Effect of Penicillamine on the Kidney

by Dr A M Davison
(*Department of Renal Medicine, St James's University Hospital, Leeds*)
Dr A T Day and Dr J R Golding
(*Department of Rheumatology, St. James's University Hospital, Leeds*)
and Dr D Thomson
(*Department of Pathology, University of Edinburgh*)

The introduction of the drug penicillamine into therapeutic use (Walshe 1956) was soon followed by the demonstration of adverse effects. The early reports of undesirable side-effects may have been due to the use of DL-penicillamine, but undoubtedly D-penicillamine is by itself capable of causing toxic reactions.

Proteinuria was reported as a side-effect of the use of DL-penicillamine (Fellers & Shahidi 1959, Adams *et al.* 1964) and this has also been detected in patients taking only the D isomer (Goldberg *et al.* 1963), Rosenberg & Hayslett 1967, Luke *et al.* 1968). Commonly the proteinuria is slight, but it may be of sufficient magnitude to produce the nephrotic syndrome. The present study was undertaken to examine the nature of the nephropathy produced by D-penicillamine in patients with rheumatoid arthritis.

Materials and Methods

Urinalysis for protein and blood was performed at least at monthly intervals in patients with rheumatoid arthritis who were receiving D-penicillamine (Distamine). No patient had hæmaturia or proteinuria at the beginning of the study. D-penicillamine was normally started in a dose of 150 mg daily and increased by 150 mg increments monthly until a satisfactory clinical response was obtained. The current regime employs tablets of 125 mg penicillamine base. The dose at which proteinuria developed is shown in Table 1. There were 12 females and 8 males aged between 25 and 65 years in the group. All had had definite and classical rheumatoid arthritis for at least one year (range 1–25 years) although 6 were seronegative. They had received D-penicillamine for a mean of eight months (range 6 weeks to 60 months) and in the 11 patients who underwent renal biopsy the time interval between stopping D-penicillamine and biopsy was one to four months except in 2 cases: Case 3 who had proteinuria 12 months after stopping penicillamine and Case 7 who had hæmaturia some 23 months after stopping penicillamine.

Renal function was assessed by creatinine clearance and total daily urinary protein excretion.

Table 1
Clinical details of patients

Case	*Duration of rheumatoid arthritis (years)*	*Maximum dose of penicillamine (mg/day)*	*Duration of penicillamine treatment (months)*	*Proteinuria at time of biopsy (g/24 h)*
1	10	375	4	0.05
2	3	150	24	0.05
3	1	250	1.5	0.06
4	4	875	48	0.10
5	3	250	9	0.11
6	22	450	14	0.20
7	4	250	2	0.27
8	25	750	6	0.35
9	3	150	1.5	0.48
10	13	900	20	0.59
11	1	500	11	0.60
12	2	450	18	0.67
13	6	300	7	0.70
14	8	300	60	1.37
15	4	625	11	1.60
16	6	1000	2	2.40
17	14	500	18	2.60
18	9	1000	7	2.90
19	4	875	5	6.00
20	4	450	7	9.10

Urinary fibrin/fibrinogen concentration was measured by the tanned red cell hæmagglutination inhibition immunoassay (Clarkson *et al.* 1970). Percutaneous renal biopsy material was examined by light, immunofluorescence and electron microscopy.

Results

Proteinuria: At the time of referral the proteinuria had resolved in some 5 patients. The mean protein excretion in the remainder was 2.5 g per 24 h (range 0.2 to 9.1 g per 24 h). Only 2 patients exceeded 3 g per 24 h.

Hæmaturia: Two patients developed hæmaturia, one macroscopic. The patient with macroscopic hæmaturia was found to have a renal calculus which was removed surgically, with resolution of his symptoms. The other patient remains with microscopic hæmaturia and continues to have good renal function.

Renal function: The creatinine clearance was normal in most patients, being less than 60 ml/min in only 4.

Complement: The serum complement (C3 and C4) in all patients was within the normal range for this laboratory.

Urinary fibrin/fibrinogen degradation products: The average maximum urinary fibrin degradation product (FDP) excretion was 1.6 mg/100 ml (range 0.20–5.0 mg/100 ml).

