error in the metabolism of the essential sulphur-containing amino acid, methionine (Carson and Neill, 1962; Gerritsen, Vaughn, and Waisman, 1962). There is now good evidence (Mudd *et al.*, 1964) to show that the basic defect in this disorder is an inactivity of hepatic cystathionine synthetase, which prevents the formation of cystathionine from homocysteine and causes disruption of the normal metabolic pathway (see Fig. 1). This results in an accumulation of homocysteine in the blood, and as this is a 'non-threshold' amino acid, it is rapidly excreted in the urine in its oxidized form, homocystine. Homocysteine may be remethylated to methionine with the aid of methyl group donors such as N⁵-methyltetrahydrofolic acid (M⁵FH₄) or betaine; the accumulation of homocysteine favours remethylation, and results in raised serum methionine levels. As cysteine cannot be formed, it now becomes an essential amino acid, and the patient depends on the dietary intake for his cysteine requirements. There also appears in the blood and urine the mixed disulphide of homocysteine and cysteine. Homocysteine and cysteine are converted to their oxidized forms, both enzymatically and non-enzymatically, and unless specific precautions are taken at the time of venepuncture to protect the S-H groups, these amino acids are quickly converted to the S-S form. In this work these compounds have been estimated in their oxidized form and will hereafter be referred to as homocystine, cystine, and their mixed disulphide.

Such patients present a distinctive clinical picture. The majority have fair hair, a malar flush, poor peripheral circulation with well-marked livido reticularis. Ectopia lentis is present except in the very young, and mental retardation is common. Cardiovascular disturbances are present in older children; thrombotic episodes occur in about 40 to

50 % of all ages. Skeletal changes vary from pes cavus and genu valgum in young children to typical Marfan-like features in pre-adolescents.

In general, there are three possible therapeutic approaches to homocystinuria—restriction of substrate (low methionine diet), replacement of missing products (cystine), and supplementation with coenzymes (pyridoxine) (Fig. 1). A further approach to treatment has been advocated by Carey, Fennelly, and FitzGerald (1966, 1968) and Perry *et al.* (1968) who have attempted to lower plasma homocysteine levels by encouraging remethylation to methionine by the use of folic acid and choline, respectively. The present survey relates to a trial of pyridoxine therapy in homocystinuric patients.

Method of Study

Eleven patients with homocystinuria, aged 9 months to 29 years from seven families, have been treated with pyridoxine (vitamin B₆) supplements. This vitamin was given orally three times a day in tablet form. Initial dosage varied from 300–450 mg. daily, with the exception of a 9-month-old infant who was given a dose of 150 mg. daily. Patients were permitted an unrestricted diet without cystine supplements.

The biochemical response to therapy was monitored by blood amino acid studies, using a Standard Technicon Amino Acid Analyser, with particular reference to levels of methionine, homocystine, the mixed disulphide of homocystine/cystine, and cystine. As treatment was carried out mainly on an out-patient basis, estimations of overnight fasting concentrations were not practicable; blood samples were taken routinely approximately 3 hours after breakfast.

Owing to the capacity of homocystine to bind on to plasma proteins, blood samples were centrifuged and the plasma deproteinized as soon after venepuncture as possible with dry sulphosalicylic acid (40 mg./1 ml. plasma). The dipotassium salt of ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant, and deproteinized samples were stored at -20 °C. until analysed.

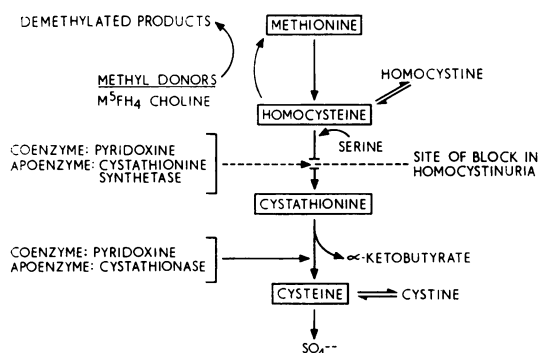


FIG. 1.—Schematic summary of main katabolic pathway of methionine.

