Serum Sulphydryl Changes in Rheumatoid Coalworkers' Pneumoconiosis Patients Treated with D-penicillamine

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The pathogenesis of the complicated forms of coalworkers' pneumoconiosis has been the subject of various hypotheses. Of these, silica dust and tubercle bacilli have been thought some of the more probable etiological agents.

However, following the initial observations of Caplan (1953), who discovered an association between the incidence of rheumatoid arthritis and the presence of numerous small rounded radiological lung opacities, an autoimmune pathogenesis of the complicated pneumoconioses has been proposed. Further serological studies (Caplan *et al.* 1962) have since indicated that in addition to the 'classical' Caplan's nodules, other radiological types of progressive massive fibrosis were also associated with the rheumatoid diathesis. Immunohistological investigations (Wagner & McCormick 1967) revealed deposition of rheumatoid factor within the pneumoconiotic lesions, and thus provided further evidence of rheumatological involvement.

The use of penicillamine for the treatment of rheumatoid lung disease in patients not exposed to dust (Lorber 1966) prompted the consideration of its possible usefulness in the treatment of rheumatoid-complicated pneumoconiosis. The improvement in clinical, radiological, physiological and serological parameters was also accompanied by a corresponding rise in serum sulphydryl (SH) levels. Serum SH levels have been shown to be decreased in rheumatoid arthritis and in other connective tissue diseases (Lorber *et al.* 1964).

In initiating a study into the potential therapeutic use of penicillamine in coalworkers with rheumatoid-complicated pneumoconiosis, it was therefore thought that an investigation into the changes in serum SH levels would be of particular interest.

Materials and Methods

A total of 9 patients, divided into groups of 5 and 4, were admitted for penicillamine treatment. The criteria for selecting patients were rapidly progressive or newly developed pneumoconiotic lesions, preferably of the Caplan type, and clinical or serological evidence of rheumatoid involvement.

The treatment regime consisted of 1.2 g of D-penicillamine per day for the first three months, rising to 1.8 g/day for the final three months. Ascorbic acid (200 mg/day) and pyridoxine (40 mg/day) supplements were also given.

The patients served as their own controls, with pre- and post-treatment evaluation of selected clinical, physiological, radiological, hæmatological, serological and biochemical parameters.

Blood for serum SH determinations was collected in nitrogen-filled tubes and analysed as soon as possible the same day in order to minimize oxidative changes. Serum SH levels were measured spectrophotometrically using Ellman's (1959) reagent: 5-5′ dithiobis-(2-nitrobenzoic acid) (DTNB). The method was modified to allow discrimination between 'fast' and 'slow' reacting SH groups. 'Total' serum SH groups were measured in 10 mmol DTNB in 0.1 mol phosphate buffer, pH 7.6, and 'fast' reacting serum SH groups in 1 mmol DTNB in 0.1 mol phosphate buffer, pH 6.5, each for a 5-minute reaction time period. The level of 'slow' reacting SH was obtained by calculating the difference between the 'total' and 'fast' reacting serum SH levels.

Results

Spectrophotometric measurement of penicillamine, a low molecular weight thiol, at both pH 7.6 and pH 6.5, demonstrated virtually no

Table 1

Serum sulphydryl levels (μmol/l), mean values

		Rheumatoid coalworkers (N=9)			
	Normals (N=11)	*Level before penicillamine treatment*	*Maximal level during penicillamine treatment*	*Change in serum SH*	*Percentage of 'total' increase*
'Total' (fast+slow) reacting serum SH	471	367	520	+153	100
'Slow' reacting serum SH	449	332	470	+138	90
'Fast' reacting serum SH	22	35	50	+ 15	10

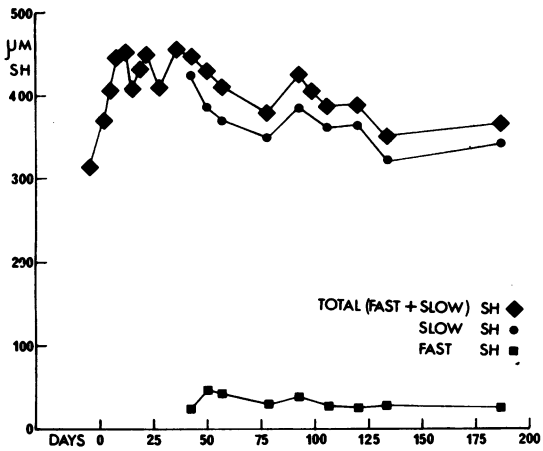

Fig 1 *Effect of penicillamine on serum SH levels. Group 1: (N=5) mean values indicated. Serum SH expressed as μmol/l*

