The Effect of Chelation Therapy on the Amino Aciduria and Peptiduria of Wilson's Disease

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Wilson's disease is characterised by excess urinary excretion of free amino acids and small molecular weight globulins, but there is also an increased output of peptides[1]. Some uncertainty, however, still exists as to whether this is due to a reduced proximal reabsorption of peptides from the glomerular filtrate or to an increase in their plasma concentration secondary to an excess breakdown of skeletal tissue in this disease, or to a combination of both factors. If the peptiduria is secondary to renal tubular dysfunction, any improvement from treatment should parallel that in urinary amino acid loss. On the other hand, if the peptiduria is due to a metabolic lesion, less correlation is to be expected. This article describes an investigation of the excretion of free amino acids and peptides in 11 patients with Wilson's disease, both before the start of therapy with penicillamine or trientine 2HCl[2] and approximately two years later, after the patients had shown a good clinical response.

Methods

Concentrated early morning specimens of urine were obtained from the 11 cases of Wilson's disease shortly before therapy and after two years of treatment with penicillamine in eight cases or trientine in three. Details of the patients and their treatment are to be found in Table 1. The mean duration of therapy was 22.5 ± 4.9 months (mean ± SD). The collection period was from 18.00 hours until 10.00 hours the following day. To avoid possible errors due to loss of urine in severely ill patients, the results of urinary analyses of free amino acids and of amino acids derived from peptide hydrolysis have been expressed as μmol of amino acid/mmol of creatinine. The use of creatinine as a reference compound was considered preferable to corrections based on calculated body surface area, in view of the considerable disparity in nutritional status of different patients before therapy and in individual patients before and after treatment. Before the post-therapy urine collections were made the chelating agent was stopped for three days, to avoid analytical complications from excretion of the drugs and their metabolites. The patients were on normal diets but care was taken to avoid excess collagen or gelatine that might influence the output of hydroxyproline-containing peptides. Analytical methods have previously been described in detail[1]. In this series of studies urinary peptides were determined by ligand-exchange column chromatography[3].

Urinary output of free amino acids was distributed in a log-normal manner and the results have been expressed as mean and range. Excretion of peptides did not significantly differ from a normal distribution and the results have been expressed as mean ± the standard deviation. In the statistical analysis of the results the Wilcoxon signed rank test was used for free amino acid output and the paired t-test for amino acids derived from peptide hydrolysis.

Results

The output of free amino acids and of amino acids derived from peptide hydrolysis before and after therapy is given in Table 2. The mean total output of free amino acids fell to 43 per cent of the pre-treatment figure, and the excretion of amino acids derived from peptides to 68 per cent. Twenty individual amino acids were measured, and the mean rise in concentration of each before treatment was approximately twofold. Sixteen showed a statistically significant fall in output, whereas of the 18 amino acids derived from peptide hydrolysis all except methionine showed a significant reduction. The fall in the total of free amino acids and of amino acids derived from oligopeptides was highly significant for both (P<0.001 and P<0.01 respectively). The improvement due to therapy was, in both cases, purely quantitative; there was no significant change in the percentage of individual amino acids in relation to the total output.

Figure 1 shows the percentage change in free amino acid excretion and of that derived from peptide hydrolysis.
Table 1. Details of cases of Wilson’s disease investigated.

| Case No. | Sex | Age at onset (months) | Age at diagnosis (months) | Start of therapy (months) | Penicillamine g/day (months) | Trientine 2HCl g/day (months) | CNS and eyes | Clinical Features |
|----------|-----|-----------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|--------------|------------------|
| 1        | F   | 16                    | 17                        | 17                        | 1.25                        | 24                          | Severe intention tremor. Kayser-Fleischer rings | Nil | Normal | Minimal tremor, left hand |
| 2        | F   | 14                    | 14                        | 14                        | 1.25                        | 24                          | Kaysers-Fleischer rings only | Chronic aggressive hepatitis | Normal | No evidence of active liver disease |
| 3        | M   | 26                    | 27                        | 27                        | 1.25                        | 24                          | Mild intention tremor. Kayser-Fleischer rings. Cataracts | Nil | Great clearance 52 ml/min. Hypercalciuria | Arthropathy both knees, otherwise symptom-free |
| 4        | F   | 10.5                  | 10.8                      | 11                        | 1.0                         | 14                          | Kaysers-Fleischer crescents only | Chronic aggressive hepatitis | Fructosuria | Symptom-free |
| 5        | M   | 15.5                  | 16.5                      | 16.5                      | 0.75                        | 24                          | Kayser-Fleischer rings only | Chronic active hepatitis | Normal | Symptom-free |
| 6        | M   | ?                     | 9                         | 9                         | 0.75                        | 24                          | Pre-symptomatic | Pre-symptomatic | Normal | Symptom-free |
| 7        | M   | ?                     | 12.5                      | 12.5                      | 0.75                        | 9                           | Kayser-Fleischer rings only | Hepatomegaly | Normal—developed penicillamine nephropathy | Symptom-free. Renal lesion cleared |
| 8        | M   | ?                     | 11                        | 11                        | 0.75                        | 9                           | Nil | Hepatomegaly | Normal—developed penicillamine nephropathy | Symptom-free. Renal lesion cleared |
| 9        | M   | 18                    | 18                        | 18                        | 1.5                         | 12                          | Kayser-Fleischer rings only | Hepatic disease. Haemolysis | Galactosuria | Symptom-free |
| 10       | F   | 15                    | 15                        | 15                        | 1.5                         | 6                           | Dystonia. Drooling. Kayser-Fleischer rings | Nil | Slight proteinuria | Dysarthria. Coombs test positive. Haemolytic anaemia. Lupus nephritis |
| 11       | M   | 11                    | 16                        | 16                        | 1.0                         | 24                          | Dysarthria. Drooling. Involuntary movements. Kayser-Fleischer rings | Slight proteinuria | Symptom-free |

