

Historical Perspectives on Penicillamine

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In 1942 Abraham and his colleagues[1] discovered that acid hydrolysates of penicillin gave a strong blue-violet coloration with the ninhydrin reagent. They also found that about 59 per cent of the total nitrogen of the pure penicillin preparation could be estimated as amino-nitrogen after one hour's hydrolysis with N/10 sulphuric acid using the Van Slyke method. These observations indicated that the substance responsible for the results of the ninhydrin and Van Slyke amino-nitrogen reactions was an integral part of the penicillin molecule. The following year they were able to report its isolation as a crystalline hydrochloride[2]. To this amino acid, obtained as a degradation product by acid hydrolysis of penicillin, they gave the name penicillamine. The chemical structure was determined by Chain in 1949[3]. It is closely related to the natural amino acid cysteine, with a sulphhydryl group on the beta carbon atom of the carbon chain in which two methyl groups replace the hydrogen atoms in the beta carbon position (Figure 1). An asymmetric carbon atom is present in the penicillamine molecule producing D, L or DL isomers—the letters referring to its steric configuration and not to the direction of its optical rotation, as the D form is not

necessarily dextro-rotatory. Since animal experiments have shown that the L-enantiomorph is the most toxic, only D-penicillamine, either as the hydrochloride or free amino acid, is now used therapeutically in man.

Animal Toxicity

Acute Toxicity

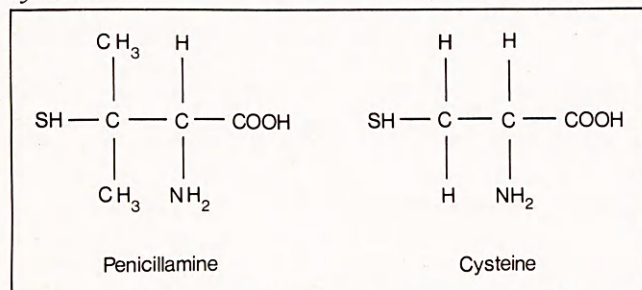
The LD₅₀ of DL-penicillamine for the rat was 365 mg/kg body weight given orally in a single dose. Death usually occurred within 24 hours following running fits, shrieking, salivation and convulsions. D-penicillamine produced no toxic signs even when administered at a dose of 1200 mg/kg[4]. When DL-penicillamine, dissolved in 0.9 per cent saline, was given intraperitoneally, the LD₅₀ was calculated to be 334 mg/kg, death usually occurring within 3 to 12 hours. Experiments conducted at Dista Laboratories confirmed that oral D-penicillamine was not lethal to most rats though some died following a dose of 10,000 mg/kg body weight (W. H. Lyle, 1979, personal communication). The oral LD₅₀ for L-penicillamine, however, was 400 mg/kg body weight. When these animals were injected with penicillamine intraperitoneally, the LD₅₀ for the L-enantiomorph was 350 mg per kg, while up to 660 mg/kg body weight of D-penicillamine failed to kill them.

Antipyridoxine Activity

Wilson and du Vigneaud (1950)[5] investigated the role of penicillamine as a possible metabolic antagonist. They found that the addition of L-penicillamine to the diet of young rats inhibited their growth, and frequently produced convulsions, 37 of the 42 animals dying within 10 weeks.

In 1957 Kuchinskas and du Vigneaud[6] observed that skin changes in the rats were similar to those reported for vitamin B6-deficient animals. Addition of pyridoxine to the diet reversed this growth inhibition and other ab-

Fig. 1. The structural formula of penicillamine, (molecular weight 149), a sulphur containing amino acid (beta, beta-dimethyl cysteine), a structural analogue of cysteine.



normalities. They also observed that rats given L-penicillamine excreted large amounts of xanthurenic acid after a test dose of tryptophan, the excretion being suppressed when pyridoxine was administered simultaneously. This led them to speculate that the toxic effects of L-penicillamine were mainly due to the drug reacting with pyridoxal-5-phosphate to form a thiazolidine compound, which reduced the activity of the coenzyme in its metabolic role. These observations have been confirmed by other workers[7-9]. Though D-penicillamine similarly reacted with pyridoxal phosphate and biochemical evidence of B6 deficiency was detected in rats following administration of this isomer[10], it was a less potent pyridoxine antagonist than the L-isomer[5, 11].