Results

Biochemical. Of 11 patients with homocystinuria, 6 responded biochemically to pyridoxine therapy by showing complete disappearance or great reduction of homocystine in the plasma, a lowering of methionine and of the mixed disulphide homocystine/cystine, and an increase of cystine in the plasma. The mixed disulphide was readily separated on the analytical system used, taking the position of the non-biological amino acid, norleucine. However, because the pure compound was not available, quantitation was not possible. Nevertheless, it was possible to adduce the fact that though the peak corresponding to this dipeptide became reduced in intensity as a result of therapy, it never completely disappeared from the plasma chromatogram. At the time of writing, these 6 patients have been under treatment from 21 to 63 weeks. The 5 who failed to respond were all treated for at least 6 weeks, and the dose

of pyridoxine was increased from 450 mg. to 600 mg. daily. In any one family all affected children responded in like manner, suggesting the existence of the same basic defect in each sib.

The Table shows the recorded plasma levels of homocystine, cystine, and methionine, before and after 1 week of treatment with pyridoxine. In those patients who responded to pyridoxine, a marked lowering of the plasma homocystine level was noted, with a coincidental rise in cystine. The long-term biochemical results recorded in 2 patients who responded to pyridoxine are shown in Fig. 2. As will be seen from this illustration, increasing the daily amount of pyridoxine in 2 of these patients resulted in a further fall in the concentration of plasma homocystine. The fluctuations noted in the plasma homocystine levels, independent of changes in therapy, can perhaps be accounted for by variations in the patient's diet and lack of standardization in terms of time of administration of therapy and time of blood sampling. The abrupt cessation of treatment in one pyridoxine-responsive patient, whose treatment lapsed on three separate occasions as a result of failure to renew his pyridoxine prescriptions, resulted in a rapid rise in plasma homocystine concentration, with a corresponding decrease when treatment started again.

Recently the dose of pyridoxine has been gradually reduced in an attempt to define the lowest effective daily requirement. It has been possible to reduce the maintenance dose of pyridoxine in 5 of the patients to 150 to 250 mg. daily and still retain biochemical control. This phase of the study is still proceeding.

At no time was a sustained rise in plasma cystine noted in those who failed to respond to therapy. An occasional early but temporary decrease in

TABLE

Change in Serum Levels of Homocystine, Cystine, and Methionine After 1 Week on Oral Pyridoxine (μ Moles %)

| Case No. | Age (mth.) | Family | Pyridoxine (mg. daily) | Homocystine | | Cystine | | Methionine | |
|----------|------------|--------|------------------------|-------------|-------|---------|-------|------------|-------|
| | | | | Before | After | Before | After | Before | After |
| 1 | 9 | I | 150 | 1.9 | 0.6 | 1.3 | 2.0 | 3.0 | 3.5 |
| 2 | 5 | I | 300 | 3.1 | 0 | 0.8 | 2.9 | 5.7 | 3.9 |
| 3 | 10 | I | 300 | 10.5 | 2.9 | 0 | 2.6 | 4.3 | 4.3 |
| 4 | 18 | II | 300 | 20.1 | 3.1 | 0.85 | 3.7 | 5.4 | 4.9 |
| 5 | 22 | III | 450 | 8.3 | 1.5 | Trace | 4.7 | 6.6 | 5.3 |
| 6 | 8½ | IV | 300 | 7.3 | 2.0 | 2.0 | 4.6 | 5.0 | 3.5 |
| 7 | 20 | V | 450 | 19.6 | 9.9 | 0 | 0 | 4.8 | 5.3 |
| 8 | 15 | VI | 400 | 7.3 | 8.6 | 0.4 | 0 | 15.5 | 22.1 |
| 9* | 29 | VI | 450 | 16.5 | 22.9 | Trace | Trace | 9.6 | 8.4 |
| 10* | 7 | VII | 300 | 6.9 | 4.1 | 0 | Trace | 4.4 | 3.3 |
| 11* | 10 | VII | 300 | 13.4 | 12.6 | 0 | 0 | 6.2 | 4.1 |

*2 weeks after therapy.

