The conquest of Wilson’s disease

J. M. Walshe

Formerly Dept of Neurology, The Middlesex Hospital, London (Retired)

Correspondence to: J. M. Walshe, Huntingdon, UK
E-mail: penicillamine@waitrose.com

When (Samuel Alexander) Kinnier Wilson described a new disease involving the liver and the lenticular nucleus of the brain in 1912, he was unable to recommend any form of treatment, although that remained his life long ambition (Wilson, 1912; Fig. 1). Until the underlying cause of the disease was understood, this inevitably remained a somewhat forlorn hope. A role for copper, as a possible pathogenic agent, was suggested the year after Wilson’s original publication when Rumpel (1913) reported finding excess copper in the liver of a patient who had died of this newly described disease. But this observation was not followed until, in 1948, (John) Cumings (1948) demonstrated that copper is present in excess both in the brain and liver of patients with Wilson’s disease. This observation led Cumings to suggest that treatment with the newly developed chelating agent British antilewisite (Dimercaprol) might arrest the progress of the disease.

It is generally held, not unreasonably, that all major advances in therapeutics are made by the multi-national pharmaceutical companies. There are, however, a few exceptions to this rule as illustrated in ‘Orphan Drugs’ by Fred Karch (1982). Accounts of seven attempts to introduce new drugs, discovered by individual research workers, are presented here. There is, therefore, still a role for the individual researcher to make significant advances, although these are likely to remain confined to the treatment of rare diseases. The large pharmaceutical companies will, by the very nature of free market economics, confine their research and development programmes to common diseases offering the prospect of large profits. The cost of introducing a new drug is now so large—an average figure as high as 800 million dollars has recently been suggested—that no other course is possible.

Wilson’s disease illustrates this state of affairs. All treatments now available were introduced as a result of individual or small team research. The story did not begin in 1948 when Cumings suggested that treatment with Dimercaprol might arrest the course of Wilson’s disease by eliminating copper from the body. When World War II started in 1939 it was feared that Hitler would attack the British Isles with the arsenical war gas ‘lewisite’ and a team of workers in Oxford, under the direction of Sir Rudolph Peters, Professor of Biochemistry, started the search for an antidote, publishing the results of this work in 1945 (Peters and Stocken, 1945). The molecule they designed, dimercaptopropanol, was a short-chain alcohol with two substituted sulphhydryl (–SH) groups. These gave dimercaptopropanol the ability to bind arsenic in a tight five-membered ring, rendering it non-toxic. This compound later became known as British antilewisite (BAL). It was BAL that Cumings hypothesized might arrest the progress of Wilson’s disease. Four years later Cumings (1951), in London, and Denny Brown and Porter (1951), working independently at the Boston City Hospital, reported favourably on this form of treatment. Whilst this represented a significant advance in the treatment of Wilson’s disease, it soon became apparent that repeated courses of BAL had a decreasing effect and were also associated with a high incidence of toxic reactions, besides being painful through having to be administered by deep intramuscular injection. Thus, whilst a very significant advance in the management of Wilson’s disease, BAL was clearly not the final answer.

Research into Wilson’s disease really took off in the early 1950s. Working separately, Bearn and Kunkel (1952) and Scheinberg and Gitlin (1952) reported a deficiency or absence of the serum copper carrying protein, caeruloplasmin, in patients with Wilson’s disease, although the exact significance of this has never become apparent (Fig. 2). At the same time a number of attempts to improve treatment were made using high protein diets, steroids and the chelating agent ethylenediamine tetraacetic acid. None of these proved beneficial.
My own involvement in this story came a few years later but, in practice, dates to 1951 when studying disturbances of amino acid metabolism found in patients with liver disease, in Professor Charles Dent’s metabolic unit at University College Hospital, using the technique of paper chromatography which had recently been introduced into clinical biochemistry. The key observation came about during the investigation of a patient undergoing partial liver resection for a hepatocellular carcinoma confined to the left lobe. Post-operatively, he was found to excrete an amino acid not previously found in urine. Its movement in the solvent systems used suggested that it was a sulphur amino acid closely related to cysteine. Using alternative solvent systems to study the movement of the amino acid it was possible to postulate that its formula was dimethyl cysteine. Professor Dent pointed out that dimethyl cysteine was a breakdown product of penicillin, better known as penicillamine (Fig. 3). Armed with this information, a search of the patient’s notes showed that, post-operatively, he had been treated with penicillin. It was then possible to show that when given a large dose of penicillin, I also excreted this new compound and later studies showed that this was true of all patients so treated (Walshe, 1953). Clearly this finding, whilst interesting, shed no new light on abnormalities of liver function and the observation appeared to be of no great significance.