Lathyrogenic Properties

Another consequence of feeding penicillamine to rats was dermolathyrisms[12, 13], produced by both the D and DL-isomers, though mainly the latter. Experiments showed the induction of skin fragility, inhibition of wound healing and a decrease in tensile strength, accompanied by a marked increase in the amount of neutral salt soluble collagen and a drop in insoluble material. Similar effects on wound healing were observed by other workers in young rats[14, 15] and guinea-pigs[16]. Hoffman and his colleagues[17] showed that DL-penicillamine reduced the elastic properties of rat lung, while in the rabbit it increased lung compliance, and inspiratory and vital capacities[18]. This effect of penicillamine has also been demonstrated in tendon, but not in blood vessels or the gut[19]. These investigators concluded that penicillamine prevented cross-linking of newly synthesised collagen and to a lesser extent depolymerised incompletely cross-linked insoluble collagen. This probably occurred by interaction of the drug with the aldehydes present in the tropocollagen[20]. Osteolathyrisms has also been induced by penicillamine in weanling rats[21, 22], and in the fetus by feeding the drug to pregnant rats[23]. Jacobus *et al.*[24] observed that the dosage required to produce the bony lesions was far in excess of that which was employed clinically.

Early Clinical Application in Man

Chelation of Heavy Metals

Walshe (1953)[25] in a study of disturbances of amino acid metabolism following liver injury, was the first to identify penicillamine in the urine of patients with advanced liver disease receiving parenteral penicillin therapy. Since this compound was excreted in the reduced (-SH) state he deduced that the thiol group might prove active in the chelation of copper. Further investigations confirmed that penicillamine prompted the urinary excretion of copper, both in healthy people and in patients with Wilson's disease[26, 27].

The principal advantage of penicillamine over dimercaprol was that it retained its activity when given by mouth and was tolerated in larger doses calculated on the basis of available -SH groups. Penicillamine forms

chelate with other divalent cations such as lead. Boulding and Baker (1957)[28] first demonstrated that administration to patients with lead poisoning induced a urinary output of over 2000 µg of lead per litre, with striking remission of symptoms.

Disulphide Exchange with Cystine

Tabachnick and his co-workers (1954)[29] observed that penicillamine reacted readily with cystine with the formation of a mixed disulphide which they designated penicillamine-cystine. It was not until almost ten years later that this observation was used in the treatment of patients with cystinuria[30]. The excessive urinary excretion of cystine was shown to be reduced or abolished according to the dose of penicillamine administered. The resulting penicillamine-cystine disulphide in the urine was more soluble than cystine and offered a new therapeutic approach to the prophylaxis of urolithiasis in cystinuria. In fact, penicillamine produced dissolution of cystine calculi in these patients when added to a previously ineffective conventional therapeutic programme of forced fluids and alkali[31].

Effects in Rheumatoid Disease

Humoral Immune Effects

Deutsch and Morton (1957)[32] showed that human serum macroglobulins could be dissociated *in vitro* by sulphhydryl (-SH) compounds such as mercaptoethanol and cysteine. The following year depolymerisation of IgM rheumatoid factor was produced by these reducing thiol compounds[33]. Soon after, Ritzman *et al.* (1959)[34] discovered that penicillamine worked similarly when given to a patient with Waldenström's macroglobulinaemia, decreasing serum viscosity and abolishing gel formation.

It was Jaffe who in 1962[35] demonstrated the dissociation of rheumatoid factor in synovial fluid when penicillamine was injected into the knee joint of a patient with rheumatoid arthritis. In 1963[36] he proved that oral administration in such a patient produced a consistent reduction of circulating rheumatoid factor, accompanied by clinical improvement, but only after a latent period of a month on drug treatment. He also reported a beneficial response in a case of rheumatoid arteritis[37] and a return to normal values of laboratory parameters of disease activity[38]. While Jaffe's original observation[36] that penicillamine did produce intravascular depolymerisation of rheumatoid factor was valid, the persistence of reduced titres for months after drug withdrawal made this rationale of therapy untenable. Jaffe[39, 40] also observed a drug-induced fall in the levels of IgG and IgM. Other workers confirmed that all major immunoglobulin classes fell towards normal in treated patients[41-43]. The immunoglobulin changes however, have shown no correlation with clinical improvement[43-45]. Jaffe (1975)[40], reviewing the effect of penicillamine on immune complexes, observed that they declined after treatment. These effects could be mediated by a selective inhibition of immunoglobulin

synthesis or be secondary to a basic effect of the drug on the presumed primary antigenic stimulus of the disease process.