(Cases 5 and 6 and cases 7 and 8 are sibling pairs)

in each of the 11 cases of Wilson’s disease. All showed a fall in amino acid output after therapy except for one patient diagnosed in the course of a family screening programme; he was an asymptomatic boy of 9 years with a serum caeruloplasmin of zero, a plasma copper of 17 μg/dl (2.7 μmol/litre), a basal urine copper of 140 μg (2.2 μmol)/24 hours which, on giving penicillamine, rose to 1,400 μg (22.2 μmol)/24 hours. There is a significant correlation between reduction of free amino acids and that of amino acids from peptides (r = 0.80; P<0.01), showing that improvement in each parameter of the disease occurs in parallel.

Discussion

There have been some previous reports of the beneficial effects of therapy on the output of free amino acids in Wilson’s disease[4,5], but these dealt with fewer cases and were studied in less detail than in this article. A greater quantitative improvement occurred in free amino acid output than in the peptiduria. This is consistent with the results of a previous paper[1] in which it was reported that the mean free amino acid output in Wilson’s disease was almost twice that of matched controls, while peptiduria showed an increase of only 66 per cent above that of control subjects. Renal tubular dysfunction in Wilson’s disease is usually thought to be directly due to the toxic effects of copper deposits in the tubular epithelium; urinary concentrating power, H+ excretion, output of low molecular weight proteins and hypercalciuria have all been reported to be improved by effective treatment[4,6]. The close correlation between the improvement in free amino acid output and in peptide excretion supports the view that peptiduria in Wilson’s disease is an additional
### Table 2. Amino acid and oligopeptide output in Wilson’s disease before and after therapy.