Shortly after this, having been awarded a Fulbright Fellowship, it was possible to travel to the Boston City Hospital to work in Dr Charles Davidson's liver unit, part of the Harvard University Medical School. My prime line of research there was to study oxygen uptake in brain slices and evaluate the effect of various toxins, which might be found in the circulation of patients in liver coma. However, during the course of that academic year, Davidson was asked by Professor Derek Denny Brown to advise on the management of a patient with Wilson’s disease, being treated with BAL, who was going into liver failure. The patient in question had an impressive pseudosclerotic tremor and marked fluid retention. Professor Davidson gave the advice, accepted at the time, on the management of fluid balance and we returned to the liver unit. On the way back the thought occurred that penicillamine had a structural formula which should chelate copper, binding this in a ring compound between the sulphydryl and the amino group. Davidson had an extraordinary range of
contacts and was able to get 2 g of penicillamine from Professor John Sheehan at the Massachusetts Institute of Technology. This raised the problem: what was a safe therapeutic dose of penicillamine? In those days there were no ethical committees from whom to seek permission and, in practice, no ethical guidelines at all on which to base decisions. It seemed right to act on one of Charles Dent’s aphorisms: ‘never give an untried compound to a patient that you are not prepared to take yourself’. Nothing untoward developed, 24 h after the ingestion of 1 g of penicillamine powder taken in solution. Fortuitously, this proved to be important when tap water was added to the penicillamine it immediately turned bright blue. This rather unexpected finding resulted from the colour reaction between an –SH group and iron in the tap water. This ad hoc toxicity test resulted in no untoward reaction so it seemed safe to give the remaining gram to Professor Denny Brown’s patient. This resulted in a significant increase in his urinary copper excretion. Again there were no toxic effects. The lack of toxicity was hardly surprising since every patient treated with penicillin excreted penicillamine in the urine. The next problem was to find further supplies of penicillamine as the initial dosing had failed to elicit any cupriuresis—at first sight a very disappointing result. However, remembering the ability of cysteine to auto-oxidize to cystine and lose its –SH group, it seemed probable that penicillamine might show a similar reaction. When the MSD preparation was tested with ferric chloride, no blue colour formed which clearly showed that the compound did not have a free –SH group. Presumably the dimethyl cysteine had auto-oxidized to tetramethyl cystine. After some further correspondence, MSD admitted their mistake and hoped that this would not interfere with my research! Fortunately, Prof Sheehan was able to put me in touch with Mann’s Fine Chemicals who were able to offer 50 g of penicillamine acetonide together with instructions on how to derive free penicillamine from this sample for further clinical trials. I brought this back to England on completion of my year as a Fulbright Fellow. Penicillamine obtained from penicillin is the D-isomer of the aminoacid. Synthetic penicillamine consists of the D- and L-isomers. The L-isomer is toxic.

