MEGEL, H., RAYCHAUDHURI, A., SHEMANO, I., BEAVER, T. H. & THOMAS, L. L. (1975). *Ibid.*, **149**, 89–93.
SMITH, M. J. H. & FORD-HUTCHINSON, A. W. (1975). *Agents and Actions*, **5**, 318–321.
VANE, J. R. (1973). *Adv.Biosciences*, **9**, 395–411.
WALKER, J. R., SMITH, M. J. H. & FORD-HUTCHINSON, A. W. (1976). *Agents and Actions*, in the press.
WALKER, J. R., SMITH, M. J. H., FORD-HUTCHINSON, A. W. & BILLIMORIA, F. J. (1975). *Nature*, **254**, 444–446.

Fluorimetric determination of cephalexin

R. H. BARBHAIYA*, P. TURNER, *Department of Clinical Pharmacology, St. Bartholomew's Hospital, London EC1A 7BE, U.K.*

Previous methods for the quantitative measurement of cephalexin include ultraviolet spectrophotometry (Marrelli, 1972; Kirschbaum, 1974) and hydroxamate colour formation (Mays, Bangert & others, 1975). Because these methods are of low sensitivity, microbiological assays have generally been used for the determination of cephalexin at the low concentrations, encountered in biological fluids. On alkaline hydrolysis the β-lactam ring of cephalexin opens and the corresponding cephalosporoate is formed which on heating yields a fluorescent derivative (Cohen, Funke & Puar, 1973; Indelicato, Norvilas & others, 1974; Yamana, Tsuji & others, 1974). This has been made the basis of a fluorimetric assay procedure.

Cephalexin monohydrate of reference standard quality (Lilly) was used for fluorimetric determinations. All other chemicals used were of analytical grade.

Citrate buffer pH 5·0: 42·0 g citric acid was dissolved in 204·0 ml of 2 N NaOH and the mixture was diluted to 1 litre with double distilled water.

Formaldehyde (10·0 ml) was mixed with 990·0 ml citrate buffer pH 5·0 to make 1% formaldehyde solution in the buffer.

Quinine solution (1·0 μg ml^{-1}) was prepared in 0·1 N sulphuric acid as a standard fluorescence solution.

Method in aqueous solution. 1·0 ml aqueous solution containing cephalexin was pipetted into a test tube containing 0·5 ml N NaOH; 10 min later 0·5 ml N HCl was added followed by 3·0 ml of pH 5·0 citric acid-sodium hydroxide buffer containing 1% formaldehyde. A fluorescent product was obtained by heating for 30 min at 100° the tubes being covered with loosely fitting polythene balls to minimize evaporation. After the tubes had been cooled at 20° for 10 min, the fluorescence intensity was measured using a Baird Atomic Fluoripoint Spectrofluorimeter with a Xenon lamp at 420 nm (excitation 345 nm).

Method for plasma. To 1·0 ml plasma, 0·8 ml 20% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 3000 rev min^{-1} (10 min). 1·0 ml of supernatant was pipetted into a test tube containing 0·5 ml 2 N NaOH and after 10 min 0·5 ml 2 N HCl was added, followed by 3·0 ml citric acid-sodium hydroxide buffer (pH 5·0) containing 1% formaldehyde. A fluorescent product was obtained from this solution and its intensity measured under the conditions described for the aqueous solution above. A blank value was determined by treating cephalexin free plasma in the same way.

The excitation and emission spectra of the fluorescent product are shown in Fig. 1. Profiles of the product formed from analytical cephalexin and from cephalexin obtained from plasma are identical.

Variation of fluorescence with pH suggested that measurements should be made at pH 5. At this value the fluorescent derivative is formed after between 30 and 50 min incubation at 100°. The necessity to incorporate 1% formaldehyde in the buffer solution is shown by a drop in fluorescent yield under the assay conditions when the formaldehyde is omitted (from 120 to 65 units).

A linear dependence of fluorescence intensity on cephalexin concentration is obtained between zero and 20 μg ml^{-1} in the aqueous solution and in the pooled human plasma (regresium equation is $y = 8{\cdot}7 + 22{\cdot}4\alpha$).

The precise chemical identity of the fluorescent alkali degradation product is uncertain, but there is evidence to suggest that it may be the diketopiperazine derivative. Intramolecular nucleophilic attack of the α-amino group on the side chain of the cephalexin has been suggested as a decomposition mechanism for the formation of the fluorescent derivative (Cohen & others, 1973; Indelicato & others, 1974; Yamana & others, 1974). Addition of formaldehyde catalyses the reaction by reducing the basicity of the amino group (Jusko, 1971). Alkaline hydrolysis of ampicillin followed by heating at 100° in pH 5·0 buffer containing 1% formaldehyde results in a fluorescent derivative which possesses identical excita-

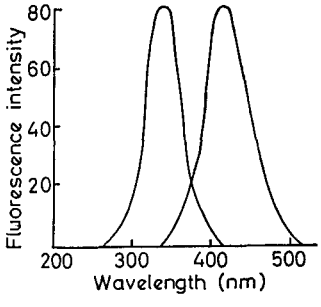

FIG. 1. Spectrophotofluorimetric excitation (345 nm) and emission (420 nm) spectra for the fluorescence product of cephalexin.

