EFFECT OF PENICILLAMINE ON SERUM IRON

BY

J. M. WALSHE and VALERIE PATSTON

From the Department of Experimental Medicine, University of Cambridge, and the Biochemical Section, John Bonnett Clinical Laboratories, Addenbrooke's Hospital, Cambridge

(RECEIVED FOR PUBLICATION MARCH 9, 1965)

In recent years there has been an increasing interest in toxic reactions to drugs, and a number of these have been reported following the treatment of Wilson's disease with penicillamine, particularly the D-L racemic mixture. Purified L-penicillamine has been shown to be a pyridoxine antimetabolite in rats (Kuchinskas and du Vigneaud, 1957; Kuchinskas, Hovarth, and du Vigneaud, 1957) and more recently evidence for this activity has been reported in man (Tu, Blackwell and Lee, 1963). The nephrotic syndrome and renal failure have also been reported following the use of D-L penicillamine (Adams, Goldman, Maxwell and Latta, 1964; Yonis and Karp, 1963) and also bone-marrow depression (Sternlieb and Scheinberg, 1964). Originally D-penicillamine was thought to be completely non-toxic but recently there have been suggestions that even this isomer, in large doses, can also act as a pyridoxine antagonist not only in rats* (Asatoor, 1964; Gibbs and Walshe, 1965) but also in man (Jaffe, Altman, and Merryman, 1964), though work in this laboratory (Gibbs and Walshe, 1965) does not support the latter observations. Surprisingly there has been little mention of the possible action of penicillamine depleting the body of other divalent cations besides copper, though zinc has received some attention (Schouwink, 1961). It has been shown that a significant fall in the serum copper occurs in patients with Wilson's disease after less than a year's treatment (J. M. Walshe, 1964, unpublished observations). Boulding mentioned the possibility of metal depletion (Boulding, 1961) and suggested that a 'trace metal cocktail' should be given weekly to all patients on maintenance doses of penicillamine. However, there are no published reports to suggest that this precept has been followed, and none of the reports on the use of penicillamine in the treatment of Wilson's disease have included any data on serum iron levels (Sternlieb and

Scheinberg, 1964; Richmond, Rosenoer, Tompsett, Draper, and Simpson, 1964; Goldstein, Randall, Gross, Rosevear, and McGuckin, 1962; Walshe, 1960). We have therefore investigated the effect of maintenance doses of penicillamine, given to patients with Wilson's disease, on the concentration of serum iron, iron-binding capacity, and haemoglobin formation, and compared these with the changes in the serum copper and clinical status of the patients.

Material and Methods

Ten patients with Wilson's disease (3 male and 7 female) were selected because samples of their serum before the start of treatment were available in the deep freeze; all were clinically and biochemically typical of Wilson's disease and all had Kayser-Fleischer rings at the start of treatment. The mean duration of time between tests was 19.2 months.

Serum iron and iron-binding capacity were estimated by the methods of Ramsay (1957a, b) and serum copper by a modification of the dithiocarbamate method already described (Walshe, 1963). All patients received D-penicillamine in doses varying from 900 to 1,800 mg. daily.

Results

The details are given in the accompanying Table. Briefly it can be seen that there was no significant change in the mean concentration of iron in the serum, or in the mean haemoglobin values. There was, however, a fall in the percentage saturation of the transferrin, which was due to a rise in the total iron-binding capacity of the sera from 377 $\mu\text{g.}/100$ ml. to 479 $\mu\text{g.}/100$ ml. Two patients call for special mention, J.Ba. and A.W. When J.Ba. was seen at one of her routine visits 29 months after she had started penicillamine treatment she was found to have severe hypervitaminosis D, with vomiting, weight loss, a raised blood urea, and polyuria, and this probably accounted for her low haemoglobin which rapidly returned to normal when the hypercalcaemia was controlled by vitamin D withdrawal

* Dose in rats was 300 mg./kg., equivalent to approximately 20 g./day for an adult patient.