On returning home I resumed my post as an assistant on the Medical Unit at University College Hospital, in Charles Dent’s Metabolic Unit. The next step was to find patients with Wilson’s disease and prepare free penicillamine packed into capsules for administration to prospective patients. Finding compliant cases was less of a problem than might have been expected. My father, Sir Francis Walshe, was able to persuade colleagues to send patients for clinical trials. Two came from Dr Dennis Brinton at the National Hospital for Nervous Diseases, Queen Square and one from Dr Michael Ashby at the Archway Hospital. No questions were asked about ethics or safety. How things have changed! The results of these initial experiments showed that this new drug did indeed have real potential for future treatment (Walshe, 1956a). All patients excreted large amounts of copper after a single test dose, unlike other compounds that were tried. Possible toxicities, after long-term use, were discussed but none of these have proved problematic. After the trial, Dr Brinton’s two patients resumed treatment with BAL. One died shortly afterwards from oesophageal haemorrhage and the other was restarted on penicillamine some years later, presumably because of a poor response or intolerance to continued BAL. The patient from Dr Ashby remained under my care and became the first person with Wilson’s disease to receive long-term treatment with penicillamine. The dosage that it was then possible to give, 450 mg daily, would now be considered too small but after ~9 months her severe Parkinsonian syndrome started to improve and, although some symptoms and signs persisted, she improved sufficiently to get married and bring up three children. More than 50 years later, she remains on the same drug having suffered no adverse effects.

In 1957, I moved from University College Hospital to Professor McCance’s Department of Experimental Medicine in Cambridge, with an Honorary Consultant appointment at Addenbrooke’s Hospital (Fig. 4). During the 1960s, the use of penicillamine became the accepted treatment of choice for patients with Wilson’s disease (Boudin et al., 1963; Lange, 1963; Sternlieb and Scheinberg, 1964; Deiss et al., 1971). In 1961 Schouwink, a Dutch neurologist, submitted an MD thesis in which he demonstrated that zinc salts could effectively block the absorption of copper from the gut and, therefore, might offer an alternative treatment for Wilson’s disease (Schouwink, 1961). The zinc did not, however, promote the excretion of copper via either...
bile or urine so its action in achieving decoppering would be very slow. Later, this treatment was taken up in Holland by Hoogenraad et al. (1979) and eventually in the USA by Brewer et al. (1981), who is an enthusiastic supporter of this treatment. The FDA, however, have only licensed the use of zinc salts for maintenance treatment after initial decoppering with a chelating agent. Zinc has the advantage of being a much cheaper treatment than a chelating agent and has fewer toxic side-effects. As an initial treatment its effects will, inevitably, be slow as it only creates a negative copper balance by limiting copper absorption from the gut. There has been some controversy about the priority of the discovery of this treatment between Hoogenraad (1998) and Brewer (1999a), but in fact it was Schouwink (1961) who made the initial observation. My own experience with this therapeutic approach has been disappointing (Walshe, 1983b; Walshe and Munro, 1995; Yonetani and Walshe, 2000). Meanwhile, inevitably, adverse effects of penicillamine were reported (Walshe, 1968), the most serious being an immune reaction leading to systemic lupus erythematosus (SLE) and immune complex nephritis, neither of which had been anticipated (Walshe, 1956b). These toxic reactions, though uncommon, have led to controversy over the use of penicillamine (Brewer, 1999b; Walshe, 1999). An example of the problem of penicillamine toxicity was one such patient who presented with penicillamine nephropathy. If treatment with penicillamine was to be continued, death from renal failure seemed probable but, if stopped, the symptoms of Wilson’s disease would also probably prove fatal. BAL induced only a very poor cupriuresis in his case and caused great distress from pain and fever, and was therefore not an option.

The search for a new chelating agent meant finding a compound that was non-toxic, could be given orally, and would induce copper excretion in the urine comparable with that induced by penicillamine. Somewhat unscientifically, using rats, a number of compounds, whose formulae looked promising, were assayed but none provided useful results. I then approached Dr Hal Dixon in the University Department of Biochemistry and he immediately suggested the use of triethylene tetramine, pointing out that it had a very similar formula to two biologically occurring amines, spermine and spermidine, and should therefore be non-toxic. However, this known industrial chemical, used in epoxyresin hardener, is intensely alkaline with pH 14 and would, therefore, need to be neutralized to around pH 7.2. As this commercially available chemical was only ~60% pure it was hoped that an acid could be found that would produce a pure crystalline salt for clinical use. This proved a real problem and eventually it was necessary to settle for the dihydrochloride, which remained in solution with the other impurities, from which a final impure product was obtained by freeze drying. This was highly hygroscopic and had to be stored over phosphorus pentoxide. It tasted rather like stale biscuits. However, when administered to rats it proved non-toxic and induced a significant cupriuresis.

