On the effects of antirheumatic drugs on protein sulphydryl reactivity in human serum

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Hall and Gillan (1979), using single point estimations, suggest that stimulation of sulphydryl-disulphide exchange reactions in vivo may distinguish 'specific antirheumatic' drugs from non-steroidal anti-inflammatory agents (NSAIA's) in the treatment of rheumatoid arthritis (RA).

We feel that the significance of this 'rate reaction' is questionable since the increase they observed in untreated rheumatoid patients compared with normal subjects increased further away from normality with specific antirheumatic drug therapy. Moreover patients satisfied with NSAIA's alone usually have a lower level of disease activity than those requiring specific antirheumatic drugs. Thus, the differences in reaction rate observed between the various groups in Fig. 1 of Hall & Gillan's paper may simply be a reflection of differences in disease severity.

As the authors point out, Gerber et al (1967) have shown that high dose aspirin affects sulphydryl reactivity which perhaps suggests that sulphydryl exchange reactions do not differentiate between specific antirheumatic and NSAIA's. To evaluate this further we have made use of a similar, though not directly comparable, reaction in making serial assessments on patients with RA during their first 6 months' treatment with both specific antirheumatic drugs and aspirin.

Sulphydryl concentrations were measured spectrophotometrically immediately after each clinic visit by diluting serum 1:10 with phosphate buffer (pH 7·4), incubating for 15 min at 37°C and mixing 2 ml of the dilution with 0·5 ml of 1 mM 5,5¹-dithiobis(2-nitrobenzoic acid), (DTNB). This solution was read (440 nm) against a blank containing 2 ml diluted serum mixed with buffer (0·5 ml). The reaction was allowed to proceed at 37°C until equilibrium was reached (10–15 min).

The mean results (± s.e.) for groups of 15 patients on each drug at each clinic visit are shown in Fig. 1. All patients had classical or definite RA (ARA Criteria), shown to be active as defined by the presence of at least three of the following: (a) tenderness of >6 joints. (b) swelling of >3 joints. (c) morning stiffness >45 min. (d) articular index > 20. (e) ESR >28 mm h^{-1}.

The drug regimes were: D-penicillamine, 125 mg day^{-1} increasing to 500 mg day^{-1} by week 8. Hydroxychloroquine, 200 mg twice daily. Sodium aurothiomalate, 50 mg per week until 1 g has been given, then 50 mg per month i.m. Alclofenac, 1 g three times daily. Salazopyrine, 1·5 g day^{-1} increasing to 3 g day^{-1} by week 8.

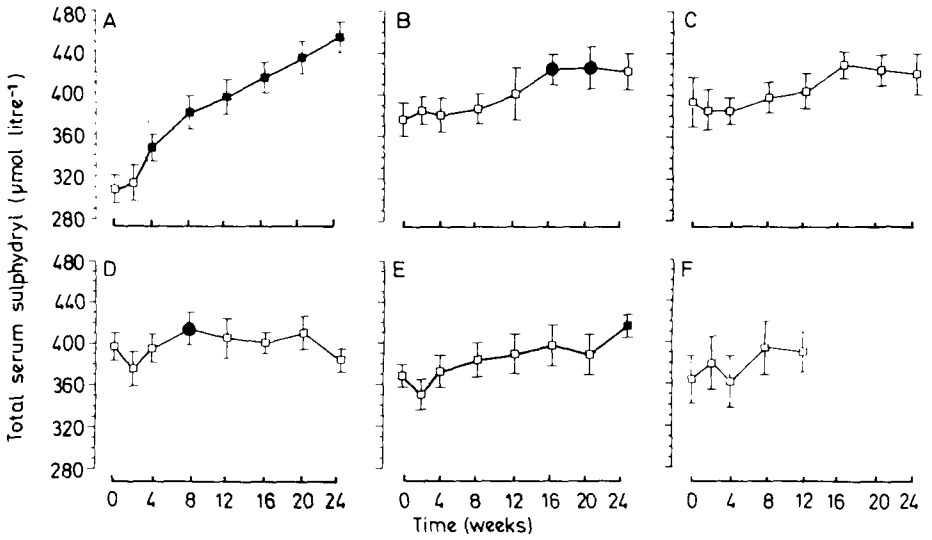

FIG. 1. Serum total sulphydryl concentrations (μ mol $litre^{-1}$) following specific antirheumatic and non-steroidal anti-inflammatory drug therapy. A. D-Penicillamine. B. Sodium aurothiomalate. C. Salazopyrine. D. Hydroxychloroquine. E. Alclofenac. F. Aspirin. ● Significant change ($P<0·05$) from week 0 (Wilcoxon Rank Sum Test for paired data). ■ Significant change ($P<0·01$) from week 0.