Effects on Lymphocyte Function

Roath and Wills (1974)[46], investigating the effects on DL-penicillamine *in vitro* on cultured human lymphocytes stimulated with phytohaemagglutinin (PHA), noted a dose related inhibition of lymphocyte transformation. Schumacher *et al.* (1975)[47] have shown that pre-exposure of mouse spleen T-cells to D-penicillamine *in vitro* resulted in striking inhibition of the cellular immune response as measured by the sheep erythrocyte rosette technique. They also observed drug induced inhibition of human lymphocytes stimulated by soluble mitogens[48]. This inhibition of PHA-induced lymphocyte transformation was confirmed by Maini and Roffe (1977)[49], but only after using high D-penicillamine concentrations. However, augmentation of PHA responses was observed at lower drug concentrations in cultures of diluted whole blood from healthy and untreated rheumatoid patients, compared with inhibition in penicillamine-treated patients. They speculated that the therapeutic action of the drug might be through modulation of T-cell function *in vivo*. Kendall and Hutchins (1978)[50], experimenting on mouse spleen cells stimulated in culture by concanavalin A, observed that the true effect of penicillamine was an enhancement of lymphocyte transformation. This effect was reversed when it was in the medium long enough to deplete the cystine supply, the rate of this depletion being dependent on the penicillamine concentration. Hence any apparent inhibition after several hours was caused by this secondary mechanism.

Immune Effects in Animal Models

Altman and Tobin (1965)[51] observed suppression of the primary immune response in rabbits treated with DL-penicillamine beginning one day prior to sensitisation with human serum albumin. This contrasted with their earlier study in which the drug, if administered for 28 days before sensitisation, resulted in an accelerated immune responsiveness[52]. Hubner and Gengozian[53], using mice treated with the drug for three weeks before typhoid injection, showed a depression of the humoral antibody response. Liyanage and Currey (1972)[54], on the other hand, found no difference in inhibition of immune responses between controls and treated rats.

Penicillamine has also not been shown to have any consistent effect in conventional animal models of inflammation, such as adjuvant-induced arthritis[55]. Klamer *et al.* (1968)[56] showed a modest inhibition by DL-penicillamine in rats; Liyanage and Currey (1972)[54] demonstrated failure of D-penicillamine to modify adjuvant arthritis in their animals. Arrigoni-Martelli and Bramm (1975)[57], while noting no effect on the primary reaction to the adjuvant injection, observed an enhancement of the secondary lesions when D-penicillamine was administered from day 15 to day 30

following adjuvant injection. They stressed the importance of the dosing regime used in their experiments, secondary lesions generally being accepted as an expression of cell-mediated immunity[58].

Arrigoni-Martelli *et al.* (1976)[59] using the rat paw pertussis model first described by Willoughby (1966)[60], confirmed that D-penicillamine enhanced the cell-mediated response, while indomethacin suppressed it. This model, therefore, may be useful to distinguish the effects of anti-inflammatory drugs from those like penicillamine which have a specific anti-rheumatoid activity. Similar results were obtained when inflammation was induced in sensitised animals by the intrapleural injection of pertussis vaccine[61].

Recently, Hunneyball *et al.* (1977)[62] reported inhibition of synovitis by oral administration of D-penicillamine in a different animal model—chronic antigen-induced experimental arthritis in rabbits[63]. Since T-cell-mediated immunity is of importance in this model of experimental immune synovitis[64], their results indicate that penicillamine might be acting at the cellular level.

Metabolism in Man

Gibbs and Walshe (1971)[65] studied the fate of orally administered ³⁵S DL-penicillamine in six patients with Wilson's disease. Absorption from the gut was rapid, with a peak blood level one hour after ingestion followed by a rapid fall in concentration, though there were detectable amounts for up to 48 hours. Initially, clearance from the plasma approximated that of creatinine, and a mean of 73 per cent of the total dose was excreted in the urine by 24 hours. After this, because the drug became protein-bound, very little urinary excretion occurred. However, it was recoverable in the urine for three months after administration, suggesting binding to body constituents with a slow turnover rate[66]. Autoradiographic studies showed that the drug was excreted mainly as free penicillamine and penicillamine-cysteine disulphide. In cystinuric patients, apart from the mixed disulphide[67], chromatographic analysis indicated that the drug was excreted in the urine as penicillamine disulphide[68]. Perret *et al.* (1976)[69] identified a further, though minor, urinary metabolite on the chromatogram as S-methyl D-penicillamine. A balance study revealed that the only significant faecal metabolite was the internal disulphide and the overall recovery in urine and faeces averaged 47 per cent[70]. The deficit of about 50 per cent was explained by using ¹⁴C-D-penicillamine in a normal subject. Only half the dose was absorbed from the gut, some 35 per cent being excreted in the faeces over a three-day period, the deficit of 15 per cent probably resulting from counting errors.