| Amino Acid | Free amino acid output (μmol/mmol creatinine) before therapy Mean and range | Free amino acid output (μmol/mmol creatinine) after therapy Mean and range | P | Oligopeptide output (μmol/mmol creatinine) of constituent amino acids Mean ± SD before therapy | Oligopeptide output (μmol/mmol creatinine) of constituent amino acids Mean ± SD after therapy | t-test | P |
|------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|--|---|
| asp        | 45.2 (1.2–257.5)                                                                                   | 6.7 (1.3–29.2)                                                                                   | >0.01 | 94.0 ± 17.0                                                                                     | 58.2 ± 12.9                                                                                     | 3.52 | <0.01 |
| thr        | 116.4 (11.8–475.2)                                                                                  | 27.9 (5.7–75.3)                                                                                  | <0.02 | 21.1 ± 7.6                                                                                      | 12.9 ± 5.6                                                                                      | 3.44 | <0.01 |
| ser        | 151.3 (5.5–453.3)                                                                                   | 33.8 (2.6–89.0)                                                                                  | <0.05 | 28.7 ± 7.6                                                                                      | 19.0 ± 7.3                                                                                      | 3.69 | <0.01 |
| asn        | 31.5 (0.7–136.8)                                                                                   | 9.3 (0.6–38.8)                                                                                   | <0.05 |                                                                                                 |                                                                                                 |     |    |
| glu        | 169.7 (1.8–565.0)                                                                                   | 55.9 (29.4–130.1)                                                                                | <0.05 |                                                                                                 |                                                                                                 |     |    |
| glu        | 11.3 (1.2–31.6)                                                                                     | 9.4 (1.5–22.0)                                                                                   | <0.10 | 148.3 ± 23.3                                                                                     | 98.7 ± 15.6                                                                                     | 2.73 | <0.05 |
| pro        | 3.3 (0–26.6)                                                                                       | 1.3 (0–12.7)                                                                                     | <0.10 | 40.4 ± 9.5                                                                                       | 31.0 ± 10.8                                                                                     | 3.59 | <0.01 |
| gly        | 481.0 (125.0–1620.0)                                                                               | 233.0 (78.0–981.0)                                                                               | <0.01 | 132.0 ± 18.7                                                                                     | 99.8 ± 20.6                                                                                     | 3.17 | <0.02 |
| als        | 138.8 (22.7–432.0)                                                                                 | 43.1 (18.4–86.7)                                                                                 | <0.01 | 30.7 ± 7.7                                                                                       | 22.2 ± 8.3                                                                                      | 4.04 | <0.01 |
| val        | 7.8 (2.0–14.3)                                                                                      | 2.8 (0.4–8.6)                                                                                   | <0.002 | 15.4 ± 10.6                                                                                      | 4.7 ± 4.1                                                                                       | 2.81 | <0.02 |
| asys       | 41.1 (7.6–77.5)                                                                                     | 11.1 (4.6–20.2)                                                                                 | <0.002 | 12.5 ± 10.1                                                                                      | 3.4 ± 2.9                                                                                       | 2.72 | <0.05 |
| met        | 14.8 (0.6–39.5)                                                                                     | 8.0 (3.0–19.5)                                                                                  | <0.05 | 2.6 ± 2.2                                                                                       | 2.1 ± 1.9                                                                                       | 1.71 | <0.10 |
| ile        | 2.7 (1.0–7.5)                                                                                      | 1.7 (0.4–5.9)                                                                                   | <0.05 | 5.3 ± 4.5                                                                                       | 3.2 ± 2.9                                                                                       | 3.82 | <0.01 |
| leu        | 8.4 (1.8–23.7)                                                                                      | 4.1 (0.6–11.3)                                                                                  | <0.05 | 10.1 ± 4.7                                                                                       | 6.0 ± 4.1                                                                                       | 4.47 | <0.01 |
| tyr        | 38.7 (8.1–147.8)                                                                                    | 15.8 (6.0–35.3)                                                                                 | <0.01 | 6.1 ± 5.5                                                                                       | 2.0 ± 1.8                                                                                       | 3.30 | <0.01 |
| phe        | 16.9 (3.8–51.5)                                                                                     | 7.3 (2.4–15.3)                                                                                  | <0.05 | 5.8 ± 5.3                                                                                       | 3.5 ± 3.5                                                                                       | 2.27 | <0.05 |
| orn        | 6.9 (2.7–14.3)                                                                                      | 3.1 (1.6–6.3)                                                                                   | <0.01 |                                                                                                 |                                                                                                 |     |    |
| lys        | 39.4 (2.7–122.9)                                                                                    | 17.4 (1.0–68.5)                                                                                 | <0.05 | 10.2 ± 5.8                                                                                       | 7.5 ± 4.9                                                                                       | 3.03 | <0.02 |
| his        | 192.0 (39.0–405.0)                                                                                 | 103.8 (32.0–208.0)                                                                              | <0.05 | 5.5 ± 5.0                                                                                       | 2.2 ± 1.9                                                                                       | 3.61 | <0.01 |
| 3-methis   | 22.1 (11.7–29.7)                                                                                    | 26.9 (22.4–34.1)                                                                                | >0.10 | 4.8 ± 4.6                                                                                       | 3.0 ± 2.6                                                                                       | 2.39 | <0.05 |
| arg        | 2.4 (0.4–6.3)                                                                                       | 2.5 (0.1–8.9)                                                                                   | >0.10 | 80.5 ± 16.2                                                                                      | 64.4 ± 8.3                                                                                      | 2.19 | <0.05 |
| hypro      |                                                                                                   |                                                                                                 |       | 659.0 ± 131.0                                                                                    | 447.0 ± 122.9                                                                                    | 4.20 | <0.01 |

| Total      | 1535.0 (330.3–4352.2)                                                                               | 659.0 (156.7–1041.0)                                                                              | <0.001 |

![Fig. 1](image.png)

**Fig. 1.** Free and combined amino acid output in 11 cases of Wilson’s disease before and after chelation treatment. Results are expressed as the percentage of excretion after therapy as related to that before therapy. The two regression lines show the close correlation between the two sets of values (r = 0.80: P < 0.01).