* Correspondence.

tion and emission spectra to those obtained with cephalexin (Barbhaiya & Turner, unpublished observations).

As cephalexin does not appear to undergo metabolism in man (Kirby, DeMaine & Serill, 1971; Brogard, Pinget & others, 1975) this fluorimetric assay may prove to be satisfactory for kinetic studies and routine estimations of the drug in human plasma.

The authors wish to thank Lilly Research Centre Limited for a donation of cephalexin and Mr D. Hall for helpful discussions. The kind help of Dr Annemarie Hedges in the preparation of this manuscript is also acknowledged.

June 21, 1976

REFERENCES

BROGARD, J., PINGET, M., DORNER, M. & LAVILLAUREIX, J. (1975). *J. clin. Pharm.*, **15**, 666–673.
COHEN, A., FUNKE, P. & PUAR, M. (1973). *J. pharm. Sci.*, **62**, 1559–1561.
INDELICATO, J., NORVILAS, T., PFEIFFER, R., WHEELER, W. & WILHAM, W. (1974), *J. medl Chem.*, **17**, 523–527.
JUSKO, W. J. (1971). *J. pharm. Sci.*, **60**, 728–732.
KIRBY, W. M. M., DEMAINE, J. B. & SERILL, W. S. (1971). *Postgrad. med. J.*, **475**, 41–46.
KIRSCHBAUM, J. (1974). *J. pharm. Sci.*, **63**, 923–924.
MARRELLI, L. P. (1972). *Ibid.*, **61**, 1647–1648.
MAYS, D. L., BANGERNT, F. K., CANTRELL, W. C. & EVANS, W. G. (1975). *Analyt Chem.*, **47**, 2229–2234.
YAMANA, T., TSUJI, A., KANAYAMA, K. & NAKONO, O. (1974). *J. Antibiotics*, **27**, 1000–1002.

LETTERS TO THE EDITOR

Acute rheumatoid arthritis developing in a patient treated with salmon calcitonin: evidence against a major anti-inflammatory action of this hormone

N. J. Y. WOODHOUSE*, S. M. MOHAMEDALLY, F. SAEDNEJAD, *Department of Medicine, King's College Hospital Medical School, Denmark Hill, London, SE5 8RX, U.K.*

Calcitonin (CT) is known to reduce the increased vascular permeability produced by dextran in the rat paw. This anti-inflammatory action is independent of the fall in serum calcium and is inhibited by β-adrenergic blockade (Reisterer & Jaques, 1970). Salmon CT has also been shown to reduce carrageenan oedema and the severity of Freund's adjuvant arthritis in rats. Inhibition of prostaglandin release was postulated as a possible mechanism for these effects (Velo, De Bastiani & others, 1975). The same authors also reported clinical improvement in some patients with rheumatoid arthritis treated with salmon CT. These results suggest a possible therapeutic role for CT in certain inflammatory disorders. The present report, however, describes the development of acute rheumatoid arthritis in a patient whose Paget's disease was being successfully controlled by daily subcutaneous injections of salmon CT.

The patient, age 67 years, presented in 1973 complaining of pain in the left forearm and tibia which had been present since 1945. Deformity of the affected bones was first noticed in the mid-1950s and Paget's disease was diagnosed in 1958 by radiographs and bone biopsy. Progressive pain in the ankle joint and deformity of the tibia necessitated an arthrodesis in 1964 and a tibial oesteotomy in 1967. The tibia remained painful and she regularly consumed panadol and DF 118 (dihydrocodeine tartrate) tablets. Previous medication included butazolidine, sodium fluoride and mithramycin. Temporary relief of bone pain had occurred with the

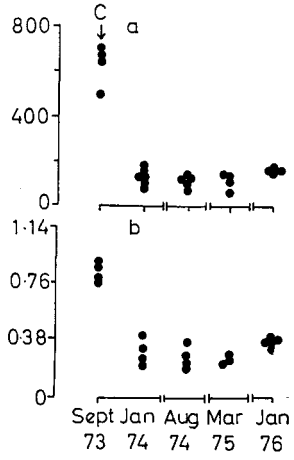

FIG. 1. Patient with active Paget's disease before and during therapy with salmon calcitonin. The individual a-serum alkaline phosphatase concentrations (IU litre^{-1}) are shown (normal range 30–85) and b-urinary total hydroxyproline concentrations (mM day^{-1}) (normal range 0·11–0·34). C-Control values.

* Correspondence.