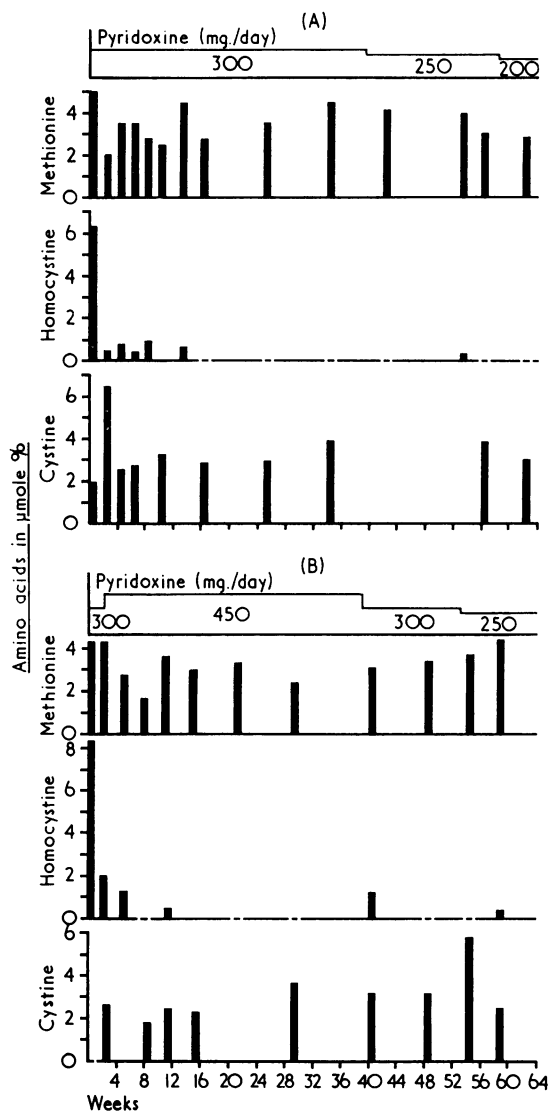


FIG. 2.—(A) Case 3. Response of plasma homocystine, cystine, and methionine to oral pyridoxine. (B) Case 6. Response of plasma homocystine, cystine, and methionine to oral pyridoxine.

plasma homocystine was present, associated with the appearance of very small quantities of cystine, but these changes were not maintained. Fig. 3 illustrates the plasma homocystine, cystine, and methionine concentration in a patient who failed to respond to pyridoxine.

Haematological. Serum folic acid levels in 9 patients, estimated before treatment with pyrid-

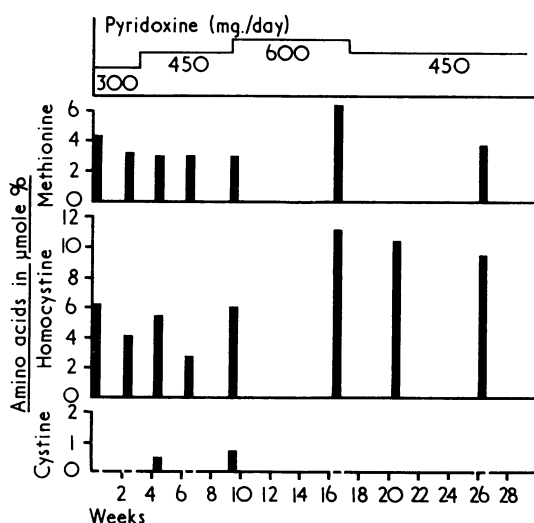


FIG. 3.—Case 10. Response of plasma homocystine, cystine, and methionine to oral pyridoxine.

oxine, varied from 3.9 ng./ml. to 7.5 ng./ml., with a mean level of 5.7 ng./ml. (normal range 4.6–15 ng./ml., with the majority of levels falling between 4.6 and 9 ng./ml., using a modification of the technique described by Waters *et al.* (1961)). In 3 patients pretreatment levels of folic acid were significantly reduced after therapy with pyridoxine.

One patient (Case 6), who responded to pyridoxine, complained of 'pins and needles' after 24 weeks of therapy. His serum folic acid level at that time was found to be 1.6 ng./ml., the level at the start of treatment being 6.8 ng./ml. Hb and indices were within the normal range; bone-marrow aspiration showed a normoblastic reaction, with normal white cell and platelet series. He was nevertheless treated with folic acid and his symptoms disappeared within a few weeks. Serum folic acid levels fell from 4.8 to 2.8 ng./ml. in Case 11: this patient had no symptoms and his peripheral blood picture showed no abnormality; a bone-marrow aspiration was not done.