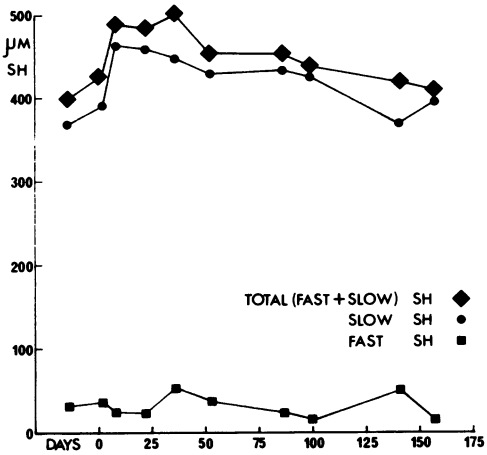

Fig 2 *Effect of penicillamine on serum SH levels. Group 2: (N=4) mean values indicated. Serum SH expressed as μmol/l*

difference in SH reactivity. Therefore, because penicillamine is equally reactive at the lower pH, it is classified as 'fast' reacting.

However, measurement of serum SH levels under the same conditions revealed that the SH reactivity at pH 6.5 was only approximately 5% of its reactivity at the more alkaline pH 7.6. Hence, about 95% of the SH groups present in serum, virtually all confined to the serum protein fraction, are classified as 'slow' reacting.

Measurement of the pretreatment serum SH levels showed the 'total' and 'slow' reacting serum SH to be considerably reduced in the patients compared to normal males of comparable age (Table 1).

Subsequent treatment with penicillamine caused an increase in 'total' serum SH, maximal levels exceeding even those of normals, being attained in periods varying from two to four weeks. Thereafter 'total' SH levels tended to decline (Figs 1 and 2).

Levels of 'fast' reacting serum SH levels remained low, despite the patients' intake of penicillamine. The predominant increase in serum SH was contributed by the 'slow' reacting SH, characteristic of the normal serum protein SH reactivity pattern and level.

Discussion

The lowering of serum SH in rheumatoid disease correlates with disease activity (Lorber *et al.* 1971) and with high rheumatoid factor titres (Kosaka 1970). Studies on the nature of these serum SH changes have shown that quantitative and qualitative alterations in the albumin fraction are predominantly responsible (Thomas & Evans 1975).

The importance of decreased albumin levels as a discriminant feature in rheumatoid disease has been emphasized by Wilding *et al.* (1975), and appears related to the increase rate of catabolic degradation (Ballantyne *et al.* 1971). It is of interest in this connexion that the formation of mixed disulphides between mercaptalbumin and cysteine causes conformational changes which may well influence the rate of degradation (Ohkubo 1969). Indeed, the characteristic micro-heterogeneity of serum albumin is related to its SH group and associated permutations in disulphide (SS) bond formation (Moore & Foster 1968), a state which may be dependent on protein bound copper (Klotz *et al.* 1955).

The significance of copper in rheumatoid disease and penicillamine therapy has long been a topic for speculation. Penicillamine has been shown to form ternary intermediates with albumin bound copper (Sugiura & Tanaka 1972), and copper chelates of penicillamine increase its anti-inflammatory properties (Sorenson 1976). A pathogenic role for copper has been suggested because of its ability to catalyse the formation of aggregates of IgG, and hence by immunostimulation, of rheumatoid factor (Gerber 1974). However, more subtle conformational changes have been detected in IgG structure in rheumatoids using circular dichroism studies (Johnson *et al.* 1974). These changes could well account for the increased catabolism of IgG that is also a feature of rheumatoid arthritis (Watkins & Swannell 1973).

SH group reactivity, also, is a useful method for probing changes in molecular conformation. Electrostatic and steric configurations in proteins directly influence the reactivity and availability

of their constituent chemical moieties. There exists, therefore, a population spectrum of SH groups of varying reactivities, each dependent on its own molecular environment. The division of SH groups into 'slow' and 'fast' reacting is obviously an oversimplified but nevertheless useful step towards understanding their metabolic significance.

The ability of nonsteroidal anti-inflammatory drugs to accelerate serum SH reactivity (Gerber *et al.* 1967), parallels their capacity to produce a similar effect on membrane SH groups (Famaey & Whitehouse 1975). Studies with the nonsteroidal drug alclofenac have revealed corresponding increases in the 'fast' reacting serum SH group category (Evans 1975). It would appear therefore that this class of drug is able to increase the reactivity of the albumin SH group by modifying its molecular environment.

The results of the present study indicate that, in contradistinction to the foregoing drugs, penicillamine administration causes an increase in the 'slow' reacting serum SH; i.e. it produces a more normal level and pattern of SH reactivities. It would be of interest to discover whether N-acetyl penicillamine and mercaptoethylamine, drugs which also increase serum SH levels but do not reduce the rheumatoid factor titre (Jaffe & Merryman 1968), do so by their contribution to the 'fast' or 'slow' reacting serum SH.