Figure 4 Myself out side my lab in Canham’s yard Cambridge.
The time had come for a clinical trial. When given as a single test dose to the nephrotic patient it induced an enormous cupriuresis and clearly had real potential for the treatment of Wilson’s disease (Walsh, 1969, 1973). Over the following years this crude triethylene tetramine proved to be of value in the management of an increasing number of patients who were penicillamine intolerant (Walsh, 1983a). However, it put a real load on my assistant, Kay Gibbs who had taken on the task of manufacturing the new drug and packing it into capsules for distribution to patients who needed treatment. Eventually, and probably inevitably, this ‘do-it-yourself’ pharmacological adventure ran into trouble. Two new patients, who had developed early toxic reactions to penicillamine, were referred and both were changed to the crude triethylene tetramine bought from a new supplier—both developed acute renal tubular necrosis. Why other patients supplied from the same batch did not do so remains a mystery, but all were immediately advised to stop treatment. Of the two new patients one died shortly afterwards from massive bleeding due to oesophageal varices. Examination of his kidneys confirmed the diagnosis of renal tubular necrosis. This led to an immediate search for the toxic impurity in this new batch of triethylene tetramine. Triaminotriethyamine was implicated but this had been present in all batches used over several years so was unlikely to be the culprit. However, the formula of triaminotriethylamine suggested that it might be a ganglion blocking agent and studies by Dr. Grundy in the Department of Pharmacology proved this hypothesis to be correct. Had this unexpected pharmacological action induced severe hypotensive shock? Given parenterally to rats it was certainly able to do so. The Department of Chemistry was also asked to help in the search from minute quantities of impurities but was not able to make any useful contributions.

The second patient affected was, of necessity, without treatment for several weeks during this search for a toxin and during this time her Wilson’s disease became significantly worse although renal function, which had been very severely damaged, recovered slowly. The time was not wasted, however, and Hal Dixon was able to perfect a method for purifying triethylene tetramine by crystallization from absolute alcohol. Armed with this purified compound, it was possible to restart treatment and careful monitoring of the renal function showed that the patient was able to tolerate this treatment. Over the following weeks she made excellent progress and when ready for discharge was found to be pregnant, conception being dated to a hospital visit from her husband some weeks earlier! The question now arose, would triethylene tetramine damage the foetus? Hilda Bruce, a powerful anti-copper agent. The problem of obtaining molybdate had no beneficial effects either clinically or biochemically. The mistake they made was not to realize that the affected sheep were also grazing on sulphur rich pastures and in the reducing conditions of an abomasum the second stomach of the sheep, the molybdate was reduced to tetrathiomolybdate which is a powerful anti-copper agent. The problem of obtaining pure tetrathiomolybdate was solved by Professor Stuart Laurie, an inorganic biochemist based at the De Montfort University in Leicester, who generously supplied this at a cost so modest that it could be absorbed into the laboratory budget. It was, therefore, decided to try the latter compound as a new therapy. As there were no known toxicity data on the use of this compound in man (Walsh, 1983b), there was no option but to take it myself for 4 days before administering it to the patient. This trial showed no clinical or biochemical evidence of toxicity and the way was clear for a clinical use. Professor Laurie suggested a dose of 30 mg twice a day and this, unexpectedly, resulted in a sharp increase in the serum copper concentration but only a modest increase in urine copper. There was a corresponding decrease in the trichloroacetic acid soluble fraction of the serum copper suggesting that...
the copper had become tightly bound to protein. Studies with radioactive copper showed that the isotope was cleared from the plasma much more slowly than before administration of the drug. When the radio copper was given by mouth, tetra-thiomolybdate caused almost no copper to be absorbed from the gut and in this respect it was more effective than zinc salts (Walshe, 1986). One year of treatment with thiomolybdate resulted in complete restoration of the histological picture in the patient’s liver and a fall in copper concentration to one-third of the pre-treatment level. Her clinical condition showed a corresponding improvement. This was clearly an effective new therapy. To prove that a compound is effective is a far cry from getting it licensed or of establishing a reliable source of supply. Twenty-four years later, this hurdle has still to be cleared in the UK.