Histology: Renal biopsy was performed in 11 patients. In one there was evidence of glomerular deposition of amorphous material with the

Table 2
Biopsy findings

Case	*Immunofluorescence*	*Electron microscopy*
3	Nil	●
5	G F	Normal
7	●	Normal
10	G	●
12	G F	+
15	G F	+++
16	G A M F	+
17	●	++
18	G C3	+++
19	●	+
20	●	+++

Immunofluorescence: G, IgG; A, IgA; M, IgM; C3, complement C3; F, Fibrin/fibrinogen
Electron microscopy: +, few granular small deposits; ++, several granular small deposits; +++, large number of dark granular deposits
●not examined

staining characteristics of amyloid (Case 3). In the remaining 10 specimens the appearances were either normal (4 cases) or a mild proliferative glomerulonephritis (6 cases). The proliferation, where present, was predominantly of mesangial cells with some slight increase in mesangial matrix.

Immunofluorescence: In 6 patients immunofluorescence examination of the biopsy was undertaken. In all 6 biopsies there was a fine granular deposition of IgG within glomerular capillary walls, associated in 4 cases with a weak granular deposition of fibrin-fibrinogen. It was difficult to localize the site of deposition of the IgG exactly, but it was probably subepithelial, whereas the fibrin appeared to be within the glomerular capillary lumen. Complement (C3) deposition was noted in one of the biopsies and had a similar distribution to IgG. In one case IgA was present and one of these also had IgM (Table 2).

Electron microscopy: Nine of the biopsies were examined by electron microscopy. In two no significant abnormality was detected. In the remaining seven there were granular subepithelial deposits with focal loss of epithelial foot processes. There was considerable variation both in size and frequency of deposits, but in general the deposits were very small, requiring careful search at high power to ensure detection. Although occupying the same position, they were both smaller and flatter than the typical 'humps' of poststreptococcal glomerulonephritis. There was no significant proliferation of mesangial or epithelial cells (Figs 1–3).

Discussion

This study confirms other findings that the administration of D-penicillamine to patients with rheumatoid arthritis is associated with the development of proteinuria in a significant number of patients (Jaffe 1965, Golding *et al.* 1970). The average time taken from the start of therapy to the appearance of proteinuria is eight months, which is in agreement with the results of other authors (Felts *et al.* 1968), but we would like to draw attention to the wide range of six weeks to 60 months. The dose of D-penicillamine at the time of onset of proteinuria is also variable, being as low as 150 mg daily to as much as 750 mg daily.

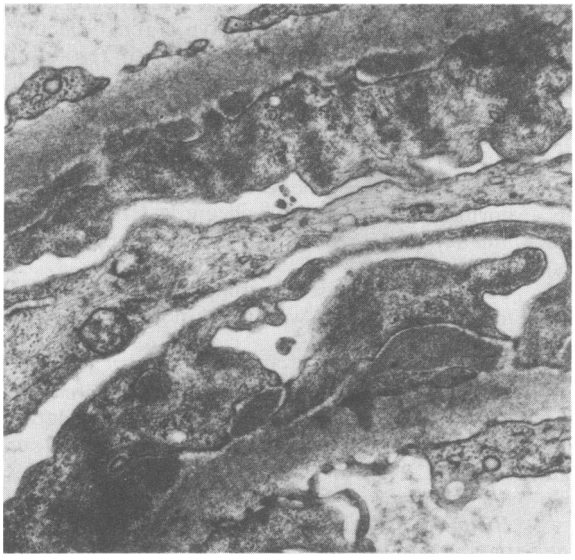

Fig 1 *Several small subepithelial deposits seen on adjacent capillary loops.* × *22 500*

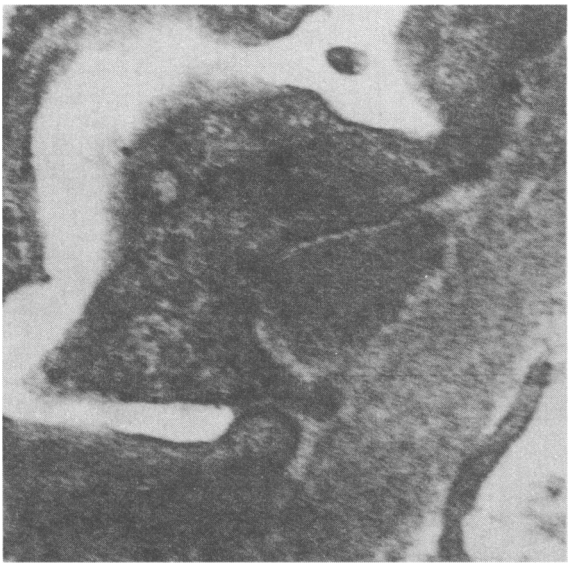

Fig 2 *Small granular deposit on subepithelial aspect of capillary basement membrane.* × *60 000*