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Aspirin, maximum tolerated dose (1·8→3·6 g day^{-1}) (12 weeks). Supplementary Nuseal aspirin was permitted as required in all the groups.

Total serum sulphydryl concentrations are consistently low in RA compared with age and sex matched normal subjects (Dixon et al 1980). With treatment with D-penicillamine, gold, and salazopyrine, return towards normality (450–600 μmol litre^{-1}) occurs along with clinical improvement, irrespective of whether the drug molecule contains a thiol group (Fig. 1). However, comparable changes are seen during treatment with aspirin and alclofenac, drugs for which clinical improvement was not generally observed (Bird et al 1980). Also minimal change in serum sulphydryl occurred despite clinical improvement following hydroxychloroquine therapy. Although total serum sulphydryl level is a useful index of disease activity following therapy with some drugs such as D-penicillamine (Dixon et al 1980), we have found serum sulphydryl concentrations less reliable in monitoring long-term clinical changes than the acute phase reactants (Bird et al 1980; Dixon et al 1980; McConkey et al 1973).

The demonstrated effect of aspirin on total sulphydryl concentrations would also substantiate the non-specificity of this reaction.

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Differential pulse polarographic determination of cephalexin after hydrolysis in neutral phosphate buffer

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Cephalosporins having a substituted methyl group in the 3-position, or having another polarographically reducible group, can be determined by differential pulse polarography (d.p.p.) (Fogg et al 1979a). Cephalexin is one of a small group of cephalosporins that have an unsubstituted 3-methyl group and that are not reduced polarographically. Several degradation products of cephalexin, however, have been shown to give d.p.p. peaks and in some cases the products responsible for particular peaks have been identified (Fogg et al 1979a, b). In the present work the possibility of using the height of one of these peaks to determine cephalexin after hydrolysis under controlled conditions has been investigated.

A fluorimetric procedure for the determination of cephalexin based on sequential hydrolysis in 0·3M sodium hydroxide solution then in pH 5 buffer containing formaldehyde has been developed (Barbhaiya & Turner 1977). The fluorescent hydrolysis product, 2-hydroxy-3-phenyl-6-methylpyrazine (Barbhaiya et al 1978), is also given by other α-aminobenzylcephalosporins, such as cephradine and cephaloglycin, under similar conditions (Barbhaiya & Turner 1977) and also by α-aminobenzylpenicillin (ampicillin) on hydrolysis at pH 4 (Barbhaiya et al 1978). The d.p.p. peak of 2-hydroxy-3-phenyl-6-methylpyrazine has been identified in d.p. polarograms of degrading cephalexin solutions by comparison with a known sample, and has been used to study its formation in solutions of different pH (Fogg et al 1979b). In the present work its formation under conditions used in the fluorimetric assay has been followed by d.p.p. and a d.p.p. procedure for determining cephalexin based on this reaction has been assessed.

In our recent d.p.p. study of the degradation of cephalexin (Fogg et al 1979b) a high yield of a compound, believed to be a carbonyl compound, responsible for a d.p.p. peak at −1·26 V was observed on carrying out the degradation at 80 °C in pH 7·4 phosphate buffer for 1 h. A reliable d.p.p. procedure for the determination of cephalexin based on the formation of this compound has been developed here.

A DC polarographic procedure for the determination of cephalexin at the 10^{-3} M level after hydrolysis in 5M hydrochloric acid solution at 80 °C for 15 min has been described (Squella et al 1978). This procedure has also been studied here with a view to adapting it for use with the differential pulse mode at lower concentrations.

Measurements were made with a PAR 174 polarographic analyser (Princeton Applied Research Corp.). For d.p.p. operation, a forced drop time of 0·5 s, a scan rate of 5 mVs^{-1} and a pulse height of 50 mV were used. Three-electrode operation was employed with a dropping mercury electrode, a platinum counter electrode and a saturated calomel reference electrode. The water-jacketted polarographic cell was kept at 25 °C. Solutions for polarography were deoxygenated with nitrogen gas which had previously been passed through a vanadium-(II) scrubber.

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