In the third patient (Case 7) serum folic acid levels fell from 6.9 ng./ml. to 4.2 ng./ml. while on treatment with pyridoxine. This patient was given a trial of pyridoxine when in hospital for enucleation of an eye. She received heparin after operation in an attempt to avoid post-operative thrombosis to which these patients are subject. Unfortunately, as a result of this therapy, haemorrhage occurred into the eye socket, followed by infection. A transfusion was given, but the patient became anaemic within a few weeks of the

transfusion without any evidence of further bleeding. A bone-marrow puncture showed a megaloblastic erythropoiesis. The significance of these haematology findings in terms of pyridoxine therapy are however questionable, as this patient was receiving anticonvulsant treatment with phenytoin and phenobarbitone, a form of therapy known to predispose to a megaloblastic reaction (Reynolds *et al.*, 1966).

Studies of platelet stickiness, using the method as described by McDonald *et al.* (1964), were performed on 9 patients. The results indicated that platelet stickiness of a minor degree was present in 6 of the 9 patients before treatment with pyridoxine. All the tests were performed by the same person under the same physical conditions. Platelet stickiness studies were undertaken on 8 patients at each visit while under treatment. No correlation with improvement in biochemical status was found, and no consistent trend was noted.

Clinical. Owing to the short period of observation and follow-up, it has not been possible to determine any alteration in the intelligence of the patients studied. However, the *petit mal* fits which were occurring daily in one patient (Case 10) ceased after 2 weeks of therapy with pyridoxine; this patient was biochemically unresponsive to therapy. The parents of two other children who were both biochemically responsive to pyridoxine reported that their general behaviour had greatly improved while on treatment.

Discussion

Coenzyme supplementation in homocystinuria was first suggested by Spaeth and Barber (1965). The same authors later (Barber and Spaeth, 1967) reported the biochemically successful treatment of 3 patients in 2 families, with pyridoxine and an unrestricted diet. Homocystine was eliminated from the plasma and urine, methionine levels reverted to normal, and cystine appeared in the plasma, the results being consistent over a 6-month period of therapy. This was followed a few months later by a communication from Hooft, Carton, and Samyn (1967) who successfully treated one homocystinuric child with pyridoxine, but with only partial success in another patient similarly treated. Failure of pyridoxine therapy in two further patients was also reported by Turner (1967). Chase, Goodman, and O'Brien (1967) found no decrease in serum methionine in patients treated with intramuscular pyridoxine for 1 week, but levels of homocystine were not given. Perry *et al.* (1968) and Hagberg and Hambræus (1968)

have also reported unresponsiveness to pyridoxine therapy in a total of 6 further patients. However, Cusworth and Dent (1969) found that of 12 patients with homocystinuria, 7 responded with complete disappearance of homocystine from the serum and reduction in serum methionine to normal levels, 2 had an intermediate response with lowering of serum homocystine and methionine levels, and 3 had no response to treatment.

The results recorded in the 11 patients studied in this series show two clearly defined types of response, which would explain the apparent discrepancy in results reported by the above investigators. These observations point to the fact that there exist two basic defects in the enzyme cystathionine synthetase causing the biochemical and clinical features of homocystinuria. In those who are pyridoxine resistant, the basic defect seems to be a deficiency of apoenzyme, as has been demonstrated by Mudd *et al.* (1964) in a liver biopsy from a homocystinuric patient. In those patients who respond to large doses of pyridoxine, the disorder appears to constitute yet another example of a pyridoxine dependency syndrome. Several such disorders are now known, i.e. pyridoxine-dependent convulsions (Hunt *et al.*, 1954); xanthurenic aciduria (Tada *et al.*, 1967); cystathioninuria (Frimpter, Haymovitz, and Horwith, 1963); and pyridoxine-responsive anaemia (Horrigan and Harris, 1964). For example, when patients with cystathioninuria are given excess pyridoxine, the excretion of cystathionine in the urine is greatly reduced but returns as soon as pyridoxine therapy is withheld. Frimpter (1965) has demonstrated by *in vitro* studies in 2 such patients using liver homogenates that the greatly reduced activity of the apoenzyme cystathionase could be largely restored by the addition of excess pyridoxine to the system.

Pyridoxal-5-phosphate (PLP) is the most important intracellular active form of vitamin B₆. It acts as an essential coenzyme for many apoenzymes concerned in the biosynthesis, interconversion, and degradation of amino acids. There are differences in the mode of binding of apoenzyme to coenzyme, and the nature of these differences at the molecular level is still not clearly defined. It appears, however, that every side chain of the PLP molecule plays a role in the binding process, and their relative importance varies from one apoenzyme to another. Specific SH groups have been implicated in a few apoenzymes, and blocking of this group in some cases completely prevents binding to coenzyme and in others simply reduces binding capacity (Snell, 1958).