Some Clinical Applications of Limited Value

Scleroderma

Harris and Sjoerdsma (1966)[71] were the first to demonstrate the effect of D-penicillamine on human collagen obtained by punch biopsy of the skin. They

presented evidence that penicillamine-treated patients with Wilson's disease, cystinuria and rheumatoid arthritis had increased solubility of dermal collagen and decreased intramolecular cross-linking. They noted that concentrations of soluble collagen in the dermis of untreated patients with scleroderma were below normal and mooted the possible clinical application of the drug in this condition. Fulghum and Katz (1968)[72] treated five patients with advanced systemic sclerosis with a maximum of 4 g D-penicillamine daily for up to 11 months. They observed no improvement of joint mobility, while adverse effects occurred in all patients. Other workers similarly obtained little or no clinical response to the drug[73, 74]. Winkelmann *et al.* (1971)[75], using lower doses in 10 patients for up to 18 months, observed that five patients were subjectively better, with softer skin and decreased stiffness, while two patients had an improvement in pulmonary function as measured by an increase in tidal volume. Despite these generally disappointing results, Moynahan (1973)[76] reported benefit in the treatment of patients with morphea (localised scleroderma). The following year he described[77] the successful use of penicillamine in 14 cases of morphea in children as well as patients with keloid. Herbert and his co-workers (1974)[78] found a high proportion of reducible aldimine cross-links only in patients with active scleroderma. Since D-penicillamine could cleave the labile cross-links of newly synthesised collagen, drug therapy might be useful only in active cutaneous disease. This view was reinforced by Jayson *et al.* (1977)[79] who concluded that D-penicillamine was of limited value for the cutaneous features of progressive systemic sclerosis but probably of no value for the vascular and visceral manifestations of the disease.

Primary Biliary Cirrhosis

In a study of the relation between cirrhosis and trace metal content of the liver, Hunt *et al.* (1963)[80] discovered that in primary biliary cirrhosis the mean copper content of the liver was 30 times greater than normal. Administration of penicillamine to two patients with advanced disease showed a marked increase in urinary copper excretion, though both subsequently died. Leeson and Fourman (1967)[81] in a single case report on a patient with primary biliary cirrhosis and renal tubular acidosis described the response to penicillamine prescribed six months before she died. There was a fall in serum copper and the urinary excretion of copper almost doubled. These workers argued that earlier treatment in this condition might retard its evolution. In 1977, Jain *et al.*[82] reported a randomised controlled trial of D-penicillamine in primary biliary cirrhosis. They suggested that accumulation of hepatic copper in this disorder may damage hepatocytes and provoke collagen synthesis. In their study 19 patients received the active treatment and 13 received placebo. Some of the patients treated with penicillamine showed a significant reduction in serum aspartate transaminase concentrations and a fall in mean liver copper concentration. Liver histology demonstrated an im-

provement in cholestasis but the histological stage of the disease remained similar in both groups. They concluded that D-penicillamine might help by chelating copper, though whether it could prevent collagen deposition in primary biliary cirrhosis was unpredictable, since the type of cross-linking of this collagen was unknown.

Active Chronic Hepatitis

Alexander and Kludas (1969)[83] used D-penicillamine hydrochloride in two patients with active chronic hepatitis, with good improvement, as did Wiontzek (1970)[84] in a single case. In 1971 Lange *et al.*[85] and Schnack[86] showed improvement in biochemical tests of liver function and histology in their treated patients. Subsequently, Alexander and Willie (1974)[87] confirmed beneficial effects in 15 of their 34 patients, though cirrhotic changes were uninfluenced. Promising results were reported by Wildhirt in 60 patients, with biochemical improvement in 38 per cent of cases[88]. Nevertheless, he cautioned that adverse effects were an important limitation of treatment in a quarter of the patients.

In 1977, Stern *et al.*[89] in a controlled trial, randomly allocated D-penicillamine to 18 patients, comparing it with prednisolone in 17 patients receiving maintenance therapy after the disease had been brought under biochemical control with larger doses of corticosteroids. Penicillamine treatment was associated with a greater frequency of adverse effects and there were no significant differences in liver function tests between the two groups of patients remaining after one year. However, they thought penicillamine seemed a useful alternative treatment if prednisolone was contra-indicated or if it failed to control the disease.