Another advance in the 1980s was the rediscovery of the use of BAL by Scheinberg and Sternlieb (1984). They showed that in certain severely dystonic patients, an intensive course of BAL could induce a remission that could then be maintained by conventional chelation therapy. I have also found this a most useful addition to the therapeutic armamentarium now available for the treatment of a small number of patients with Wilson’s disease (Yonetani and Walshe, 2001). It may well be that, being fat soluble and non-polar, BAL works in these individuals by being more readily able to cross the blood-brain barrier. However, it must be admitted that there is still a small number of patients who fail to respond to any of the available therapies and the reason for this remains unclear. It may be a particularly unfavourable mutation or combination of mutations for with approaching 300 of these, and most patients being compound heterozygotes, the possible permutations and combinations are enormous. Starzl and colleagues (1982) introduced liver transplantation for patients with Wilson’s disease in hepatic failure and this has become a valued treatment. However, while the transplanted patients no longer have Wilson’s disease and therefore do not need chelation therapy, in exchange they have to take lifelong anti-rejection therapy which may well have more side-effects than conventional chelation.

The latest chapter in this story, although as yet it has no therapeutic implications, is the discovery of the mutant gene by three separate groups in 1993. This was shown to be on chromosome 13q14, the product being a P-type ATPase, 140-kDa copper-transporting enzyme (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993).

Despite all these advances, problems remain. Why is there such a wide spectrum of response to treatment? As reported by Walshe and Yealland (1993), whilst the majority of patients responded well to treatment, a significant minority remained with significant disability despite adequate decoppering and 11 out of 137 died—the reason for which was not apparent. In a later analysis of this problem (Walshe, 2007), the cause of death in 67 patients, out of a total of 300, was analysed. Of these 67 patients, 32 presented with neurological symptoms, 11 with hepatic, 10 with a mixture of hepatic/neurological picture, haemolysis in six and eight had died before a definitive diagnosis was made. No patients treated presymptomatically were involved. The principal cause of death was late diagnosis, followed by poor compliance, the onset of malignant disease and accidents together with the small number who failed to respond to any form of treatment.

My personal approach to treatment has remained to start with penicillamine and cover the first 3 months with alpha tocopherol, as a free radical scavenger. Pyridoxine is only needed in growing children, pregnancy and intercurrent illness; 50mg once a week is sufficient (Gibbs and Walshe, 1966) (Fig. 5).

If there is an initial sharp increase in symptoms penicillamine must be stopped and treatment changed to trientine. BAL can be used for patients with severe dystonia and can on occasion reverse an apparently progressive illness. Tetrathiomolybdate is not commercially available in this country and its future use in therapy remains in doubt, however it can be of use in severely disabled patients who are responding poorly to chelation treatment. Personally, I have little experience with zinc therapy and, as detailed above, I have been disappointed with the response to this drug in the few patients in whom I have used it.

It must be stressed that all available treatments for Wilson’s disease have been introduced as the result of work motivated and led by one researcher or a small group of academics. None can be laid at the door of the giant multi-national pharmaceutical companies though, no doubt, they have made profits from taking on the manufacture of these discoveries without having to suffer the expense of research and development or of obtaining a product licence. First, BAL was designed by the Oxford team of Peters, Stocken and Thompson. Then came the introduction of penicillamine, followed by Schouwink’s discovery of the ability of zinc salts to block copper absorption from the gut. In the 1970s, there was the introduction of trientine, first suggested by Hal Dixon and pioneered for clinical use in Cambridge, followed by liver transplantation and, finally tetrathiomolybdate, now heavily backed by Brewer in the USA (Brewer et al., 1991). Presumably the ultimate treatment will be based on gene manipulation, but this seems distant at present. It is hoped that this saga will be followed by similar developments in other single gene mutational diseases of the nervous system.

References


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