The development of proteinuria is not associated with marked glomerular pathology. Light microscopy reveals only minor proliferative changes, but the techniques of immunofluorescence and electron microscopy reveal frequent deposition of immunoglobulins in a subepithelial site. This is in agreement with other reports (Jaffe 1968, Hayslett *et al.* 1968, Dische *et al.* 1976), in which subepithelial deposits have been detected. However, these deposits are small, and together with their subepithelial position, this probably accounts for the lack of mesangial or endothelial reaction and the relatively minor changes visible on light microscopy. The deposition of fibrin/fibrinogen within glomerular capillary walls is minimal, and this finding is supported by the presence of only small concentrations of fibrin degradation products in the urine. Unlike some previous reports (Dische *et al.* 1976) we have only detected complement (C3) deposition in one of six biopsies. The serum complement (C3 and C4) concentrations were normal in all patients, but this does not exclude an immune-complex ætiology as the serum complement only reflects the balance of the production and utilization of the various complement components. In this study there did not appear to be any relationship between the dose of penicillamine and the size or number of deposits. However, patients with the largest numbers of deposits appeared to have the greatest degree of proteinuria.

Hæmaturia has not been a common finding in these patients. In one it was due to a condition presumably unrelated to penicillamine therapy, while in the second it has not been associated with any deterioration in renal function as has been reported in other cases.

The pathogenesis of these deposits is uncertain. It is possible that penicillamine acts as a haptene with subsequent antibody formation and immune complex deposition. This would be supported by the fact that there is usually a latent period between commencing therapy and the development of proteinuria, although in some instances the latent period is excessive. To date, however, penicillamine has not been detected within the glomerular deposits. It is also possible that penicillamine acts on polymeric forms of immunoglobulins, such as rheumatoid factor, splitting them into smaller and more soluble forms which would more readily become deposited within the glomerular capillary walls. Although penicillamine can degrade immunoglobulins *in vitro* (Virella 1971), there is no evidence for this *in vivo*; against this hypothesis is in addition the fact that the proteinuria produced by penicillamine is not restricted to patients with rheumatoid arthritis but occurs in patients with cystinuria (Hayslett *et al.* 1968, Stephens & Watts 1971) and lead poisoning (Goldberg *et al.* 1963). An alternative explanation for the development of an immune complex glomerulonephritis might be that it is due to effects of penicillamine on antibody production. Some experimental animals taking penicillamine have a diminished and delayed antibody production (Altman & Tobin 1965, Hubner & Gengozian 1965). This would favour the production of small, soluble immune complexes, in conditions of antigen excess, and this

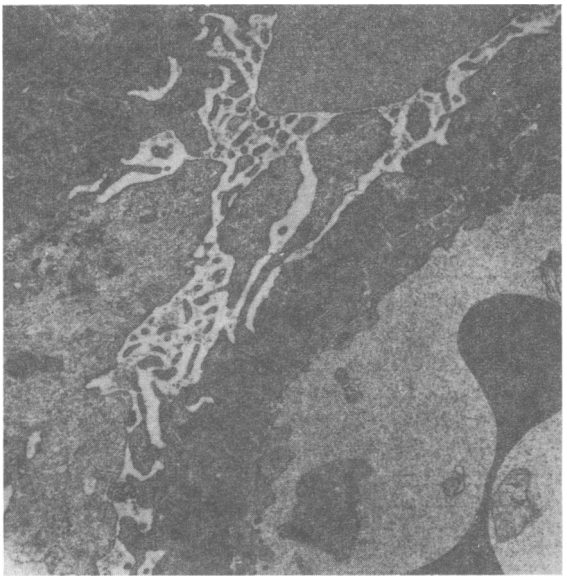

Fig 3 *In addition to small subepithelial deposits there are spikes of basement membrane protruding on the subepithelial aspect.* × *8 000*

type of complex is known to be particularly associated with glomerulonephritis (Dixon *et al.* 1961).

Summary

Twenty patients with rheumatoid arthritis developing proteinuria when being treated with penicillamine have been studied. In 5 the proteinuria was mild and resolved rapidly. Eleven of the remaining patients have undergone renal biopsy. One was found to have amyloidosis, and in the other 10 there was evidence of an immune complex type of injury, manifested by a granular immunoglobulin deposition within glomerular capillary walls associated with subepithelial deposits, found on electron microscopy. The proteinuria associated with this was mild (mean 2.6 g per 24 h) and occurred on average some eight months after commencing therapy (range 6 weeks to 60 months).