Adverse Effects

Since the early American experience that clinical use of synthetic DL-penicillamine was associated with greater toxicity than the D isomer[90, 91] only D-penicillamine, as prepared from penicillin in this country, has been used. It is generally recognised, particularly in the treatment of rheumatoid disease, that lower and more gradually established maintenance doses reduce the frequency of adverse reactions[92-94]. Nevertheless, these constitute the biggest drawback of the drug and careful monitoring of patients is required[95].

Hypogeusia

In 1965 Jaffe[38] reported an alteration or impairment in the sense of taste of his rheumatoid patients treated with D-penicillamine. This occurred in approximately 25 per cent of the arthritis patients, compared with only 4 per cent of those with Wilson's disease[92]. It was thought that penicillamine-induced copper deficiency, in patients other than with Wilson's disease, might be responsible for decreased taste acuity[96]. However, Jaffe (1968)[92] observed this to be a reversible phenomenon, whether or not the drug was discontinued. Moreover, copper sup-

plements made no significant difference and did not prevent taste loss[97].

Skin

Various types of skin eruptions have been described, the commonest being sensitivity reactions in 30 per cent of patients[98], occurring in the early days or weeks of treatment. These early rashes could be managed by temporary withdrawal of penicillamine and restarting the drug under steroid cover if necessary[99, 100]. A less common late rash occurred after six months or more of treatment[101]. It consisted of itchy, scaly lesions, unresponsive to local steroid therapy, taking weeks to disappear after stopping the drug. Another type of skin lesion was reported in patients with Wilson's disease who received more than 2 g of penicillamine daily[99]. The affected skin, especially over pressure points, was friable, with bleeding following slight trauma. Various bullous lesions have been described as epidermolysis bullosa[102, 103] and pemphigus[104, 107]. Beer and Cook[102] postulated that hyaluronidase, which is inhibited by copper, was activated when tissue levels of this metal were depressed by penicillamine therapy, leading to these skin lesions. Scheinberg (1968)[98], however, suggested they were probably due to the effect of the drug on skin collagen, first described by Nimni and Bavetta (1965)[12].

Systemic Lupus Erythematosus and other Autoimmune Syndromes

Since Walshe (1968)[108] reported two patients with Wilson's disease who developed a positive LE cell test on penicillamine, other authors have had a drug-induced syndrome, with fever, rash, arthralgia, pericarditis and pleurisy, not only in patients with Wilson's disease[109, 110] but also cystinuria[111] and rheumatoid arthritis[112]. Some of these workers speculated that penicillamine, acting as a hapten, combined with epidermal proteins that contain cystine and induced cellular sensitisation[109, 111]. This rare syndrome has been characterised by raised titres of circulating antinuclear and even anti-DNA antibodies[113]. Penicillamine has also been associated with cases of polymyositis[114], myasthenia gravis[115], thyroiditis[116] and Goodpasture's syndrome[117]. These immunologically dependent adverse effects, including pemphigus[118], associated with circulating autoantibodies, have usually resolved on discontinuation of the drug.

Kidney

Proteinuria was first recognised as an adverse effect of the DL racemic mixture[119, 120, 91], though it was not long before it was detected in patients taking only the D isomer[121, 122, 92]. The frequency of this complication occurred in some 20 per cent of patients with long-term therapy[97]. Studies of renal biopsies by light microscopy[123] and both electron microscopy and

immunofluorescence[124] suggested an immunologically induced nephropathy. Several workers confirmed an immune complex disease resembling membranous glomerulonephritis[125-127] though penicillamine itself has not been detected in the lesion. Withdrawal of the drug usually led to insignificant traces of proteinuria within 12 months, even in those patients who had developed the nephrotic syndrome[128].

Blood

Perhaps one of the most common and potentially serious adverse effects described has been thrombocytopenia due to bone marrow suppression[95]. The Multicentre Trial Group noted a mean fall of 27 per cent in the platelet count, though recovery was rapid on stopping the drug[97]. Thrombocytopenia was often dose related and patients could sometimes be successfully maintained on low doses[101]. Leukopenia may also occur[98] and has even caused death[129]. Though there have been cases of full recovery from agranulocytosis[130] and aplastic anaemia[131], blood dyscrasias accounted for 14 of 18 reported fatalities[132]. It is thought that patients previously treated with gold may be more likely to develop such adverse effects when penicillamine is prescribed[133, 134].

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