TABLE
SERUM IRON AND COPPER LEVELS AFTER PENICILLAMINE

Subject	Age at First Test (yr.)	Sex	Months Between Tests	Penicillamine Dose (mg./day)	Serum Fe (μ g./100 ml.)	Saturation of Transferrin	Haemoglobin (g./100 ml.)	Serum Cu (μ g./100 ml.)	Response to Treatment
L.H.	10	F	4	1,000	136 109	83 30	11.1 12.5	42 28	Symptom free
W.McC.	42	M	7	1,350	138 114	42 25	14.8 —	50 20	Symptom free
C.B.	17	F	8	1,350	93 111	20 17	14.3 13.2	97 4	Much improved
J.H.	13	F	18	1,350	122 148	28 33	13.2 13.0	33 19	Symptom free
N.Y.	15	F	24	1,800	112 154	32 30	— 15.0	17 6	Much improved
F.Ty.	15	F	26	1,200	175 89	42 11	14.5 13.4	45 13	Symptom free
A.K.	14	M	27	900	147 100	40 23	13.2 14.0	32 17	Much improved
H.L.	15	F	28	1,350	111 102	— 26	12.7 14.0	38 17	Symptom free
A.W.	22	M	34	1,200	37 93	8 20	— 14.4	56 24	Much improved
J.Ba.	26	F	29	900	94 116	23 46	13.4 8.0	44 33	Much improved
			6	1,350	— 61	— 29	— 11.7	— 21	
Means			19.2	1,250	116.5 108.8 $\pm$ 11.8 $\pm$ 7.8	35.2 26.4	13.4 12.9	45.3 18.4 $\pm$ 6.67 $\pm$ 3.0	
Normal range					80-180 male 60-160 female	30-45 adult		90-150	

and steroid therapy. The relatively rapid synthesis of haemoglobin in a patient with a history of prolonged vomiting, weight loss, and renal impairment may well have taxed the body stores of iron leading to the subsequent fall in the concentration of this metal in the serum to 61 μ g./100 ml. 6 months later. During this time no iron preparation was given and penicillamine was continued at an increased dosage. The very low serum iron found in A.W. before the start of penicillamine could have been due to previous treatment with BAL but was more probably due to a chronic staphylococcal osteomyelitis of the spine which led to his admission to hospital. Again the serum iron levels returned to normal without additional iron treatment. Contrary to the apparent stability of the serum iron in the face of chelation therapy, there was a sharp and significant fall in the serum copper from 45.3 ± 6.7 μ g./100 ml. to 18.4 ± 3.0 μ g./100 ml. Corresponding with the fall in the serum copper there was a marked clinical improvement in all patients whether the symptoms were neurological (J.Ba., C.B., J.H., W.McC., H.L., A.K., A.W., and N.Y.) or hepatic (L.H. and F.T.).

Discussion

These results leave little doubt that the serum iron and haemoglobin formation are not significantly affected by prolonged treatment with D-penicillamine, either in children, adolescents, or adults. In only one patient was there a fall in the concentration of serum iron to the lowest level of the normal range as determined by the method employed (60 to 160 μ g./100 ml. for females), and in this case the clinical picture was obscured by moderately severe vitamin D intoxication. Similarly, except for this one patient, haemoglobin levels were also well main-

tained. There was, however, a fall in the percentage saturation of the transferrin due to a rise in the serum concentration of this protein. While this could have been an indication of slow progression of the liver lesion of Wilson's disease, it seems unlikely in view of the general clinical improvement of all the patients, even those presenting with liver damage. Corresponding with the clinical improvement there was a return to normal of the serum flocculation tests and transaminases. On the other hand all patients showed a progressive and highly significant fall in the serum copper levels, and these observations, taken in conjunction with findings of a fall in the serum caeruloplasmin reported elsewhere (Walshe, 1964), leave little doubt that this treatment is effective in depleting the abnormal body stores of copper which are found in this disease. Failure to achieve and maintain such a fall in the serum copper and caeruloplasmin (cf., Richmond *et al.*, 1964) probably means inadequate maintenance dosage of D-penicillamine and re-accumulation of excess copper stores. It is interesting that, as with iron, penicillamine does not appear to be able to deplete the body of copper for normal requirements of caeruloplasmin formation (Walshe, 1964) or in the formation of hair (Gibbs and Walshe, 1965). Thus the stability of iron stores for normal haemoglobin formation does not necessarily mean that the pathological accumulations which are found in haemochromatosis or haem siderosis would necessarily be resistant to treatment with penicillamine. However, the therapeutic possibilities of penicillamine in these conditions seem to have attracted remarkably little attention, which is perhaps surprising as there is still no effective chelating agent for iron which is active when given by mouth (Gross, 1964).