The index of copper-induced renal tubular dysfunction[7] and not part of the primary metabolic defect[8]. The excess peptides have a high content of hydroxyproline, which is associated with the breakdown of skeletal tissue; indeed, the highest degree of peptiduria in Wilson’s disease is found in those rare cases in whom there is a combination of severe skeletal disease and renal tubular dysfunction (Asatoor and Walsh, unpublished). In these cases the peptiduria is due to a combination of metabolic and renal tubular defects. An analogy in the case of free amino acid output would be in those patients in whom there is a combined failure of amino acid metabolism from severe hepatic disease and a renal tubular absorption defect, such as might occur in the hepatic form of Wilson’s disease. A disorder of renal tubular function is, however, more common in Wilson’s disease than either overt skeletal breakdown or liver failure. It can now be stated with reasonable confidence that while bone or liver disease may be contributory to the peptiduria of Wilson’s disease, reduced absorption of peptides in the glomerular filtrate is a more important and more constant factor. Hydroxy-prolinuria is a useful index of the severity of bone breakdown and turnover in most cases of skeletal disease, but clearly becomes unreliable in the rare patient with a combination of bone disease and a Fanconi type of proximal renal tubular defect. The most common example of this unusual combination is to be found in Wilson’s disease[9].

### Summary

1. There is an excess urinary output of free amino acids and of urinary peptides in most cases of untreated Wilson’s disease. Studies of 11 patients have shown that both these abnormalities are greatly improved by two years of standard chelation therapy.
2. The reduction in excretion of both free amino acids and peptides is purely quantitative, there being no significant change in the percentage composition of amino...
acids, either free or combined, in relation to their total urinary output.

3. Arguments are advanced that the peptiduria of the disease is usually due to a proximal renal tubular reabsorption defect, but in rare cases it may be due to excess bone breakdown or even to a combination of bone and renal tubular disease in the same patient.

References
1. Asatoor, A. M., Milne, M. D. and Walshe, J. M. (1976) Clinical Science and Molecular Medicine, 51, 369.
2. Walshe, J. M. (1982) Lancet, 1, 649.

‘Pinched with Straightness of Tyme’

If the presence of a number of editions of Euclid in the College library evokes expressions of surprise, it is only necessary to recall that Harvey had laid it down that, besides medical books, those on geometry among others would be suitable additions; moreover, some editions of Euclid are to be found in the Dorchester Library—a general one. The earliest translation of Euclid into Latin was made in about 1130, and first printed at Venice in 1482. It was the first and highly successful attempt to produce a long mathematical work illustrated by diagrams. This edition is not represented in the library, but the first in Greek and English, respectively published in 1533 and 1570, arc. The English translation is the work of Henry Billingsley, an alderman and later Lord Mayor of London, who worked from the Greek edition, although he is thought to have had the Latin by his side. There are those who think that the translation was made by John Dee. At least he wrote the introduction to the various books and added annotations throughout the body of the text.

The well-known theorem of Pythagoras (‘33 Theoreme. The 47 Proposition’) is described in these words: ‘In rectangle triangles, the square which is made of the side that subtendeth the right angle, is equal to the squares which are made of the sides containing the right angle.’

To it the following note has been added: ‘This most excellent and notable Theoreme was first invented of the great philosopher Pythagoras, who for the exceeding joy conceived of the invention thereof, offered in sacrifice an Oxe, as recorde Hierone, Proclus, Lycius and Vitruvius. And it hath bene commonly called of barbarous writers of the latter time Dulcarnon.’ This was because the two squares which contain the right angle roughly represent horns, and Dulcarnon comes from an Arabic word meaning ‘the possessor of two horns’.

John Dee’s most important contribution to the first English edition of Euclid was undoubtedly the preface, in which he outlines the entire state of science as it was known in the sixteenth century. That Dee was under some pressure when he wrote it is clear from its conclusion, but whether from his manifold interests as alchemist, astrologer and mathematician or because he was being pressed for copy by his publisher, is less clear. However, he wrote ‘I have been pinched with straightness of tyme: that, no way, I could so pen downe the matter (in my Mynde) as I determined: hopying of convenient layures . . .

The book itself is a remarkable production, of which the College copy, acquired after 1912, is a fine example. There is the book-plate of John Eustace Anderson inside the front cover, the signature of Thomas O’Keefe on the title-page, and on the back of the title-page that of George Carter ‘his book 1682’. Nothing is known of any of these owners and it would be interesting to know the name of the first owner(?) who paid the ‘Pret. 16d’. The same title-page had been used by John Day, the printer, in four earlier books from 1559 on, and was also used afterwards for twelve other books. It is elaborately emblematic, containing figures of various philosophers, and of females representing Geometry, Astronomy, Arithmetic and Music. At the foot is Mercury, a sedate figure of a corpulent and full bearded citizen of middle age, which is very unmercurial, but perhaps it is intended to be a portrait of the printer.

Euclid’s Elements is the work of a mathematician who has almost given his own name to the science of geometry. He lived at Alexandria in the time of the first Ptolemy, 323-283BC; he is not to be confused with the Socratic philosopher of the same name who lived at Megara in the fifth century BC. It is the oldest mathematical text-book in the world still in common use today, about a thousand editions and translations having been published. To quote from The Legacy of Greece: ‘No work presumably except the Bible has had such a reign; and future generations will come back to it again and again as they tire of the various substitutes for it.’

Leonard Payne