In pyridoxine dependency syndromes the extracellular vitamin pool is adequate, and the inactivity of the holoenzyme system at fault is a specific one. This may be the result of an abnormality in the structure of the apoenzyme, perhaps because of a substitution of one amino acid for another at a specific site in the polypeptide chain. The effect of pyridoxine therapy may be to achieve some degree of binding by simple mass action, thus restoring biochemical equilibrium.

Proof of pyridoxine dependency in homocystinuria must await detailed studies in both those patients responsive and those unresponsive to pyridoxine. For example, the cystathionine synthetase activity of liver homogenates assayed in the presence of excess pyridoxal-5-phosphate, before and after therapy with large doses of pyridoxine, should show a marked increase in cystathionine synthetase activity in the pyridoxine-dependent patient, and little or no change in those whose plasma homocystine and cystine levels remain unaltered with pyridoxine therapy.

Recently, Uhlendorf and Mudd (1968) have reported the ability of fibroblasts from normal skin punch biopsies to produce cystathionine synthetase, while the fibroblasts of homocystinuric patients produce very little or none. Clearly the use of skin biopsies would be a more practical approach for this study.

Other than the biochemical improvement noted in 6 of the 11 patients, the only clinical benefit recorded was improved behaviour in 2. Cessation of *petit mal* fits occurred in 1 other patient who was, however, biochemically unresponsive to pyridoxine. Increased platelet stickiness in patients with homocystinuria has been reported by McDonald *et al.* (1964). No consistent changes were recorded in this parameter during the course of therapy with pyridoxine. Studies of platelet adhesiveness are, however, notoriously subjective, and rigorous standardization of technique is essential if the results are to have any significance. Many apparently unrelated factors are found to influence platelet stickiness, and it is not possible to attribute to this aspect of the study any real degree of significance.

Carey *et al.* (1966, 1968) have reported low fasting levels of serum folic acid (2.6–4.6 ng./ml.) in homocystinuric patients. Folic acid absorption and clearance tests were performed and over-utilization of folate was found. These workers concluded that this was due to attempts on the part of the homocystinuric patient to remethylate homocysteine to methionine for which the folic acid derivative N⁵-methyltetra-hydrofolic acid acts

as a methyl donor. Though very low serum folic acid levels were not recorded before treatment in the 11 patients studied here, none the less values were in the low normal range. What appears, however, to be of considerable practical importance is the fact that serum folic acid levels were found to decline significantly in 3 patients while under treatment with pyridoxine. Clearly, further studies are required to elucidate the relation between low serum folic acid values and high doses of pyridoxine in homocystinuric patients.

The fact that homocystinuria appears to be a slowly progressive disease makes attempts at treatment worth while at any age. Low methionine diets with cystine supplements have been successful in correcting the abnormal biochemistry but are very unpalatable. Recently, a low methionine balanced amino acid mixture* has become available and will be extremely useful in conjunction with foods low in methionine, particularly for newborn infants. Such a diet seems unnecessary if the patient is responsive to pyridoxine therapy, and all homocystinuric patients irrespective of their age should be given the opportunity of a trial with this vitamin.

Summary and Conclusion

Eleven patients with homocystinuria were given a trial of treatment with high doses of oral pyridoxine. Biochemically 6 patients responded, as shown by the return of their plasma amino acid pattern to normal or near normal. 5 showed no response. The same type of biochemical response was recorded in all affected members of a family.

It is suggested that in homocystinuria two different genetically determined basic defects exist in the cystathionine synthetase system, producing the same biochemical and clinical picture. In those patients who are biochemically unresponsive to pyridoxine, the defect appears to be a deficiency of the apoenzyme, cystathionine synthetase. On the other hand, in patients who respond biochemically to pyridoxine, the disorder seems to represent a further example of a pyridoxine dependency syndrome.

It is too early to say if treatment with pyridoxine will prevent the sequelae of this disorder, and only by prolonged follow-up of the younger patients will this become known. The possibility of a folic acid deficiency arising while on treatment is stressed.

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* Scientific Hospital Supplies Limited (Liverpool).

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