Summary

We have studied the effect of maintenance doses of D-penicillamine (mean 1,250 mg./day for 19.2 months) on the concentrations of serum iron and copper and on the haemoglobin values of 10 patients with Wilson's disease.

The mean serum iron before the start of treatment was 116.5 µg./100 ml. and after treatment it was 108 µg./100 ml. There was also a small fall in the level of haemoglobin from 13.4 to 12.9 g./100 ml.

There was a fall in the percentage saturation of the transferrin from 35.2 to 26.4% due to a rise in the total iron-binding capacity from 377 to 479 µg./100 ml.

There was a highly significant fall in the concentration of mean serum copper from 45.3 µg./100 ml. to 18.4 µg./100 ml., which corresponded with a marked clinical improvement in all cases.

It is concluded that D-penicillamine does not deplete essential body stores of iron.

We wish to thank all those physicians who have most generously allowed us to study patients under their care.

REFERENCES

- Adams, D. A., Goldman, R., Maxwell, M. H., and Latta, H. (1964). Nephrotic syndrome associated with penicillamine therapy of Wilson's disease. *Amer. J. Med.*, **36**, 330.
- Asatoor, A. M. (1964). Pyridoxine deficiency in the rat produced by D-penicillamine. *Nature (Lond.)*, **203**, 1382.
- Boulding, J. E. (1961). Treatment of Wilson's disease with D-penicillamine. In *Wilson's Disease: Some Current Concepts*, ed. J. M. Walshe and J. N. Cumings, pp. 224-226. Blackwell, Oxford.
- Gibbs, K., and Walshe, J. M. (1965). Copper content of hair in normal families and those with Wilson's disease. *J. med. Genet.*, **2**, 181.
- Goldstein, N. P., Randall, R. V., Gross, J. B., Rosevear, J. W., and McGuckin, W. F. (1962). Treatment of Wilson's disease (hepatolenticular degeneration) with DL-penicillamine. *Neurology (Minneapolis)*, **12**, 231.
- Gross, F. (1964). *Iron Metabolism: an International Symposium*. Springer, Berlin.
- Jaffe, I. A., Altman, K., and Merryman, P. (1964). The antipyridoxine effect of penicillamine in man. *J. clin. Invest.*, **43**, 1869.
- Kuchinskas, E. J., and du Vigneaud, V. (1957). An increased vitamin B₆ requirement in the rat on a diet containing L-penicillamine. *Arch. Biochem.*, **66**, 1.
- , Hovarth, A., and du Vigneaud, V. (1957). An anti-vitamin B₆ action of L-penicillamine. *ibid.*, **68**, 69.
- Ramsay, W. N. M. (1957a). The determination of iron in blood plasma or serum. *Clin. chim. Acta*, **2**, 214.
- (1957b). The determination of total iron-binding capacity of serum. *ibid.*, **2**, 221.
- Richmond, J., Rosenoer, V. M., Tompsett, S. L., Draper, I., and Simpson, J. A. (1964). Hepato-lenticular degeneration (Wilson's disease) treated by penicillamine. *Brain*, **87**, 619.
- Schouwink, G. (1961). *De Hepato-cerebrale Degeneratie*. G. W. Van der Wiel, Arnhem.
- Sternlieb, I., and Scheinberg, I. H. (1964). Penicillamine therapy for hepatolenticular degeneration. *J. Amer. med. Ass.*, **189**, 748.
- Tu, J., Blackwell, R. Q., and Lee, P.-F. (1963). DL-penicillamine as a cause of optic axial neuritis. *ibid.*, **185**, 83.
- Walshe, J. M. (1960). Treatment of Wilson's disease with penicillamine. *Lancet*, **1**, 188.
- (1963). Filterable and non-filterable serum copper. 1. The action of penicillamine. *Clin. Sci.*, **25**, 405.
- (1964). Endogenous copper clearance in Wilson's disease: a study of the mode of action of penicillamine. *ibid.*, **26**, 461.
- Yonis, I. Z., and Karp, M. (1963). Chelating agents in Wilson's disease. *Lancet*, **2**, 689.