The significance of serum SH changes in rheumatoid patients treated with penicillamine, with respect to its mechanism of action, has been raised previously (Pavelka 1971). Studies on the binding of penicillamine to serum proteins (Planas-Bohne 1973) have shown that it is mostly bound to mercaptalbumin via the formation of mixed disulphides; thus one may expect a lowering of serum SH levels.

Hypotheses concerning the therapeutic role of penicillamine in the treatment of rheumatoid disease have generally emphasized its role as a metabolic inhibitor: either by dissociation or synthesis inhibition of the rheumatoid factor; or by preventing the formation of its presumed antigenic stimulus, aggregated IgG. However, the restoration of apparently normal serum SH levels both in quantity and quality suggests an alternative mechanism; as a promoter of normal molecular conformation. This would result in normalized albumin-tryptophan-binding properties (Aylward 1975) and an IgG nonantigenic structure. Such a mechanism would also be in keeping with the delayed effect that is characteristic of penicillamine treatment.

Studies on the renaturation of disulphide-linked proteins have indicated that, in the presence of decreased levels of low molecular weight thiols, an accumulation of incorrectly disulphide-linked metastable intermediates may occur (Creighton 1974). Thus it is proposed that penicillamine, either as a thiol or copper chelate, acts as a conformational catalyst in the formation of the 'correct' disulphide bonds during protein synthesis. The relative metabolic inertness of penicillamine may well be a relevant factor to its role in molecular therapy.

REFERENCES

Aylward M (1975) *Current Medical Research and Opinion* **3**, 249
Ballantyne F C, Fleck A & Carson Dick W (1971) *Annals of Rheumatic Diseases* **30**, 265
Caplan A (1953) *Thorax* **8**, 29
Caplan A, Payne R B & Withey J L (1962) *Thorax* **17**, 205
Creighton T E (1974) *Journal of Molecular Biology* **87**, 563
Ellman G L (1959) *Archives of Biochemistry and Biophysics* **82**, 70
Evans P H (1975) *Current Medical Research and Opinion* **3**, 268
Famaey J-P & Whitehouse M W (1975) *Biochemical Pharmacology* **24**, 1609
Gerber D A (1974) *Arthritis and Rheumatism* **17**, 85
Gerber D A, Cohen N & Giustra R (1967) *Biochemical Pharmacology* **16**, 115
Jaffe I A & Merryman P (1968) *Annals of Rheumatic Diseases* **27**, 14
Johnson P M, Watkins J, Scopes P M & Tracey B M (1974) *Annals of the Rheumatic Diseases* **33**, 366
Klotz I M, Urquhart J M, Klotz T A & Ayers J (1955) *Journal of the American Chemical Society* **77**, 1919
Kosaka S (1970) *Journal of Experimental Medicine* **100**, 349
Lorber A (1966) *Nature (London)* **210**, 1235
Lorber A, Bovy R A & Chang C C (1971) *Metabolism* **20**, 446
Lorber A, Pearson C M, Meredith W L & Gantz-Mandell L E (1964) *Annals of Internal Medicine* **61**, 423
Moore W E & Foster J F (1968) *Biochemistry* **7**, 3409
Ohkubo A (1969) *Journal of Biochemistry* **65**, 879
Pavelka K (1971) In: Rheumatoid Arthritis: Pathogenic Mechanisms and Consequences in Therapeutics. Ed. W Muller, H Harwerth & K Fehr. Academic Press, London; p 365
Planas-Bohne F (1973) *Research in Experimental Medicine* **161**, 289
Sorenson J R J (1976) *Journal of Medicinal Chemistry* **19**, 135
Sugiura Y & Tanaka H (1972) *Molecular Pharmacology* **8**, 249
Thomas J & Evans P H (1975) *Clinica chimica acta* **60**, 237
Wagner J C & McCormick J N (1967) *Journal of the Royal College of Physicians of London* **2**, 49
Watkins J & Swannell A J (1973) *Annals of the Rheumatic Diseases* **32**, 247
Wilding P, Kendall M J, Holder R, Grimes J A & Farr M (1975) *Clinica chimica acta* **64**, 185

DISCUSSION

Dr D R Stanworth (*Birmingham*): Has Dr Evans looked for auto-antibodies against altered albumin in rheumatoid patients? If there is conformational alteration taking place there is a possibility that anti-albumins would be seen in the same way as we see antigammaglobulins.

Dr Evans: We have not looked at them but there are reports in the literature of autoimmune bodies to albumin.