Acknowledgments: Dr S M Rajah of the Regional Blood Transfusion Service kindly performed the urinary fibrin/fibrinogen estimations.

Support from the Yorkshire Kidney Research Fund is gratefully acknowledged.

REFERENCES

Adams D A, Goldman R, Maxwell H & Latta H (1964) *American Journal of Medicine* **36**, 330
Altman K J & Tobin M S (1965) *Proceedings of the Society for Experimental Biology and Medicine* **118**, 554
Clarkson A R, Morton J B & Cash J D (1970) *Lancet* ii, 1220
Dische F E, Swinson D R, Hamilton E B D & Parsons V (1976) *Journal of Rheumatology* **3**, 145
Dixon F J, Feldman J D & Vazquez J J (1961) *Journal of Experimental Medicine* **113**, 899
Fellers F X & Shahidi N T (1959) *American Journal of Diseases of Children* **98**, 699
Felts J H, King J, Stanton J & Boyce W H (1968) *Lancet* i, 53
Goldberg A, Smith J A & Lochhead A C (1963) *British Medical Journal* i, 1270
Golding J R, Wilson J V & Day A T (1970) *Postgraduate Medical Journal* **46**, 599
Hayslett J P, Beusch K G, Kashgarian M & Rosenberg L E (1968) *Laboratory Investigation* **19**, 376
Hubner K F & Gengozian N (1965) *Proceedings of the Society for Experimental Biology and Medicine* **118**, 561
Jaffe I A (1965) *Arthritis and Rheumatism* **8**, 1064
(1968) *Postgraduate Medical Journal* **44**, October Suppl., p 15
Luke R G, Briggs J D, Felly G S & Kennedy A C (1968) *Journal of Urology* **99**, 207
Rosenberg L E & Hayslett J P (1967) *Journal of the American Medical Association* **201**, 698
Stephens A D & Watts R W E (1971) *Quarterly Journal of Medicine* **159**, 355
Virella G (1971) *Experientia* **27**, 94
Walshe J M (1956) *Lancet* i, 25

DISCUSSION

Dr R C Bucknall (*Bristol*): Bacon and others in our department have carried out similar studies to those of Dr Davison (Bacon *et al.* (1976) *Quarterly Journal of Medicine*, **45**, 661). Two findings are of particular interest: (1) That there does not seem to be any correlation between the presence of subepithelial deposits and the degree of proteinuria, in that patients with less than 1 g of proteinuria had these deposits as well as those with 3 or 4 g, and (2) Bacon carried out serial studies on some of these patients, with repeat biopsies at one year after stopping penicillamine. In spite of reduction in the amount of proteinuria, the subepithelial deposits remained present in the same amount.

Dr F E Dische (*London*): May I support Dr Davison's findings? In a rather smaller series, 5 patients who developed proteinuria had a biopsy. The degree of proteinuria was much higher in general, but the lesions were precisely the same (Dische *et al.* (1976) *Journal of Rheumatology*, **3**, 145).

However, I would diverge from Dr Davison over the question of complement deposition, because we found complement in every case. I find it difficult to correlate this with his findings. Is it possible that his reagents were not sensitive?

Dr Davison: I find it just as difficult to reconcile Dr Dische's excellent report with our findings. We have studied serum complement (C3 and C4) and found them normal in all our patients. We have found significant complement deposition in only one of the biopsies. I cannot explain the difference in our immunofluorescence findings to that reported by other groups.

Dr J Glyn (*London*): Has Dr Davison – or anyone else – tried to recover penicillamine from these complexes? In other words, have any attempts been made to identify the real evidence that penicillamine is involved in the complexes by a direct test?

Dr Davison: We have not tried to do that, though it would be technically possible. I believe much will depend on the formation of the complex.

Dr H Berry (*London*): Up to now most people have said that penicillamine lesions are unrelated to impairment of creatinine clearance. I noticed that 4 of Dr Davison's patients had impairment of creatinine clearance. Is this attributable to penicillamine in his experience, or to an increase of renal pathology?

Dr Davison: Four patients had a creatinine clearance of less than 60 ml/min. Creatinine clearances were not carried out on our patients before therapy, so it is difficult to say whether they started in a normal range and decreased to 60 ml/min. I cannot answer the question without observing what happens when they stop therapy and whether the clearance subsequently rises. There are so many causes in rheumatoid patients for an impaired creatinine clearance that it is difficult to attribute it to the drug alone.