studies of caries incidence with computer evaluation of the multiple factors involved would provide a more reliable picture than cross-sectional studies. Most factors, such as plaque, food debris, and calculus, and the importance of trace elements in foodstuffs, are discussed in relation to resistance.

Finn's paper reviews heredity in relation to caries resistance in human and animal families, and the possible mechanism for genetic transmission. Caries resistance in relation to individual tooth surfaces is reported by Backer-Dirks, and König deals with resistance in experimental animals and the hypothesis of immune tolerance.

Brudevold, McCann, and Grøn relate the chemistry of enamel to caries resistance and conclude that the greater resistance of the surface layers of enamel is due to a high degree of mineralization, accumulation of trace elements especially fluoride, and a low concentration of carbonates and water.

The physical features of caries-resistant teeth are

discussed by Darling, and Frank follows this with a study of the ultrastructure, using electron microscopy.

Jenkins discusses the relation between saliva, plaque, and caries-resistant enamel, and Hardwick discusses the effects of trace elements in nutrients. The bacterial flora and negative association of lactobacilli in resistant mouths is presented by Snyder, and the possible role of saliva in enamel maturation, protection, and repair is reported by Wah Leung.

The effect of diet on plaque and the metabolism of nutrients in relation to caries is dealt with by Hartles.

In this excellent symposium modern concepts and knowledge regarding resistance to caries are discussed in a series of papers embracing the various aspects of the subject. The papers are well referenced and the ensuing discussions are faithfully reported.

This is an important book for all interested in the aetiology and prevention of dental caries; the literature is well reviewed, and the need for further studies is made apparent.

Paediatric Research Society

The 7th meeting of the Paediatric Research Society (Secretary, B. D. Bower) was held at Alder Hey Children's Hospital, Liverpool on March 12 and 13, 1965.

The following papers were read:—

'Recent work on the rubella virus.'

By Kevin McCarthy.

'Acid-base abnormalities in infants with congenital heart disease.'

By Richard Jones.

'The histological approach to nephritis.' Jean Bouton.

'The value of peritoneal dialysis in children.'

By Tom McKendrick.

'Changes in the plasma protein electrophoretic pattern occurring during diuresis in acute glomerulo-nephritis.'

By John MacLaurin.

'Some observations on plasma and urine electrolyte and urea levels in the newborn.' By Constance Forsyth.

On March 12 visits were made to the Neonatal Surgical Unit, the Cardiorespiratory Unit, and the Cleft Palate Unit at Alder Hey Hospital.

Errata: Vol. 40, December, 1965

'Electroencephalographic Studies in Infants and Children with Hypothyroidism' by R. Harris *et al.*

Page 616, beginning of second column, 6th line, the sentence should have read,

'In the present study, the patients were children in whom the maturation of the central nervous system was incomplete and, as shown by Bradley *et al.* (1960) in the rat, the effect of thyroid hormone deprivation is greater on the immature brain than the mature brain from both the EEG and the histological aspects.'

'Effect of Penicillamine on Serum Iron'

Page 651. It is regretted that an unfortunate error occurred with the name of the second author of the paper 'Effect of Penicillamine on Serum Iron'. This author's name should have been Valerie Clarke, not Valerie Patston.