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.

January 18, 1980

REFERENCES

Bird, H. A., Dixon, J. S., Pickup, M. E., Lee, M. R., Wright, V. (1980) Ann. Rheum. Dis. in the press

Dixon, J. S., Pickup, M. E., Lowe, J. R., Hallett, C., Lee, M. R., Wright, V. (1980) Ibid. in the press

Gerber, D. A., Cohen, N., Giustra, R. (1967) Biochem. Pharmacol. 16: 115–123

Hall, N. D., Gillan, A. H. (1979) J. Pharm. Pharmacol. 31: 676–680

McConkey, B., Crockson, R. A., Crockson, A. P., Wilkinson, A. R. (1973) Q.J. Med. 42: 785–791

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

A. G. FOGG*, N. M. FAYAD, R. N. GOYAL†, *Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, U.K.*

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.

* Correspondence.
† On leave from Roorkee University, India.

The phosphate buffer was prepared by adjusting a 0·5 M disodium hydrogen phosphate solution to pH 7·4 by addition of 0·5 M potassium dihydrogen phosphate solution.

Procedure. Into 10 ml calibrated flasks add aliquots (<5 ml) of neutral cephalexin solution (standards or samples) containing less than 30 μg of cephalexin, dilute the solutions to 10 ml with pH 7·4 buffer, and heat to 100 °C for 1 h. Transfer the cooled solution to the polarographic cell, deoxygenate the solution (5 min) and obtain the d.p. polarogram between −1·1 and −1·4 V at 25 °C.

The calibration graph obtained for the range 0·2–3 μg ml^{-1} of cephalexin in the measured solution was rectilinear with a coefficient of variation (10 determinations) at the 2 μg ml^{-1} level of 3·6%. The heating time (at 100 °C) must be controlled (see Fogg et al 1979b). Although negligible error occurs for times between 55–75 min, peak heights obtained after 45 and 90 min heating were 10% and 5% low respectively. Removal of dissolved oxygen from the solution before hydrolysis had no effect on the height of the peak subsequently obtained. Such peak heights, however, remained constant for 2·5 h at 25 °C.

The degraded solutions gave a positive test with 2,4-dinitrophenylhydrazine. Further the peak at −1·26 V is decreased in steps on the drop-wise addition of Schiff's reagent, and disappears completely on the addition of an excess amount of this reagent or a few drops of 45% sodium bisulphite solution. The d.p.p. peaks of the other degradation products that are present, including 2-hydroxy-3-phenyl-6-methylpyrazine, are unaffected by such reagents.

The effect of the mercury height on the limiting current using DC polarography, shows that the wave is largely but not fully diffusion controlled, and probably has some residual solution kinetic character: this might be expected if the carbonyl compound is an aldehyde. The temperature coefficient of the wave is small (1·5% per degree) and the peak is rather broad indicating a high degree of irreversibility in the reduction reaction. The peak potential is independent of pH.

Attempts at using the hydrolysis conditions of Barbhaiya & Turner (1977) were unsuccessful and led to unreliable results when the resultant solutions were examined by differential pulse polarography. Under these conditions 2-hydroxy-3-phenyl-6-methylpyrazine is the product (Barbaiya et al 1978). The same unsatisfactory results were obtained using the hydrolysis conditions of Squella et al (1978).

The recommended procedure based on hydrolysis in pH 7·4 buffer provides a reliable and precise method of determining cephalexin. The initial diketopiperazine derivative formed, 2-[6-phenyl-2,5-dioxo-3-piperazinyl]-5,6-dihydro-5-methyl-2H-1,3-thiazine-4-carboxylic acid, and possibly certain other degradation products, however, if present in the cephalexin sample would produce the carbonyl compound responsible for the peak at −1·26 V on hydrolysis (Fogg et al 1979b) and would interfere. The former compound, however, can be tested for by polarographing the solution before heating at 100 °C, as it gives a d.p.p. peak at −0·9 V and its concentration could be determined and allowed for. It is envisaged, therefore, that as well as for the determination of low concentrations of cephalexin in degradation free systems, the d.p. polarographic method could be used with advantage in degradation and metabolic studies.

Although the compound responsible for the d.p.p. peak used in the recommended procedure is known to be a carbonyl compound, it has not so far been identified. We suspect that this is formed when 3-hydroxy-4-methyl-2(5H)-thiophenone, recently isolated from degraded cephalexin solutions (Dinner 1977), degrades to give hydrogen sulphide (Fogg et al 1979b). Further studies are in progress to identify this and other degradation products giving polarographic peaks.

The authors wish to thank Dr C. Burgess of Glaxo Operations (U.K.) Ltd. for the provision of samples and useful discussion. One of us (RNG) thanks the University of Roorkee for study leave and the British Council for financial support.

November 30, 1979

REFERENCES

Barbhaiya, R. H., Turner, P. (1977) Br. J. Clin. Pharmacol. 4: 427–431

Barbhaiya, R. H., Brown, R. C., Payling, D. W., Turner, P. (1978) J. Pharm. Pharmacol. 30: 224–227

Dinner, A. (1977) J. Med. Chem. 20: 963–965

Fogg, A. G., Fayad, N. M., Burgess, C., McGlynn, A. (1979a) Anal. Chim. Acta 108: 205–211

Fogg, A. G., Fayad, N. M., Burgess, C. (1979 b) Ibid. 110: 107–115

Squella, J. A., Nunez-Vergam, L. J., Gonzalez, E. M. (1978) J. Pharm. Sci. 67: 1466–7